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Review article

Fennel (*Foeniculum vulgare* Mill.) in Polish herbaria of the 16th-18th centuries: An ethnopharmacological analysis in the context of contemporary knowledge

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ABSTRACT

*This study aimed to analyse the use of common fennel (*Foeniculum vulgare* Mill.) in Polish herbaria of the 16th-18th centuries and to compare historical ethnopharmacological knowledge with contemporary scientific data. The research involved a theoretical ethnopharmacological analysis based on the works of renowned Polish botanists such as S. Falimirz, H. Spiczynski, M. z Urzędowa, S. Syreński and K. Kluk. Digitised versions of herbaria, alongside modern scientific publications, were employed to assess the pharmacological properties of fennel. The primary objective was to compare historical data with contemporary publications, particularly those detailing fennel's chemical composition and pharmacological properties, including its key active compounds such as anethole, fenchone, and limonene. The results of the study indicated that fennel has traditionally been used as a remedy for the digestive and respiratory systems, a use that is corroborated by contemporary scientific data on its antimicrobial, antispasmodic, anti-inflammatory, and antioxidant properties. Historical records suggest that fennel was employed to alleviate pain and treat coughs, colic, and bloating. The study also revealed a significant nutritional content in fennel fruits, including proteins (15.8 g), fats (14.9 g), and carbohydrates (36.6 g) per 100 g, as well as essential oils, which contribute to its medicinal properties. Particular attention was given to the high concentration of trans-anethole (up to 83.1%) in sweet fennel, making it effective in treating stomach disorders, bloating, and other gastrointestinal issues. The findings underscore the importance of fennel as a modern herbal remedy with the potential for use in treating serious conditions such as oxidative stress and cancer. The study opens up opportunities for further research into the potential of this plant in modern medicine.*

Keywords:Antimicrobial properties, botany, digestive disorders, fennel, phytopreparations, traditional medicine,

INTRODUCTION

Fennel (*Foeniculum vulgare* Mill.) is a plant that has been an important part of traditional medicine in many cultures for centuries due to its healing properties. Its medicinal characteristics, such as antiseptic, anti-inflammatory and antispasmodic properties, have been known since ancient times, and it has been used to treat various diseases, including digestive and respiratory disorders. In view of this, fennel is a valuable plant not

only from a medical, but also from a cultural and historical point of view.

The relevance of the study is to compare historical knowledge about fennel with modern scientific data. In the 16th-18th centuries, fennel was widely used in Polish herbalists to treat a variety of ailments, which emphasizes its importance in folk medicine at that time (Komilova *et al.*, 2020). This study aims to analyze the use of fennel in Polish herbaria and compare this

historical data with modern scientific knowledge about the chemical composition and medicinal properties of the plant. Specific problems that this study seeks to address include the uncertainty in how ancient ethnopharmacological knowledge can be reconciled with the latest scientific discoveries. In addition, it is important to consider how changes in the growing conditions of fennel affect its chemical composition and, consequently, its efficacy for medicinal purposes. Another important issue is the need to investigate possible contraindications and side effects of fennel, as modern approaches to medicine emphasize the safe use of herbal remedies, especially in the context of modern diseases and therapies.

Common fennel (*Foeniculum vulgare* Mill.) is one of the most widely used medicinal plants, employed for centuries in various traditional medical systems, particularly in Europe. Its healing properties are extensively described in the studies of numerous botanists and herbalists from the 16th-18th centuries, including Falimirz(1534), Spiczyński(1542), Urzędowa(1595), Syreński(1613) and Kluk(1786). These scholars included fennel in their herbals as a remedy for digestive issues, respiratory ailments, and to alleviate inflammation. Their works made significant contributions to the development of European ethnopharmacology and continue to attract the attention of modern researchers seeking to uncover new applications for fennel based on historical knowledge. Traditionally, fennel has been used to treat a wide range of conditions, such as bloating, colic, coughs, and various digestive disorders (Jadid *et al.*,2023; Zotaj *et al.*, 2024).

Contemporary scientific research continues to contribute new insights into the study of fennel. Notably, Benabdallah *et al.*(2022) and Kishore and Verma(2022) have established that the primary biologically active components of this plant, such as anethole, fenchone, and limonene, are

responsible for its pharmacological properties, including anti-inflammatory, antispasmodic, and antibacterial effects. An important aspect is fennel's potential in cancer treatment, as demonstrated by the research of Amiza *et al.*(2022). Trans-anethole, the main component of fennel, has shown the ability to inhibit tumour cell growth and induce apoptosis (programmed cell death) in cancer cells. Although these studies have been conducted primarily at the preclinical level, they open new possibilities for the use of fennel in oncology and warrant further confirmation through clinical trials.

In addition to its anti-inflammatory and antispasmodic properties, fennel also possesses potent antioxidant activity. As noted in the research by Noreen *et al.*(2024), the essential oils of fennel, particularly limonene, exhibit high antioxidant potential, making this plant a promising agent for preventing oxidative stress and combating oncological diseases. Investigating the antioxidant properties of fennel is a crucial area of research, as it may contribute to the development of new phytotherapeutic agents for the treatment and prevention of various diseases, including chronic conditions associated with oxidative stress.

Despite significant advancements in fennel research, there remain certain unresolved questions requiring further investigation. For instance, Telci *et al.*(2009) highlight variations in the chemical composition of fennel based on climatic conditions, growth stage, and the origin of the raw material, which can influence its pharmacological activity and stability. This underscores the need for additional research to ensure the standardisation of fennel as a medicinal raw material and enhance its efficacy across diverse applications.

Furthermore, fennel has found its niche not only in medicine but also in cosmetics due to its antimicrobial and antioxidant properties. Its extracts are utilised in skincare products as they contribute to reducing inflammation and promoting skin rejuvenation, as detailed in the research of Badgujar *et al.*(2014).

These attributes further underscore the versatility of fennel as a plant with a broad spectrum of applications.

This study aimed to conduct a comprehensive analysis of common fennel (*Foeniculum vulgare* Mill.) based on Polish herbaria from the 16th-18th centuries and contemporary scientific research, to compare ethnopharmacological knowledge with current data on the pharmacological properties of this plant.

The research objectives encompass: conducting a historical analysis of 16th to 18th-century herbaria to explore traditional uses of fennel; comparing the pharmacological properties of fennel described in ancient sources with contemporary scientific data; and evaluating the potential of fennel for further research in modern medicine and cosmetics.

MATERIALS AND METHODS

The research into common fennel (*Foeniculum vulgare* Mill.) in Polish herbaria of the 16th-18th centuries was conducted in the format of theoretical ethnopharmacological analysis, based on existing historical and contemporary sources at the University of Agriculture in Krakow Faculty of Biotechnology and Horticulture.

The foundation for the historical analysis consisted of Polish herbaria of the 16th-18th centuries, created by prominent botanists of the Renaissance and later periods. Specifically, the research sample included works by the following authors: Falimirz (1534), Spiczyński (1542), Urzędowa (1595), Syreński (1613), and by Kluk (1786).

The following key electronic resources and software were employed to analyse historical sources, enabling access to digital versions of ancient herbaria and contemporary research articles: Polona – the National Digital Library of Poland, Jagiellonian Digital Library – the digital library of Jagiellonian University, Google Books – a

database of digital books, and Europeana – the European digital platform.

The following software was employed for text processing and analysis: Adobe Acrobat Reader was used to view, annotate, and work with digital PDF files of ancient herbaria. Zotero, a bibliographic management software, was used to organise research sources and create bibliographic references. NVivo, a qualitative data analysis software, enabled the coding and analysis of the content of digital herbaria, identifying key terms related to fennel, and comparing them across different sources.

The following databases were utilised for searching and collecting scientific literature: PubMed was employed to locate contemporary research articles on ethnopharmacology, pharmacognosy, and medicine related to the biochemical composition and modern uses of fennel. Google Scholar served as the primary tool for searching scientific papers, articles, and reviews, enabling the identification of the most relevant sources for comparative analysis of fennel. ScienceDirect, a database specialising in medical and pharmacological publications, complemented the data on the chemical properties and pharmacological applications of fennel.

The search queries included the following terms: “*Foeniculum vulgare*”, “fennel”, “ethnopharmacology”, “pharmacognosy”, “biochemical composition”, “pharmacological uses of fennel”, and various combinations of these terms. The primary criteria for inclusion in the sample were: articles containing original data on the biochemical composition and uses of fennel; publications in peer-reviewed scientific journals up to 2024; and open-access sources to ensure data verification. To compare and analyse the use of fennel in contemporary ethnopharmacology, scientific publications highlighting the biochemical composition, pharmacological properties, and modern medicinal applications of fennel were examined. Particular attention was paid to the key active compounds of the plant, such

as anethole, fenchone, and limonene, which corroborate traditional knowledge regarding the plant's medicinal properties.

A comparative method was employed to analyse data by contrasting ancient (16th-18th centuries) and modern pharmacological knowledge. The comparison was based on examining the similarities and differences between the properties of fennel described in ancient herbals and their scientific validation through contemporary research. The selection of materials for analysis was based on several inclusion criteria: Polish herbaria had to contain specific descriptions of the medicinal use of fennel; modern scientific articles had to illuminate the plant's chemical composition and pharmacological properties; ancient works where fennel was mentioned only in passing or without specific medical characteristics were excluded.

No physical equipment was used, as the research was entirely theoretical.

RESULTS AND DISCUSSION

Ethnopharmacology and contemporary pharmacology

Ethnopharmacology is considered an interdisciplinary field of science that combines natural and humanities disciplines. Its primary aim is to study and describe traditional knowledge concerning the use of medicinal plants and other natural materials in various ethnic groups, and to assess their efficacy from the perspective of modern pharmacology. Ethnopharmacology seeks to determine whether contemporary scientific data supports or refutes the traditional knowledge of plants used for medicinal purposes (Schapovalova *et al.*, 2022).

Pharmacology, the science of the effects of medicinal drugs on living organisms, began to develop significantly in the 19th century when biomedical sciences opened new avenues for studying plants, animals, and minerals used in traditional medicine. Consequently, pharmacology investigates substances that, through chemical reactions,

can stimulate or inhibit physiological processes in the body, influencing antigens or other pathogenic agents (Pencheva & Danyliv, 2022; Sanli & Ok, 2023).

Throughout the 19th century, considerable attention was given to plant extracts, particularly quinine, digitalis, and morphine, which formed the basis for further research in pharmacology. The rapid advancement of pharmacology as a science was driven by the discovery of new classes of drugs in the first half of the 20th century, which, in turn, led to significant breakthroughs in the treatment of various diseases (Gerasymchuk *et al.*, 2021; Somasiri *et al.*, 2024). At the present stage, ethnopharmacology primarily focuses on researching traditional medicines of non-European peoples; however, it also draws upon the experience of European traditional medicine as recorded in herbaria of the 16th-18th centuries, which serves as a fundamental source of knowledge regarding the use of medicinal plants. Documenting and restoring this knowledge are crucial tasks for ethnopharmacology.

Analysis of the use of fennel in Polish herbaria of the 16th-18th centuries

A study of common fennel (*Foeniculum vulgare* Mill.), based on an examination of Polish herbaria of the 16th-18th centuries, has confirmed the plant's significant role in traditional Polish medicine. It was widely used for treating gastrointestinal ailments, respiratory diseases, and to alleviate symptoms of inflammation and swelling. Falimirz, Spiczynski, Urzędowa, Syreński and Kluk emphasized fennel's role as a versatile medicinal remedy.

In folk medicine, fennel was used to relieve spasms, relieve inflammation, and as an expectorant for coughs (Topchubaeva *et al.*, 2020; Smiyan *et al.*, 2015). These properties have not lost their relevance in modern science, although today these statements are also supported by the results of clinical studies and scientific experiments. From a

modern point of view, fennel is a nutritious plant rich in vitamins, minerals, and fiber. According to the USDA, 100 g of fresh fennel contains 15.8 g of protein, 14.9 g of fat, and 36.6 g of carbohydrates. These nutritional components make fennel an important element of the diet, providing the body with essential macronutrients that support overall health and energy balance. The vitamin C it contains has powerful antioxidant properties that help protect cells from damage and support the immune system.

Fennel is also recognized by the European Food Safety Authority as a health benefit, particularly for improving digestion and supporting gastrointestinal health. The properties of fennel that help relieve bloating and discomfort are supported by modern scientific research. Fennel contains compounds such as anethole, which has an antispasmodic effect, reduces bloating and helps to normalize intestinal motility (Zubtsova & Skliar, 2023). Modern approaches to health emphasize the fight against inflammation, and fennel plays an important role in this context due to its anti-inflammatory properties.

Falimirz (1534), in his work "About Herbs and the Power of Them", describes fennel as one of the key remedies for improving digestion. He recommended it for the treatment of colic and bloating, while also emphasising its diuretic properties. The use of fennel for kidney cleansing and reducing swelling indicates its widespread application in addressing fluid imbalance within the body. Furthermore, Falimirz highlights fennel's ability to alleviate coughs, underscoring its role in the treatment of respiratory ailments, particularly colds and coughs.

Spiczyński (1542), in his work "About Local and Overseas Herbs and the Power of Them", pays particular attention to fennel's ability to treat stomach ailments and stimulate appetite. The author highlights its versatility, emphasising fennel's antiseptic properties. Its use for wound healing and

relieving abdominal pain in children is corroborated by contemporary scientific research. Modern studies suggest that these properties of fennel can be attributed to the presence of anethole in its essential oil, which exhibits potent anti-inflammatory and calming effects.

Similar findings regarding the properties of fennel are presented in "HerbarzPolski" by Urzędowa (1595), who also devotes significant attention to the treatment of digestive disorders, particularly colic and diarrhoea. The author recommends fennel to improve well-being after heavy meals, indicating its importance for maintaining digestive health. Additionally, he notes that fennel is effective in combating coughs and colds, particularly as an expectorant.

Syreński (1613), in his work "Herbarium", describes fennel as a remedy for improving appetite and treating stomach disorders. He recommends using fennel seeds to alleviate abdominal pain and prevent bloating. Modern science supports these properties of fennel, as it contains compounds such as fenchone and limonene, which have antispasmodic effects and promote digestion. Furthermore, Syreńskidescribes fennel as an effective remedy for treating eye infections, which is supported by contemporary knowledge of the antimicrobial properties of anethole.

Kluk(1786), in his work "Plant Dictionary", summarises the knowledge about fennel accumulated by the end of the 18th century and systematically categorises information about medicinal plants. He confirmed its significance as a medicinal plant for treating gastrointestinal disorders, inflammation, and respiratory infections. An important addition is the mention of fennel's use in veterinary medicine, indicating its value not only in human medicine but also in animal husbandry and agriculture.

These works demonstrate that fennel was an exceptionally important plant in early modern Polish medicine. Its versatility and medicinal properties were repeatedly

confirmed and have found support in contemporary scientific research.

Contemporary data on fennel (chemical composition and nutritional value)

Foeniculum vulgare, commonly known as fennel, has been extensively analysed to reveal its chemical composition, which exhibits a rich profile of nutrients and bioactive compounds. Chemical analysis by Bernáth *et al.* (1999) demonstrated that the chemical components within fennel vary depending on the source, plant morphotype, climate, and harvest stage. Per 100g of fennel fruit, there is approximately: 36.6 g of carbohydrates; 15.8 g of proteins; 14.9 g of fats; 8.8 g of water; 8.2 g of ash (containing 19 mg of iron, 1.2 g of calcium, 385 mg of magnesium, 1.7 g of potassium, 487 mg of fluorine, 88 mg of sodium, and 28 mg of zinc) 15.7g of fibre. Every 100g of fruit contains a certain amount of vitamins: niacin (6 mg), thiamine (0.41 mg), as well as vitamin A (135 IU) and riboflavin (0.35 mg), and a determined energy value of 1,440 kJ (Table 1). The primary constituents of fennel fruits are starch, mucilage, sugars, tannins, and essential oils. The most significant essential oils include oleic, petroselinic, palmitic, and linoleic acids. The studied plant also contains approximately 50% trans-anethole, 10-30% limonene, 10-20% fenchone (imparting a bitter taste to the fruits), 12-16% α -pinene with β -pinene, α -tujone, myrcene, estragole, and 1,8-cineole, and 3-11% α -phellandrene (Table 2). Fennel has a relatively high content of methyl chavicol (47.09%), as well as limonene (29.07%), α -terpinene (2.5%), fenchone (13.43%), fenchylacetate (1.95%), and cis- β -ocimene. Sweet varieties have a higher content of anethole (50-80%) and significantly lower concentrations of fenchone (5%, may be completely absent), with comparatively more safrole, limonene, pinene, and estragole.

Table 1: Chemical composition and nutritional value of different parts of

fennel: shoots, leaves, stems, and inflorescences (USDA, USA)

Fennel components	Per 100g edible portion	Fennel components	Per 100g edible portion
Energy (kcal)	19	Iron (mg)	2.7
Fats (g)	0.3	Vitamin A (mg)	783
Proteins (g)	1.4	Vitamin B1 (mg)	0.03
Carbohydrates (g)	2.8	Vitamin B2 (mg)	0.11
Dietary fibre (g)	2	Niacin (mg)	0.2
Potassium (mg)	395	Vitamin B6 (mg)	0.1
Calcium (mg)	38	Vitamin C (mg)	93
Magnesium (mg)	12	-	-

Source: compiled by the author based on Bernáth *et al.* (1999).

The high potassium content is particularly noteworthy, as potassium plays a crucial role in maintaining fluid balance and cellular functions. Fennel is also a good source of dietary fibre, which is well-known for its benefits in digestion, while its high phosphorus and magnesium content contributes to improved bone health and metabolic processes.

Table 2: Essential compounds obtained by distillation from sweet and bitter fennel fruits

Compound	Bitter fennel (%)*	Sweet fennel (%)**
Trans-anethole	55.0-75.0	79.8-83.1
Fenchone	12.0-25.0	4.6
Estragole	Max 6.0	3.9-5.1
α -pinene	1.0-10.0	0.3-3.6
Limonene	0.9-5.0	2.2-3.8
α -pinene/limonene	>1.0	-
CIS-Anethole	0.5% (max)	-
Anisaldehyde	2.0 (max)	-

Source: compiled by the author based on Baser *et al.* (2006).

The essential oils of both bitter and sweet fennel (Table 2) contain a range of

biologically active compounds whose concentrations vary depending on the plant's maturity stage and the time of year the fruits were harvested. Particularly significant are the differences in the concentrations of compounds such as trans-anethole and fenchone, as these influence the plant's taste and medicinal properties. Trans-anethole is the primary component of sweet fennel essential oil and is responsible for its sweet, anise-like flavour. The presented data show that trans-anethole concentration remains high at all stages of fruit maturation, especially in ripe and dry samples, where its content reaches 78.25% (January 2001) and 77.26% (May 2001). Fenchone, which is responsible for the bitter taste of bitter fennel, exhibits a significantly different

pattern. Its content is slightly lower in ripe and dry fruits, however, the maximum concentration of fenchone is observed in green fruits, where its proportion reaches 16.98% (May 2001). Such variations in chemical composition are important for the culinary and medicinal applications of fennel, as different concentrations of active components can influence the therapeutic efficacy of preparations or the flavour of spices produced from this plant (Table 3).

Table 3: Composition of the essential oil from three categories of fruits of *Foeniculum vulgare* var. *dulcis*

Compounds	Essential oil composition					
	January 2001			May 2001		
	Green	Mature	Dry	Green	Mature	Dry
α -Pinene	1.27	0.28	1.19	1.16	0.41	0.29
Mircene	0.93	0.50	0.72	0.95	0.49	0.41
Limonene	5.26	2.74	3.67	6.70	3.32	2.32
Fenchone	15.08	15.14	13.98	16.98	14.9	4.03
Metylchavicol	2.56	2.50	2.45	3.57		
Cis-Anethole	-	-	-	-	-	-
Trans-Anethole	73.81	77.67	78.25	69.67	76.01	77.26
β -Ocimene	-	-	-	-	-	-
γ -Terpinene	0.57					
Endo Fenchyl-acetate	-	-	-	-	-	-
ExoFenchyl-acetate	-	-	-	3.35	4.24	-

Source: compiled by the author based on Stefanini *et al.* (2006).

Therefore, the nutritional and chemical analysis of *Foeniculum vulgare* highlights its value as both a medicinal plant and a food additive. Its abundance of essential oils, particularly trans-anethole, indicates its anti-inflammatory, antimicrobial, and antioxidant properties. The high content of dietary fibre, vitamins, and minerals makes fennel a popular choice in traditional medicine, especially for improving digestion and supporting overall health. The data presented in the tables corroborate the historical use of fennel, as recorded in ancient herbaria of the 16th-18th centuries, and confirm its significance in modern medicine and nutrition.

The efficacy of fennel in treating diseases

Fennel (*Foeniculum vulgare*) has been used for centuries as an effective medicinal herb, particularly for the treatment of digestive and respiratory ailments. Its widespread use is supported not only by historical evidence but also by contemporary scientific research, which confirms the efficacy of this plant.

One of the primary historical and contemporary uses of fennel has been in the treatment of digestive disorders. Current research indicates that the active components of fennel, such as anethole and fenchone, possess spasmolytic properties, which help to relax the smooth muscles of the gastrointestinal tract. A study conducted by Portincasa *et al.* (2016), Rafieian *et al.* (2024) demonstrated that regular consumption of

fennel significantly alleviated the symptoms of irritable bowel syndrome, leading to an improved quality of life for patients. Fennel also plays a significant role in the treatment of respiratory ailments. Contemporary studies support these properties due to the high content of essential oils and flavonoids, which possess antibacterial and anti-inflammatory properties.

Fennel cultivation conditions, including soil type and climate, can significantly affect its chemical composition and, consequently, its medicinal properties. According to Telci *et al.* (2009), variations in these conditions can alter the concentration of active compounds in fennel, such as essential oils, flavonoids, and other bioactive components, which directly affects its therapeutic effects. The type of soil, in particular its pH, organic matter and mineral content, can affect plant growth and the synthesis of chemical compounds contained in fennel. For example, acidic soil can stimulate the accumulation of certain trace elements or compounds that contribute to the effectiveness of fennel when used as a medicinal plant. Although fennel is a widely used herb with numerous medicinal properties, its excessive use can cause side effects and contraindications. In addition, excessive consumption of fennel can lead to gastrointestinal disorders such as bloating, nausea, or even diarrhea, especially when using large doses. For people with sensitive digestive tracts or those with gastrointestinal conditions, fennel should be used with caution (Allaberdiev *et al.*, 2021; Niyazbekova *et al.*, 2023).

Furthermore, fennel has demonstrated effectiveness in treating infant colic. A randomised controlled trial by Attarha *et al.* (2008) showed that fennel significantly reduces colic in infants due to its spasmolytic properties. Fenchone, one of the active components of fennel, has been shown to decrease spasms in the smooth muscles of the intestines, alleviating pain and discomfort. Other studies have also indicated that fennel helps to reduce bloating,

particularly when consumed regularly as a tea or infusion.

Thus, it is evident that the efficacy of fennel in treating disorders of the digestive and respiratory systems is supported by both historical and contemporary scientific evidence. Its antibacterial, antispasmodic, and anti-inflammatory properties render fennel a significant component of modern phytotherapy, particularly in the management of gastrointestinal and respiratory disorders.

Differences between historical and contemporary perspectives on fennel usage

Fennel is known for its antiseptic and healing properties, which is why it has been used to treat wounds. Fennel is known to have been used to treat cuts and wounds, as its extracts help reduce inflammation and accelerate healing. This is also confirmed by medical records, which describe its use for external use, in particular in the form of compresses or decoctions for washing wounds. Another important use of fennel is as an expectorant. Due to its anti-inflammatory and antiseptic properties, fennel has been widely used to treat respiratory diseases, in particular to relieve cough and shortness of breath. It has been used as a natural sputum expectorant, making it an important element in the treatment of colds, bronchitis, and other respiratory conditions.

Combining these diverse medical uses, fennel in Polish folk medicine of the 16th-18th centuries was extremely important as a versatile remedy used to solve a wide range of health problems. Its use in the treatment of digestive disorders, wound healing, and respiratory diseases demonstrates a deep understanding of the multifunctionality of this plant. Due to its diverse properties, fennel gained popularity in folk medicine as a remedy that could help treat everyday ailments, improve quality of life, and maintain overall health. Its significance in historical Polish medicine can be assessed

not only through its specific therapeutic effects, but also through its role in shaping the holistic picture of folk medicine of the time.

While most properties of fennel described in ancient herbaria have been corroborated by modern science, new aspects of this plant's use have emerged. For instance, contemporary research, notably by Chebet *et al.* (2021) and Noreen *et al.* (2024), highlights fennel's potential in combating cancer cells due to its antioxidant properties. Such properties were unknown during the Renaissance but are now actively investigated in the context of oncology. The antioxidant properties of limonene and other components of fennel's essential oil make it a promising agent for preventing oxidative stress (Belov *et al.*, 2005; Akha *et al.*, 2014). It inhibits the spread of cancer cells and promotes apoptosis.

Based on the results of the study, it can be concluded that fennel remains a valuable medicinal resource in contemporary medicine. Its multifaceted properties, supported by both historical records and modern research, indicate its potential for further investigation, particularly in the fields of oncology and cosmetology. The findings from this study of fennel (*Foeniculum vulgare* Mill.) corroborate its significant role in traditional medicine and contemporary phytotherapy, particularly in the treatment of digestive and respiratory disorders, as well as in cosmetic applications. This research aligns with other scientific studies that underscore fennel's rich chemical composition and broad spectrum of pharmacological properties.

A study by Akbari *et al.* (2023) investigated the phenolic composition and antioxidant activity of various fennel populations. Their results revealed high concentrations of phenolic compounds, such as flavonoids, which are responsible for the plant's significant antioxidant properties. These conclusions are consistent with results from other research, which also identified fennel as being rich in flavonoids and phenolic

compounds. Antioxidant activity is a crucial component of fennel's therapeutic efficacy, as it reduces oxidative stress levels, which is particularly beneficial for patients with chronic conditions such as irritable bowel syndrome and bronchitis (Kosmuratova *et al.*, 2021). The significance of this research lies in demonstrating that different fennel populations have varying phenolic compound content, which opens avenues for further selective breeding of the most valuable populations for medicinal applications. This is consistent with research that has also highlighted the importance of standardising the bioactive compound content in different fennel varieties.

Abdesslem *et al.* (2021), Mehra *et al.* (2021) investigated the chemical composition and biological activity of essential oils in fennel, using ethanolic extracts from both organic and conventional crops. Their findings indicated that organic fennel contained higher concentrations of bioactive compounds such as trans-anethole and fenchone, which are primary components of essential oils. This finding aligns with other research that has also identified elevated levels of these compounds in fennel, which are crucial for its antibacterial and antispasmodic effects. A significant aspect of this research is the comparison of fennel grown under different conditions, confirming that organically grown plants exhibit higher biological activity.

A comprehensive review of the therapeutic and pharmacological potential of fennel was conducted by Kooti *et al.* (2015), focusing on its use in traditional medicine for the treatment of digestive and respiratory diseases. The review confirms that fennel is employed in the management of conditions such as bloating, cramps, cough, and bronchitis, which is consistent with the findings of this study. Notably, the research provides an in-depth examination of the antibacterial activity of fennel, which is crucial for its application in treating respiratory infections. This study underscores the significance of fennel in

contemporary medicine, affirming that its therapeutic potential could be harnessed for the development of new medicinal products based on natural components. Thus, the conclusions align with the data presented in this study and open new avenues for further clinical research.

The article of Bernáth *et al.* (1999) is fundamental, as it investigates the patterns of essential oil accumulation in fennel fruits at various stages of the plant's development. The study demonstrates that essential oil accumulates in the later stages of fruit maturation, which is linked to histological changes in the plant's structure. These results are significant for the research, as they confirm that the timing of harvesting the plant significantly affects the quantity and quality of essential oil, which may influence its therapeutic properties. Furthermore, the findings illustrate, that different stages of growth and harvesting of fennel can impact the activity of its components, thereby opening new avenues for optimising the production of fennel oil.

Baser and Kirimer (2006), Stefanini *et al.* (2006) conducted a study on the essential oils of plants in the *Lamiaceae* family, which includes fennel. The composition of the essential oils from various plants in this family was examined, revealing significant differences in chemical composition depending on climatic conditions and geographical location. These findings confirm that the essential oil of fennel may have a variable composition, as noted in the current study. The composition of fennel essential oil and its antifungal activity were studied by MimicaDukić *et al.* (2003), who examined the impact of distillation conditions on the composition and antifungal properties of fennel essential oil. It was found that different distillation conditions can alter the composition and activity of the oil, which corresponds with data on the variability of essential oils based on cultivation and processing conditions. This study underscores the importance of technological aspects in the processing of

plant materials to optimise their therapeutic properties.

Diao *et al.* (2014), Ilić *et al.* (2019) investigated the enhancement of yield and antimicrobial activity of fennel essential oil through the grinding of seeds. It was established that pre-grinding fennel seeds could significantly increase the yield of essential oil and its antimicrobial activity. This conclusion aligns with data indicating that the mechanical processing of raw materials can substantially influence their bioactive properties.

Karami *et al.* (2023), Amudhamathi *et al.* (2024) conducted a study on the role of landforms in habitat suitability and connectivity of *Moringa peregrina* in southeastern Iran, highlighting the critical influence of environmental factors on the distribution and growth potential of medicinal plants. Their findings emphasize the importance of ecological conditions, such as soil composition, climate, and topography, in determining the quality and yield of bioactive compounds in medicinal species. This research is particularly relevant to study, as it underscores the significance of environmental factors in the cultivation and chemical composition of *Foeniculum vulgare* (fennel).

Given fennel's well-documented pharmacological properties, including its digestive, respiratory, and antimicrobial benefits, the insights from Karami *et al.* (2023) can aid in optimizing cultivation strategies to enhance the production of key bioactive compounds such as trans-anethole, fenchone, and limonene. Their study also suggests that habitat connectivity plays a crucial role in genetic diversity and plant resilience, which may have implications for sustainable fennel cultivation and the preservation of its medicinal qualities.

The analysed sources indicate a high medicinal and pharmacological value of fennel. Future research could focus on investigating the optimal conditions for the cultivation, processing, and extraction of

essential oil to maximise the preservation and enhancement of its bioactive properties. Particular attention should be given to the technological processes that may influence the chemical composition of the essential oil, as well as the potential applications of fennel in the treatment of infectious diseases.

CONCLUSION

The study revealed that *Foeniculumvulgare* (fennel) possesses significant pharmacological properties, as confirmed by both ethnopharmacological knowledge and contemporary research. Polish herbaria describe fennel as an effective remedy for digestive disorders, respiratory diseases, and as a natural antiseptic. Fennel contains a rich chemical composition, including essential oils such as trans-anethole, fenchone, and limonene, which exhibit anti-inflammatory, antimicrobial, and antioxidant activities. Important qualitative indicators include a high content of dietary fibre, minerals such as magnesium, potassium, and phosphorus, as well as vitamins, all of which contribute to overall health enhancement and the maintenance of normal bodily functions. The research confirmed that fennel exhibits significant effectiveness in treating digestive disorders, such as colic and bloating, due to its antispasmodic and anti-inflammatory properties. Furthermore, its application in respiratory diseases has also proven effective, attributed to its antibacterial and expectorant properties.

CONFLICT OF INTEREST STATEMENT

The author declare that he has no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Review article

Documenting conservation status and medicinal potential of selected non-edible fruit-bearing plants in the Bangladesh Agricultural University Botanical Garden

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ABSTRACT

Non-edible fruit-bearing plants are particularly fascinating due to their remarkable adaptability to diverse ecological conditions, their utilization in traditional medicine, and their potential contributions to pharmaceutical development. A total of 38 non-edible fruit plant species, spanning 36 genera and 20 families, were documented. The Euphorbiaceae family exhibited the highest diversity, with six species, followed by Fabaceae, Lecythidaceae, Malvaceae, and Bignoniaceae, each represented by three species. Families such as Annonaceae, Celastraceae, Clusiaceae, Fagaceae, and Rubiaceae had two species each, while ten families included only a single species. At the genus level, Lithocarpus and Mallotus were the most prominent, each represented by two species, while the other 34 genera were represented by a single species. The study revealed that 76% of the species were indigenous, whereas 24% were exotic. In terms of conservation status, 8% of the species were classified as vulnerable, 3% as endangered, 52% as least concern, 8% as data deficient, and 29% had not been evaluated globally. The plants were used to treat a wide range of common diseases, including cancer, cardiovascular, gastrointestinal, and respiratory disorders, as well as infections, and more. This paper provides the conservation status and collective information on the medicinal uses of these non-edible fruit plants.

Key words: BAUBG, conservation status, medicinal uses, non-edible fruit-bearing plants

INTRODUCTION

Plants have been an essential source of medicine throughout human history, playing a pivotal role in traditional and modern therapeutic systems. Fruit plants have long been recognized for their medicinal value, and their significance in both traditional and modern healthcare continues to grow. Fruits are a rich source of essential vitamins and

minerals, antioxidants, dietary fiber, and natural bioactive compounds, e.g., alkaloids, flavonoids, and tannins, all of which are vital for maintaining health and preventing diseases (Ashrafuzzaman *et al.*, 2021, Kumar *et al.*, 2023). Across various cultures, fruit plants have traditionally been used to treat a wide variety of ailments such as skin disorders, infections, inflammation, and digestive

problems (Ragasa *et al.*, 2014; Shilpi *et al.*, 2016). While much research has focused on edible fruit plants due to their direct nutritional benefits, non-edible fruit plants remain an underexplored yet potentially rich source of bioactive compounds. These plants often contain unique phytochemicals that confer significant medicinal properties, including antimicrobial, antioxidant, anti-inflammatory, and anticancer activities.

Bangladesh is home to a diverse range of tropical and subtropical fruits. In addition to edible fruits, the country also has a significant variety of non-edible fruits. These non-edible fruits are less favored by the community compared to edible ones due to their unpleasant odor, lack of palatability, limited nutritional value, and a general lack of awareness regarding their potential uses. As a result, people are less concerned about their conservation in nature. Non-edible fruits are not consumed directly because they often contain toxic compounds that can be harmful to human health. However, many of these fruits are still utilized in traditional medicine in various forms, such as pastes, powders, and extracts. Despite their toxicity or unpalatable nature, they contain bioactive compounds with therapeutic potential when processed correctly. Extensive research has been conducted globally on the medicinal uses of non-edible fruits among various indigenous communities (Biswas *et al.*, 2018; Kumar *et al.*, 2023). Despite their promising potential in traditional medicine and pharmaceutical applications, these non-edible fruits remain largely undocumented. This study aims to analyze the conservation status and compile the medicinal potential of selected non-edible fruit-bearing plants found in the BAU Botanical Garden, with the goal of establishing a foundation for future pharmacological research.

STUDY AREA

The Bangladesh Agricultural University Botanical Garden (BAUBG), established in 1963, has been dedicated to the collection and conservation of plant species. BAUBG is located on the west bank of the Old Brahmaputra River and covers an area of 25 acres. Geographically, it lies at E90° 26' 29.6" and N24° 43' 26.8" at an elevation of 29 meters above the mean sea level. The region experiences a tropical monsoon climate, with summer humidity ranging from 80% to 90%, and winter humidity between 60% and 70%. The average annual rainfall in the area is approximately 2,000 mm.

A survey was conducted at the Bangladesh Agricultural University Botanical Garden (BAUBG) between July 2019 and October 2021 to identify non-edible fruit-bearing plants with documented medicinal properties. Plant specimens were collected, processed, and preserved following standard herbarium techniques (Das, 2021) during field visits. These specimens underwent thorough examination in the laboratory of the Department of Crop Botany at Bangladesh Agricultural University. Their taxonomic identity was verified through expert consultation and published literature (Leeratiwong *et al.*, 2011). Binomial nomenclature was updated using two widely accepted botanical databases: Plants of the World Online (<https://powo.science.kew.org/>) and World Flora Online (<http://www.worldfloraonline.org/>). The conservation status of the identified species was determined using the International Union for Conservation of Nature (IUCN) Red List of Threatened Species (<https://www.iucnredlist.org/>). The major medicinal uses of the collected species were compiled from several authoritative sources, including *Indian Medicinal Plants* (Kirtikar and Basu, 1999), *Encyclopedia of Flora and Fauna of Bangladesh* (Ahmed *et al.*, 2009 a,b), *Vascular Flora of Chittagong and the*

Chittagong Hill Tracts (Uddin and Hassan, 2018), *Traditional Uses of Ethnomedicinal Plants of the Chittagong Hill Tracts* (Uddin and Rahman, 2006), and *Medicinal Plants of Bangladesh* (Yusuf *et al.*, 1994). The identified genera and species are listed alphabetically, with each taxon's valid name, conservation status, nativity, and medicinal uses detailed in the text.

RESULTS OF SURVEY AND DISCUSSION

A total of 38 plant species, belonging to 20 families, were documented from BAUBG, Mymensingh. For each species, information on the local name, scientific name, family, conservation status, nativity, and medicinal uses was recorded (Tables 1 & 2). Among these 38-plant species, photographs of 32 species are presented in Plate I and Plate II, with 16 species featured in each plate. The family Euphorbiaceae was the most diverse, comprising six identified species. Other prominent families, including Bignoniaceae, Fabaceae, Lecythidaceae, and Malvaceae, were each represented by three species, highlighting the rich floral diversity of the region. Additionally, families such as Annonaceae, Celastraceae, Clusiaceae, Fagaceae, and Rubiaceae each contained two species, whereas ten other families were represented by only one species each (Table 1, Figure 1.a). Among the documented plant genera, *Lithocarpus* and *Mallotus* were the most represented, with two species each, while the remaining 36 genera were represented by a single species each (Table 1). The nativity of the species shows that the majority (76%) of the plants documented in the region are native and 24% of the species were classified as exotic (Table 1, Fig. 1.b). This high proportion of native plants suggests a well-preserved local flora that contributes to the ecological balance and biodiversity of the region. Indigenous plants are often adapted to local conditions, supporting native fauna and

maintaining ecological stability. However, the presence of 24% exotic plant species is noteworthy. While some exotic species may integrate into the ecosystem without causing harm, others can become invasive, outcompeting native species and disrupting local ecosystems. Exotic species often lack natural predators or controls in their introduced environment, which can lead to their rapid proliferation. The relatively high proportion of exotic species highlights the need for careful monitoring and management to prevent potential ecological impacts. Conservation efforts should prioritize the protection and restoration of indigenous plant populations while managing exotic species to prevent them from becoming invasive.

The study reveals a significant disparity in the conservation status of species. The majority (52%) are categorized as least concern, reflecting a relatively stable state for these species. However, the presence of species in categories such as vulnerable (8%) and endangered (3%) highlights ongoing conservation challenges and the need for targeted measures to address threats to biodiversity. The high percentage of species categorized as not evaluated (29%) underscores a critical knowledge gap in our understanding of biodiversity. This gap hinders effective conservation planning and suggests that additional resources and research efforts are essential to assess these species' statuses. Similarly, the 8% of species classified as data deficient signals a need for more robust data collection and monitoring systems to inform conservation decisions. Among these non-edible fruits species, *Heritiera fomes* (EN) of Malvaceae family was most recently assessed for the IUCN Red List of Threatened Species in 2008 at the global level (<https://www.iucnredlist.org/>). So, protecting these plants and their habitats should be a priority.

Although fruits are non-edible but have a wide range of potential uses following paste, oil,

extracts, or other modes of preparation. Many non-edible fruit species have traditional uses in medicine. These species may contain bioactive compounds that can be used to treat various ailments. In the present findings, most non-edible fruit were used to treat common diseases such as Cancerous; Cardiovascular and Liver; Dermatological; Gastrointestinal; Helminthiasis and Diabetes; Infectious; Inflammation and pain; Respiratory; Sexual and Anti-oxidative; Urogenital diseases. The most commonly reported medicinal uses of non-edible fruit plant species were categorized as- gastrointestinal purposes, with 15 species; inflammation and pain, 11 species; helminthiasis and diabetes, 10 species; dermatological conditions, 9 species; infectious diseases, 9 species; and respiratory purposes, 8 species (Table 3). The frequent use of plants for gastrointestinal treatments aligns with findings in other regions, where plants with digestive health benefits are highly valued. The use of these plants for inflammation, pain relief, and infectious diseases suggests they may contain bioactive compounds with therapeutic potential, which could be further developed into pharmaceutical products. This highlights the need for pharmacological studies to isolate and understand the compounds responsible for these medicinal effects.

CONCLUSION

Detailed observations reveal that many of these non-edible fruit species possess noteworthy medicinal properties, paving the way for their potential application in plant-based therapeutics. Future research should prioritize assessing the ecological impacts of exotic species, developing effective conservation strategies to protect the region's unique flora, and scientifically validating the medicinal properties of these plants through clinical trials.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Table 1: Inventory of Non-Edible Fruit-Bearing Plants in the Bangladesh Agricultural University Botanical Garden

Sl No.	Local Name	Scientific name	Family	Cons. Status	Nativity
1.	Amur	<i>Aglaia cucullata</i> (Roxb.) Pellegr.	Meliaceae	DD	Indigenous
2.	Kakra	<i>Aporosa cardiosperma</i> (Gaertn.) Merr.	Euphorbiaceae	VU	Indigenous
3.	Agarwood	<i>Aquilaria malaccensis</i> Lam.	Thymelaeaceae	NE	Indigenous
4.	Kathali chapa	<i>Artabotrys hexapetalus</i> (L.f.) Bhandari	Annonaceae	NE	Indigenous
5.	Fish poison tree	<i>Barringtonia asiatica</i> (L.) Kurz	Lecythidaceae	LC	Exotic
6.	Moos	<i>Brownlowia elata</i> Roxb.	Malvaceae	VU	Indigenous
7.	Sultana Champa	<i>Calophyllum inophyllum</i> L.	Clusiaceae	LC	Indigenous
8.	Mayna kanta	<i>Catunaregam longispina</i> (Link) Tirveng.	Rubiaceae	NE	Indigenous
9.	Dahur/Dagor	<i>Cerbera odollam</i> Gaertn	Apocynaceae	NE	Indigenous
10.	Javanikapu	<i>Cleidion javanicum</i> Blume	Euphorbiaceae	NE	Indigenous
11.	Bowler gach	<i>Cordia dichotoma</i> G.Forst.	Boraginaceae	LC	Indigenous
12.	Naglingam	<i>Couroupita guianensis</i> Aubl.	Lecythidaceae	LC	Exotic
13.	Kalabos	<i>Crescentia cujete</i> L.	Bignoniaceae	LC	Exotic
14.	Singra	<i>Cynometra ramiflora</i> L.	Fabaceae	LC	Indigenous
15.	Tamal	<i>Diospyros montana</i> Roxb.	Ebenaceae	NE	Exotic
16.	Gilalota	<i>Entada rheedii</i> Spreng.	Fabaceae	NE	Indigenous
17.	Behala bot	<i>Ficus lyrata</i> Warb.	Moraceae	LC	Exotic
18.	Gustva/dadra	<i>Gustavia augusta</i> L.	Lecythidaceae	LC	Exotic
19.	Sundori	<i>Heritiera fomes</i> Buch-Ham.	Malvaceae	EN	Indigenous
20.	Chalmogra	<i>Hydnocarpus kurzii</i> (King) Warb.	Achariaceae	DD	Indigenous
21.	Kigelia	<i>Kigelia africana</i> (Lamk.) Benth.	Bignoniaceae	LC	Exotic
22.	Kali batna	<i>Lithocarpus acuminatus</i> (Roxb.) Rehder	Fagaceae	DD	Indigenous
23.	Boro batna	<i>Lithocarpus elegans</i> (Blume) Hatus. ex Soepadmo	Fagaceae	LC	Indigenous
24.	Roktan	<i>Lophopetalum wightianum</i> Arn.	Celastraceae	LC	Indigenous
25.	Mahua	<i>Madhuca longifolia</i> (L.) J.F.Macbr.	Sapotaceae	NE	Indigenous
26.	Pitali/Latim	<i>Mallotus nudiflorus</i> (L.) Kulju & Welzen	Euphorbiaceae	LC	Indigenous
27.	Sindur	<i>Mallotus philippensis</i> (Lam.) Müll.Arg.	Euphorbiaceae	LC	Indigenous
28.	Nageshwar	<i>Mesua ferrea</i> L.	Clusiaceae	VU	Indigenous
29.	Gandhi-gazari	<i>Miliusa velutina</i> (Dunal) Hook.f. et Thoms.	Annonaceae	LC	Indigenous
30.	Cuajilote	<i>Parmentiera aculeata</i> (Kunth)	Bignoniaceae	LC	Exotic

		Seem.			
31.	Koronj	<i>Pongamia pinnata</i> (L.) Pierre	Fabaceae	LC	Indigenous
32.	Salacia	<i>Salacia diandra</i> Thwaites	Celastraceae	NE	Indigenous
33.	Hurmoi	<i>Shirakiopsis indica</i> (Willd.) Esser	Euphorbiaceae	LC	Indigenous
34.	Udal	<i>Sterculia villosa</i> Roxb.	Malvaceae	LC	Indigenous
35.	Kuchilla	<i>Strychnos nux-vomica</i> L.	Loganiaceae	NE	Indigenous
36.	Bon narangy	<i>Suregada multiflora</i> (A. Juss.) Baill.	Euphorbiaceae	LC	Indigenous
37.	Piralu	<i>Tamilnadia uliginosa</i> (Retz.) Tirveng. & Sastre	Rubiaceae	LC	Indigenous
38.	Bhuikakur	<i>Trichosanthes tricuspidata</i> Lour.	Cucurbitaceae	NE	Exotic

EN – Endangered; VU – Vulnerable; LC - Least Concern; DD - Data Deficient; NE - Not Evaluated

Table 2. List of Non-Edible Fruit plants with medicinal uses

Sl No.	Scientific name	Medicinal uses	References
1.	<i>Aglaia cucullata</i> (Roxb.) Pellegr.	Skin diseases, Dysentery, Anti-inflammatory	Das <i>et al.</i> , 2005
2.	<i>Aporosa cardiosperma</i> (Gaertn.) Merr.	Antibacterial	Abdul <i>et al.</i> , 2024
3.	<i>Aquilaria malaccensis</i> Lam.	Laxative, Carminative, Asthma	Yusuf <i>et al.</i> , 1994
4.	<i>Artabotrys hexapetalus</i> (L.f.) Bhandari	Cholera, Cardiac stimulant	Yusuf <i>et al.</i> , 1994
5.	<i>Barringtonia asiatica</i> (L.) Kurz	Stomach ache, Rheumatism	Ragasa <i>et al.</i> , 2014
6.	<i>Brownlowia elata</i> Roxb.	Skin ailments, Rheumatism, Urinary problem	Hasnat <i>et al.</i> , 2019
7.	<i>Calophyllum inophyllum</i> L.	Astringent, Rheumatism	Yusuf <i>et al.</i> , 1994
8.	<i>Catunaregam longispina</i> (Link) Tirveng.	Gastrointestinal, Hepatic problems Anti-inflammatory	Timalsina <i>et al.</i> , 2021 Kirtikar & Basu, 1999
9.	<i>Cerbera odollam</i> Gaertn	Anti-cancerous, Antifungal	Saxena <i>et al.</i> , 2023
10.	<i>Cleidion javanicum</i> Blume	Anti-infectious	Phumthum & Balslev, 2020
11.	<i>Cordia dichotoma</i> G.Forst.	Cough, Chest diseases	Yusuf <i>et al.</i> , 1994
12.	<i>Couroupita guianensis</i> Aubl.	Anti-inflammatory, Anti-ulcer, Anti-cancer	Gousia <i>et al.</i> , 2013
13.	<i>Crescentia cujete</i> L.	Anti-inflammatory, Antibacterial	Parvin <i>et al.</i> , 2015.
14.	<i>Cynometra ramiflora</i> L.	Antioxidant	Sookying <i>et al.</i> , 2013
15.	<i>Diospyros montana</i> Roxb.	Fever, Pneumonia, Diarrhea	Yusuf <i>et al.</i> , 1994
16.	<i>Entada rheedii</i> Spreng.	Anti-ulcerogenic, Antimicrobial	Okba <i>et al.</i> , 2018
17.	<i>Ficus lyrata</i> Warb.	Anti-diabetic, Anticancer, and antimicrobial	Khan, 2017
18.	<i>Gustavia augusta</i> L.	Vomiting	Rovira <i>et al.</i> , 1999

19.	<i>Heritiera fomes</i> Buch-Ham.	Anti-diabetic, Anti-oxidative	Sultana <i>et al.</i> , 2022
20.	<i>Hydnocarpus kurzii</i> (King) Warb.	Leprosy, Skin diseases, Cancer	Yusuf <i>et al.</i> , 1994
21.	<i>Kigelia africana</i> (Lamk.) Benth.	Skin disorders, Cancer, Gynecological complaints	Nabatanzi <i>et al.</i> , 2020
22.	<i>Lithocarpus acuminatus</i> (Roxb.) Rehder	Skin infection, Scabies	Singh <i>et al.</i> , 2015
23.	<i>Lithocarpus elegans</i> (Blume) Hatus. ex Soepadmo	Skin infection, Scabies	Singh <i>et al.</i> , 2015
24.	<i>Lophopetalum wightianum</i> Arn.	Antibacterial, Antifungal	Bharadwaj <i>et al.</i> , 2018
25.	<i>Madhuca longifolia</i> (L.) J.F.Macbr.	Bronchitis, Diabetes	Jodh <i>et al.</i> , 2022
26.	<i>Mallotus nudiflorus</i> (L.) Kulju & Welzen	Rheumatism	Jena <i>et al.</i> , 2024
27.	<i>Mallotus philippensis</i> (Lam.) Müll.Arg.	Anthelmintic, Bronchitis, Rheumatism	Yusuf <i>et al.</i> , 1994
28.	<i>Mesua ferrea</i> L.	Purgative, Asthma	Sharma <i>et al.</i> , 2017
29.	<i>Miliusa velutina</i> (Dunal) Hook.f. et Thoms.	Anti-inflammatory, Anti-bacterial	Phrompanya <i>et al.</i> , 2024
30.	<i>Parmentiera aculeata</i> (Kunth) Seem.	Diabetes, Asthma, Diarrhea	Santiago Ruiz <i>et al.</i> , 2021
31.	<i>Pongamia pinnata</i> (L.) Pierre	Skin diseases, Piles	Al Muqarrabun <i>et al.</i> , 2013
32.	<i>Salacia diandra</i> Thwaites	Diabetes	Karunaratne, 2013
33.	<i>Shirakiopsis indica</i> (Willd.) Esser	Gastritis	Mokmued <i>et al.</i> , 2021
34.	<i>Sterculia villosa</i> Roxb.	Diuretic, Urinary problem, Rheumatism	Yusuf <i>et al.</i> , 1994
35.	<i>Strychnos nux-vomica</i> L.	Diabetes	Bhati <i>et al.</i> , 2012
36.	<i>Suregada multiflora</i> (A. Juss.) Baill.	Sore, Stomach troubles	Yusuf <i>et al.</i> , 1994
37.	<i>Tamilnadia uliginosa</i> (Retz.) Tirveng. & Sastre	Antidiarrheal, Antimicrobial, Anti-inflammatory, Antidiabetic	Kalita <i>et al.</i> , 2023
38.	<i>Trichosanthes tricuspidata</i> Lour.	Anti-Inflammatory	Ahuja <i>et al.</i> , 2019

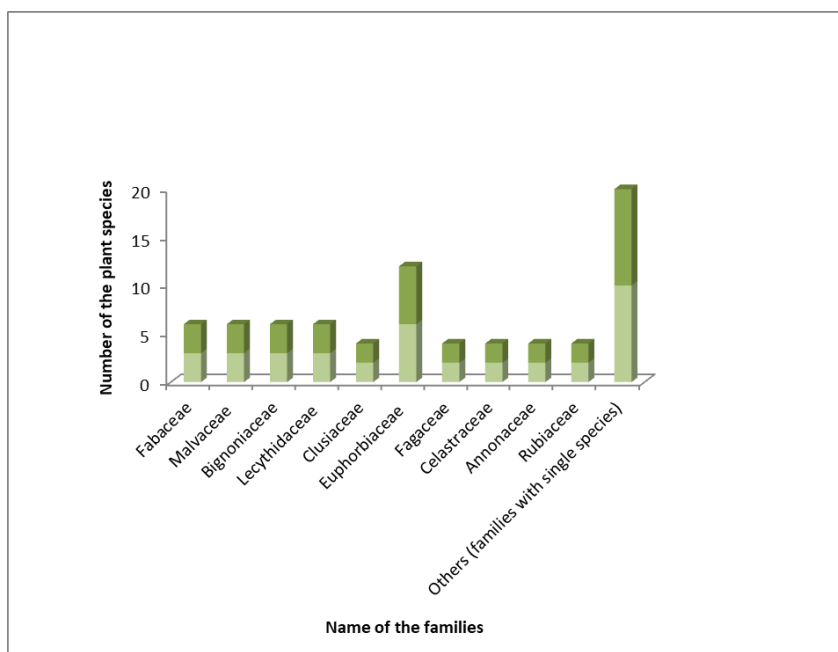


Figure 1(a): Family wise distribution of selected non-edible fruit plant species in BAUBG.

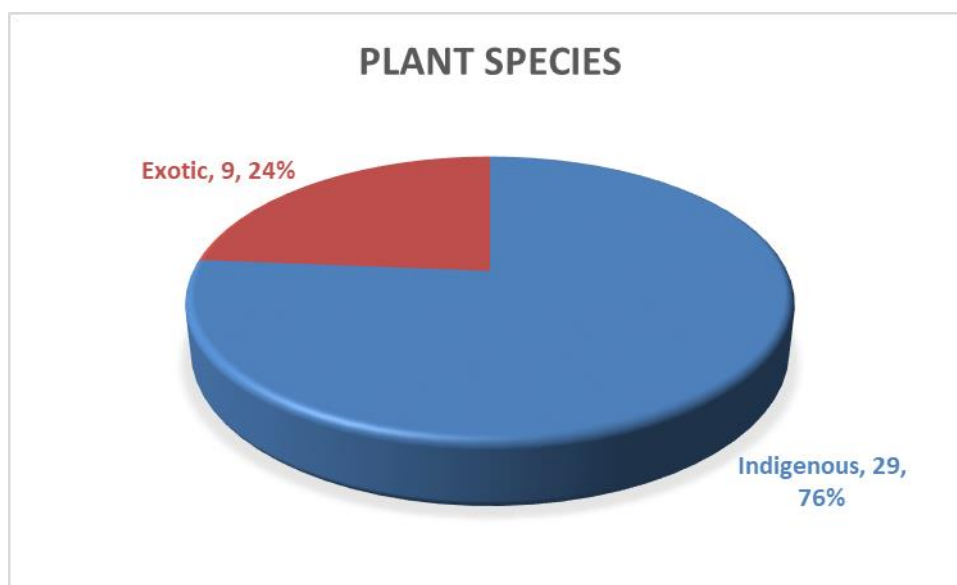


Figure 1(b): Indigenous vs. Exotic trees in BAUBG

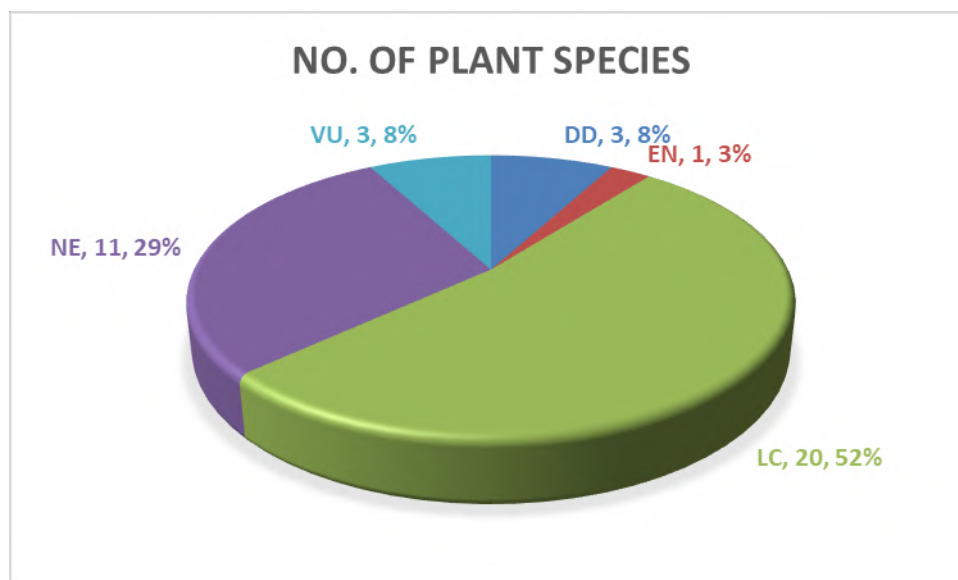


Figure 1(c): IUCN red list categories of the plant species where, EN– Endangered; VU– Vulnerable; LC- Least Concern; DD- Data Deficient; NE- Not Evaluated

Table 3: Diseases grouped by major diseases categories

Category	Common diseases/Medical terms	No. of species used
Cancerous	Cancer	5
Dermatological	Skin diseases, Scabies, Leprosy	9
Gastrointestinal	Stomach disorders, Stomach ulcer, Appetite, Diarrhea, Cholera, Acidity, Vomiting, Dysentery, Gastric troubles, Carminative, Astringent, purgative, Laxative	15
Helminthiasis and Diabetes	Anthelmintic, Diabetes	10
Infectious	Malarial fever, Viral fever, Bacterial and Fungal diseases	9
Inflammation and pain	Inflammation, Rheumatic pain	11
Respiratory	Cough, respiratory disorders, Asthma, Bronchitis, Pneumonia	8
Sexual and Anti-oxidative	Gynecological disorders, anti-oxidative	3
Urogenital	Urinary problems, Diuretic, diaphoretic, Piles	3

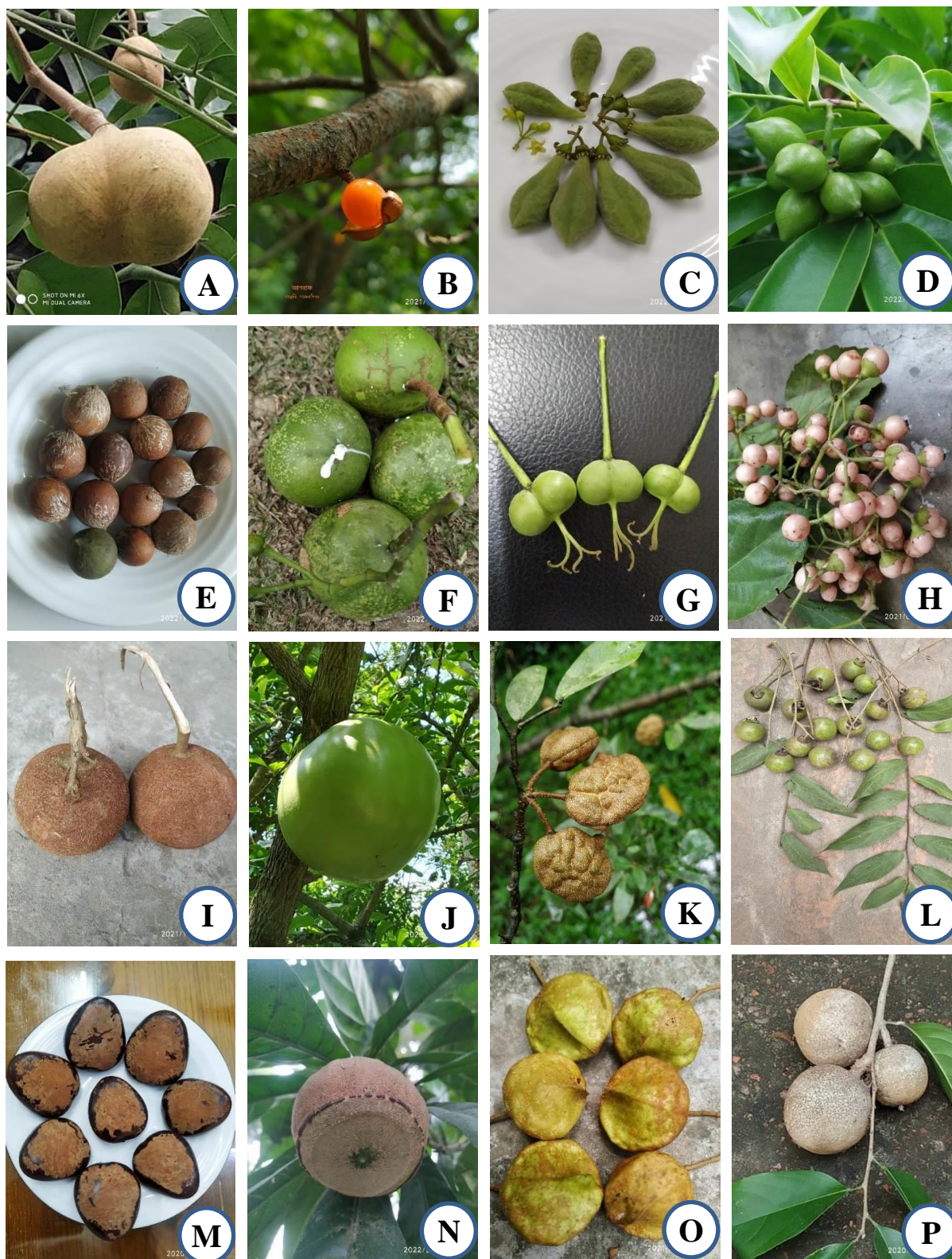


Plate-I: (A) *Aglaia cucullata* (B) *Aporosa cardiosperma* (C) *Aquilaria malaccensis* (D) *Artabotrys hexapetalus* (E) *Calophyllum inophyllum* (F) *Cerbera odollum* (G) *Cleidon javanicum* (H) *Cordia dichotoma* (I) *Couroupita guianensis* (J) *Crescentia cujete* (K) *Cynometra ramiflora* (L) *Diospyros montana* (M) *Entada rheedii* (N) *Gustavia augusta* (O) *Heritiera fomes* (P) *Hydnocarpus kurzii*

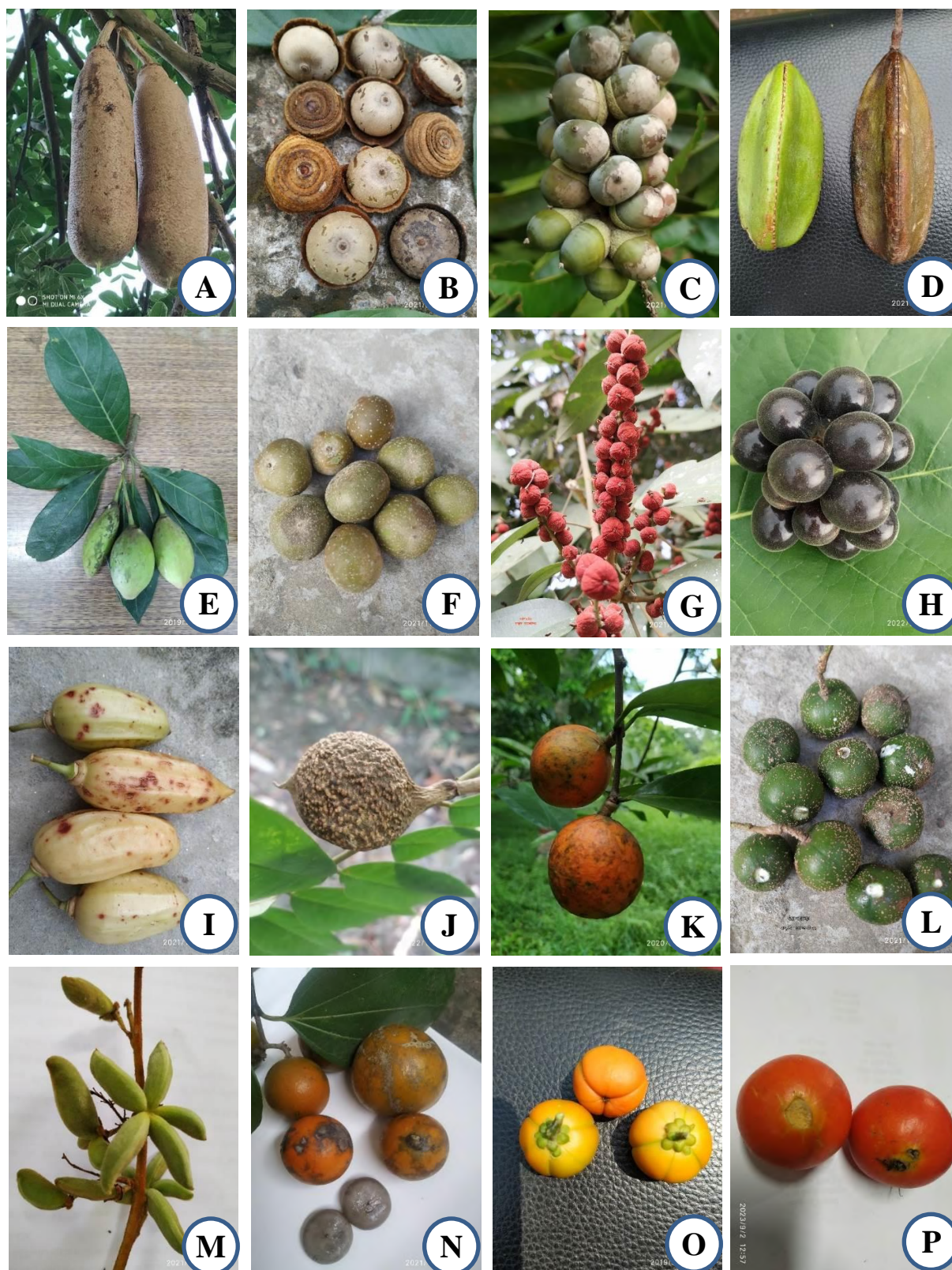


Plate-II: (A) *Kigelia africana* (B) *Lithocarpus elegans* (C) *Lithocarpus acuminatus* (D) *Lophopetalum wightianum* (E) *Madhuca longifolia* (F) *Mallotus nudiflorus* (G) *Mallotus philippensis* (H) *Miliusa velutina* (I) *Parmentiera aculeata* (J) *Pongamia pinnata* (K) *Salacia diandra* (L) *Shirakiopsis indica* (M) *Sterculia villosa* (N) *Strychnos nux-vomica* (O) *Suregada multiflora* (P) *Trichosanthes tricuspidata*

Review article

Medicinal plant genetic resources of Bangladesh exhibiting anti-dengue activity: A review

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ABSTRACTS

Dengue fever, caused by the arthropod-borne dengue virus, has experienced a global increase, with the number of cases rising from 500,000 to 5.2 million and over 5000 deaths within the last two decades. Although dengue incidence in Bangladesh fluctuated from 1964 to 1999, peaking in 2000, it has since spread, impacting thousands of lives and causing diseases. There is currently no (or limited availability of) effective dengue vaccination and antiviral medication. A comprehensive review was conducted to identify the local medicinal plants with anti-dengue activity. A total of 73 species belonging to 42 families possess anti-dengue properties; some also have insecticidal properties, especially against Aedes species. Medicinal plants possess different types of secondary metabolites, for example, phenolic derivatives, alkaloids, flavonoids, terpenoids, and polysaccharides with compound-specific dengue preventing mechanisms. The conversion of these medicinal plants into prescription drugs to treat dengue infections requires more clinical investigation.

Key words: Alkaloids, dengue, flavonoids, polysaccharides, terpenoids, total phenolics

INTRODUCTION

Dengue fever is the most emerging viral human disease caused by the arthropod-borne dengue virus (DENV), which belongs to the genus *Flavivirus* in the family *Flaviviridae* (Goel *et al.*, 2004). Dengue incidence has surged globally, with the World Health Organization (WHO) reporting a tenfold increase from 500,000 to 5.2 million cases between 2000 and 2019; the year 2019 experienced an all-time high, with cases reported in 129 countries (<https://www.who.int/emergencies/disease-outbreak-news/item/2023-DON498>). Since the start of 2023, more than 5000 dengue-related deaths and nearly a historic five million cases have been reported in more than 80 countries and territories due to continuous transmission and an unanticipated increase in dengue infections (<https://www.who.int/emergencies/disease-outbreak-news/item/2023-DON498>). With

40% of the world's population living in areas at risk for transmission, dengue virus infection is a leading cause of illness in the tropics and subtropics (<https://www.cdc.gov/dengue/training/cme/cm/page52245.html>). About 8 million people worldwide may be afflicted by dengue and malaria in 2080 (Hillary *et al.*, 2024). The dengue virus is spread through two species of *Aedes*, *A. aegypti* and *A. albopictus*, mosquitoes (Hossain *et al.*, 2023). When an infected mosquito bites a human, it transmits the disease. There are four serotypes of DENV: DENV-1, DENV-2, DENV-3, and DENV-4. Among these, DENV-2 is more lethal than any other serotype (Saleh and Kamisah, 2021). Infection of one serotype confers permanent immunity to that serotype and only temporary immunity to the other serotypes; subsequent infections with a different serotype raise the risk of developing severe

dengue (Reich *et al.*, 2013). Symptoms of dengue fever include fever, headache, joint, and muscle pain (Gubler, 2006). Chronic symptoms include dengue fever to severe hemorrhagic, characterized by low platelet levels and blood plasma leakage, also known as dengue shock syndrome (Pigili and Runja, 2014). First infections with DENV-1 or DENV-3 often led to more severe illness than main infections with DENV-2 or DENV-4 (Tang *et al.*, 2012).

Dengue incidence in Bangladesh was intermittently reported from 1964 to 1999. The first significant dengue outbreak happened in 2000, and since then, it has spread throughout the country, affecting thousands of people's quality of life and creating diseases (Hossain *et al.*, 2023). According to the most recent official statistics (November 22, 2024), at least 438 individuals died from connected issues in 2024, while 84,826 patients were admitted to hospitals across the country (https://dashboard.dghs.gov.bd/pages/heoc_dengue_v1.php). The climatic conditions of Bangladesh are favourable for dengue infection. Although dengue is endemic in Bangladesh, the current dengue surge is unusual in terms of seasonality and the early sharp increase compared to previous years, where the surge typically starts around late June (Hossain *et al.* 2023). The case fatality rate (CFR) so far this year is relatively high compared to previous years for the full-year period. The pre-monsoon *Aedes* survey shows that the density of mosquitoes and the number of potential hotspots are at the highest level in the past five years (WHO, 2023). Hossain *et al.* (2023) recently conducted a thorough analysis of the entire dengue situation, including illness burden, clinical spectrum, seroprevalence, circulating serotypes/genotypes, and spatial distribution since the first outbreak was documented in Bangladesh.

Dengue fever does not currently have a specific treatment. Therefore, with the rapid expansion of dengue disease, it is imperative to create efficient preventative and control strategies, such as antiviral medications and dengue vaccinations. However, there is currently no effective dengue vaccination or

antiviral medication (Zandi *et al.*, 2011; Tang *et al.*, 2012) or limited availability (a Sanofi Pasteur-developed dengue vaccine has been approved in 24 nations and included in public vaccination programs in Brazil and the Philippines) (Hossain *et al.*, 2023). Typically, dengue patients get supportive care until they fully recover without particular therapeutic interventions. Traditional herbal remedies have been thoroughly studied as an alternative treatment due to the adverse (or disastrous) side effects of synthetic medications and the increasing resistance of bacteria to conventional antimicrobial therapy. About 80% of the population in developing countries, especially in Asia and Africa, use natural products from plants for their primary healthcare (WHO, 2020). Plants and chemicals produced by them continue to be valuable sources for developing novel antiviral medications due to their high accessibility in nature and anticipated low side effects. Several medicinal plants have been shown to have significant antiviral properties at various stages of viral development, and many have been used to treat viral infections in both humans and animals, including DENV infection (Ganjhu *et al.*, 2015; Sarwar *et al.*, 2022; Firuj *et al.*, 2023). Bangladesh is rich in medicinal plant genetic resources (Sarwar, 2020); therefore, this study aims to identify the medicinal plants of Bangladesh, plant parts used, and active constituents that could be used for dengue disease treatments.

METHODOLOGY

A narrative literature review was conducted on the possibility of using medicinal plants in Bangladesh to treat dengue. Different electronic sources of databank – PubMed, SpringerLink, MDPI link, ResearchGate, Google and Google Scholar, ScienceDirect, etc.—were searched for the article. Bangladeshi articles were searched in the BanglaJol database (an exclusive database of Bangladeshi Journals) with a combination of keywords “dengue”, “antiviral”, “medicinal plants”, “Bangladesh”, etc. Articles published in other languages except English and editorials and comments not

subjected to peer review were excluded. Internationally recognised websites such as Plants of the World Online (<https://powo.science.kew.org/>), the International Plant Names Index (<https://www.ipni.org/>), and World Flora Online (<https://www.worldfloraonline.org/>) have been checked for current nomenclature and family delimitation.

RESULTS AND DISCUSSION

Medicinal plants of 73 species belonging to 42 families could be used to treat dengue fever (Table 1). With seven species (9.59% of total), the family Lamiaceae emerged as the largest source of anti-dengue plants, followed by Fabaceae (8.22%; six species), Asteraceae and Zingiberaceae (5.48%; four species each) (Figure 1). Twenty-seven families were represented by a single species each (Table 1). In other publications, Lamiaceae is also identified as one of the most useful families for treating dengue and other viral diseases. For example, according to Saleh and Kamisah (2021), 54 species from 31 families exhibited anti-dengue activity; the most prominent representatives were the Lamiaceae (10.5%), Asteraceae (9.9%), Aristolochiaceae, and Loganiaceae (each 7.2%).

In terms of plant parts used, more than one plant part was used for 18 plant species (24.66% of the total), followed by the whole plant (13.70%; 10 species) (Table 1; Figure 2). Individually, leaves were the most common (36.99%; 27 species) plant part used, followed by fruits (5.48%; 4 species) (Table 1; Figure 2).

Medicinal plants possess different types of secondary metabolites, for example, phenolic derivatives (4-hydroxy panduratin, geraniin, α -mangostin, methyl gallate), alkaloids (castanospermine, hirsutine, palmatine), flavonoids (chartaceones, pectolinarin and acacetin-7-O-robinosides, catechin, 5-hydroxy-7-methoxy-6-methyl flavanone), terpenoids (betulinic acid 3 β -caffeate, lupeol acetate, celastrol, andrographolide), and polysaccharides (fucoidan, carrageenan, galactomannan)

(Table 1; Altamish *et al.*, 2022; Dhiman *et al.*, 2022); although the mechanism of preventing dengue infections is different (Figure 3) and compound-specific. Saleh and Kamisah (2021) enumerated 51 bioactive substances in total with different degrees of anti-dengue activity, including seven polysaccharides, 14 flavonoids, 17 phenolics and flavonoid glycosides and derivatives, two alkaloids, a saponin, six terpenoids, and four phenolic acids. Quercetin, one of several compounds, showed significant anti-DENV-2 inhibitory effects (Zandi *et al.*, 2011). More importantly, both DENV and SARS-CoV viral infections are inhibited by quercetin (Saleh and Kamisah, 2021). The plant bioactive compounds demonstrate an antiviral response either directly or via inducing immunomodulatory response cascades against DENV at various stages of infection, such as inhibition of viral entry and/or viral cell adhesion, viral adsorption, intracellular replication, proliferation, and cellular metabolism (Figure 3; Loaiza-Cano *et al.*, 2021; Dhiman *et al.*, 2022). Certain medicinal plants, such as *Houttuynia cordata* and *Boesenbergia rotunda* and their bioactive compounds effectively inhibit both DENV and SARS-CoV infections, despite their distinct viral families, structures, entry mechanisms, replication, and pathogenicity (Saleh and Kamisah, 2021). Flavonoids also facilitate the entry of infectious virus particles into phagocytes, immune cells that generate several proinflammatory cytokines involved in the pathophysiology of severe dengue (Loaiza-Cano *et al.*, 2021). Additionally, plant metabolites aid in lowering the antigen burden and regulating several host immunological components (Dhiman *et al.*, 2022). Plant-mediated immunomodulation results in increased platelet counts, phagocytic pathway activation, and controlled cytokine production.

The insecticidal qualities of medicinal plants, such as *A. paniculata*, *A. calamus*, *A. indica*, *C. citratus*, etc., are another important feature that makes them a viable and environmentally benign alternative to

controlling *Aedes* mosquitoes and dengue infection (Table 1; Senthil-Nathan, 2020; Saleh and Kamisah, 2021; Dhiman *et al.*, 2022). Medicinal plant extracts have larvicidal, pupicidal, ovicidal, and adulticidal properties, and act as repellents, toxins, feeding deterrents, growth retardants, and bite protectors. These extracts target cellular mechanisms, potentially disrupting vector mosquitoes' functions by disturbing proteins, ion channels, nucleic acids, and other cellular components; reducing enzyme levels, affecting metamorphosis and body tissues like epithelium layer, muscles, midgut, and nervous systems (Hillary *et al.*, 2024). Medicinal plant extracts, as bioinsecticides, are effective alternatives to chemical insecticides in integrated mosquito management programs due to their multiple modes of action and synergic compounds, which lower the possibility of development of resistance to it (Demirak and Canpolat, 2022). These products of plant origin offer the combined benefits of being affordable, safe for non-target creatures, biodegradable, and environmentally benign. Moreover, some medicinal plants are still utilised in traditional ways for their anti-dengue qualities and have not yet been studied for scientific validation (Table 1; Dhiman *et al.*, 2022).

CHALLENGES AND FUTURE DIRECTIONS

The absence of an effective and widely accessible dengue vaccine and antiviral medications, along with difficulties in outbreak prediction and management in Bangladesh, coupled with fluctuating incidence, necessitates localized strategies and the utilization of medicinal plants with anti-dengue properties. Constraints for the integration of traditional medicine into the modern healthcare system could be divided into organizational, legal, policy, and socio-cultural. The efficacy, safety, uniformity, and quality of conventional medicine are sometimes a matter of debate. Traditional approaches to prescribing medicinal plants with anti-dengue properties are yet to be scientifically validated. Extensive research is, therefore, required to assess immunologic

potentials, phytochemical richness, and clinical probing of these plants for effective medicine development. Moreover, comparison among medicinal plants (and/or phytochemicals) could be an interesting topic for commercial biopesticides development. Collaboration between traditional medicine practitioners and modern pharmaceutical researchers should be fostered to integrate traditional knowledge with contemporary science effectively.

CONCLUSION

This review shows the enormous potential of Bangladeshi medicinal plants, available in other parts of the world as well, for the treatment of dengue. Several interesting medicinal plants have been identified that could be developed into prescription medications to treat dengue infections. To create a successful medication, further clinical research is needed to evaluate the immunologic potential of plants and their phytochemical diversity as well.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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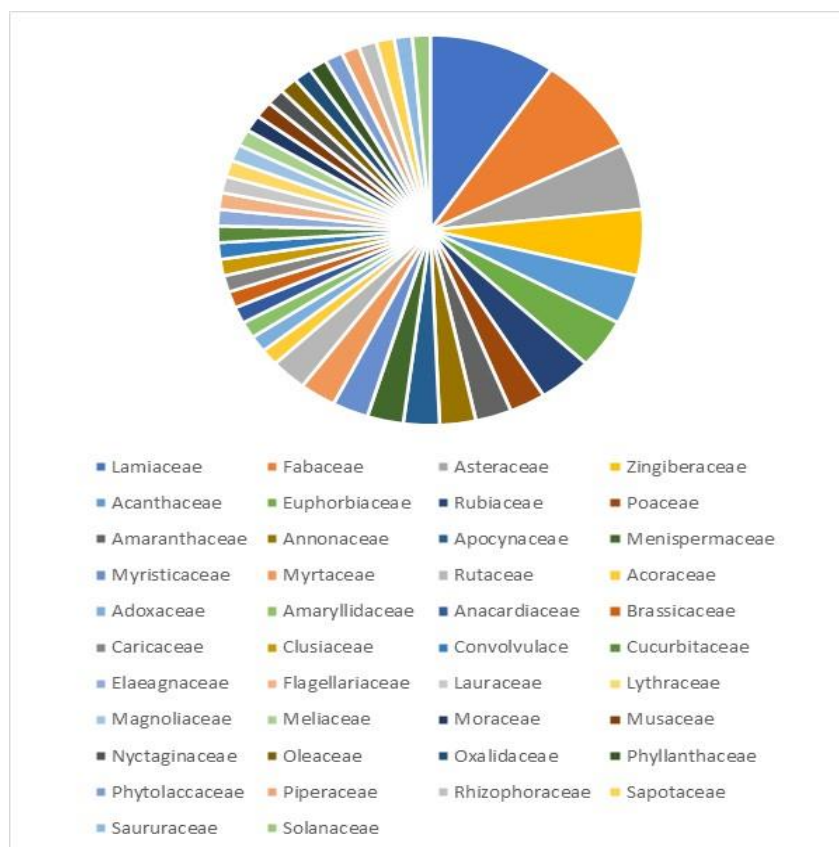


Figure 1. Family-wise distribution of medicinal plants in Pie graph.

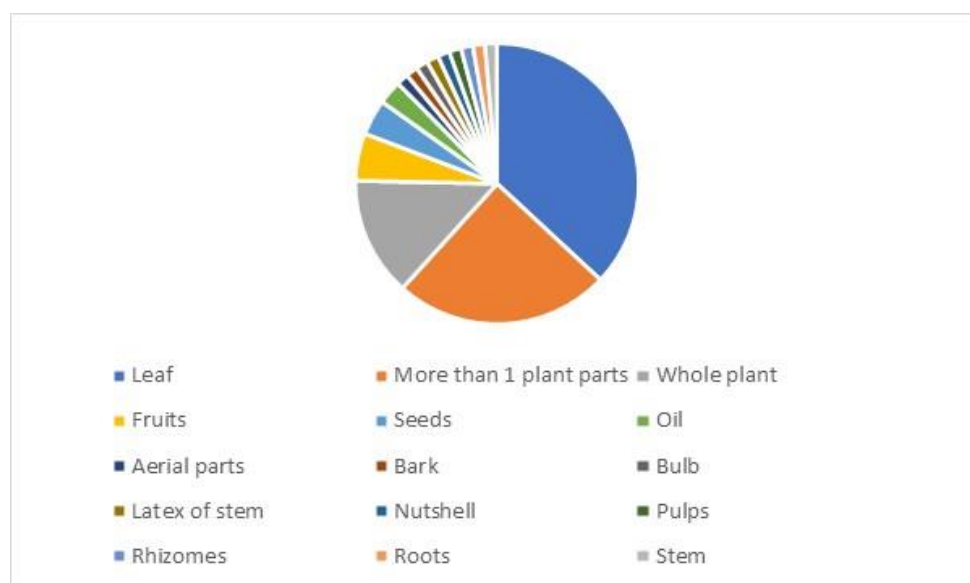


Figure 2. Plant parts use of medicinal plants in Pie graph.

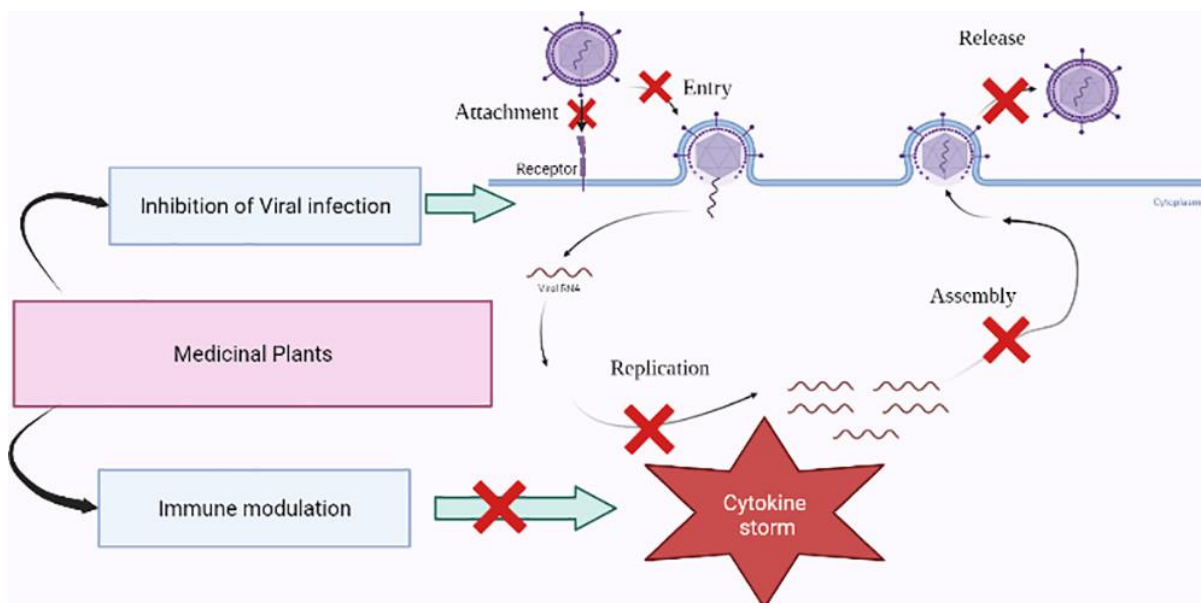


Figure 3. Illustration of dengue virus inhibition mechanisms by medicinal plants (adopted from Altamish *et al.*, 2022).

Table 1: List of the medicinal plants used against dengue.

Sl. No.	Bangla/Common Name	Scientific name	Family	Part used	Active constituents	Reference
1.	Kalmegh	<i>Andrographis paniculata</i> (Burm.f.) Wall. ex Nees*	Acanthaceae	Whole plant	Diterpneoid, Andrographolides	Divyaa <i>et al.</i> , 2020
2.	Sabah Snake Grass	<i>Clinacanthus nutans</i> (Burm.f.) Lindau	Acanthaceae	Leaves, Whole plant	-	Julsrigival <i>et al.</i> , 2021
3.	Bashak	<i>Justicia adhatoda</i> L.	Acanthaceae	Arial parts	-	Wilson <i>et al.</i> , 2021
4.	Botch	<i>Acorus calamus</i> L.*	Acoraceae	Leaves, Roots	β-asarone, Acoric acid, Calamusin D	Rosmalena <i>et al.</i> , 2019
5.	Elder	<i>Sambucus nigra</i> L.	Adoxaceae	Flower, Leaves	Alkaloids, Flavonoids, Coumarins	Julsrigival <i>et al.</i> , 2021
6.	Malancha	<i>Alternanthera philoxeroides</i> (Mart.) Griseb.	Amaranthaceae	Whole plant	Flavonoids, Saponins	Altamish <i>et al.</i> , 2022
7.	Shalinche/Carpet Weed	<i>Alternanthera sessilis</i> (L.) DC.	Amaranthaceae	Leaves	-	Saleh and Kamisah, 2021
8.	Garlic	<i>Allium sativum</i> L.	Amaryllidaceae	Bulb	Diallyl disulfide (DADS)	Altamish <i>et al.</i> , 2022
9.	Cheshunt	<i>Anacardium occidentale</i> L.	Anacardiaceae	Nutshell	Cardol Triene	Loaiza-Cano <i>et al.</i> , 2021
10.	Atta/Custard apple	<i>Annona reticulata</i> L.	Annonaceae	Bark, Fruit	-	Saleh and Kamisah, 2021
11.	Soursop	<i>Annona muricata</i> L.	Annonaceae	Fruit	-	Saleh and Kamisah, 2021
12.	Chatim	<i>Alstonia scholaris</i> (L.) R.Br.	Apocynaceae	Leaves	Alstotides	Julsrigival <i>et al.</i> , 2021
13.	Nayantara	<i>Catharanthus roseus</i> (L.) G.Don	Apocynaceae	Whole plant, Leaves	-	Islam <i>et al.</i> , 2014
14.	Assamlata	<i>Mikania cordata</i> (Burm.f.) B.L.Rob.	Asteraceae	Leaves	-	Rahmatullah <i>et al.</i> , 2009
15.	Bikashlata/Bittervine	<i>Mikania micrantha</i> Kunth	Asteraceae	Leaves	-	Bradacs <i>et al.</i> , 2011
16.	Tridhara	<i>Tridax procumbens</i> L.	Asteraceae	Stem	Flavonoids	Bhuiyan <i>et al.</i> , 2020
17.	Cucushim	<i>Vernonia cinerea</i> (L.) H.Rob	Asteraceae	Leaves	Phenolics (gallic acid)	Bhuiyan <i>et al.</i> , 2020
18.	Field Mustard	<i>Brassica campestris</i> L.	Brassicaceae	Oil	-	Saleh and Kamisah, 2021
19.	Papaya	<i>Carica papaya</i> L.	Caricaceae	Leaves, Fruits	Quercetin	Sarker <i>et al.</i> , 2021
20.	Mangosteen	<i>Garcinia mangostana</i> L.	Clusiaceae	Fruit pericarp	α-mangostin	Altamish <i>et al.</i> , 2022
21.	Sweet potato	<i>Ipomoea batatas</i> (L.) Lam.	Convolvulaceae	Leaves	-	Saleh and Kamisah, 2021
22.	Bitter gourd	<i>Momordica charantia</i> L.	Cucurbitaceae	Roots, Fruits	Potent protein	Desai <i>et al.</i> , 2020
23.	Seaberry	<i>Hippophae rhamnoides</i> L.	Elaeagnaceae	Leaves	-	Divyaa <i>et al.</i> , 2020
24.	Bishkatali	<i>Cladogynos orientalis</i> Zipp. ex Span.	Euphorbiaceae	Whole plant	-	Klawikkan <i>et al.</i> , 2011
25.	Dudhia/Asthma-plant	<i>Euphorbia hirta</i> L.	Euphorbiaceae	Leaves, stems, roots	-	Fiscal, 2017
26.	Multigreen	<i>Breynia androgyna</i> (L.) Chakrab. & N.P.Balabr.	Euphorbiaceae	Leaves	β-sitosterol	Joshi <i>et al.</i> , 2023
27.	Black Bean	<i>Castanospermum australe</i> A.Cunn. ex Mudie	Fabaceae	Seeds	Castanospermine	Altamish <i>et al.</i> , 2022
28.	Licorice	<i>Glycyrrhiza glabra</i> L.	Fabaceae	Root	Glycyrrhizin	Babbar <i>et al.</i> , 2023
29.	Ipil Ipil	<i>Leucaena leucocephala</i> (Lam.) de Wit	Fabaceae	Seeds	Galactomannan	Altamish <i>et al.</i> , 2022
30.	Khair	<i>Senegalia catechu</i> (L.f.) P.J.H.Hurter & Mabb.	Fabaceae	Leaves	Pep-RTYM, Catechin, Quercetin	Altamish <i>et al.</i> , 2022
31.	Sonalata	<i>Senna alexandrina</i> Mill.	Fabaceae	Leaves	Phenolic glycosides	Dhiman <i>et al.</i> , 2022
32.	Methi/Fenugreek	<i>Trigonella foenam-graecum</i> L.	Fabaceae	Leaves	-	Deep <i>et al.</i> , 2018
33.	Bon Chad	<i>Flagellaria indica</i> L.	Flagellariaceae	Whole plant	-	Klawikkan <i>et al.</i> , 2011
34.	Musk Basil	<i>Basilicum polystachyon</i> (L.) Moench	Lamiaceae	Whole plant	-	Altamish <i>et al.</i> , 2022
35.	Bhat/Glory bower	<i>Clerodendrum viscosum</i> L.	Lamiaceae	Leaves	-	Rahmatullah <i>et al.</i> , 2011
36.	Patabahar/Coleus	<i>Coleus scutellarioides</i> (L.) Benth.	Lamiaceae	Leaves	-	Gascon, 2011
37.	Wild Pudina/Wild mint	<i>Mentha arvensis</i> L.*	Lamiaceae	Leaves	-	Gascon, 2011
38.	Tulsi/Holy Basil	<i>Ocimum tenuiflorum</i> L.	Lamiaceae	Leaves	-	Chand <i>et al.</i> , 2018
39.	Java Tea	<i>Orthosiphon aristatus</i> (Blume) Miq. var. <i>aristatus</i>	Lamiaceae	Leaves	-	Saleh and Kamisah, 2021
40.	Nishinda	<i>Vitex negundo</i> L.	Lamiaceae	Leaves	-	Gascon, 2011

41.	Avocado	<i>Persea americana</i> Mill.	Lauraceae	Fruits	(2 R,4 R)-1,2,4-trihydroxyheptadec-16-yne (THHY)	Altamish et al., 2022
42.	Dalim/Pomegranate	<i>Punica granatum</i> L.	Lythraceae	Fruits	Punicalagin	Loaiza-Cano et al., 2021
43.	Magnolia	<i>Magnolia officinalis</i> Rehder & Wilson	Magnoliaceae	Bark, Seed cones	Honokiol (Lignan Biphenol)	Fang et al., 2015
44.	Neem	<i>Azadirachta indica</i> A.Juss.*	Meliaceae	Leaves	Azadirachtin	Parida et al., 2002
45.	Velvet leaf	<i>Cissampelos pareira</i> L.	Menispermaceae	Aerial parts	Cissampelo flavone	Babbar et al., 2023
46.	Gulancha/Heart-leaved moonseed	<i>Tinospora cordifolia</i> (Willd.) Hook.f. & Thomson	Menispermaceae	Leaves, Stems	Alkaloids, Glycosides, Steroids, Flavonoids	Deep et al., 2018
47.	Hauili	<i>Ficus septica</i> Burm.f.	Moraceae	Leaves, Stem, Fruit, Heartwood	Alkaloids, Lignans, Triterpenes, Flavonoids, And Steroids	Altamish et al., 2022
48.	Banana	<i>Musa × paradisiaca</i> L.	Musaceae	Latex of stems	-	Saleh and Kamisah, 2021
49.	Nutmeg	<i>Myristica fatua</i> Houtt.	Myristicaceae	Seeds	Artesunic acid, Homoeogonol, Myristicin	Rosmalena et al., 2019
50.	Nutmeg	<i>Myristica fragrans</i> Houtt.	Myristicaceae	Leaves	-	Saleh and Kamisah, 2021
51.	Guava	<i>Psidium guajava</i> L.*	Myrtaceae	Leaves, Bark, Young fruits	-	Deep et al., 2018; Batoro & Siswanto, 2017
52.	Jamrul	<i>Syzygium samarangense</i> (Blume) Merr. & L.M.Perry	Myrtaceae	Leaves	5-hydroxy- 7- methoxy- 6-methylflavanone (FN5Y)	Altamish et al., 2022
53.	Punarnava Red	<i>Boerhavia diffusa</i> L.	Nyctaginaceae	Leaves, Stem	tannins, phenols, Flavonoids, Others	Babbar et al., 2023
54.	Sheuli, Shefali	<i>Nyctanthes arbor-tristis</i> L.	Oleaceae	Leaves	β-Sitosterol, Calceolarioside A	Morshed et al., 2022
55.	Kamranga/Carambola	<i>Averrhoa carambola</i> L.	Oxalidaceae	Leaves, Barks, Fruit	2-dodecyl-6-methoxycyclohexa -2,5-diene-1,4-dione	Wei et al., 2018
56.	Bhui amla	<i>Phyllanthus amarus</i> Schumach. & Thonn.	Phyllanthaceae	Whole plant	-	Altamish et al., 2022
57.	Southern pokeweed	<i>Phytolacca bogotensis</i> Kunth.	Phytolaccaceae	Fresh leaves	-	Bussmann & Sharon, 2006
58.	Chuijhal	<i>Piper retrofractum</i> Vahl.*	Piperaceae	Whole plant	-	Klawikkan et al., 2011
59.	Lemon grass	<i>Cymbopogon citratus</i> (DC.) Stapf.*	Poaceae	Oil	Geranial, Geranyl acetate	Rosmalena et al., 2019
60.	Banar-mul	<i>Vetiveria zizaniodes</i> L.*	Poaceae	Roots	Khushimol, α-vetiverone, β-vetiverone, ethyl 4-(4- methylphenyl)-4- pentenoate	Firuj et al. 2023
61.	Garjan	<i>Rhizophora apiculata</i> Blume	Rhizophoraceae	Whole plant	-	Klawikkan et al., 2011
62.	Cinchona	<i>Cinchona</i> spp.	Rubiaceae	Bark	-	Sarah et al., 2020
63.	Bishphal	<i>Pavetta tomentosa</i> Roxb. ex Sm.	Rubiaceae	Leaves	Saponins, Flavonoids and Alkaloids,	Pratheeba et al., 2019
64.	Thankuni	<i>Tarenna asiatica</i> (L.) Kuntze ex K.Schum.	Rubiaceae	Leaves	Saponins, Favonoids and Alkaloids,	Pratheeba et al., 2019
65.	Lemon	<i>Citrus limon</i> (L.) Osbeck*	Rutaceae	Pulps	-	Hajdu & Hohmann, 2011
66.	Euodia	<i>Euodia species</i>	Rutaceae	Leaves	-	Kandowangko et al., 2011
67.	Miracle fruit	<i>Synespalum dulcificum</i> (Schumach. & Thonn.) Daniell.	Sapotaceae	Leaves	-	Gascon, 2011
68.	Aishta nagini	<i>Houttuynia cordata</i> Thumb.	Saururaceae	Whole plant	Chlorogenic acid	Klawikkan et al., 2011
69.	Bon tepari	<i>Physalis angulata</i> L.	Solanaceae	Leaves	-	Bradacs et al., 2011
70.	Chinese Ada/Fingerroot	<i>Boesenbergia rotunda</i> (L.) Mansf.	Zingiberaceae	Rhizomes	Cardamonin	Kiat et al., 2006
71.	Holud/Curcuma	<i>Curcuma longa</i> L.*	Zingiberaceae	Rhizomes, Leaves	Curcumin	Ichsyani et al., 2017
72.	Ada/Ginger	<i>Zingiber officinale</i> L.*	Zingiberaceae	Rhizomes, Leaves	-	Sharma et al., 2015
73.	Bon Ada/Ginger	<i>Zingiber purpureum</i> Rosc.	Zingiberaceae	Rhizomes, Leaves	-	Kandowangko et al., 2011

* Plants with insecticidal properties

Review article

Specific features of cannabidiol metabolism and excretion during long-term consumption of cannabis

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ABSTRACT

The purpose of this study was to characterize the key patterns of cannabidiol metabolism and elimination. To fulfil this purpose, the study employed the latest scientific sources for the last 5-6 years from authoritative scientometric databases and archives, using bibliographic and bibliosemantic research methods. Cannabidiol has considerable therapeutic potential in the treatment of neurodegenerative diseases and neuropsychiatric disorders. Due to its properties, cannabidiol can be effective in treating conditions such as Alzheimer's disease, Parkinson's disease, multiple sclerosis (especially in young people), post-traumatic stress disorder, schizophrenia, etc. However, apart from therapeutic effects, it is necessary to consider the principles of drug metabolism and elimination. This substance is absorbed in the gastrointestinal tract after oral administration. The percentage of cannabidiol absorbed depends on the form of intake (capsules, oils, or food) and can range within 6-20%. The metabolism of cannabidiol is a multi-stage and complex process involving absorption in the digestive tract, intensive metabolism in the liver under the influence of digestive enzymes and cytochrome P450, and subsequent excretion of metabolites through the bile into the intestines. Understanding these processes is important for the optimised use of cannabidiol for medical purposes, considering its bioavailability and possible interactions with other drugs. The metabolism of cannabidiol is largely dependent on isoenzymes, specifically CYP2C19 and CYP3A4, which oxidise cannabidiol to active and inactive metabolites such as 7-hydroxycannabidiol (7-OH-CBD). Additional enzymes, such as CYP1A1, CYP1A2, CYP2C9, and CYP2D6, are also involved in this process, but their contribution is less significant.

Keywords: Elimination, hepatobiliary system, law, narcotic substances, resistance,

INTRODUCTION

Cannabis, a plant with a rich history of use in folk medicine, is renowned for its therapeutic properties. Containing over one hundred chemical compounds, most notably cannabinoids like tetrahydrocannabinol (THC) and cannabidiol (CBD), cannabis has been employed to treat pain, inflammation,

anxiety, and insomnia. Its leaves, flowers, and seeds have long been used in traditional remedies, while the hemp variety has been valued for its fiber in textiles and other industrial products. The growing recognition of cannabis as both a medicinal and industrial resource continues to shape modern health discussions. Recently, the popularization

of CBD has gained momentum worldwide, including in Ukraine, driven by increasing scientific research, demand for alternative therapies, and the widespread use of cannabinoids in countries like the USA, Italy, Switzerland, Germany, Canada, and Georgia.

For a long time, CBD was regarded as an exclusively narcotic substance in Ukraine. However, cannabis was legalized for medical use in 2024, as stated in the 2023 Draft Law No. 7457. Historically, cannabis has been used for various purposes, but its place in modern medicine remains controversial. Ukrainian law traditionally prohibits cannabis-related activities, with exceptions for scientific research. In contrast, researchers such as Babak and Kabrera-Lapitska (2022) highlight the growing legal discussions surrounding cannabis and its potential medical applications. Shevchuk and Hasiuk (2023) emphasize that CBD, unlike THC, does not produce typical narcotic effects like euphoria or altered consciousness. This makes it a promising candidate for medical use. Researchers like Hurtova and Kasianenko (2021) focus on CBD's therapeutic potential in treating neurological diseases such as epilepsy, Parkinson's, and Alzheimer's. They note that, since 2017, CBD derived from cannabis has been recognized as an effective treatment for these conditions.

Furthermore, the legal aspects of CBD are explored by scholars like Hurtova and Kasianenko (2021), who suggested that countries such as France and Switzerland, where medical cannabis is regulated through a doctor's prescription, could serve as models for Ukraine's legislative approach. Kalieniuk (2021) argues that cannabis use should be viewed not as a personal issue, but as a societal concern. Dispelling the myth that CBD is merely a recreational drug, researchers such as Stepaniuk and Lozova (2024) stress the need to provide evidence

of its therapeutic properties. The metabolism of CBD is another critical area of study. Radish and Yevtushenko (2020) note that genetic factors, age, and health conditions can affect how individuals metabolize CBD, influencing its therapeutic effects. Despite the growing body of evidence, the exact mechanisms of CBD's action remain unclear.

Thus, most researchers have paid considerable attention to legal issues related to the use of cannabinoid substances for medicinal purposes, and only a few have raised the complexity of cannabidiol metabolism in the human body, the mechanisms of their effects on a healthy body and in pathology. The complexity and relevance of the problem lies in the fact that very few researchers have considered the elimination or excretion of cannabidiol by the human body. The purpose of this study was to analyse the key processes of CBD metabolism. The objectives of the study were to investigate the most common legislative documents on regulating the distribution of cannabis-based drugs; to find the benefits and dangers of CBD; and to investigate the specifics of CBD elimination.

MATERIALS AND METHODS

To fulfil the purpose set, the bibliographic and bibliosemantic research methods were used as the basis. The approach to the use of scientometric databases such as Scopus, Web of Science, PubMed, Google Scholar, as well as other scientific archives, including Research Gate, demonstrated a broad and comprehensive analysis of the current professional literature on the issues under study. The choice of searching by title rather than by topic helped to accurately identify relevant research topics, which contributed to the accuracy and relevance of the information obtained. The absence

of language restrictions helped to cover a wide range of scientific literature and consider different opinions and approaches to the problem under study. The literature search was conducted using keywords and phrases related to cannabidiol metabolism and elimination. The data obtained were thoroughly analysed, which helped to identify the key trends, problems, and areas for further investigation in this area. Bibliographic and bibliosemantic methods, as well as modern scientometric databases, helped to obtain comprehensive and up-to-date information on the metabolism and elimination of cannabidiol, which underlies further research and practical applications in this area. A series of terms, concepts, phrases, and keywords were used to search for scientific information: “cannabidiol”, “cannabis”, “marijuana”, “analgesics”, “narcotic substances”, “law”, “law and order”, “permission”, “draft law”, “medicine”, “palliative medicine”, “headache”, “neurology”, “mental disorders”, “panic”, “fear”, “anxiety”, “stress”, “metabolism”, “elimination”, “excretion”, “absorption”, “half-life”, “liver”, “gallbladder”, “urination”, “hepatobiliary tract”, “pharmacokinetics”, “pharmacodynamics”, “diabetes mellitus”, “oncology”, “insulin resistance”, “sphingolipids”, “psoriasis”, “skin accumulation”, “toxicity”, “coronavirus”, “antioxidant properties”, “metabolic regulators”, “cytochrome P450”.

Considering that the study of cannabidiol metabolism and elimination is a complex process and should be carried out under special conditions, a selection of the latest literature from the last 5-6 years on the pharmacodynamics and kinetics of cannabidiol, as well as legal issues related to this substance, has been compiled. In most sources, scientists performed preclinical studies on rats (*in vivo* animal models). To obtain the results, a dose of

cannabidiol was experimentally administered by intravenous injection, orally or by inhalation. After that, blood samples were taken at different time intervals to determine the concentrations of cannabidiol and its metabolites. Bile collection by gallbladder cannulation. Furthermore, faecal samples were collected to determine the excretion of metabolites. The content and effect of cannabidiol on cellular structures were investigated using microscopy. Subsequently, standard cultivation of hepatocytes and other relevant cell lines was performed. Specific inhibitors were used to determine the role of individual enzymes (CYP450 enzyme inhibitors).

In the legal aspect, the following documents were analysed to investigate the legality and appropriateness of cannabidiol use in a more structural way: the Law of Ukraine No. 60/95-VR “On Narcotic Drugs, Psychotropic Substances and Precursors” (1995), the Law of Ukraine No. 123/96-VR “On Medicinal Products” (1996), the Draft Law of Ukraine No. 5596 “On Amendments to Certain Legislative Acts of Ukraine Regarding the Regulation of the Circulation of Cannabis for Medical, Scientific, and Scientific-Technical Purposes” (2021). These laws and draft laws are the principal regulations governing the circulation of drugs and the possible use of medical cannabis in Ukraine. They define the legal framework, restrictions, and requirements for the production, storage, use, and control of these substances.

RESULTS AND DISCUSSION

CBD products that have not been approved by regulatory authorities have gained considerable popularity as self-medication for various diseases due to widespread, but often unfounded, beliefs about their medicinal properties. Many people resort to CBT in the hope of

alleviating symptoms of anxiety, pain, insomnia, and other medical problems. However, due to the lack of scientifically proven data, the use of such products without proper consultation with medical professionals can be dangerous. These products may have an unknown composition, which creates additional risks for consumers. The lack of control by official authorities means that the quality and safety of such products are not absolutely guaranteed to the public, and they may contain impurities or have an inaccurate concentration of active substances. This complicates the assessment of their efficacy and safety, increasing the risk of adverse side effects.

Recently, the popularity of CBD has increased, which has led to the emergence of many CBD products on the market. Research includes both experimental and clinical trials aimed at investigating its effects on cognitive and anxiety disorders, movement disorders, and pain inflammation. However, existing research does not provide sufficient high-quality evidence on the effectiveness of CBD in the treatment of these conditions. In addition, not enough is known about its safety and the exact functions and dosage for each particular disorder or disease. Many people use these products to treat conditions such as chronic pain, anxiety, depression, and even epilepsy, despite the lack of reliable scientific evidence of the effectiveness of CBD in these cases (Britch *et al.*, 2021; Dashi *et al.*, 2015). Since these products are not properly regulated, there is an elevated risk that they may contain impurities or incorrect doses of the active ingredient, which can lead to unpredictable health consequences, and no one will be held responsible for this.

In 2024, Ukraine legalized the use of medical cannabis for various conditions, including cancer and post-traumatic stress disorder (PTSD) (Draft

Law No. 7457, 2023). This step is aimed at alleviating pain, stress, and other symptoms in patients, including those affected by the war. The United Nations (UN) established the International Narcotics Control Board. This organization has emphasized the significance of the availability of medicines containing narcotic substances for patients who need them, following international standards and requirements (International Narcotics Control Board, 2024). The law also regulates the cultivation of hemp. It is allowed to grow technical hemp varieties with a THC content of less than 0.3% for industrial purposes, while for medical purposes the permissible THC level may be higher. This draft law was accompanied by a considerable number of discussions and amendments. Specifically, the law prescribes the creation of an electronic register of the Ministry of Agrarian Policy and the approval of medical protocols for the use of cannabis to treat certain diseases. However, there is some criticism about possible contradictions in the law that could affect doctors and patients.

The legalization of medical cannabis in Ukraine, effective from 2024, holds significant potential for the agricultural, pharmaceutical, and industrial sectors. The nation's favourable climate and soil conditions make it ideally suited for cannabis cultivation, presenting an opportunity for agricultural diversification. Additionally, the growing demand for cannabis-based medicines could stimulate the pharmaceutical industry, positioning Ukraine as a key player in the European market. The industrial sector stands to benefit from the processing of hemp for textiles, biofuels, and other products, potentially leading to new manufacturing processes and products (Aliakperova *et al.*, 2023).

Beyond its medical applications, the legalization of CBD in Ukraine offers

considerable economic prospects, particularly in the agricultural sector, where large-scale hemp cultivation is permitted, with the expectation of increasing agricultural productivity and creating new markets for Ukrainian farmers. The pharmaceutical sector is also poised for growth, as cannabis-based medicines become more widely accepted (Vyshka *et al.*, 2014). Furthermore, industries such as textile production and biofuels, which utilize hemp fibres, are expected to experience an increase in production and innovation (Burlaka *et al.*, 2024). Collectively, these sectors present Ukraine with a unique opportunity to diversify its economy, attract foreign investment, and create new job opportunities.

Pharmacological studies have shown that lower doses of CBD (e.g., 10-20 mg) can effectively reduce anxiety in individuals with social anxiety disorder, whereas higher doses (e.g., 100-300 mg) may be needed to address generalized anxiety disorder (García-Gutiérrez *et al.*, 2020; Castillo-Arellano *et al.*, 2023). In one clinical trial, doses ranging from 5 mg to 400 mg per day were tested, demonstrating that while a moderate dose of 200 mg daily led to a marked reduction in anxiety symptoms in patients with generalized anxiety disorder, higher doses did not yield significantly better results, suggesting a dose-dependent effect (García-Gutiérrez *et al.*, 2020). For pain management, a dose of 10-20 mg CBD has been shown to significantly reduce pain and inflammation in chronic pain models (Britch *et al.*, 2021). In contrast, for neurodegenerative diseases like Parkinson's and Alzheimer's, higher dosages (50-100 mg) have been more effective in reducing symptoms and improving patient quality of life (Shevchuk and Volos, 2023; Lako *et al.*, 2023).

CBD affects various ion channels, including sodium and calcium channels. This can have a considerable impact on neural excitability and signal transmission in the nervous system. CBD's effect on ion channels contributes to its therapeutic properties, such as pain relief and seizure reduction. CBD also interacts with metabotropic receptors such as serotonin and adenosine receptors. The effect on these receptors may explain the anxiolytic and antidepressant properties of CBD, as serotonin receptors play a key role in the regulation of mood and anxiety (Fig. 1).

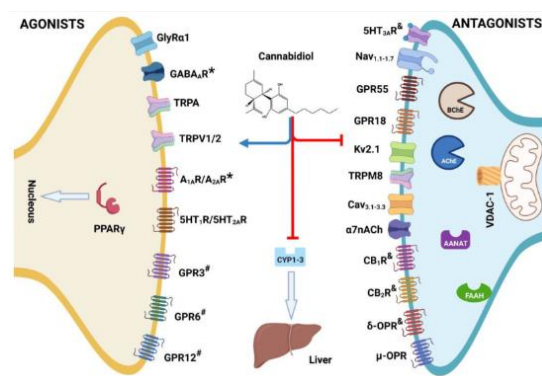


Fig. 1 : Molecular structure of cannabidiol and its relationship with receptors and synapses

Source: compiled by the authors based on Castillo-Arellano *et al.* (2023)

Increased doses of CBD can activate TRPV1 receptors, reducing anxiolytic effects. Blocking these receptors may enhance CBD's anxiolytic properties, reducing anxiety and stress (Fig. 2). CBD can also affect the levels of anandamide, an endocannabinoid that plays an important role in regulating mood and anxiety. The FAAH enzyme breaks down anandamide, reducing its levels in the brain. By inhibiting FAAH, CBD can increase the concentration of anandamide, which enhances its anxiolytic and antidepressant effects (Pivtoraiko *et al.*, 2022).

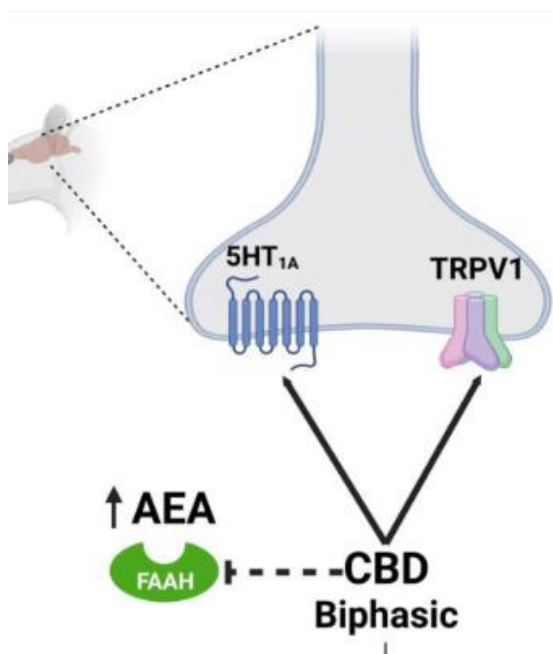


Fig. 2 : Schematic representation of the effect of cannabidiol on brain receptors in anxiety disorders

Source: compiled by the authors based on Castillo-Arellano *et al.* (2023)

These mechanisms underline the complexity of the action of CBD and the significance of understanding its dose-dependent effects. The choice of dose can have a considerable impact on its effectiveness in treating anxiety and other conditions. Blocking the TRPV1 receptor or inhibiting the FAAH enzyme may be potential strategies to enhance the anxiolytic effects of high doses of the compound.

Portillo *et al.* (2024) found that CBD disrupts tryptophan catabolism, potentially impairing fetal cognitive development through alterations in serotonin and kynurenine pathways. The cited study demonstrates that CBD considerably inhibits serotonin transport in the placenta, reducing its level by approximately 60% even at the lowest concentrations tested. This suggests that the compound may interfere with the

natural mechanism of serotonin transport, which is important for foetal development.

CBD has been associated with metabolic disorders like diabetes and obesity, primarily through its interaction with cannabinoid receptors CB1 and CB2, which influence various physiological and immune processes (Charytoniuk *et al.*, 2021; Gochman *et al.*, 2023). Activation of the CB2 receptor is associated with anti-inflammatory effects and may have therapeutic value in the treatment of inflammatory and autoimmune diseases. Anandamide, also known as N-arachidonylethanolamine, is one of the main endocannabinoids. It binds to both CB1 and CB2 receptors but has a higher affinity for CB1. Anandamide is involved in the regulation of mood, memory, appetite, and pain. It is metabolised by the enzyme FAAH, which breaks down anandamide into less active compounds. 2-arachidonoylglycerol (2-AG) is another important endocannabinoid that binds to both CB1 and CB2 receptors (Chayasirisobhon, 2021). It is involved in the regulation of many physiological processes, such as inflammation, immune response, and neuroprotection. 2-AG is metabolised via the enzymes monoacylglycerol lipase (MAGL) and α/β -hydrolase domain-6 (ABHD6).

CBD and cannabigerol (CBG) have anti-tumor effects, reducing glycolysis and lipid synthesis, and inducing oxidative stress in cancer cells (Mahmoud *et al.*, 2023). These cannabinoids can reduce the activity of the MAPK/ERK pathway, which plays a significant role in cancer cell proliferation, and inhibit angiogenesis, the formation of new blood vessels necessary for tumor growth.

CBD is primarily metabolized in the liver by cytochrome P450 enzymes, with CYP3A4 and CYP2C19 playing the most significant roles in its biotransformation. These enzymes oxidize CBD to primary metabolites, such as 7-hydroxycannabidiol

(7-OH-CBD) and 7-carboxycannabidiol (7-COOH-CBD), which may also be biologically active and contribute to its therapeutic effects. Additionally, CBD interacts with other drugs through inhibition of CYP450 enzymes, particularly CYP3A4 and CYP2C19, potentially altering the concentration and efficacy of other medications (Chan and Duncan, 2021; Nasrin *et al.*, 2021).

According to Martinez Naya *et al.* (2024), while CYP2C19 and CYP3A4 are the primary enzymes involved in CBD metabolism, other enzymes like CYP1A1, CYP1A2, CYP2C9, and CYP2D6 contribute to a lesser extent. 7-OH-CBD, one of the main active metabolites, is formed through hydroxylation by these enzymes and shows 38% lower plasma levels than CBD, indicating reduced bioavailability or faster excretion. After its formation, 7-OH-CBD can undergo additional transformations in the liver, producing further metabolites. In a similar manner, the hepatoprotective and choleretic properties of herbal compounds such as *Achillea* and *Tanacetum* are contingent on the liver for their metabolism and biliary excretion. The present study thus demonstrates the pivotal role of the liver in the metabolism and excretion of compounds, a finding that is further highlighted by the case of both CBD and the herbal compounds mentioned above (Kazakova *et al.*, 2024).

CBD and its metabolites are primarily excreted via feces, with key metabolic steps including hydroxylation, oxidation, and conjugation. These processes help prepare the metabolites for excretion, with glucuronidation and sulfation ensuring their water solubility. This facilitates their removal through bile or urine.

CBD metabolites are primarily excreted through the intestines, following secretion into the bile and passage through the gallbladder (Mamontov *et al.*, 2023).

Most of the CBD metabolites are excreted from the body through the intestine, which emphasises the important role of the hepatobiliary pathway in the excretion of CBD. After the liver secretes the metabolites of the CBD into the bile, they pass through the gallbladder and enter the intestines. There, the metabolites continue their journey through the digestive system and are eventually eliminated from the body. This process is the main and key one for the effective circulation and elimination of CBD from the body. It emphasises the significance of the liver and gallbladder in the metabolism and excretion of cannabis-like substances. Knowledge of this mechanism is important for understanding the pharmacokinetics of CBD and for developing optimised regimens for its use, ensuring the effectiveness and safety of treatment.

A comprehensive understanding of the metabolism of CBD is essential for the accurate determination of its pharmacokinetic properties and the optimal utilisation of its therapeutic potential. Given the pivotal role of the hepatobiliary pathway in the excretion of CBD, it is equally important to explore the biological effects of CBD itself. CBD is renowned for its anti-inflammatory, antioxidant, and neuroprotective properties, and has consequently attracted considerable attention in the field of medical research with regard to its potential for the treatment of a wide range of conditions.

CBD is one of the key cannabinoids with considerable potential in medical research due to its biological properties, which include anti-inflammatory, antioxidant, and neuroprotective effects. However, to fully understand its pharmacokinetics and metabolic profile, it is necessary to investigate in detail the metabolic processes associated with this compound. CBD has anti-inflammatory,

antioxidant, and neuroprotective biological properties. CBD can reduce inflammation, which is useful in the treatment of various inflammatory diseases such as arthritis, Crohn's disease, etc. CBD exhibits antioxidant activity, helping to neutralise free radicals, which helps protect cells from oxidative stress and damage. Another undoubted fact is that it can help protect neurons and maintain the health of the nervous system, which is promising in the treatment of neurodegenerative diseases such as Alzheimer's and Parkinson's (Zotaj *et al.*, 2024).

The legal status of CBD remains unclear internationally, with some researchers questioning its marketed health benefits (Brunetti *et al.*, 2020; Aliekperova *et al.*, 2020). Considering the above facts, it is necessary to agree with Brunetti *et al.* (2020) and Aliekperova *et al.* (2020) as no regulatory framework for the regulation of cannabis-like substances has been identified. Despite the significant therapeutic potential of cannabidiol and other cannabinoids, their legal status stays ambiguous and often uncertain in many countries. Furthermore, the above researchers note that the practices of other countries such as the United States of America, the Netherlands, etc., cannot be used as an example in this case, as there is still a lack of information on the positive and negative effects of CBD, and some positive results of experimental data are overestimated. Furthermore, most of the studies have only undergone preclinical trials of the CBD and have not passed the clinical stage, which is scientifically incorrect.

CBD's anti-inflammatory and analgesic properties may aid recovery and improve performance in athletes (Schouten *et al.*, 2022; Sunda and Arowolo, 2020). Furthermore, CBD can help athletes cope with stress and improve sleep quality, which also has a positive

impact on recovery and preparation for competition. The *in vivo* studies described by the scientist in his paper focus on the impact of CBD on living organisms, which allows for a more comprehensive understanding of its effects in real life. According to the data obtained, CBD has therapeutic and health-improving properties. However, the results of the study in the cited paper suggest more of a rehabilitative effect of CBD. It is impossible to agree with these researchers on the usefulness of CBD in sports, as these substances can clearly be considered doping for athletes, which is prohibited. In common with the present study, the *in vitro* studies conducted by Zhou *et al.* (2025) on cell cultures allow for a detailed analysis of the mechanisms of action of CBD at the molecular level. The results of these studies suggest the potential of CBD to improve skeletal muscle metabolism, reduce inflammation, accelerate tissue regeneration, and promote anabolism. Human trials show that CBD supplementation promotes muscle recovery, reducing pain and improving well-being. It was expected that CBD was effective in increasing the expression of metabolic regulators in the muscles of obese mice, such as Akt and glycogen synthase kinase-3 (GSK-3). Treatment with CBD in rodents also reduced muscle inflammation after eccentric exercise by affecting nuclear factor kappa B (NF- κ B). In models of muscular dystrophy, CBD reduced the levels of inflammatory markers such as interleukin-6 (IL-6) and tumour necrosis factor alpha (TNF- α). Analogously, in obese rodents, CBD reduced inflammatory processes, specifically by affecting cyclooxygenase-2 (COX-2) and NF- κ B.

In turn, Saraswat *et al.* (2023) highlighted data on the metabolism and mechanism of action of cannabidiol in coronavirus infection in their studies. Co-administration of CBD with intravenous

ramdivir may be a promising strategy to improve treatment efficacy in patients with COVID-19 by providing longer-lasting active levels of ramdivir or another drug in the body. This can be particularly useful for maintaining therapeutic drug concentrations and improving clinical efficacy.

Jarocka-Karpowicz *et al.* (2020) describe the relationship between psoriasis-affected skin and the use of cannabis-like substances such as CBD in this case. In contrast, the cited study investigates the anti-inflammatory and antioxidant effects of CBD and analogous substances. As a result of study, Jarocka-Karpowicz *et al.* cited findings differing from the present study, namely that CBD compounds can accumulate in the skin and are only partially eliminated. CBD has demonstrated the ability to modulate the redox state of cells and affect phospholipid metabolism, which may be important for psoriasis therapy and skin protection from UV radiation. These findings point to the potential therapeutic potential of CBD to reduce inflammation and oxidative stress in the skin, which may be useful for treating psoriasis and protecting the skin from the harmful effects of UV rays. Further research may help to determine the optimised regimens for the use of CBD to maintain skin health and treat dermatological diseases. In patients with psoriasis, CBD helps to reduce oxidative stress and lipid peroxidation, which can help to reduce inflammation and cell damage (Hartmane *et al.*, 2024; Rubins *et al.*, 1992). In healthy people, CBD affects these processes in a slightly different way, emphasising the difference in metabolic pathways between healthy and psoriasis-affected cells.

Berk *et al.* (2022) and Talwar *et al.* (2023) presented data analogous to the above. CBD affects metabolic processes and inflammation in various organs, and

its role in regulating sphingolipid levels is one of the potential mechanisms for improving insulin sensitivity. Sphingolipids play a key role in cellular signalling pathways and may contribute to the development of insulin resistance (Belov *et al.*, 2005). However, the author refutes the long-term presence of cannabinoids in the skin. Considering the data obtained and the literature reviewed by these researchers, we can fully agree that CBD has a pronounced anti-inflammatory effect in the pathogenesis of psoriasis, but these theses require further in-depth investigation. The results suggest a direct influence on the metabolism and development of sphingolipid molecules and all fatty components of the skin.

The pharmacokinetics of CBD includes studies of its absorption, distribution, metabolism, and excretion. Absorption is next: CBD can be administered by various routes, including oral administration, inhalation, sublingual administration, and transdermal patches. The rate and efficiency of absorption depends on the method of administration. CBD is metabolised in the liver, mainly by cytochrome P450 enzymes (CYP3A4 and CYP2C19) (dos Santos *et al.*, 2023). Several metabolites are formed as a result of metabolism, including 7-OH-CBD and other compounds. The main routes of elimination of CBD and its metabolites from the body include excretion through the kidneys in the urine and through the intestines in the faeces. The elimination half-life of a CBD may vary depending on the route of administration, dose, and individual characteristics of the human body. Interaction with other drugs may also affect the metabolism and excretion of CBD, which is important to consider when using it for therapeutic purposes.

The metabolism of CBD is a multistructural and complex process involving several CYP450 isozymes (Le Bacquer *et al.*, 2024; Spelta *et al.*, 2024).

The main metabolites are 7-OH-CBD, which has a 38-45% lower amount of cannabis-like substance in plasma compared to the original CBD. Further metabolism in the liver leads to the formation of additional metabolites, which are excreted mainly in the faeces and to a lesser extent in the urine. An understanding of these metabolic pathways is crucial for the optimised and beneficial use of CBD for therapeutic purposes and for predicting possible interactions with other drugs.

The results of the study of the pharmacokinetics and metabolism of FDCs provide a foundation for the formulation of recommendations for specific population groups, including children and elderly patients. It is imperative to consider age-related differences in liver enzyme activity, body composition, and potential comorbidities when developing these recommendations. In pediatric patients, the metabolism of CBD may be slower due to the underdevelopment of liver enzymes in younger children, which matures with age (Tutchenko *et al.*, 2024; Rudyk *et al.*, 2024). This can necessitate lower starting doses and gradual dose escalation to monitor for adverse effects, especially in conditions like pediatric epilepsy. Since data on the safety and efficacy of CBD in children is still limited, careful monitoring is required. Additionally, variations in the absorption and bioavailability of CBD may be observed in this age group, depending on their body composition and the rate of enzyme maturation.

For elderly populations, CBD metabolism may be altered due to reduced liver function, which commonly occurs with aging, as well as changes in body fat composition that can affect the distribution and elimination of the compound (Komilova *et al.*, 2024). Therefore, a lower initial dose and gradual titration are recommended for older

patients, with careful attention to the potential for drug interactions, particularly with medications metabolized by cytochrome P450 enzymes such as CYP3A4 and CYP2C19. Elderly individuals are also more susceptible to side effects like sedation and hypotension, making it crucial to adjust dosages accordingly (Niyazbekova *et al.*, 2023). Given the increased risk of polypharmacy in this demographic, monitoring for interactions and individualizing treatment plans will ensure both safety and efficacy. Further research is necessary to establish comprehensive age-specific dosing guidelines and evaluate the long-term safety of CBD in these vulnerable populations.

The analysis shows that most researchers point out that the lack of proper regulation and control by official bodies means that the quality and safety of such products can vary substantially. This creates a risk of not only ineffective treatment, but also potential side effects or interactions with other medications. Nevertheless, the CBD market continues to grow, attracting increasingly more consumers looking for alternative treatments. It is important to emphasise the need for further scientific research to determine the true capabilities and limitations of the CBD, as well as to develop clear recommendations for its safe use.

Thus, despite the potential benefits of CBD, the need for further research and controlled clinical trials stays urgent. This will help to ensure the proper level of safety and effectiveness of the CBD products, as well as to develop clear recommendations for their use. It is important to conduct further studies to confirm these results in clinical settings and to assess possible risks and interactions between the CBD and other pharmaceuticals. The prospects for additional investigation of the metabolism

of CBD involving cytochrome P450 are particularly valuable. This will enable the development of optimised treatment regimens and ensure patient safety when using these drugs simultaneously.

CONCLUSIONS

The potential of medical cannabis to significantly improve the treatment of various diseases in Ukraine is considerable; however, it is essential to overcome the legislative, social, and regulatory barriers that currently impede its use. In order to optimise its medical use, it is first necessary to gain a thorough understanding of its metabolism, particularly its interactions with other drugs that are metabolised by cytochrome P450 enzymes. Although the pharmacological parameters of cannabinoids, including metabolism and excretion, remain insufficiently understood, further research is required to investigate these aspects and the long-term safety and efficacy of cannabidiol. An investigation into the role of TRPV1 receptors and FAAH inhibition may facilitate an enhanced understanding of the anxiolytic effects of cannabidiol. It is imperative that further research be conducted on the endocannabinoid system in order to facilitate the advancement of therapeutic applications.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Review article

The nagoya protocol and biodiversity conservation: legal insights on access and benefit-sharing for medicinal and aromatic plant resources

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ABSTRACT

This study examines the emergence of a rights-based perspective for biodiversity conservation through the Convention on Biological Diversity, which grants states sovereign rights over natural resources and emphasizes fair and equitable benefit-sharing (FEBS) from their commercial use. The Nagoya Protocol, a critical international legal framework, addresses unregulated access to genetic resources by establishing Access and Benefit-Sharing (ABS) mechanisms based on Prior Informed Consent (PIC) and Mutually Agreed Terms (MAT). The study explores how these mechanisms protect indigenous rights and promote biodiversity conservation, particularly in developing nations rich in biodiversity. Using a comparative analysis of ABS frameworks in India, Kenya, and Mexico, the research identifies diverse legal approaches and challenges such as enforcement, transparency, and compliance. The findings reveal that while the Protocol strengthens indigenous rights and ensures fair compensation for traditional knowledge, implementation gaps persist. The study concludes that enhancing ABS frameworks is essential for equitable benefit-sharing, safeguarding indigenous rights, and supporting sustainable development and biodiversity conservation.

Keywords: Access and Benefit Sharing (ABS), Convention on Biological Diversity (CBD), Genetic Resources and Indigenous People, Mutually Agreed Terms (MAT), Prior Informed Consent (PIC)

INTRODUCTION

Biodiversity, defined as the variety of life across genes, species, and ecosystems, is fundamental to ecological balance, human development, and so-economic well-being. However, rapid population growth and human activities such as deforestation, habitat destruction, and climate change have led to significant biodiversity loss, even in biodiverse-rich nations like India. To address this, international efforts like the Convention

on Biological Diversity (CBD, 1992) and the Nagoya Protocol (2010) were established, emphasizing conservation, sustainable use, and fair and equitable benefit-sharing (ABS) of genetic resources. The Nagoya Protocol introduced legal mechanisms such as Prior Informed Consent (PIC) and Mutually Agreed Terms (MAT) to protect indigenous traditional knowledge and ensure equitable benefit-sharing.

This research aims to critically examine the legal frameworks governing ABS measures, focusing on the requirement for obtaining PIC from indigenous communities. It seeks to evaluate how these mechanisms address biodiversity conservation and sustainable use while respecting indigenous rights and sovereignty. Through a comparative analysis of ABS frameworks in India, Kenya, and Mexico selected for their biodiversity richness and membership in the Like-Minded Mega-Diverse Countries the study explores the effectiveness of ABS mechanisms, their challenges, and their implications for indigenous rights and biodiversity conservation in developing nations.

Evolution of access and benefit sharing (ABS) mechanism and legal framework

During the colonial period, the biological resources of colonized nations were utilized without distributing any resulting benefits to the indigenous populations. This utilization led to immense socio-economic detriment for the colonies. As the industrial revolution began in the mid-18th century, the use of biological resources expanded from consumption to the derivation of usable commercial products. The colonizers were not only interested in the economic value but also the medicinal properties of the biological resources. (Scarlett, 2022).

Despite this exploitation, there were positive developments such as unrestrictive interactions and exchange of seeds between farmers leading to the evolution of new plant varieties. This led to the emergence of divide between the industrialized countries equipped with advanced technologies and Bio-diversity rich nations unfolding legal protection to the creators of new plant varieties.

Initiating analysis of legal perspectives, it is crucial to engage in an economic examination and comprehend the priorities of plant genetic

resources users. The paramount goals of these users, for instance, multinational seed producers, strive to achieve in order to optimize profits encompass unrestricted access to genetic resources, ownership rights over the evolved product, and unregulated, extensive and open market operations. By the mid-1980s, these users had secured the first two objectives and were working towards the third, i.e., open market. Simultaneously, provider states, rich in biodiversity, together with their local and indigenous people, began to perceive that the current legal structure regulating biodiversity affords limited potential to protect their biodiversity and guarantee advantages in the trade of genetic resources (Conaghan, 2023). This recognition of their justified interests in the “fair and equitable sharing of benefits” arises from Access and Benefit Sharing trade on global platform.

During this phase, IPR's emerged as a new concept. Additionally, bio-diverse nations began experiencing tension due to the continued open access to their territories' genetic resources. This tension occurred on two levels - internationally between states, and domestically within states between governments and Indigenous and Local Communities (ILCs) those who have cultivated and preserved biodiversity for generations. The tension stemmed from the unregulated access to genetic resources and associated TK, as well as the lack of benefit-sharing with the providers of these resources and knowledge. (Moraru, 2023).

The history of the Nagoya Protocol on ABS, an important international legal development, should not be examined in isolation. It is essential to consider the broader developments that occurred during the last two decades of the 20th century, which played a significant role in shaping its evolution. In 1987, the Brundtland Commission proposed a concept of

“Sustainable Development” in its report “Our Common Future” as a means to both protect the global environment for future generations as well as enable socio-economic progress (Colella *et al.*, 2023).

The Uruguay Round of negotiations, which began in 1986, addressed all outstanding trade policy issues to facilitate necessary reforms in international trade. Originally, intended to conclude in 1990, in order to resolve every issue necessitated extending the process for nearly four additional years. This resulted in the 1994 signing of the Marrakesh Agreement which led to the establishment of the WTO and the TRIPS Agreement (Preeg, 2012). In 1992, the international community convened at the UN Conference on Environment and Development in Rio de Janeiro, Brazil. Several conventions were adopted during this conference, including the UNFCCC and the CBD.

Post World War II and the adoption of the UDHR in 1948, the latter half of the 20th century experienced an increased emphasis on rights-based approaches to law. The development of rights as a concept strengthened efforts regarding sovereignty over natural resources and can be viewed as a foundational aspect of benefit-sharing within access and benefit-sharing legal frameworks (McNeilly, 2023).

Representatives from the genetic resource’s conservers considered indigenous communities as the holders of the holders of genetic resources found within their lands. It was proposed that any benefits derived from the utilization of these genetic resources and traditional knowledge, of which the indigenous communities are the rightful holders, should be shared with them.

Case studies exploring access and benefit-sharing during this period revealed a gap in

existing law regulating access and benefit-sharing processes. For example, the Kani Tribe case study involved a drug named Jeevani, derived from the genetic resource of the ‘Arogyapacha plant and Kani Tribe’s traditional medicinal knowledge. This case demonstrated a voluntary benefit-sharing mechanism established between the research institute (TBGRI) and the Kani Tribe. During this controversy, India’s domestic legal framework was insufficient to address the questions arising from access and benefit-sharing processes (Heinrich *et al.*, 2020). The mentioned Table 1 represents the key details of the Kani Benefit-Sharing Case, including the discovery of bio-resource, research to extract genetic material, commercialization of Jeevani drug, and financial arrangements of the drug involving the Kani tribe.

Access and Benefit Sharing’ mechanism under Nagoya protocol

The Nagoya Protocol on Access and Benefit Sharing (ABS) is a significant international agreement that seeks to guarantee “the fair and equitable sharing of benefits arising from the utilization of genetic resources” (Morgera, 2015). The primary objective of this protocol is to ensure that the benefits derived from the utilization of genetic resources, particularly those originating from developing nations, are shared fairly and equitably. The comprehensive reading to the text of the protocol makes it evident that the protocol establishes a flexible framework that necessitates national-level actions for effective implementation. The overarching goal is to guarantee that the preservation of biological diversity and the sustainable utilization of its components are accomplished through suitable access to genetic resources, technology transfer, and funding mechanisms (Colella *et al.*, 2023).

The transition from the Convention to the Guidelines, and to the Protocol, represented a shift from non-binding principles to those with legal force. The Nagoya Protocol on Access and Benefit Sharing (ABS), which became legally binding on its signatory countries in 2014, obliges many states to establish domestic legislation on ABS, with many states currently in the process of developing a comprehensive framework of standards to regulate access to genetic resources and ensure equitable benefit-sharing (Morgera, 2016).

The adoption of the Nagoya Protocol in 2010, there was a significant wave of ABS legislation between 2010 and 2015, demonstrating the immediate impact of the international agreement. Countries such as Brazil, which enacted its ABS law in 2015, the Philippines and Vietnam in 2015 and 2017 respectively, and Peru, which had already implemented ABS measures by 2009, all reflected the growing momentum sparked by the protocol. This period marked a critical phase where the majority of countries recognized the need to align their national policies with global biodiversity and benefit-sharing standards.

From 2016 onwards, the steady expansion of ABS legislation continued, with China (2014), Indonesia (2018), Turkey (2017) and Uganda (2015) among the countries that enacted laws in response to the evolving international framework. This trend indicates that even several years after the protocol's adoption, the push for compliance and the establishment of ABS frameworks remained strong. Ethiopia and Mozambique, with their ABS laws enacted in 2006 and 2007 respectively, reflect a broader adherence to environmental governance norms that predated the protocol but still align with its principles.

‘Fair and equitable sharing of benefits’ expression under Nagoya protocol

The interpretation of “fair and equitable sharing of benefits” as mentioned in the Nagoya Protocol focuses on defining the terms “fair” and “equitable.” The term “fair” indicates to procedurally sound access and benefit-sharing (ABS) transactions, indicating said transactions must adhere to relevant law and regulations. On the contrary, “equitable” relates to economic fairness essential to the benefit-sharing mechanism, suggesting the sharing of benefits between the user and provider nations (NBA, 2021).

A significant critique of the Protocol’s text is its omission of the term “traditional knowledge” from the provision on the objective. However, this omission does not diminish the importance of traditional knowledge and its holders. A comprehensive reading of the Protocol, particularly in conjunction with articles 8(j) and 15 of the CBD, indicates that the objective of fair and equitable benefit-sharing inherently includes the access to and sharing of traditional knowledge associated with genetic resources.

The Protocol mandates that all international instruments related to ABS must align with and support the objectives of the CBD and the Nagoya Protocol. This requirement applies to both existing instruments at the time the Protocol came into force and any future agreements. The primary goal is to restrict the freedom of parties in negotiating ABS contracts or creating new international instruments, ensuring that they remain consistent with the objectives of the CBD and the Protocol.

Extent of Nagoya Protocol

The Nagoya Protocol addresses genetic resources referred to in Article 15 of the CBD and aims at sharing benefits arising from their

utilization. This law deals with the access and benefit sharing arrangements related to genetic resources and the TK associated with it. As Human genetic resources is expressly excluded from the scope of CBD likewise excluded from the protocol's scope.

The Protocol similarly excludes marine genetic resources found in the high seas or within the Antarctic Treaty Area. Article 15 of the CBD, which recognizes the "sovereign rights of states over their natural resources" indicates that the genetic resources governed by the Protocol must fall within the national jurisdiction of member states. As a result, the Nagoya Protocol does not extend to bioprospecting activities conducted in areas outside national jurisdiction (Richerzhagen, 2014).

The Protocol explicitly does not extend to genetic resources acquired before the CBD entered into force. It remains ambiguous on whether the benefit-sharing obligations outlined in the Protocol apply to the ongoing use of genetic resources obtained in the period between the CBD's entry into force and the Protocol's implementation (Morgera, 2016).

Secondly the Protocol is also silent on its application to genetic resources obtained before the enforcement of the CBD. The protocol does not specify whether benefit-sharing obligations under the agreement apply to continued uses of genetic resources obtained between the CBD and the subsequent protocol.

Jurisprudential justification for 'fair and equitable sharing of benefits' under Nagoya Protocol

The jurisprudential rationale behind the "fair and equitable sharing of benefits arising" out of the utilization of Genetic Resources (GRs) and associated knowledge is firmly rooted in the principles of ethics, morality, and equity,

as prescribed in the CBD. This rationale is rooted in the Hohfeldian framework of rights and duties, where a right is understood as a positive claim against another, directly corresponding to a duty (Cook, 1919). In the context of ABS, the principle of "sovereign rights of states over their natural resources" suggests a corresponding obligation on other states and legal entities to respect these rights, thereby preventing the unauthorized use of these resources. Access to such resources is granted only when the conditions of Prior Informed Consent (PIC) and Mutually Agreed Terms (MAT) are complied.

The rights given to provider states, and in some cases, their Indigenous and Local Communities (ILCs), to ensure "fair and equitable sharing of benefits from the use of genetic resources and related traditional knowledge" are a significant legal effort to address the historical injustice caused by biopiracy.

The notion of what is "fair and equitable" reflects a moral imperative rather than simply a hard-coded lawful requirement. It encompasses an array of factors, including the nature of the biological resource, its availability and contribution to the benefits derived, and its cultural, ecological, and economic worth. This notion offers the negotiating parties a broad scope to share benefits in unique manners, which can be completely non-monetary. Nonetheless, it requires parties to be guided by the awareness or 'conscience' that ethical and fair exchanges necessitate adopting a conscionable mechanism.

The contention of what constitutes a "fair and equitable share" in each ABS case may vary as negotiations unfold. Still, a conscionable and ethical approach is bound to reconcile differences regarding the content and scope of fair and equitable sharing. This approach, thus forms the heart of the ABS jurisprudence

seeking to redress historical injustices suffered by the providers of GRs and associated TK.

Intellectual property rights of indigenous communities vis-à-vis ABS mechanism

The sphere of biodiversity and IPR of Indigenous people calls for extensive scrutiny as the current universal IP Law regime encourages uniformity and harmonization of IP law standards. This primarily lessens barriers to legitimate trade and bestows private ownership rights to patentees over higher life forms, resulting in potential conflicts with the CBD that establishes sovereign rights of states over natural resources.

Currently, the grant of IPR over life forms strengthened by elements like the TRIPS Agreement, catalyzes innovation, resolving immediate economic and food security interests (Modic *et al.*, 2019). However, researchers argue a long-term adverse impact on biodiversity as IPRs incentivize the development of genetically modified plant varieties posing serious ecological risks. Owning patents on GMOs effectively hands over the ownership of 'biological information' to private individuals and corporations, leading to an unchecked commercialization of flora or fauna.

Several major pharmaceutical companies actively source bioresources from developing countries, utilizing these resources for drug development, particularly in areas of traditional medicine and plant-based compounds. Novartis, a Swiss multinational, has sourced bio-resources from countries like India and Brazil, leveraging indigenous knowledge for drug development, especially in traditional medicine. Bayer, the German pharmaceutical giant, has been involved in sourcing plant-based compounds and other bio-resources from South America and

Africa, using them in pharmaceuticals and agrochemicals. Astra Zeneca engages in bio-prospecting across Latin America and Asia, focusing on genetic resources found in these biodiversity-rich areas to develop new drugs, particularly in oncology and respiratory diseases.

Glaxo Smith Kline (GSK) has a history of sourcing bio-resources from developing nations, particularly in Southeast Asia and Africa, where it focuses on natural products for vaccines and infectious disease treatments. Sanofi, a French multinational, sources bio-resources from tropical countries like Madagascar and other African nations to develop antimalarial drugs and other therapeutic compounds derived from natural resources. Pfizer, an American pharmaceutical company, collects marine and terrestrial bio-resources from developing nations, including countries in Southeast Asia and the Caribbean, for drug discovery and research purposes.

Roche, another Swiss pharmaceutical company, sources bio-resources from various developing countries, focusing on plant extracts and natural compounds used in cancer treatment and other chronic diseases. Johnson & Johnson, known for its interest in traditional medicine, sources bio-resources from Latin America and Africa, particularly botanicals and other natural products, for use in skincare, consumer health, and pharmaceuticals. These companies' involvement in sourcing bioresources reflects their reliance on the rich biodiversity of developing nations for innovative drug development and highlights the importance of equitable access and benefit-sharing agreements.

The patenting process converts a genetic resource into a private property post successful creation through anthropogenic genetic intervention. The new patented

product produced from the biological resources future generation becomes private property, resulting in the sole ownership of a few multinationals controlling a large portion of valuable IPR-protected technology.

This shift threatens indigenous traditions, typically involving passing down seeds from one generation to another to maintain genetic diversity. With patented plant varieties, traditional practice becomes illegal, making indigenous people liable for royalty payments and redefining their practices. This hinders protection of biodiversity and is detrimental to traditional rights of holders in developing countries.

The existing IP laws have a very limited scope with respect to extending legal protection to Traditional knowledge and Biodiversity. TRIPS which is a WTO's agreement provides for a minimum standard of protection to IPR's (Phillips, 2016).

Our current IPR regime favours developed nations housing large corporate giants conducting extensive R&D ventures. Consequently, these conglomerates generate substantial number of profits and obtain patent right over the medicinal properties of genetic resources originating from the developing nations (Maskus, 2018). Thus, conclusion can be drawn here that these countries are unfamiliar with the idea of commercializing their resources with the IP regimes.

In the complex world of international law, the benefits and responsibilities arising from ABS arrangements among indigenous communities can often intersect with IPR. These legal complexities arise especially due to differing objectives of the CBD and WTO's TRIPS Agreement (Gaia and Grian, 1998).

CBD seeks to ensure "fair and equitable sharing" of benefits that come from the

utilization of genetic resources, whereas TRIPS grants individual property rights to results of research involving the genetic modification of such resources. Thus, when genetic resources from bio-diversity rich communities are accessed by technology rich nations, patent rights governed by TRIPS are typically sought for any resultant innovation (Bossche, 2020). The benefits accrued from patent rights, whether monetary or otherwise, are then subject to sharing under the CBD.

This dichotomy can be especially convoluted when patent rights are transferred to third parties for commercialization. Such transfers yield wide-ranging benefits, such as joint ownership over patents, share in licensing fees, and others, which need to be equitably shared between the user (patent holder) and the provider (Biological resource owner) country. Fulfilling this obligation has its challenges (Díaz, 2005).

The Nagoya Protocol on ABS aims to ensure compliance with ABS laws of state parties, proposing the need for amendments to TRIPS that would oblige patent applicants to disclose the source and the country from which the said resource had been accessed. This would help in preventing Biopiracy and erroneous grant of patents, creating a harmonious relation between TRIPS and the CBD.

The Access and Benefit Arrangements can result into obtaining of various IP Rights. Patent rights may be obtained when inventions result from genetic resources is sourced from provider countries, and agreements can include clauses that require users to notify providers when filing patents. Trademark rights may also be involved when symbols related to access genetic resources are used in branding, necessitating mutual agreements on usage limitations. Copyright protection can apply to the written records, documents, diagrams, or databases generated from research on genetic resources.

Furthermore, when traditional knowledge from indigenous communities is shared, it can be considered a trade secret, warranting confidentiality to protect this valuable information.

Thus, it is imperative to have ABS agreements that address these potential intellectual property rights and lay out equitable mechanisms for the sharing of

Comparative analysis

The comparative study of the ABS (Access and Benefit-Sharing) legal frameworks in India, Mexico, and Kenya (Table 2) reveals the varied approaches and challenges faced by these countries in implementing the Nagoya Protocol, focusing on the subject matter of access and benefit-sharing, requirements of Prior Informed Consent (PIC), fair and equitable sharing of benefits, and measures to strengthen compliance and ensure transparency.

India's ABS legal system is primarily governed by the Biological Diversity Act, 2002, and the accompanying rules and guidelines. The subject matter of ABS in India includes plants, animals, micro-organisms, and their genetic material, excluding value-added products. The framework mandates that both domestic and foreign users obtain approval from relevant authorities, such as the National Biodiversity Authority (NBA), to access biological resources. India places a strong emphasis on PIC and Mutually Agreed Terms (MAT) to ensure fair benefit-sharing, with benefits often taking the form of joint ownership of intellectual property, technology transfer, or direct monetary compensation (Gill, 2021). However, India faces challenges in enforcement and compliance, especially regarding the transparency of approvals and ensuring that benefits are adequately shared. The country has not yet fully designated

resulting benefits. It is also necessary for the providers to enhance their understanding of these matters for them to negotiate favourable conditions when consenting to access their genetic resources. Ultimately, the realization of the objective of "fair and equitable sharing of benefits" will occur only when indigenous communities become capable of identifying their interests and obtain resources that reflect the value of their contribution.

checkpoints for monitoring compliance, relying instead on existing bodies such as the patent office.

Kenya's ABS framework is characterized by its emphasis on community involvement and the recognition of traditional knowledge. The legal system, governed by various laws including the Environmental Management and Co-ordination Act and the Protection of Traditional Knowledge and Cultural Expressions Act, requires PIC and MAT to be negotiated between users and resource providers, including local communities, private managers, and state agencies. As per National Environment Management Authority, Kenya's system allows local communities significant control over access to their resources and traditional knowledge, which must be respected by users (National Environment Management Authority, 2014). Challenges in Kenya include limited resources and technical capacity to enforce compliance, difficulties in ensuring that benefits reach intended beneficiaries, and complex negotiations between multiple stakeholders.

Mexico's ABS framework integrates a strong focus on traditional knowledge and the rights of indigenous communities. Mexican laws emphasize the direct involvement of communities in negotiating access agreements, ensuring that benefit-sharing

arrangements reflect their needs and expectations. However, Mexico struggles with fragmented legal governance, inconsistent enforcement, and varying levels of awareness among communities, which can impede effective implementation (Angón, 2019). The country has also faced challenges in monitoring compliance and ensuring transparency in ABS processes, including the designation of checkpoints and issuing internationally recognized certificates of compliance.

While India, Kenya, and Mexico all seek to uphold the principles of the Nagoya Protocol, their ABS frameworks reflect their distinct socio-legal contexts. India's approach is highly regulated and state-driven, Kenya's is community-centric with a strong role for local knowledge holders, and Mexico emphasizes traditional knowledge protection. Common challenges across these countries include weak enforcement mechanisms, insufficient transparency, and the need for more robust compliance measures, highlighting the ongoing need for improvements in ABS governance to achieve the Protocol's objectives effectively.

CONCLUSION

The Convention on Biological Diversity (CBD) marked an initial step in establishing an international legal regime for access and benefit-sharing (ABS) of genetic resources, while the Nagoya Protocol represents a more robust, legally binding framework to extend legal protections to genetic materials and associated traditional knowledge. Historically, biological diversity and genetic resources were predominantly accessed and commercially exploited by developed nations without equitable benefit-sharing, a disparity that persisted until the CBD's

implementation. The CBD introduced the concept of ABS into international law, aiming to address imbalances between developed and developing nations by ensuring fair and equitable sharing of benefits derived from genetic resources. The Nagoya Protocol, enacted in 2014, further advanced this objective by establishing a comprehensive framework for bilateral agreements based on prior informed consent and mutually agreed terms, extending protections to derivatives of genetic resources and pathogens.

The Protocol strengthens the position of provider countries, particularly developing nations, by safeguarding their interests and promoting equitable benefit-sharing. However, the diverse domestic approaches to ABS regulation across countries underscore the necessity for international cooperation and knowledge exchange to effectively operationalize the Protocol's provisions. This study highlights the Protocol's role in enhancing the rights of indigenous communities and advancing biodiversity conservation through ABS mechanisms. A comparative analysis of ABS frameworks in India, Kenya, and Mexico reveals varying strategies and challenges, including issues of enforcement, transparency, and compliance. The research underscores the importance of refining ABS frameworks to ensure equitable benefit distribution, respect for indigenous rights, and the promotion of sustainable development and biodiversity conservation.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Table 1: The table Represents the key details of the Kani Benefit-Sharing Case

Aspect	Details
Location	Kerala, India
Population	Approx. 18,000 Kani tribal people
Livelihood	Handicrafts, gathering and selling forest produce
Year of Discovery	1987
Discovery	Arogyappacha plant with anti-fatigue properties
Research Institutes Involved	All India Co-Ordinated Research Project on Ethnobiology (AICRPE), Tropical Botanic Garden and Research Institute (TBGRI)
Drug Developed	Jeevani
Year of Market Readiness	1994
Technology Transfer	1996 to Arya Vaidya Pharmacy (Coimbatore) Ltd.
License Fee	Rs. 10 lakhs (approx. \$25,000)
Royalty Agreement	2% on future drug sales
Trust Formed	Kerala Kani Samudaya Kshema Trust (1997)
Trust Members	9 members, all tribals
Initial Earnings	\$50,000
Benefit Distribution	50% of license fee and royalties to Kani tribals
Total Amount to Trust	₹ 5,19,062
Criticisms	Choice of private-sector company, Low license fees
Resolution Attempts	Formation of Trust, Structured and transparent process in Benefit sharing by State Government

Table 2: Comparative analysis of ABS

Country/Region	Legislation/Regulation	Description	Key Provisions
India	Biological Diversity Act, 2002; Biological Diversity Rules, 2004; National Biodiversity Authority (NBA); State Biodiversity Boards (SBBs); Biodiversity Management Committees (BMCs)	Regulates access to biological resources and traditional knowledge, ensuring fair and equitable sharing of benefits. Establishes national and state-level authorities for biodiversity management.	Establishes access regulations, benefit-sharing agreements, and management authorities at national and state levels.
Kenya	Environmental Management and Coordination Act (EMCA), 1999; Wildlife Conservation and Management Act, 2013; Forest Conservation and Management Act, 2016; Seeds and Plant Varieties Act, 2012; Traditional Knowledge and Cultural Expressions Act, 2016; The Constitution of Kenya, 2010	Provides frameworks for environmental management, wildlife conservation, forest management, and protection of traditional knowledge. Includes community participation in conservation and benefit-sharing.	Involves community participation in conservation efforts, establishes environmental impact assessments, and promotes equitable sharing of benefits.
Mexico	Mexican Political Constitution; Law for Sustainable Rural Development; Law on Wildlife; Law on Forestry; Law on Ecological Equilibrium and Environmental Protection; International Agreements	Regulates access to genetic resources and traditional knowledge. Oversees sustainable use of natural resources with a focus on conservation, sustainable management, and alignment with international standards.	Ensures sustainable use of genetic resources, conservation of biodiversity, and adherence to international conventions such as CBD and Nagoya Protocol.

Review article

Selected Food cum Medicines (*Ghiza e Dawa*) effective in Anaemia

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ABSTRACT

Anaemia, a widespread health problem, is considered a moderate public health issue affecting children and women in Sri Lanka. In the Unani system of medicine, a condition known as Faqr ud-dam closely resembles anemia. The Unani medical system offers a treasure of single drugs for treating and managing various ailments. This study aims to scientifically review selected Unani single drugs used as food cum medicines effective in the management of Faqr ud-dam (Anaemia). All the selected drugs contained micronutrients needed for heamopoiesis such as Fe, Zn and vitamin C and possessed pharmacological actions like anti-anaemic, heamopoiesis, heamo-protective, muharrik (exhilarant) and hepatoprotective. This review provides a comprehensive overview and analysis of the nine food –cum- medicine items used to treat anaemia in Unani medicine. This covers their nutrient contents and scientific studies in related to anaemia.

Key words: Anaemia, *Faqr ud dam*, food- cum- medicines, iron deficiency, Unani single drugs

INTRODUCTION

Anaemia represents a significant global health challenge. Within Sri Lanka, this condition has emerged as a moderate public health issue, affecting various demographic groups. Specifically, the prevalence rates among preschool children, non-pregnant women, and pregnant women stand at 33%, 39%, and 34%, respectively. World Health Organization data indicates that 39% of

Sri Lankan women between 15 and 50 years of age, who are not pregnant and constitute a crucial component of the nation's labor force, suffer from anaemia (Chathuranga *et al.*, 2014). Nutritional deficiencies among these females are mainly accountable for anaemia. Sri Lanka has limited national survey on anaemia; most of them are related age or the specific population (Sheriff *et al.*, 2021).

Anaemia is defined by WHO as a condition in which the hemoglobin content of blood is lower than normal as a result of a deficiency of one or more essential nutrients (WHO, 2008). Anaemia can cause from various factors, including nutritional deficits, infections, inflammatory conditions, and genetic disorders affecting hemoglobin. Among all potential causes, iron deficiency is globally recognized as the primary contributor to anaemia. The main causes of iron deficiency are malnutrition and blood loss. Mal absorption of iron from the diet or dietary lack of iron in veganism and other causes are increasingly common in iron deficiency. The incidence of both folate and vitamin B12 deficiency is also rising. Lack of vitamin C intake will be effects the absorption of iron from non-heam iron sources (Benson *et al.*, 2021). Iron deficiency is most prevalent worldwide, with children and adolescents facing a remarkably higher risk.

The concept of *faqr ud dam* is a condition mentioned in the Unani system of medicine and corresponds to anaemia that is described in modern science. One of the most important causes of *faqr ud dam* is dietary disorder with severe malfunction of the liver due to alteration in its temperaments (Akhtar, 2010): The Unani system of medicine has a treasure of herbs for the treatment and prevention of diseases. These play a vital role in the management of anaemia with its safety, efficacy, and availability. The treatment plan in Unani medicine compacts the cause of iron deficiency and also specially the iron rich plants used in anaemia. These single drugs possess pharmacological actions like anti-anaemic, heamoposis, *muharrrik* (exhilarant) and hepatoprotective actions.

Diet therapy comes in first in the line of treatment of the Unani system of medicine

for any ailments. There are many single drugs and compound formulations indicated for anaemia in Unani classical texts. Most of the single drugs are food cum medicines (*Ghiza e dawa*) which are known as functional foods. *Aam* (Mango), *Ananas* (Pineapple), *Anar* (Pomegranate), *Amrood* (Guava), *Tamr* (Dates), *Palak* (Spanish), *Chukandar* (Beet root), *Gul e Gudhal* (Flower of China rose) and *Gul e Surk* (Rose flower) are some of them. *Aam*, *Ananas*, *Anar*, *Amrood*, and *Tamr* are popular fruits in most parts of the world. This study aims to review the scientific evaluations of selected single drugs in relation to the management of anaemia. Further these are the food cum medicines that commonly available and frequently consumed among Sri Lankans.

MATERIALS AND METHODS

A literature search was accomplished for anaemia, *faqr ud dam* and selected Food cum medicines (*Ghiza e Dawa*) effective in anaemia in classical books and research articles. There are large numbers of foods and drugs that were named in the management of anaemia. Amongst them, some plant-origin drugs available and easily accessible all over Sri Lanka were selected for review. Journal publications in English language and published from 2005 to up to date were searched in online electronic databases PubMed, Google scholar and Research-gate for articles using the search terms 'Botanical name of the selected plants or Common name or 'Tibbi name and 'Heamoposis' or Heamo- protective' or 'Hepato protective' or 'Nutritional composition' or 'Iron deficiency anaemia'.

RESULTS AND DISCUSSION

Iron deficiency anaemia

Iron deficiency is the most widespread nutrient deficiency globally. Iron deficiency anaemia is defined by The World Health Organization (WHO) as a

condition in which the number of red cells (and consequently their oxygen-carrying capacity) is insufficient to meet the body's physiological needs. There is a recognized decrease of hemoglobin concentration below the following thresholds: <130 g/L for adult men, <120 g/L for adult non-pregnant women, and pediatric values that start at <110 g/L for ages 6–59 months and increase with age (Navya and Prasad, 2022).

Signs and symptoms of anaemia

The signs and symptoms of *faqr ud dam*, as described in the Unani system, include pallor of body complexion, edematous face, eyelids, and upper or lower limbs, and occasionally generalized swelling throughout the body with pitting edema due to *raddi bukharat* (obnoxious gases). Additional manifestations may include gingivitis, disturbed sleep or excessive somnolence, loss of appetite, indigestion, flatulence, and delayed healing of wounds or ulcers (Verma et al., 2021). Symptoms of anaemia described in the modern scientific literature are fatigue, Fast or irregular heartbeat, reduced cognitive function, dyspnea, lack of energy, pounding or "whooshing" in your ears, headache, cold hands or feet, pale or yellow skin, chest pain, weakness, and dizziness. These symptoms can be interpreted as indicative of symptomatic anaemia and may thus play a role in diagnostic and therapeutic decisions (Weckmann et al., 2023).

Prevention and management of anaemia

Dietary insufficiency of iron is treated by oral iron supplements. Oral iron is readily available, inexpensive, effective, safe, and convenient. Because of patients intolerant of oral iron or with conditions where oral iron is likely to be ineffective or harmful, the IV route is preferred (Auerbach and Adamson, 2016).

Alternatively, it could be treated with iron supplements and an iron rich diet. Iron tablets may have certain side effects such as abdominal cramping, nausea, constipation, and dark, hard stool (Varma et al., 2021). Therefore, Herbal drugs including Unani drugs got attention due to their variable role in the management of anaemia with no or negligible side effects and cost-effectiveness. In the management and prevention of anaemia Unani literature recommends light, readily digestible food to improve digestion and appetite. Most of the spices were described for treatment of anaemia as single drug for their iron content and pharmacological actions. The diet specifically prefers in anaemia are the *Ghiza e lateef* (soft diet) *Kaseerut taghzia* (high nutritious diet) and *Jaiyyad ul Kaimus* (easily absorbable) diets from plant, mineral and animal sources (Husain and Sherwani, 2023).

Single herbs effective in anaemia and used as food cum medicine

The following list of food cum medicines has been mentioned in Unani text books and research articles, for its curative effect in anaemia (Husain and Sherwani, 2023; Ahmad et al., 2019).

- *Aam* (*Mangifera indica*)
- *Amrood* (*Psidium guajava*),
- *Ananas* (*Ananas comosus*)
- *Anb* (*Vitis vinifera*)
- *Anjeer* (*Ficus carica*)
- *Apricot* (*Prunus armeniaca*)
- *Asl* (*Apis indica*)
- *Badam* (*Prunus amygdalus*)
- *Bathua* (*Chenopodium album*)
- *Bean* (*Phaseolus vulgaris*)
- *Behi* (*Cydonia oblonga*)
- *Cabbage* (*Brassica oleracea*),
- *Cauliflower* (*Brassica oleracea*)
- *Cherry* (*Prunus avium*)
- *Cholai* (*Amaranthus polygamus*)

- *Gul e Gudhal* (*Hibiscus rosa-sinensis*)
- *Gul e Gulab* (*Rosa damascena*)
- Hazelnut (*Corylus avellana*)
- *Hulba* (*Trigonella foenum*)
- Jaggery (*Caryota urens*)
- *Jujubes* (*Ziziphus jujuba*)
- *Kaddu* (*Cucurbita maxima*)
- *Kamsara* (*Pyrus pyrifolia*)
- Lemon (*Citrus limonum*)
- Lime (*Citrus aurantifolia*)
- *Mouza* (*Musa paradisiaca*)
- Mulberry (*Morus alba*)
- *Palak* (*Spinacia oleracea*),
- *Papita* (*Carica papaya*)
- Peach (*Prunus persica*)
- Plum (*Prunus ceracifera*)
- *Anar* (*Punica granatum*)
- *Shaddock* (*Citrus grandis*)
- *Tahlab* (*Spirulina platensis*)
- *Tamr* (*Phoenix sylvestris*)
- *Tarbooz* (*Citrus lanatus*),
- *Tuffah* (*Malus domestica*)
- *Zafran* (*Crocus sativus*)
- *Zaitoon* (*Oleum europium*)

Special diets recommended for anaemia

- *Zirbaj* (A sour meat dish dressed with vinegar and honey or with acid syrup, raisins, few figs were sometimes added)
- *Sikbaz* (Acid minced flesh similar to *zirbaj*)
- *Zardah* (Rice dressed with *Crocus sativa*)
- *Yakhni* (Broth prepared from meat and rice)
- *Shorbae teetar* (Bird's soup)
- *Ghost Daraj* (Meat)
- *Masoosat* (Soup prepared with aromatic spices) (Ahmad *et al.*, 2019)

The nutrient contents of Food cum medicines (*Ghiza e Dawa*) in relation to anaemia

The medicinal plants are flourishing as a main source of drugs and used in therapies in worldwide (Gunawardana and Jayasuriya., 2019). Many medicinal plants with hematopoietic effect were mention in Unani Medicine. Some of them are food cum medicine. All these are good sources of iron, zinc and vitamin C. The comparative content values of iron, zinc and vitamin C among the selected plants were shown in Fig: 01, Fig: 02 and Fig: 03 respectively. Also, these foods can help

iron deficiency anaemia through various mechanisms alone or with medications.

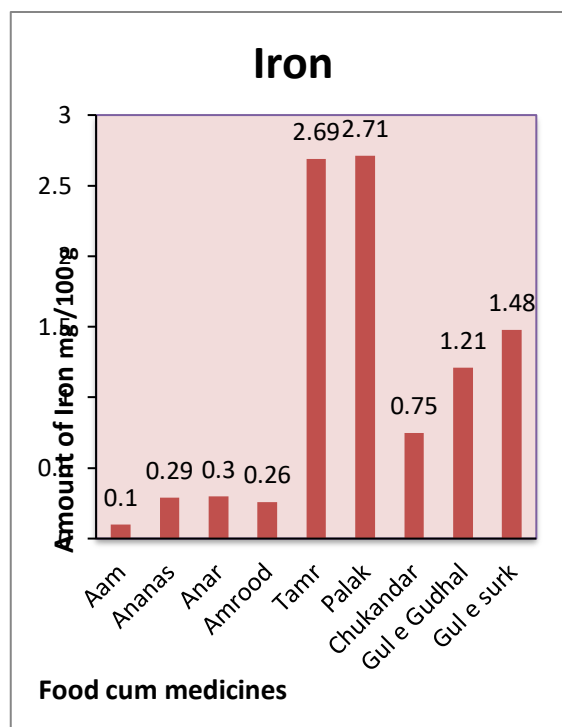


Figure 1: Iron content (mg /100 g) of selected food cum medicines effective in anaemia

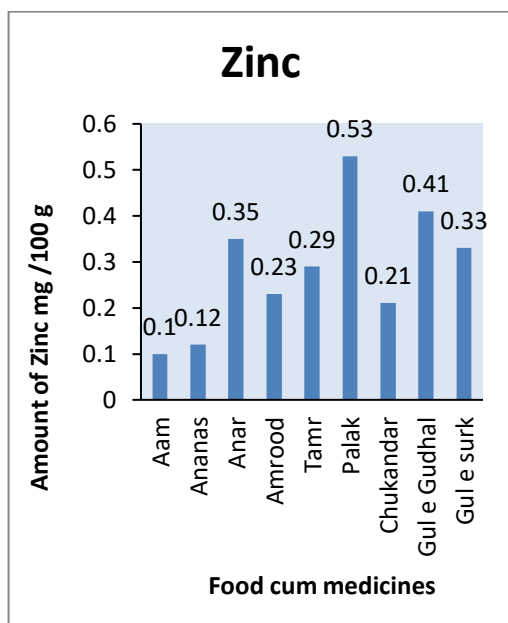


Figure 2: Zinc content (mg/100 g) of selected food cum medicines effective in anaemia

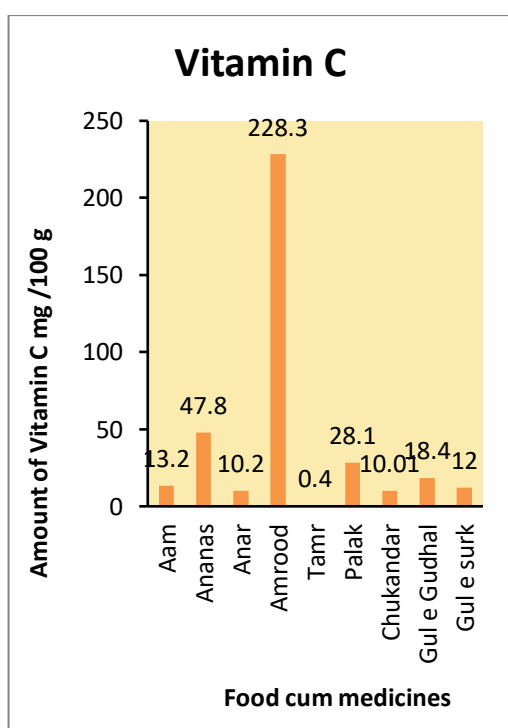


Figure 3: Vitamin C content (mg/100 g) of selected food cum medicines effective in anaemia.

Aam (Mango/ *Mangifera indica* L.)

The fruit of *Mangifera indica* has been specifically examined for its iron content

to develop natural iron products as a potential solution to iron deficiency (Saha *et al.*, 2018). It also contains small quantities of citric, tartaric, and malic acids, which enhance iron absorption. Mango juice is considered a restorative tonic for heat stroke. Furthermore, Mango kernel extract has been investigated for its hepato-protective activity. It has been suggested that mangiferin is responsible for scavenging reactive oxygen species (ROS) and free radicals involved in cellular injury of mouse liver by modulating cell growth regulators (Sarath *et al.*, 2009).

Ananas (Pineapple / *Ananas comosus* (L.) Merr)

It contains numerous volatile compounds in small quantities and complex mixtures. Pineapple is also a rich source of minerals and vitamins that provide various health benefits (Ali *et al.*, 2020). The findings of Managa and colleagues suggest that a mixed fruit juice (Beet root, pineapple, and papaya) may serve as a potential therapeutic alternative in the prevention and management of anaemia in children and women (Managa *et al.*, 2022).

Anar (Pomegranate / *Punica granatum*)

The Fruit contains several enzymes, sugar, citric acid and malic acids as well as rich in vitamin C and iron which contributes its nutritional and medicinal effects (Mirihagalla and Fernando., 2021) A pomegranate-based drug was studied in trial animals with controlled animals; the study revealed the efficacy of the trial drug in haematenic pharmacological activity and in enhancing haemopoisis thereby proved its efficacy in treating iron deficiency anaemia. The haematanic activity of the drug showed 16.41% improvement in Haemoglobin levels in experimental animals (Meenakshi *et al.*, 2018).

Amrood (Guava / *Psidium guajava*)

It contains a broad spectrum of phytochemicals including polysaccharides, vitamins, essential oils, minerals, enzymes, and proteins (Joseph and Priya, 2011). Extracts of *Psidium guajava* have been evaluated for their iron content to formulate natural iron products as a potential solution to iron deficiency, without causing the adverse effects associated with commercial iron supplements (Saha *et al.*, 2018). The hepato-protective effect of aqueous extracts of *Psidium guajava* and the phospholipid complex has been demonstrated against paracetamol-induced hepatotoxicity (D' Mello and Rana, 2010). In the groups treated with extract of Amrood (200, 400 mg/kg) and phospholipid complex (100 mg/kg), the serum levels of SGOT, SGPT, ALP, and bilirubin were decreased compared to the intoxicated control group. This finding was further validated by histopathological examination of the liver.

Tamr (Dates / *Phoenix dactylifera* L.)

The dried date flesh contains a high level of nutrients. Significantly it contains iron (10.7 mg/kg), calcium (536 mg/kg), and 9.80 mg of ascorbic acid. Dates overcome anaemia with its immense amount of iron and vitamin C content. Further, it helps in raising hemoglobin levels and improving iron stores in the human body. The study of Naveed and others proved the haematopoietic effect of date fruits in iron deficiency anaemia (Naveed *et al.*, 2023). A study that was designed to evaluate effects of date consumption and iron deficiency anaemia, revealed that the consumption of date fruit increased Hgt, Hct, and serum ferritin levels in all age groups. Iron deficiency anaemia could be controlled and prevented with cost-effective through dietary modification (Farhnaz *et al.*, 2019).

Palak (Spinach / *Spinacia oleracea* L.)

Spinach is an edible flowering plant in the family of Chenopodiaceae. The species *oleracea* Linn, is well known for its vitamin and mineral contents (Tedom *et al.*, 2020). This vegetable contains high amount of carotene (Vitamin A), calcium, iodine and ferrous ions, and it is considered suitable for children and pregnant women. A recent study proved that consuming 100 grams of *Palak* boiled in 30 °C water for 7 days will increase hemoglobin levels in mild anaemia (Natalia *et al.*, 2019). Banerjee and others proved that high bioavailability along with anti-oxidant activities of Fe content in *Palak* (Banerjee *et al.*, 2019).

Chukandar (Beet root / *Beta vulgaris*)

Betaxanthins (yellow) and betacyanins (red) are water-soluble nitrogenous pigments found in *Beta vulgaris* that serve as valuable natural food colorants. These pigments are widely used in various food products, including baked goods, yogurt, candies, ice cream, and processed meats. Numerous *in vitro* studies have demonstrated that betalains (including betaxanthins and betacyanins) derived from beetroot possess significant antioxidant properties (Kazimierczak *et al.*, 2014). Furthermore, flavonoids and phenolic compounds present in beet leaf extracts are believed to contribute to their hematinic properties.

Beta vulgaris has been identified as a natural source for pharmaceutical applications, particularly for its haematopoietic effects and potential in the treatment of anaemia (Gheith and El-Mahmoudy, 2018). Extracts from the leaves and stalks of *B. vulgaris* have shown haematopoietic and anti-anaemic effects in phenylhydrazine-induced anaemia models, as demonstrated through both *in vitro* and *in vivo* assays.

Gul e Gudhal (China rose flower/ *Hibiscus rosa-sinensis*)

Research articles and ancient literature have shown that the flowers of Ghudal possess haemo-protective activity, haemopoietic activity, anti-tumor, anti-diabetic, antioxidant activity and anti-depressant pharmacological actions. Recent research study showed that the methanolic extract of *H. rosa-sinensis* flowers are effective haemo-protective against phenylhydrazine-induced haemotoxicity in Charles foster rats. This activity of extract may be due to high phenol and flavonoid contents of *Hibiscus rosa-sinensis* L. flowers (Meena et al., 2021). Mishra and Tandon (2012) found in their research that, a significant increase in the level of hemoglobin and count of RBC with 30 days administration of aqueous extract of *Hibiscus rosa-sinensis* L. flowers in male Swiss albino mice.

Gul e surk (Rose flower/ *Rosa damascena* Mill.)

Gul-e-Surk has been extensively utilized in the food, perfume, and medicinal industries. Historically, it has been employed in the treatment of numerous conditions, including cardiovascular diseases, gastrointestinal disorders, inflammatory processes, wound healing, skin diseases, mental health issues, pregnancy-related complications, and menstrual irregularities. Consequently, it is regarded as a significant medicinal plant in various traditional systems of medicine (Davoodi et al., 2017). They highlighted that the rose flower is an excellent source of bioactive compounds such as terpenes, flavonoids, glycosides, and anthocyanins. Key constituents include citronellol, geraniol, nerol, quercetin, kaempferol, myrcene, gallic acid, and linalool. Furthermore, the distilled water extract of *Rosa damascena* flowers has demonstrated hematopoietic effects in animal models (Osama et al., 2020).

CONCLUSION

Anaemia and iron-deficiency anaemia are common medical conditions and have become global health problems. It leads to several complications. There are available treatments for anaemia, and due to adverse drug effects, the patient compliance is less in oral iron supplementation. The use of natural therapy in the treatment and prevention of disease is not only safe but also it is easily available. Since these are natural food supplements alongside provide other nutritional benefits to the individual. Therefore it is timely important of explore natural resources in healthcare.

CONFLICT OF INTEREST STATEMENT

The author declare that he has no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Review article

A review on marketing and supply chain of medicinal plants using PRISMA

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ABSTRACT

Medicinal plants represent a valuable source of drug discovery for many modern medicines. Yet very little reported work has been done on the marketing and supply chain of medicinal plants in India. This study aimed to investigate the marketing and supply chain dynamics with various stakeholders involved for medicinal plants in India. A systematic literature review was conducted using Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) framework, with data extracted from the Google Scholar database. The topic was formulated using the Population, Intervention, Control, and Outcome (PICO) technique. The study identified influential papers and authors, providing valuable insights into research trends and highlighting the need for further investigation into specific areas within this domain. This study contributes to a better understanding of the research landscape surrounding the marketing and supply chain of medicinal plants in India.

Keywords: Systematic Literature Review, PRISMA, PICO, medicinal plants, marketing, supply chain

INTRODUCTION

Medicinal plants are fundamental to numerous modern medicines. Approximately 80% of developing nations' populations rely on herbal remedies, with traditional practices deeply embedded in their cultures (Mukherjee, 2002). These plants are studied across diverse scientific fields: agricultural scientists develop improved cultivars, Ayurveda practitioners assess therapeutic efficacy, and biotechnologists explore molecular compounds for drug discovery. In India, thousands of enterprises rely on these plants for traditional medicine formulations (Ved and Goraya, 2007). The increasing global demand for high-quality, certified organic herbal products necessitates expanded commercial cultivation of medicinal herbs to bridge the supply gap

(Gaurav *et al.*, 2018). There is a significant discrepancy between the availability and demand for medicinal plants used to manufacture the nation's Ayurvedic medications (Anonymous, 2017). India can lead the global herbal medicine market by ensuring the quality of its traditional Ayurvedic, Siddha, and Unani drugs, allowing them to compete with modern medicine through lower costs and fewer side effects (Sharma *et al.*, 2008). This systematic literature review, utilizing the PRISMA guidelines, aims to provide a comprehensive analysis of the existing body of research focusing on the marketing and supply chain dynamics of medicinal plants. The specific objectives includes, identifying and synthesizing key findings, assessing the

quality and relevance of existing studies and highlighting gaps in the current literature that warrant further investigation.

METHODOLOGY OF REVIEW WORK

This Google Scholar-based study used the PICO framework to examine medicinal plant marketing and supply. It focused on research context, marketing challenges, and stakeholder roles, addressing the question: "What is the context of the articles and what are the problems with the marketing aspect of medicinal plants with various stakeholders involved?"

The study analyzed research articles, book chapters, and conference papers published between 1995 and 2024. After initial screening, 17 duplicates and 11 irrelevant articles were removed. A manual review of 135 titles and abstracts further excluded 85 articles that did not focus on the marketing and supply chain of medicinal plants, including prescribed medicines, herbal products, OTC products, and consumer behavior. The final analysis included 51 research papers.

PRISMA flow diagram for selecting the studies

This study employed a systematic literature review adhering to PRISMA-ScR guidelines. namely: (1) identification of literature, (2) screening questions and (3) eligibility criteria

Identification of literature

The literature search, conducted primarily on Google Scholar, employed various keyword

combinations using Boolean operators (or and) and phrase search methods. Synonyms, related terms, and variations of core keywords were used, including: "marketing" OR "supply chain" OR "raw materials" OR "medicinal plants" OR "herbs", "prescribed medicines" OR "herbal products", "Over The Counter products" OR "consumer behavior" OR "prescription" OR "ayurveda".

Screening questions

The initial screening process involved reviewing titles, abstracts, and keywords. Subsequently, selected articles underwent further evaluation based on these questions:

1. Do any studies discuss about raw material quality of medicinal plants and where it can be traded?
2. Is there any study done to assess the opinions of doctors and consumers?
3. Have any kind of marketing channel study been done?

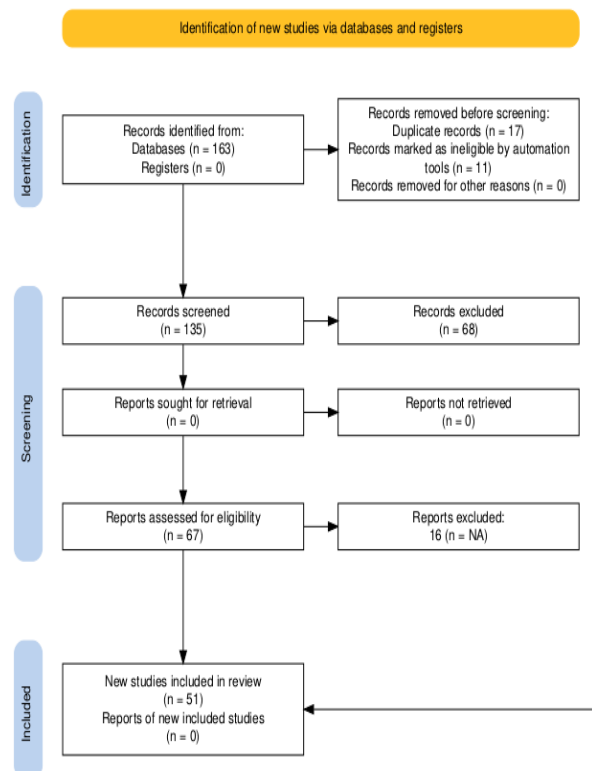
Eligibility assessment

The following inclusion criteria were then applied-

1. Does the study discuss in detail traders' opinions on the raw material of medicinal plants?
2. Does the study cover the work of various stakeholders involved in the supply chain?
3. Does the study discuss about consumers buying decisions, purchasing intentions etc?

The following study done at the Institute of Agri Business Management between October 2024 till January 2025.

Figure 1. PRISMA flow diagram describing inclusion and exclusion of studies



Supply chain of raw materials of medicinal plants

The poorly coordinated and obscure medicinal plant market chain is attributed to several factors: limited market access and transaction difficulties, information gaps, a scarcity of reliable buyers, and discriminatory and unfair pricing practices (Van de Kop and Ghayur 2006). The decentralized medicinal plant system exposes collectors and contractors to exploitation. Limited market access allows local traders to control information, financing, and marketing, driving prices down. This highlights significant power imbalances in Uttarakhand's supply chain, hindering contractors and collectors from securing fair prices and improving their financial outcomes (Van de Kop *et al.* 2006). Choosing the best raw material sources for herbal products is complex. The Analytic Hierarchy Process (AHP), a Multiple Criteria Decision Making (MCDM) tool, provides a structured approach to evaluate suppliers

based on sustainability, quality, price, and reliability (Kulshrestha *et al.* 2007). Similar research studies are mentioned in the form of Table 1..

Marketing of medicinal plants

Limited market access and reliance on middlemen distort the medicinal plant product market. Price discrepancies and local oversupply negatively impact producers. Dominant traders can manipulate prices, and farmers selling to middlemen lose all control over price and volume (Shiva 1995). The medicinal plant marketing system is unregulated and unfair, harming poor farmers and laborers. Its top-down structure limits information and benefits to local levels, leaving many, especially those at the supply chain's base, unaware of market demands and opportunities (Kala *et al.*, 2006). The Asgandh market shows a tiered structure: larger farmers have more direct market access, reducing middleman reliance, while smaller farmers engage in smaller, more localized sales (Mishra and Kotwal 2011). Similar studies are presented in the Table 2.

Herbal products of medicinal plants

The 1991 WHO guidelines, endorsed by the 6th International Conference of Drug Regulatory Authorities, mandate a thorough evaluation of herbal medicines. This evaluation includes: rigorous quality assessment of raw plant materials, preparations, and final products and efficacy assessment supported by traditional use documentation and/or animal and human studies. (Kamboj, 2000). Combined spending increases on herbal supplements from 2017 to 2019 were less than the \$1.659 billion increase in US consumer spending from 2019 to 2020. Ashwagandha (*Withania somnifera*) experienced the largest sales growth in mainstream retail, jumping 185.2% to \$31.7 million in 2020. Entering the top 40 best-selling herbs in 2018 at 34th place, ashwagandha's sales have since quadrupled, reaching 12th position in 2020, demonstrating its rapid mainstream

popularity (Smith *et al.*, 2021). Effective quality regulation in India's herbal medicine industry requires a multifaceted strategy. Despite hurdles like limited awareness and the costs of implementing GACP and GSP, the industry recognizes the importance of standardized documentation and clear QC/QA guidelines (Sarmah, 2022). Similar studies are presented in the Table 3.

Consumer perception towards herbal products

Trust and satisfaction significantly increase consumer preference for Ayurvedic products, while price negatively impacts it. Statistical analysis using t-tests and ANOVA revealed demographic differences in trust, perceived reasonable price, and satisfaction, which are key factors influencing consumer choice of Ayurvedic products (Misra *et al.*, 2022). Sentiment analysis, using supervised machine learning with the Text2Vec package, was conducted on 28,713 English tweets related to Complementary, Alternative, and Integrative Medicine (CAIM). The analysis, utilizing labeled data for individual tweet evaluation, found the most frequent hashtags to be #vitamin and #ayurveda. The overall sentiment distribution was 54% positive, 31% neutral, and 15% negative. (Ng *et al.*, 2022). Consumer buying decisions for Ayurvedic products are significantly influenced by health consciousness, social influence, product price, perceived value, and trust. (Suriyage and Jude Leon 2023). Similar studies are presented in the Table 4. This study aimed to investigate the marketing and supply chain dynamics of medicinal plants in India through a systematic literature review. The analysis revealed several key findings:

India's medicinal plant sector struggles with supply chain and marketing issues, impacting producers. Consumer trust is vital. Solutions require policy improvements in market access, value chains, and R&D, alongside industry efforts in branding, quality, and innovation.

CONCLUSION

This study provides a comprehensive overview of the research landscape surrounding the marketing and supply chain of medicinal plants in India. The findings highlight the critical importance of addressing the challenges faced by the sector to ensure the sustainable and equitable development of the herbal industry. Key challenges include limited market access, information asymmetry, power imbalances, and inadequate marketing strategies. To address these challenges, policy interventions are crucial, focusing on improving market access, strengthening value chains, empowering stakeholders, and promoting research and development. The herbal industry also needs to focus on developing strong brands, adopting good agricultural and collection practices, and investing in research and innovation. Further research is needed to investigate specific aspects of the marketing and supply chain, develop innovative solutions to address the identified challenges, and inform effective policy intervention.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Table 1: Findings on Supply chain of MAPs by various authors

Author	Parameter studied	Key Findings
Hamilton (2004)	Stakeholders, Value Chain & Market System	• Found that the structure of the value chain is poorly integrated, with no or nominal vertical links, and it is usually secretive.
Singh et al. (2005)	Participatory Rural Appraisal (PRA) tech.	• MAP farmer education and support increased cultivation interest.
Ved and Goraya (2007)	Grey reporting of raw material produce and documented	• Record-keeping in the medicinal plant sector is hampered by insufficient procedures and a lack of tracking systems in some states.
Madhavan (2008).	Supply and demand imbalances in the medicinal plant industry	• Information gaps and asset specificity hinder new medicinal plant suppliers. • Price setting is exploitative, favoring downstream actors over producers.
Lubbe and Verpoorte (2011)	Buyer expectation for MAPs	• Supply: Consistent availability of quality raw materials.

		<ul style="list-style-type: none"> • Demand: Understanding evolving consumer needs for medicinal and aromatic plants (MAPs).
Dejouhanet (2014)	Quality of material in supply chain of MAPs	<ul style="list-style-type: none"> • Ayurvedic supply chains lack traceability and transparency, quality and increasing dependence on unregulated sources.
Varshney <i>et al.</i> (2021)	lack of market transparency	<ul style="list-style-type: none"> • Traders need more than price for assessments; standardized production builds trust and meets quality standards.
Dadhich <i>et al.</i> (2024)	Promotion of MAPs by NMPB	<ul style="list-style-type: none"> • An ashwagandha marketing campaign promoted it as a "Health Promoter," using information, education, and communication (IEC) initiatives.

Table 2: Findings on marketing of MAPs by various authors

Author	Parameter studied	Key Findings
Singh (2006)	Commerce of MAPs	<ul style="list-style-type: none"> • Lack of reliable data on traded medicinal plant species, volumes, and prices stems from inadequate systematic data.
Mishra <i>et al.</i> (2009)	necessity of sustainable harvesting methods	<ul style="list-style-type: none"> • Medicinal plant collection suffers from collector competition, poor harvesting knowledge, and inadequate training.
Guleria <i>et al.</i> (2014)	major challenges faced to market the produce	<ul style="list-style-type: none"> • Major issues include insufficient processing, lack of price supports, and limited access to regulated markets.
Roosta <i>et al.</i> (2017)	Relative Market Advantage (RMA)ra	<ul style="list-style-type: none"> • Singapore, Japan, Germany, and Malaysia are identified as the most promising target markets for MAPs exports, based on a weighted average of indices.
Rathore and Mathur (2018)	Marketing channel, Value addition of MAPs	<ul style="list-style-type: none"> • MAPs offer production, distribution, and processing opportunities. Value-added products & contract farming are profitable.
Gularia & Gupta (2020)	Challenges in production and marketing of MAPs	<ul style="list-style-type: none"> • Medicinal plant cultivation is hindered by high costs, poor planting material, limited subsidies, and inadequate irrigation.
Chen <i>et al.</i> (2021)	Geographical authentication	<ul style="list-style-type: none"> • Safer environment for raw materials can be achieved by geographic authentication and protection of raw materials.
Rathore (2024)	Production and marketing of medicinal and aromatic plants	<ul style="list-style-type: none"> • Unregulated markets and poor production technology are major obstacles.

Table 3: Findings on herbal products of MAPs by various authors

Author	Parameter Studied	Key Findings
Busse (2000)	Consistent and sufficient quality of raw material	• Herbal product consistency relies on precise plant selection and standardized production.
Ahmad <i>et al.</i> (2006)	lack of a uniform quality control	• Plant-based medication's variability complicates quality control; advanced analysis is needed.
Pingali <i>et al.</i> (2013)	Effect of <i>Withania</i> on mental stress	• <i>Withania somnifera</i> extract significantly reduced aortic pressure and augmentation index compared to a placebo.
Singh <i>et al.</i> (2014)	Quality control on herbal medicine	• To minimize risks, rigorous quality control is essential throughout the entire production process, from sourcing to final product.
Maqbool <i>et al.</i> (2019)	Benefits of herbal medicine	• Ease of intake, minimal risk of deadly adverse reactions, affordability are factors which are preferred by consumers
Zahiruddin <i>et al.</i> (2020)	Effect of Ashwagandha on anxiety	• Ashwagandha leaf powder shows potential for obesity prevention due to its antianxiety, anti-inflammatory, and anti-apoptotic effects.
Smith <i>et al.</i> (2021)	Annual spending on herbal supplements in USA	• Mainstream channel, ashwagandha (<i>Withania somnifera</i> , Solanaceae) saw the biggest increase in sales
Pathak <i>et al.</i> (2024)	Sustainable cultivation of medicinal plants	• Only after dependable and economical cultivation methods have been developed should cultivation be undertaken

Table 4: Findings on consumer perception towards herbal products by various authors

Author	Parameter Studied	Key Findings
Verma (2009)	Satisfaction level of OTC users	• Customers who have used OTC health supplements earlier are somewhat satisfied
Eichhorn <i>et al.</i> (2011)	Anxiety Disorder	• Herbal remedies are common for self-treating anxiety and depression. Kava is beneficial for mild to moderate anxiety.
Ali and Yadav (2015)	Attitudes towards ayurvedic products	• Respondents report no side effects from Ayurvedic products and maintain a positive attitude towards them.
Jibril <i>et al.</i> (2019)	Consumer behaviour of herbal products	• Education and processing level, not demographics, drive herbal product preference..
Alwhaibi <i>et al.</i> (2021)	e-commerce use for purchasing herbal products	• Online drug purchases: safe, but verifying pharmacy legitimacy is difficult.
Akhgarjand <i>et al.</i> (2022)	Ashwagandha for anxiety	• Ashwagandha supplements significantly reduced tension and anxiety

Review article

**Entrepreneurial prospects of medicinal and aromatic plants in India-A
Review**

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ABSTRACT

Medicinal and aromatic plants provide livelihoods to the people in the country. These plants demanded by various industries as raw material to prepare various specific products. There is huge potential for medicinal and aromatic plants production and value addition in the country. Because medicinal and aromatic plants contain various chemical compounds. However various challenges are associated with value chain of medicinal and aromatic plants. Government initiatives and supports are helpful for small farmers and entrepreneurs to earn additional income and to sustain in this sector through production, marketing and entrepreneurial ventures. Entrepreneurship is important in medicinal and aromatic plants sector. Different agro-climatic conditions in India are suitable for cultivation of various types of medicinal and aromatic plants. This paper provides review on entrepreneurial opportunities in medicinal and aromatic plants.

Keywords: Certification, entrepreneurial prospects, medicinal and aromatic plants, value addition

INTRODUCTION

Medicinal and aromatic plants continue to play important role in ensuring health security of the nation and world as well. Medicinal plants contain a plenty of important chemical compounds which are used in many products. Tulsi, Ashwagandha and Kalonji are some important medicinal plants cultivated by farmers. Some organizations also stimulated a devoted cluster for the organic cultivation of these plants in the country. Some important medicinal and aromatic plants already have considered for the standardized package of practices and their cost of cultivation and return like Tulsi, Ashwagandha, Mentha, Shatavari, Kalmegh, Licorice, Lemongrass, Kalonji etc. Government promoting entrepreneurship through incubation support for medicinal and aromatic plants related venture. Technology Business Incubation

(TBI) has the requisite expertise in areas relevant to medicinal and aromatic plants and also intends to facilitate innovation and entrepreneurship. It promotes growth of medicinal and aromatic plants sector with technology and knowledge transfer. The incubation centre organizes sensitization workshops, boot camps and how to manage entrepreneurial venture, seminars or webinars to enhance the knowledge and skill of medicinal and aromatic plants stakeholders. Medi-Hub, Technology Business Incubation (TBI) program are designed to help start-ups to grow in a continuous manner by providing them the technology and business support system in medicinal and aromatic plants (Patel *et al.*, 2023). Growers, retailers and wholesalers, processors, collectors, and consumers are among the various stakeholders involved in the various entrepreneurial aspects including production, marketing and trade of

medicinal plants (Parvin *et al*, 2023). The use of medicinal plants by the local people residing in the Bandorban hill district for the treatment of several human ailments has been observed (Alam *et al*, 2024).

Singh *et al* (2024) revealed various raw materials derived from medicinal plants that are used in various applications. Various raw materials for pharmaceuticals, cosmetics, natural dyes and essential oils are obtained from plants. Medicinal and aromatic plants cultivated on farms for industrial requirements, many are still collected from different wild source. There are growing demand for industrial raw materials and declining wild populations' opening new avenue for farmers to cultivate important high value medicinal and aromatic plants. Improved varieties, their seed and quality of planting materials, organic as well as natural farming, incubation and start-ups in these sector play very important to support entrepreneurial ecosystem for this sector. Peoples used medicinal plants for the treatment of numerous human ailments. These plants cultivated with other crops and through different value-adding methods, to realize sustainable development goals (SDGs) and to promote the circular economy beneficial to the environment (Singh *et al*, 2024). Abadi *et al* (2021) revealed that attitude of producers' perceived behavioural, subjective norms; and perceived costs are the important drivers of intention toward these plants. Medicinal plants produced in the Himachal Pradesh state are having immense use and potential in the herbal as well as pharmaceutical sector.

Some important medicinal and aromatic plants are suitable for cultivation due to good climatic conditions and increased demands in the Himalaya of Uttarakhand state (Phondani *et al*, 2016). For the cultivation and safeguarding of medicinal plants, agroforestry provides a useful strategy (Rao *et al*, 2004). Compared to commercial crops, medicinal plants also provide remunerative to the cultivator (Das *et al*, 2016).

METHODOLOGY AND DISCUSSION

This paper attempted to review entrepreneurial perspectives of medicinal and aromatic plants. This paper is based on secondary data. The secondary data and information have been collected from different published secondary sources like published articles, research papers, annual reports, newspapers, magazines, websites etc.

Supply chain management

There is a need for a supply chain monitoring system for herbal medicine industry to ensure quality control and improve relations among and between the various herbal medicine industry value chain players (Obahiagbon and Ogwu, 2023). Seed suppliers were the highest value-adding actor for Tulsi (*Ocimum tenuiflorum*). Planning and development of a favourable ecosystem for medicinal plant production, processing, and marketing, this sector may flourish for the long term sustainability (Palash *et al*, 2021). Supply chains of medicinal plants are erratic and require sustainability in their chain like production, harvesting, processing, and its marketing. In order to become competitive in the medicinal plants global market place, value chain must become more elastic, innovative, and efficient, so it can bring to market new products (Hishe *et al*, 2016). Singh *et al* (2024) revealed that starting successful farming models requires identifying suitable crops through GIS-based agro-ecological studies. It involves assessing climatic suitability and details of Good Agricultural Practices (GAP). Supporting with this, methods such as organic cultivation and advancements in genetics and biotechnology notably improve medicinal plant quality and production.

Certification and traceability

The chemical details of the plants, active ingredient their extraction methods, country of origin, climatic and specific crops are important quality factors. Economic factors are related to demand and supply (Lubbe and Verpoorte, 2011). Medicinal and aromatic plants as source of raw materials are purchased directly from farmers by the

Traditional Medicine Service Institute (Dhuguel, 2013). Certification of organic medicinal and aromatic plants leads to important economic growth and development in the industrial sector (Cader *et al*, 2021). The current harvesting practices are unsustainable (Sharma and Kala, 2018). The certification of these plants supports the development of enterprises in the sector and reduces the risk of rejection and the self-reliance of buyers in the market (Kala, 2015). Quality certification and certification of origin encourage customers, small businesses, and entrepreneurs (Taghouti *et al*, 2022). The content in the local language as well as the use of multimedia is necessary for traditional knowledge (Kareti *et al*., 2022). According to Anonymous (2021) ensuring traceability through proper labelling and packaging of products is very essential and a crucial requirement or mandatory for organic products. The creation of a batch number also helps with traceability and identification of the products (Shrestha *et al*, 2022). Factors influencing production include an insufficient of quality planting materials, lack of appropriate agro-techniques, insufficient market information, and a lack of good agricultural practices (GAP) for organic production. Lack of value addition, there is no price parity with wild produce (Sunder *et al*, 2012).

The roles of value chain are not efficient various paths in the chain are unorganized. The medicinal and aromatic plants value chain consists of inbound logistics, transporting, storing raw and harvested plants collection at the local and state level (Chandra and Kumar, 2021). Routroy and Behera (2017) suggested inventory policy, demand forecasting was found to be important areas of agricultural supply chain

Grading, processing, value addition and packaging

Sun drying, artificial drying, and tray drying are important techniques for drying collected medicinal plants (Porwal *et al*, 2020). The post-harvesting process can involve

segregating various important parts of medicinal and aromatic plants, including plants leaves, fruits, bark, and seeds. (Phondani *et al*, 2016). Harvested plants require proper drying to conserve their quality, and for that, alternative day turning is required (Parmeshwar *et al*, 2020). Most of the time, processors grade their products in various forms, like dry and powder forms (Palash *et al*, 2021). Option of essential oil extraction can get a higher remunerative return (Thakur *et al*, 2016). Plastic and jute bags commonly used for storing as processing for fresh and dry plants, but only polythene bags are used for powder form (Palash *et al*, 2021). The important benefit of the certification of medicinal plants is enhanced quality and it leads to good prices of the plants.

Storage

The maximum amount of limonene as well as the desired constituents of citral was observed in leaves packed with nitrogen (Ebadi, 2016). Medicinal and aromatic plants are frequently stored before use it for longer time (Mahmoodi Sourestani *et al*., 2014). Storage was species-specific due to the degree of changes in phytochemical and pharmacological activity of the plants (Laher *et al*, 2013). Through oxidation and, decomposition chemical effect can be protected by the exact choice of packing material (Huyan *et al*., 2019). The longer the storage time, result some important antibacterial properties lost (Susilo *et al*, 2020). The herbs packaged in aluminium balls and aluminum plates covered with foil and stored at three degrees celsius maintained their chlorophyll content as well as fresh weight and achieved a longer shelf life. Collection from forest collecting gives the highest net benefit to the household (Astutik *et al*, 2023).

Marketing information

The trade-in medicinal and aromatic plants are unorganized, and there is no reliable information about market prices (Singh and Kumar, 2021). Consumers preferences for freshness, organic production, and traceability

over low prices of medicinal and aromatic plants should be encouraged (Kevin *et al*, 2014). A database of medicinal plants would help users readily acquire and needed information (Kumar *et al*, 2018). Chandra and Sharma (2018) proposed a marketing information system that would be helpful in linking trade of important medicinal plants. Market intelligence helps in price forecasting also helps in getting good return price for the medicinal plants (Sunder *et al*, 2012). Information technology methods have remained some adopting digital knowledge to sustain traditional knowledge of medicinal plants to preserve and disseminate traditional knowledge in the medicinal plant sector (Kareti *et al*, 2022)

Conclusion

The paper highlighted the potential of medicinal and aromatic plants to full fill the raw material requirement of different industry. The medicinal and aromatic plants are potential sources of active important chemical compounds. Production and marketing of these important medicinal and aromatic plants provides a livelihood for millions of peoples and it open new avenue for entrepreneurship in this sector. The role of government is crucial in the production, marketing, and sustainability of medicinal plant sector. The seed, bark, roots, stem and leaves are source of raw materials for the pharmaceutical, industries including herbal, and cosmetic. There is huge potential for value-added and herbal products of medicinal and aromatic plants, so farmers or growers could cultivate on a large scale to generate additional source of income, while people may get the health benefits of herbal products for improving their health. There is a need for an efficient supply chain management of these plants. In this sector there are opportunities for entrepreneurship of medicinal and aromatic plant-based resources and raw materials. Overcoming the constraints would benefit both primary producers or growers and stakeholder involve in the medicinal and aromatic plants supply chain.

CONFLICT OF INTEREST STATEMENT

The author declares that he has no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Table 1: Certification and traceability of medicinal and aromatic plants

Particular	Emphasises /Suggested	Study/Author
Certification and Traceability	Organic certification of medicinal and aromatic plants	(Cader <i>et al</i> , 2021)
	Quality certification and certification of origin	Taghouti <i>et al</i> , 2022)
	Traceability and identification of the products	Shrestha <i>et al</i> , (2022)

Review article

Allergy to green peas: Clinical manifestations, diagnosis and pathogenesis

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ABSTRACT

The study aims to analyse the clinical manifestations and pathogenesis of green pea allergy, compare the effectiveness of modern methods of diagnosing this disease, and develop recommendations. The methodology included an assessment of the pathogenetic mechanisms of green pea allergy, an analysis of the main clinical manifestations of the disease, a comparison of the main diagnostic methods, and a synthesis of the data obtained into a single whole to describe the specifics of the disease. The study determined that the main allergens of green peas are Pis s 1 and Pis s 2 proteins, which belong to the family of storage proteins and demonstrate high immunogenicity and stability to heat treatment. These proteins were found to be the main factors of cross-reactivity with other legumes such as peanuts, chickpeas and lentils. Provocative tests demonstrated the highest sensitivity and specificity (100%) of all diagnostic methods, but due to the risk of anaphylaxis, their use is limited to specialised clinics. Molecular allergology has proven to be effective in identifying specific allergenic proteins and managing cross-sensitisation. Regional and social factors also influence the prevalence of green pea allergy, including dietary habits, urbanisation and environmental conditions. In the paediatric population, allergy most often manifests itself in the form of skin reactions and anaphylaxis, which emphasises the need for early diagnosis in this group. The study confirmed the need to introduce molecular allergology to improve diagnostic accuracy and personalised treatment of green pea allergy.

Keywords: anaphylaxis, immune response, legumes, proteins, cross-reactivity,

INTRODUCTION

The relevance of green pea allergy is driven by the rising global prevalence of food allergies and their significant impact on patients' quality of life. Green peas, as a common source of vegetable protein, pose a potential risk for hypersensitive individuals. Despite their nutritional benefits, limited understanding of the clinical manifestations and pathogenesis of green pea allergy complicates diagnosis and treatment, underscoring the need for further research. The increase in green pea allergy cases, particularly severe anaphylactic reactions, is concerning, especially as pea proteins are

used in many processed foods (Oleksy-Gębczyk *et al.*, 2024; Parrinello *et al.*, 2024). The absence of a universal allergen labelling system exacerbates this issue (García-Juárez *et al.*, 2024). The prevalence of green pea allergy varies by region, with increasing plant-based protein consumption making it particularly relevant in Europe, North America, and Asia, where peas are common in vegetarian and vegan diets (Uazhanova *et al.*, 2018). This highlights the need for large-scale epidemiological studies. International organisations, such as the United Nations (UN) and European Union (EU) are working on strategies to address food allergies,

including green pea allergy, through standardised labelling, hypoallergenic food development, and public awareness (Branca, 2024). These initiatives aim to improve diagnosis, treatment, and food safety related to allergic diseases.

Current trends in allergy diagnostics include molecular allergology, which identifies specific allergenic proteins, and personalised treatment approaches (Byeon *et al.*, 2024). There is also increasing focus on new pharmacological strategies, such as monoclonal antibodies and immunotherapy, to modulate the immune response (Parrinello *et al.*, 2024). Research on legume allergies, particularly green peas, highlights several key areas. One focus is the molecular characteristics of allergens and their cross-reactivity across legumes. Taylor *et al.* (2021) analysed specific proteins in green peas, underscoring the importance of risk assessment for hypersensitive individuals. Similarly, Abu Risha *et al.* (2024) examined legume allergens, particularly pea, chickpea, and lupine, finding high cross-reactivity. Richard *et al.* (2015) explored the risks of Dun peas, which can cause anaphylaxis in children sensitised to legumes.

An important area of research is the epidemiology and prevention of legume allergies. Lisiecka (2024a) reviewed the epidemiology, prevention, and pathogenesis of these allergies, focusing on genetic and environmental factors. This aligns with Verma *et al.* (2013), who noted a global rise in legume allergies, particularly due to increased consumption, and recommended improvements in diagnostic and therapeutic approaches. Abi-Melhem and Hassoun (2023) highlighted the “hidden allergenicity” of peas, which leads to missed diagnoses and inadequate treatment, emphasising the need for better awareness among healthcare professionals. Additionally, studies on physical factors affecting allergenic properties of pulses are crucial. Sell *et al.* (2005) explored how the maturity of green peas influences allergenicity, impacting food safety. Research on cross-reactivity between peas and other foods, particularly peanuts, by

Wensing *et al.* (2003) showed a correlation between pea sensitisation and IgE antibodies to the vicilin protein. Martínez San Ireneo *et al.* (2008) examined cross-reactivity among legumes in the Mediterranean population, noting variability based on local diets, which requires tailored diagnostic approaches. Popp *et al.* (2020) identified *Pisum sativum* (Pis s) 1 as the main allergen in green peas, with key immunoglobulin E (IgE) binding sites, providing valuable diagnostic information for detecting pea allergies in children.

Despite advances in the study of food allergies, allergy to green peas remains understudied. The lack of accurate epidemiological data, imperfect diagnostic methods and a lack of personalised approaches to therapy create a gap in understanding this pathology. The study aims to analyse current approaches to the diagnosis, treatment and study of the pathogenesis of green pea allergy with a focus on identifying key issues and prospects for further research.

MATERIALS AND METHODS

In this study, the method of analysis was used to systematise information and study the methods of diagnosis, pathogenesis of the disease and clinical manifestations of green pea allergy. The comparative analysis was used to compare different approaches to diagnosis (laryngoscopy, spirometry, skin tests, immunological tests), and identify their advantages, limitations and accuracy. Synthesis was used to combine the data obtained into a holistic picture, which was used to identify systemic relationships between clinical manifestations, diagnostic methods and pathogenesis of the disease.

The study uses data and recommendations from international organisations such as the UN and EU, as well as the World Health Organisation (Food and Agriculture, 2001) and the European Food Safety Authority, which define general approaches to the study of food allergies, including green pea allergy. Statistical data on the prevalence of legume allergy among different age and regional groups was

used (Lisiecka, 2024a; Verma *et al.*, 2013). The study included an analysis of the key allergens, namely *Pis s 1* and *Arachis hypogaea* (Ara h) 1 (Popp *et al.*, 2020). The inclusion criteria for this study included peer-reviewed literature, clinical studies, and official guidelines that directly addressed the clinical symptoms, diagnosis, pathophysiology, or therapy of green pea allergy and related legume allergies. Exclusion criteria included non-scientific papers, opinion pieces with no empirical backing, and studies unrelated to green pea or legume allergies.

Deduction and induction methods were used to analyse the mechanisms of green pea allergy pathogenesis and to develop recommendations for improving diagnostic and treatment methods. The deduction method was used to draw general conclusions from already known facts, such as the principles of allergy pathogenesis, to develop current medical recommendations. Induction was used to create new approaches to studying the mechanisms of green pea allergy, as well as to improve treatment and diagnostic methods. Generalisation was to summarise the research results into common principles, and systematisation streamlined the knowledge gained, which became the basis for building a structured approach to understanding the problem.

RESULTS AND DISCUSSION

Clinical manifestations of green pea allergy

Clinical manifestations of green pea allergy vary based on individual characteristics, the method of allergen consumption, and the patient's age. Common symptoms include skin, respiratory, and gastrointestinal reactions, which may occur alone or together. These symptoms typically appear shortly after exposure but sometimes the onset may be delayed up to six hours (Mastrorilli *et al.*, 2024). In young children, skin symptoms like hives, itching, and eczema are most common, usually after consuming fresh or processed peas (Hartmane, 2024). More sensitive children

may also experience generalised skin reactions with swelling, especially in the face and lips (Smits *et al.*, 2021). Gastrointestinal symptoms, such as nausea, abdominal pain, vomiting, and diarrhoea, are frequent in school-age children and may lead to dehydration in severe cases, requiring medical attention (Hildebrand *et al.*, 2021).

In adolescents and adults, respiratory symptoms, including rhinitis, sneezing, shortness of breath, and bronchospasm, become more prominent, often triggered by inhalation of pea particles during cooking. Some may also experience generalised weakness, headache, and dizziness due to systemic allergic reactions (Webber and England, 2010). Severe allergic reactions, such as anaphylaxis, are of particular concern. Although the frequency of such reactions is relatively low, they pose a serious threat to the patient's life. Anaphylaxis is usually accompanied by a sharp drop in blood pressure, difficulty breathing due to laryngeal oedema, generalised urticaria and loss of consciousness (Dashi *et al.*, 2015; Del Carpio-Delgado *et al.*, 2023). Patients with cross-allergy to other legumes, such as peanuts or lentils, are known to have a higher risk of anaphylaxis due to shared protein allergens (Mansoor and Sharma, 2011).

Cross-reactivity with other legumes, such as lentils or chickpeas, complicates diagnosis and can lead to oral allergy syndrome. Common proteins like vicilin (*Pis s 1*) cause similar reactions in individuals with peanut, chickpea, or lentil sensitivity, making allergen identification challenging and requiring more precise diagnostic methods (Martínez San Ireneo *et al.*, 2008). The form of green peas consumed also impacts symptom development. While heat treatment may reduce allergenicity, it can sometimes alter protein structure, increasing immunogenicity (Struminska *et al.*, 2014). Additionally, ready-to-eat pea products complicate identifying the source of allergic reactions (Smits *et al.*, 2021). Social and environmental factors also affect allergies. Regions with higher green pea consumption

report more allergic reactions, particularly in children and adolescents. Some studies suggest a link between environmental pollution levels and the severity of food allergy symptoms (Sharma *et al.*, 2015). Thus, clinical manifestations of green pea allergy vary from mild skin reactions to severe conditions like anaphylaxis. Effective treatment and prevention require individualised approaches, accurate diagnosis, and patient awareness of risks.

The pathogenesis of green pea allergy is driven by Pis s 1, a storage protein with high immunogenicity that triggers an excessive immune response in susceptible individuals. Pis s 1 stimulates IgE production, binding to mast cells and basophils, leading to the release of histamine, leukotrienes, and prostaglandins, causing symptoms like itching, swelling, and bronchospasm (Smits *et al.*, 2021). Cross-reactivity with proteins from other legumes, such as peanuts, lentils, and chickpeas, complicates diagnosis, as shared epitopes like vicilin (Ara h 1 in peanuts) cause cross-reactions (Wensing *et al.*, 2003; Villa *et al.*, 2020). The immune response involves type 2 T helper cell activation, leading to IgE production and chronic mucosal inflammation. Repeated exposure activates mast cells, intensifying allergic reactions (Hildebrand *et al.*, 2021). Additionally, disruption of the gastrointestinal barrier allows Pis s 1 to trigger local immune responses, explaining gastrointestinal symptoms in some patients (Sharma *et al.*, 2015).

Genetic factors play a significant role in the pathogenesis of green pea allergy. Changes in the structure of IgE receptors or enzymes involved in inflammatory mediator metabolism can increase allergy risk. Patients with a family history of allergies are more likely to experience severe reactions, including anaphylaxis (Nowak-Węgrzyn *et al.*, 2017). While no specific gene has been definitively related to green pea allergy, polymorphisms in genes associated with the 2 T helper cell immune pathway, such as Interleukin (IL) 4, IL13, and Signal

Transducer and Activator of Transcription (STAT) 6, are frequently implicated in IgE-mediated food allergies, including legumes. Variants in the Fc epsilon receptor I alpha subunit (FCER1A) gene, which encodes the alpha chain of the high-affinity IgE receptor on mast cells and basophils, have been linked to higher allergy sensitivity. Cross-reactivity may also be influenced by minor variations in legume proteins, such as lipid transport proteins, which can trigger allergic reactions even in small amounts, increasing the risk of anaphylaxis in sensitive individuals (Skypala *et al.*, 2021). Epidemiological data highlight regional differences in allergy incidence, with higher rates in countries with high legume consumption, suggesting the impact of dietary habits on sensitivity development (Crespo *et al.*, 1995). In conclusion, green pea allergy involves complex mechanisms, including immune activation, impaired mucosal barrier function, and genetic predisposition. Cross-reactivity with other legumes complicates diagnosis and treatment, requiring a detailed approach.

Evaluation of the effectiveness of diagnostic methods

Skin tests, such as scratch and prick tests, are the primary screening tools for allergy diagnosis, offering high sensitivity (85%) but moderate specificity (70%) in green pea allergy cases. Their advantages include rapid results and ease of use in clinical settings, though external factors like antihistamines or skin conditions may affect outcomes (Nowak-Węgrzyn *et al.*, 2017). Enzyme-linked immunosorbent assay (ELISA) for detecting specific IgE to green pea allergens achieves up to 95% sensitivity and 92% specificity. It is suitable when skin test results are inconclusive or contraindicated, despite its higher cost and equipment needs (Verma *et al.*, 2013). The oral provocation test, considered the diagnostic “gold standard,” ensures 100% sensitivity and specificity but requires specialised centres due to the risk of anaphylaxis (Mastrorilli *et al.*, 2024).

Modern molecular allergology enables the identification of specific allergenic proteins, such as Pis s 1 and Pis s 2 in green peas, helping distinguish between primary and cross-allergies. This precision reduces misdiagnoses and unnecessary dietary restrictions (Popp *et al.*, 2020). Although these proteins are both immunogenic, their allergenic potential and clinical implications differ in certain ways. Pis s 1 is the most common allergen, causing severe reactions such as anaphylaxis, whereas Pis s 2 causes milder symptoms including oral allergy syndrome. Their interaction is important in co-sensitised individuals because combined exposure might exacerbate immune responses and result in more complex symptom patterns. Personalised medicine, based on molecular allergology and patient-specific data, allows for tailored treatment plans, including dietary adjustments, immunotherapy, or pharmacological interventions. Furthermore, customised tactics promote the use of targeted medicines, such as anti-IgE monoclonal antibodies, and allow for dynamic management by tracking changes in sensitisation patterns over time. New pharmacological approaches, such as monoclonal antibodies (e.g., anti-IgE therapy), offer promising treatment options by modulating immune responses (Cabanillas *et al.*, 2018). Specific immunotherapy also shows potential in gradually desensitising the immune system (Wensing *et al.*, 2003). A comparative evaluation of diagnostic methods requires analysis of their key characteristics, strengths, limitations, and indications (Table 1).

The analysis of the data shows that each diagnostic method has its strengths and weaknesses. Skin allergy tests are quick and affordable but have limited specificity. ELISA tests are highly accurate but require laboratory conditions. Provocative tests provide the “gold standard” of accuracy, although they are associated with a risk of severe reactions. Molecular allergology

allows for the identification of specific allergenic proteins for a personalised approach but remains expensive. Innovations such as monoclonal antibodies and specific immunotherapy offer the prospect of modifying the immune response but require a long time to implement in practice.

The combined use of several diagnostic methods is the most effective. For instance, the initial screening with skin tests or ELISA can be supplemented by molecular allergy to identify specific allergen proteins and provocative tests are used to finally confirm the diagnosis. This approach can achieve a diagnostic accuracy of up to 98% (Jensen *et al.*, 2008). Thus, the effectiveness of diagnosing green pea allergy is greatly enhanced by the integration of traditional methods with modern technologies, such as molecular allergology and pharmacological innovations. A personalised approach is the key to accurate diagnosis and successful treatment.

Analysis of the prevalence of allergies

The prevalence of green pea allergy varies depending on geographical, social and environmental conditions, as well as genetic factors that determine the individual susceptibility to developing allergic reactions (Ibanez *et al.*, 2003, Mondal *et al.*, 2024). The global prevalence of green pea allergy is still understudied compared to other food allergens such as peanuts, milk or eggs. However, studies show that food allergies, including pea allergy, are more common in highly developed countries with a high level of urbanisation, due to changes in lifestyle and diet (Verma *et al.*, 2013). In Europe and North America, the prevalence of food allergies reaches 6-8%, including allergies to legumes, which include green peas (Lisiecka, 2024a; 2024b). In developing countries, the level of allergy to green peas is much lower. This may be due to different cultural eating habits and the lower popularity of pulses in the diet of most of the population.

There are certain regional differences in the prevalence of green pea allergy in different parts of the world. Analysing the

metric determines that the highest prevalence of green pea allergy is observed in Europe (7%) and the United States (6%), which confirms the trend towards a higher prevalence of food allergies in developed countries. This is possibly determined by the high level of consumption of green peas as part of a balanced diet and the availability of this product. At the same time, in Asia and Africa, the rates are lower (2% and 3%, respectively), which may be due to the lower popularity of this product in the diet and genetic characteristics of the population. The lowest prevalence rate (1.5%) was recorded in developing countries, which likely reflects limited access to green peas and low levels of food allergy diagnosis.

In Europe and the United States, legume allergy patients frequently react to peas, with high sensitisation levels due to the product's common inclusion in diets across all age groups (Muller *et al.*, 2022). In contrast, green pea allergy is less common in Asian countries, where traditional diets include green pulses, possibly due to earlier exposure and genetic tolerance to certain pea components (Martínez San Ireneo *et al.*, 2008). Social and environmental factors, such as urbanisation, pollution, and climate change, significantly impact the prevalence of green pea allergy (Komilova *et al.*, 2023; 2024).

Pollution and stress in urban areas can trigger immune hyperreactivity, increasing the risk of food allergies, including to green legumes (Pastorello *et al.*, 2010). Changes in dietary habits, like increased consumption of processed foods, further contribute to allergy prevalence (Pham and Rudner, 2000). Conversely, rural areas with traditional diets and lower pollutant levels may reduce sensitisation (Matheu *et al.*, 1999). Thus, green pea allergy prevalence is influenced by geographical, environmental, and social factors, which are crucial for developing effective prevention and treatment strategies and understanding the disease's pathogenesis.

International recommendations and their implementation

International organisations play a key role in developing global food allergen risk management standards to protect consumer health. The UN, EU, World Health Organisation (WHO), and European Food Safety Authority are working on recommendations to improve food safety and reduce allergen risks. The Codex Alimentarius, under the UN, provides guidelines for managing food contamination and allergen labelling, ensuring transparency for consumers (Branca, 2024). WHO supports standardising allergen management approaches and creating a global allergic reaction database, emphasising the need for testing even minimal allergen amounts and improving communication between producers, healthcare professionals, and consumers (Food and Agriculture, 2001).

NATO and Interpol, while primarily focused on security, also contribute to food protection efforts. NATO aids in improving food safety management in vulnerable regions and developing standards to protect food from chemical threats, including allergens. Interpol combats food fraud, which may involve the intentional or accidental use of allergenic ingredients. These efforts are reflected in the daily practices of food producers, including the implementation of the Hazard Analysis Critical Control Point (HACCP) system to identify and eliminate allergenic contamination (U.S. Food and Drug Administration, 2022). Manufacturers also adopt advanced testing methods based on WHO and European Food Safety Authority standards. The EU's allergen labelling system allows consumers to avoid products containing allergens, such as green pea proteins. By following the standards set by the UN, EU, and European Food Safety Authority, countries can reduce allergen risks, improving public health and confidence in food safety systems.

Research gaps and new perspectives

Previous studies on green pea allergy have improved understanding of its clinical features and diagnosis but have key

limitations. Most research focuses on small samples, neglecting ethnic, geographical, and cultural factors that affect allergy prevalence. While traditional allergens have been well-studied, green pea proteins remain understudied, and cross-reactivity with other legumes complicates diagnosis. Current diagnostic methods may miss trace amounts of allergens, highlighting the need for more precise tools. A promising area of research is exploring genetic factors influencing susceptibility to green pea allergy, which could help identify risk groups and enable personalised prevention and treatment. Studies on food processing impacts, such as heat treatment or fermentation, may reveal ways to reduce allergenicity and create safer products. Social aspects, like public awareness of allergies, also warrant further study, especially in regions with limited access to food safety knowledge.

The results of the study highlighted the main aspects of green pea allergy, including its clinical manifestations, pathogenesis and diagnostic methods. Regional differences in prevalence were identified. At the same time, the identified research gaps open new perspectives for improving prevention and diagnosis, which will be the basis for further discussion in this section. According to Smits *et al.* (2021) and Pham and Rudner (2000), green pea allergy is common among individuals also allergic to legumes like chickpeas. This aligns with the current study, which confirms frequent sensitisation to green pea proteins due to widespread consumption. Hildebrand *et al.* (2021) and Villa *et al.* (2020) highlighted significant cross-reactivity among legumes such as peas, chickpeas, lupins, and peanuts, underlining the value of molecular allergology for precise allergen identification. Jensen *et al.* (2008) further showed that sensitisation to one legume often results in reactions to others due to protein homology.

These findings correspond with the role of Pis s 1 and Pis s 2 proteins, which warrant deeper investigation. Skypala *et al.* (2021) noted the high allergenic potential of nonspecific lipid transfer proteins due to

their resistance to heat and digestion, supporting observations on Pis s 1 and Pis s 2. Similarly, Cabanillas *et al.* (2018) stressed the stability of storage and lipid transfer proteins, explaining persistent allergenicity post-cooking. Mastrorilli *et al.* (2024) highlighted the severe impact of IgE-mediated legume allergies in children, including skin reactions and anaphylaxis, findings that mirror those in the present study. Webber and England (2010) discussed the diagnostic challenges, especially with low allergen levels, underscoring the need for more sensitive tests. Muller *et al.* (2022) confirmed the high accuracy of oral provocation tests for legume allergy, while noting the risks associated with their use. Mansoor and Sharma (2011) noted a wide range of clinical manifestations of food allergy, including severe cases of anaphylaxis. Such data are confirmed by the results of the study, which indicates the need for a personalised approach to the treatment of green pea allergy. Recommendations by Nowak-Węgrzyn *et al.* (2017) on the management of food allergens are also noteworthy. Although the authors addressed other allergies, standardisation of approaches to food safety can be effective in the case of green pea allergy.

Crespo *et al.* (1995) highlighted the role of regional factors in food allergy prevalence, aligning with this study's findings that geography and dietary habits influence green pea allergy rates. Pastorello *et al.* (2010) identified IgE-binding proteins, such as lipid transport proteins in green beans, as potent allergens – consistent with this study's results on Pis s 1 and Pis s 2 in green peas, supporting their further investigation for improved diagnostics and product safety. Matheu *et al.* (1999) and Kalogeromitros *et al.* (1996) reported anaphylaxis due to lupine and lentils, attributing it to cross-reactivity – a pattern also observed in green pea allergy, underscoring the need for thorough allergological assessment. Ibanez *et al.* (2003) explored monoclonal antibodies to regulate immune response, supporting this

study's proposed pharmacological strategies for managing severe green pea allergies. Vitaliti *et al.* (2015) demonstrated that even cooking vapours from legumes can trigger severe reactions in children, aligning with this study's findings on the broad clinical spectrum of green pea allergy.

Chan *et al.* (2019) advocated molecular allergology for identifying specific allergens in cross-reactive cases, reinforcing this study's recommendation to employ such methods for accurate green pea allergy diagnosis. The results confirm that the use of modern molecular allergy techniques, improved provocation tests and geographical considerations are key to the effective diagnosis and treatment of green pea allergy. Comparison with the current literature indicates the importance of these areas while highlighting the need for further research to develop personalised approaches and novel therapeutic strategies.

CONCLUSIONS

The findings significantly advance understanding of green pea allergy, confirming the study's objective. A broad spectrum of symptoms was observed – skin, respiratory, gastrointestinal, and severe forms like anaphylaxis – varying by age, allergen exposure, and individual factors. Children, particularly with skin symptoms, were a key focus. Pis s 1, a highly immunogenic storage protein, induces IgE production and triggers allergic cascades. Notable cross-reactivity with legumes such as peanuts, chickpeas, and lentils was identified. Skin tests remain a primary screening tool due to accessibility, though specificity is limited. ELISA showed up to 95% accuracy for detecting IgE to Pis s 1 and Pis s 2, while provocation tests offer 100% sensitivity and specificity. Molecular allergology enhances precision in allergen identification and cross-reactivity management.

The prevalence of green pea allergy is influenced by regional, social, and cultural factors, with urbanisation, dietary habits, and environmental pollution affecting sensitisation levels. This highlights the need

for tailored diagnostic and prevention approaches. Molecular methods should be more widely used to identify specific allergens and manage cross-sensitisation. Personalised treatment plans, including immunotherapy and monoclonal antibodies, should be developed based on individual characteristics. International standards for allergen labelling should account for even minimal allergenic protein amounts. These findings can directly influence real-world allergy management by encouraging the use of molecular diagnostics to properly identify particular green pea allergens, providing more precise dietary advice and decreasing unnecessary food restrictions. Furthermore, the emphasis on tailored treatment approaches promotes the development of safer, more targeted therapies for patients who are at high risk of severe allergic reactions.

Despite significant findings, the study has limitations, including a potentially unrepresentative patient sample and reliance on available diagnostic methods, which may have affected allergen detection. Cross-reactivity aspects, particularly in food processing, were not fully explored. Practical implementation of recommendations requires further research in clinical settings.

CONFLICT OF INTEREST STATEMENT

The author declares that she has no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Table 1: Characteristics and comparison of methods for diagnosing green pea allergy

Diagnostic method	Sensitivity	Specificity	Advantages	Disadvantages	Indications for use
Skin allergy tests	85%	70%	Quick results, accessibility, low cost	Possible false-positive results due to cross-reactivity; limitations in patients with skin diseases or on antihistamine therapy	Initial screening in patients with suspected allergies
Immunofluorescence assay (IFA) (IgE to Pis s 1, Pis s 2)	90-95%	87-92%	High precision, the ability to perform without the risk of allergic reactions	High cost, need for specialised equipment	Use in difficult cases or when skin testing is not possible
Oral provocation test	100%	100%	The “gold standard” of diagnostics, the highest accuracy	Risk of anaphylaxis, need for specialised conditions	Confirmation of diagnosis in controversial or complex cases
Molecular allergology	High	High	Identification of specific allergenic proteins, accurate cross-reactivity analysis	High cost, limited availability	Differential diagnosis of allergies, personalised approach
Monoclonal antibodies	Not defined	Not defined	Modulate the immune response, and reduce the risk of severe reactions	Cost and duration of treatment	Treatment of complex forms of allergy
Specific immunotherapy	High	High	Adapts the immune system to the allergen, long-lasting effect	Long-term therapy, the need for regular monitoring	Treatment of confirmed green pea allergy

Source: compiled by the author based on comparative analysis of data (Verma *et al.*, 2013; Sell *et al.*, 2005; Popp *et al.*, 2020; Mastroilli *et al.*, 2024; Skypala *et al.*, 2021).

Review article

**Beneficial microbial diversity in the rhizosphere of
Casuarina equisetifolia L. – A mini review**

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ABSTRACT

Numerous studies have demonstrated that diverse biological activities of microbial populations existing in rhizosphere have major impact on the growth and yield of plants significantly. The rhizosphere is the physical location in soil where plants and microbes interact. *Casuarina equisetifolia* L. is a multifunctional actinorhizal tree that grows quickly. Local people rely on *Casuarina* plants for non-wood forest products, small-scale timber, and fuel and are being used increasingly for rehabilitating deforested watersheds and other degraded landscapes. This brief review described the advantageous effects of several microorganisms found in the rhizosphere of *C. equisetifolia*, which contribute to the development, biomass, and enhancement of forest seedling quality by providing macro and micronutrients.

Keywords: AM fungi, *Casuarina equisetifolia*, *Frankia*, litter decomposers, rhizosphere

INTRODUCTION

The rhizosphere, that is, the tiny area surrounding and impacted by plant roots, is a hot spot for different kinds of microorganisms and is considered as one of the most intricate ecosystems on Earth. The rhizosphere is home to a variety of organisms including viruses, bacteria, fungi, oomycetes, nematodes, protozoa, algae, and arthropods.

Plants are invaded by an incredible number of microorganisms that can reach cell densities much higher than the number of plant cells. Additionally, there are many more microbial genes in the rhizosphere than that of plant genes. Numerous studies have shown that a wide range of microorganisms associated

with plants may significantly impact plant growth and development, nutrition, illnesses, productivity, seed germination, and seedling vigor. In keeping with the nomenclature for microorganisms, the aggregate communities of microbes associated with plants are called the plant microbiome (Gevers *et al.*, 2012).

Casuarina equisetifolia trees are frequently utilized to restore degraded areas. In India, China, Egypt, Tunisia and Senegal, they are utilized as windbreaks to repair sand dunes and safeguard nearby crops (Sayed, 2011). These trees are utilized in agroforestry systems to boost agricultural productivity and enhance soil fertility (e.g, intercropping with legumes) in India and China (Zhong *et al.*, 2010). In

India, *Casuarina* trees are also utilized to produce hardwood for home building and paper pulp wood, as well as smokeless fuelwood with a high calorific value. In Asia, *C. equisetifolia* trees have created shelter belts that have been extremely protective during tsunami and typhoons (Zhong *et al.*, 2010).

Numerous studies have examined the positive benefits of rhizosphere organisms on plant development and health and they include protozoa, mycorrhizal fungi, nitrogen-fixing bacteria, plant growth promoting rhizobacteria (PGPR), biocontrol microbes, and mycoparasitic fungi (Mendes *et al.*, 2013). Majority of the farmers keep growing the *C. equisetifolia* trees continuously for three cycles lasting up to up to 9 years. Consequently the harvested biomass has a high rate of soil nutrient removal. Deficit illnesses and soil sickness are caused by this loss of nutrients. There are evidences that the rhizosphere of *C. equisetifolia* contains beneficial microorganisms like *Azospirillum*, *Azotobacter*, *Phosphobacterium*, AM fungi, and *Frankia*. These microbes are known to enhance the growth of *Casuarina* seedlings as well as trees planted in farm forestry (Rajendran, 2016). In this context, several studies have been carried out to explore the beneficial microorganisms existing in the rhizosphere of *C. equisetifolia* and evaluate their role in the growth and development in various parts of the world. The present work focused on the particular features of the advantageous microbes found in rhizosphere of *C. equisetifolia*.

Rhizosphere of *C. equisetifolia*

It is interesting to note that a number of pioneer actinorhizal plant species in the *Casuarinaceae* family may form extended rows of continuous rootlets in clusters throughout their root system (Arahou and Diem, 1997). This special root development may therefore provide *C. equisetifolia* growing in nutrient-poor soils with an adaptive mechanism to maximize the uptake of insoluble nutrients needed for growth and nitrogen fixation.

Beneficial microbial populations of *C. equisetifolia*

There are many different microorganisms in the rhizosphere, or the zone of impact around plant roots. The rhizosphere soil of *C. equisetifolia* cultivated in several agroclimatic zones in Tamil Nadu, India, was examined by Rajendran *et al.* (1999) and the results showed that the population of *Azospirillum* (3.3×10^6 g/dry soil), *Phosphobacteria* (2.5×10^4 g/dry soil) and *Actinomycetes* (6.4×10^4 g/dry soil) were found in sandy clay loam soil. Panda (2010) estimated that 39.0×10^3 of bacterial and 43.8×10^2 of fungal populations were found in rhizosphere of *C. equisetifolia* in Ganjam district of Orissa, India. At species level, Vinod *et al.* (2014) identified *Streptomyces roseiscleroticus*, *S. flavochromogenes*, *S. vastus* and *S. pragueaeneses* in rhizosphere of *C. equisetifolia* in Port Blair, Andaman, (Ojha and Arya, 2018) found *Aspergillus niger* and *Trichoderma viride* from Maharaja Sayajirao University of Baroda of Gujarat, India. Huang *et al.* (2020) noted *Bryometopus*, *Codonosigidae*, *Oligohymenophorea* and *Saccharomycetes* in Hainan Province, China and Lin *et al.* (2022a) reported *Acinetobacter nosocomialis*, *Bacillus cereus*, *Enterobacter cloacae*, *Enterobacter hormaechei*, *Enterobacter* sp., *Ochrobactrum* sp., *Pantoea* sp., *Pseudomonas* sp. in Hainan Province, China.

It was stated that the microenvironment has a significant impact on soil microbial populations, which are a crucial part of the soil ecological system (Mu *et al.*, 2007). The activity and variety of the soil microbial population are influenced by soil characteristics, and soil microorganisms progressively enhance soil quality by breaking down organic materials (Chen *et al.*, 2016). The various microbial populations found in the rhizosphere of *C. equisetifolia* have unique biological effects that contribute to the growth and development of *C. equisetifolia*.

It is widely known that the associative symbiotic diazotrophic bacterium *Azospirillum* can fix nitrogen from the atmosphere and generate compounds that promote growth. Recently, this bacterium has been shown to be an effective biofertilizer for a variety of agricultural crops by increasing the inorganic nitrogen requirements of the crops. Although these bacteria are widely distributed, their numbers are typically modest, with the exception of the rhizosphere where a two to three fold increase may occur. For their growth, they need available carbon molecules, sufficient quantity of inorganic nutrients including phosphorus, calcium, magnesium, molybdenum, and optimal pH. Likewise, phosphobacteria are microbes that can dissolve insoluble phosphorus and provide it to plants in a form that they can use. Phosphobacteria include members of *Pseudomonas*, *Micrococcus*, *Bacillus* and *Flavobacterium* (Krishnamoorthy, 2002). A class of filamentous bacteria known as actinomycetes contributes significantly to the encouragement of plant development by increasing the availability of nutrients, boosting plant defenses, and generating beneficial compounds. These are the major component of the rhizosphere and play a key role in soil nutrient cycling (Singh et al., 2018).

Several studies have proved that the above said microbial populations were significantly increased the growth and development of *C. equisetifolia* when applied individually or in combination with each other. For example, Rajendran et al. (2003) evaluated that the seedling of *C. equisetifolia* inoculated with combined application of *Azospirillum* + *Phosphobacterium* + AM + *Frankia* produced the maximum growth, biomass and quality. Likewise, Rajendran and Devaraj (2004) reported that combined inoculation of *Azospirillum* + *Phosphobacterium* + AM + *Frankia* and *Phosphobacterium* + AM + *Frankia* recorded maximum height, 20.56% and 17.53% increase over the control

respectively at 24 months after planting in *C. equisetifolia*. Also, statistically there was no difference in the height between the triple inoculation of *Azospirillum* + *Phosphobacterium* + *Frankia* and *Azospirillum* + AM + *Frankia*.

Kumaravelu et al. (2004) studied the impact of combined inoculation of *Azospirillum* + *Phosphobacterium* + AM fungi has significantly increased the growth and nutrient uptake by *C. equisetifolia* in field condition. They have found that the growth was 193% increase over control in inoculated plants. Saravanan et al. (2012) estimated that the highest total biomass was recorded in the seedlings inoculate with the combined application of *Azospirillum* + *Trichoderma* + *Pseudomonas* (54.87% increase over control), followed by *Azospirillum* + *Pseudomonas* inoculated seedlings (49.17% increase over control) and *Azospirillum* inoculated seedlings (47.34 increase over control) of *C. equisetifolia*. Gunasekara et al. (2016) noted that seedlings which were treated with *Frankia* sp. (one of Actinomycetes) showed the nodule formation ability in *C. equisetifolia*. Additionally, the plants treated with *Frankia* exhibited the highest levels of root and shoot growth.

Frankia as an endosymbiont

Frankia are symbiotic, nitrogen-fixing bacteria that are found in the root nodules of actinorhizal plants. They are members of the class Actinomycetales, which has over 200 species spread across 25 genera. Typically, actinorhizal plants are pioneer species that invade nitrogen-poor areas that are unsuitable for other plants for growth. *Frankia* is reported to exist in the majority of soils, even in areas where there have long been no appropriate host plants. It is interesting to note that non-host soils can occasionally support a higher nodulating *Frankia* population than host-containing soils (Diagne et al., 2014).

The N₂ fixing actinomycetes *Frankia*, which creates root nodulation, are associated with *C. equisetifolia*. Rajendran and Jothibas (2006) observed that nodules were found at 0-30 cm soil depth in all the sites examined in various location of Tamil Nadu, India and root nodulation in trees (72.8 – 88%) was recorded in the sandy clay loam soil with nodular size at an average of 5 – 6.8 cm in diameter in three years old *Casuarina* plantation. Tani *et al.* (2003) identified a brown coloured *Frankia* nodule in sandy soil at Okayama in Japan with the nodular size about 1.5 cm size and 33 mg fresh weight. Karthikeyan (2016) isolated two different types of *Frankia* from two different study sites in Tamil Nadu, India: brown – pale yellow coloured nodules in rhizosphere of *C. equisetifolia* grown in Cuddalore and pink – light brown coloured nodules in Nagapatinam. They were 1.5 and 0.9 cm in diameter respectively.

It was reported that, *C. equisetifolia* is one of the non-leguminous trees that can fix nitrogen from the atmosphere by a symbiotic interaction that has been formed between the plant's roots and the soil microorganism *Frankia*. Within the nodules, the nitrogenase enzyme, which is produced by *Frankia*, catalyzes the conversion of atmospheric nitrogen to organic form. It was estimated that the *Casuarina* fixed 60 kg of nitrogen per hectare per year through *Frankia* (Karthikeyan *et al.*, 2013).

Many studies have been conducted to enhance the growth and development for *C. equisetifolia* by using *Frankia*. Artificial treatments of *Frankia* nodule crush enhanced *C. equisetifolia* growth and biomass production by 30–40% and the increase was caused by *Frankia* which is closely linked to improved accumulation of nitrogen (Rajendran *et al.*, 2003; Rajendran and Devaraj 2004; Rajendran and Jothibas, 2006; Saravanan *et al.*, 2012; Karthikeyan 2016). Similarly, improved growth of *C. equisetifolia* rooted stem cuttings

inoculum with *Frankia* was observed in both nursery and field conditions (Karthikeyan *et al.*, 2013). Compared to uninoculated control seedlings, *C. equisetifolia* seedlings infected with *Frankia* and cultivated in degraded coir pith as a potting media in a root trainer exhibited more nodulation and nodular biomass and seedlings showed 35.33% increase in dry weight over the uninoculated control seedlings (Saravanan *et al.*, 2012). It was also reported that rooted stem cuttings of *C. equisetifolia* infected with *Frankia* exhibited better growth and biomass under high CO₂ (Karthikeyan, 2017).

Mycorrhizae

A large number of actinorhizal plants may also establish mycorrhizal relationships and this tripartite symbiosis (host plant–*Frankia*–mycorrhiza) gives them a tendency to grow even in marginal and unfavourable soils (Dawson, 2008). Certain species have a high degree of adaptation to polluted soils, flooded terrain, desert areas, high salinity and severe p^H. Because of these characteristics, many actinorhizal trees are pioneer species that spread to damaged regions; they are often employed to re-vegetate various landscapes or prevent desertification, and they serve crucial ecological functions. The symbiotic relationship between fungus and root system of higher plants is called mycorrhiza, which literally means root fungus.

Arbuscular mycorrhizal fungus (AM) in rhizosphere of *C. equisetifolia*

The association of Arbuscular Mycorrhizae (AM) fungi is endotrophic, and has been called to as Vesicular-Arbuscular Mycorrhiza (VAM) in the past. This nomenclature has now been abandoned in favor of AM since not all fungus produce vesicles. The uptake of phosphorus and other micronutrients is significantly influenced by *Arbuscular mycorrhizae* (AM) in higher plants (Rajendran and Devaraj, 2004).

Rhizosphere of *C. equisetifolia* shows that the association of different species of AM fungi. A study carried out by Rajeswari *et al.* (1999) in various locations of Tamil Nadu, India revealed that 250/100 g of spore density with 53.88% of AM infection was found in Coimbatore, 301/100 g and 85.50% in Kanyakumari and, 340/100 g and 75% in Marakkanam respectively. The results of this study also stated that *Glomus* was found as dominant genus among AM fungi identified in all the study sites. There are several other studies also confirmed that *Glomus* was the dominant genus in rhizosphere of *C. equisetifolia* grown in different regions such as Chinnathachur in Villupuram, India (Senthilkumar *et al.*, 2010), Western Morocco (Hibilik *et al.*, 2021), Eastern coastline of Karaikal, India (Das and Sivashri, 2022) and North Sumatra, Indonesia (Delvian and Hartanto, 2022).

In the various age groups of *C. equisetifolia* plantations, the beneficial impacts of AM fungus was examined in Tamil Nadu, India (Rajendran, 2001; Munusamy *et al.*, 2010) and AM fungi are effectively used when this species is planted in deteriorated soils. Diagne *et al.* (2014) conducted a study to evaluate the impact of AM fungi to rehabilitate the salinized soil by growing *C. equisetifolia* and the results of this study revealed that *Glomus aggregatum* and *G. fasciculatum* inoculation enhanced the growth of *C. equisetifolia* in ten months after inoculation. Zhong *et al.* (2014) also discovered that mycorrhizal fungi enhanced *C. equisetifolia* development by increasing seedling nutrient intake, as well as abiotic stress tolerance and survival.

Litter decomposing microorganisms

Litter decomposition is influenced by environmental variables and also by physical-chemical characteristics of the parts such as stem wood, leaves, roots, etc. of the species studied and decomposing organisms present in the soil. The maximum degree of decomposition ascribed to the appropriate

temperature, moisture, rainfall, micro-fungal population and improved soil aeration (Pande *et al.*, 2002). Decomposers are creatures that consume, digest, and decompose dead living things. In the nutritional cycles, they are critically necessary. All living organisms, including humans, are essentially borrowing the components of their bodies. After they die, they must be recycled so that other plants and animals may make use of them. Significant amounts of nutrients are present in *C. equisetifolia* needle litter, and their release is impacted by the decomposition of the litter by a range of microorganisms that are active in different environments (Uma *et al.*, 2014). Despite the litter of *C. equisetifolia* is colonized by a wide variety of microorganisms, although only a few number of species hold dominating positions. Panda *et al.* (2007) noted *Trichoderma*, *Aspergillus* and *Penicillium* were predominant occurrence in litter and have been found to be effective decomposers. Uma *et al.* (2014) reported eight dominant fungal species have been found in the rhizosphere of *C. equisetifolia* and they were *Alternaria alternata*, *Aspergillus niger*, *Penicillium* sp., *Fusarium* sp., *Rhizopus nigricans*, *Trichoderma viride* and *Curvularia eragrostidis*. They have also found two bacterial species viz. *Pseudomonas fluorescens* and *Azospirillum brasilense* were also present in the litter. Zhang *et al.* (2020) identified six bacteria (*Pseudomonas*, *Curtobacterium*, *Jatrophihabitans*, *Mycobacterium*, *Actinomyces* sp. and *Mucilagini bacter*) in Hainan Island, China. Lin *et al.* (2022b) reported two bacteria (*Pseudomonas* and *Sphingomonas*) and two fungi (*Phaeophleospora* and *Trichaptum*) from Guilinyang coastal area of Haikou, China.

Litter-decomposing microbes are essential to the growth of *C. equisetifolia* because they promote soil improvement and nutrient cycling. The organic matter in leaf litter is broken down by these microbes, returning vital nutrients like phosphorus and

nitrogen to the soil for the tree to absorb. They also aid in improving the structure of the soil, which promotes healthier roots and trees in general (Ngom, 2020). The capacity of *C. equisetifolia* to fix atmospheric nitrogen through a symbiotic interaction with *Frankia* is well-known. However, other vital elements like potassium and phosphorus are released as microbes break down litter, and they are equally vital for the growth of the tree (Uma *et al.*, 2014).

CONCLUSIONS

This review primarily presents the main conclusions from numerous studies carried out globally on the beneficial microorganisms found in the rhizosphere zone of *C. equisetifolia* and their useful function in enhancing growth and providing advantages for nutrient cycling. This review concludes that both small and large-scale farmers can increase plant productivity by using these advantageous rhizosphere microbiome, especially in dry and semiarid regions and the usage of chemical fertilizers in farm forestry may be reduced if these microorganisms are used to enhance growth. More research is required to assess the biological efficacy mechanism of this rhizosphere microbiota to provide theoretical evidence.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Review article

Illegal harvesting and trade of medicinal and aromatic plants: A criminal justice approach

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ABSTRACT

The illegal trade of Medicinal and Aromatic Plants (MAPs) threatens biodiversity, local economy, and traditional knowledge systems worldwide. Many MAP species are threatened by unsustainable harvesting due to herbal remedy demand. This paper explores illegal MAPs collection and trade in Latin America, Africa, and South Asia, where MAP-rich ecosystems are depleting. Certain species' high market value, rural economic difficulties, and organised crime syndicates are key drivers. The environmental and social repercussions are severe, destroying biodiversity, ecosystems, and indigenous cultures. This paper examines CITES and CBD, revealing enforcement and intergovernmental collaboration shortcomings. Criminal networks use corrupt institutions and weak rules to evade law enforcement. Judicial examples show the necessity for tougher punishments and legal professional knowledge.

Keywords: Biodiversity loss, criminal networks, illegal trade, medicinal and aromatic plants

INTRODUCTION

Medicinal and Aromatic Plants (MAPs) include several species renowned for Ayurveda, Traditional Chinese Medicine (TCM), and Unani medicine, as well as modern medicines and cosmetics, use MAPs for their fragrance, medicinal, and cosmetic characteristics. Modern and traditional medicine use them, as does the cosmetics industry. The rising number of people seeking herbal and natural remedies to improve their health is driving global demand for MAPs (Riaz *et al.*, 2021). To meet MAP demand, unsustainable methods like over-harvesting and gathering have evolved (Rathore, 2024). Several species are at risk of extinction or population decline due to this. MAP trafficking is a lucrative industry worldwide (Pathak *et al.*, 2024). These criminal-backed enterprises threaten the local ecology and animals. These plants

provide food and income to the locals, but illegal commerce threatens their survival. Particular plant species are threatened by illegal commerce for this reason. Combating illicit activities like MAP harvesting and trade is an important function of the criminal justice system. It is the aim to ensure that these things do not happen. The problem can be addressed by the government through a criminal justice approach that includes international coordination, rigorous enforcement of legal frameworks, and prosecution of criminals. This approach can solve the issue. Law enforcement agencies around the world need to step up their efforts to crack down on organised crime syndicates and the illegal trade of goods. More immediate and severe legal action is required in this case. This article discusses current regulatory frameworks, the criminal justice system's role in fighting this trade, and unlawful MAP harvesting. This study

examines police and judicial responses to unlawful MAP trading and how to improve institutional and legal systems. The paper's study highlights gaps in policies and enforcement. This article further examines how the criminal justice system handled this big issue through relevant court cases.

Geographic hotspots for illegal harvesting

Some of these ecosystems are subject to illicit MAPs gathering due to their plentiful fauna and lack of enforcement. Recently, the illegal traffic in Latin American, African, South, and Southeast Asian natural species has skyrocketed. Illegally collecting aromatic and rare medicinal plants is frequent in the South American Amazon and India's Western Ghats (Silalahi *et al.*, 2023). This happened in these regions. In certain locations, declining plant numbers have caused species extinction or are close to it.

Key drivers of illegal trade

Illicit MAPs collection and trade are among the causes. The high value of certain plant species motivates people to find them. Wild Ginseng, Sandalwood, Yarshagumba Caterpillar fungus are popular worldwide (Pandey, 2022). Economic pressures from within rural communities may force them to collect illegal MAP (Pathak, 2024). Organised crime groups profit from the illicit MAP trade, making regulation and control harder. Inadequate enforcement and legislative frameworks worsen the situation. As uncontrolled or poorly supervised harvesting procedures increase, authorities are finding it tougher to detect and prohibit criminal activity (Singh *et al.*, 2024). This makes banning unlawful firms harder for authorities. Lack of resources, corruption, and insufficient coordination among flora and fauna protection authorities make it impossible to enforce current limitations in some locations.

Environmental and social impacts

Illicit MAPs collection has serious environmental impacts. Overharvesting plant species can damage ecosystems, cause

biodiversity loss, and kill indigenous plant types. Overuse usually causes these side effects. Many MAPs are needed to maintain ecological balance. Eliminating these MAPs could devastate ecosystems and other species (Pathak *et al.*, 2024). Illicit commerce destroys indigenous peoples' cultural practices and knowledge, harming society. These communities have long benefited from these plants' healing and economic benefits. Corruption in the sector exploits local harvesters. While traffickers and intermediaries split the profits, they work for low wages. Illegal MAPs harvesting is becoming a global issue that requires legislative changes, increased enforcement, and international cooperation. Cutting the problem's scope necessitates this (Pathak *et al.*, 2024).

Legal framework for protection of medicinal and aromatic plants

National and International legislation is needed to protect aromatic and medicinal plants. Due to their economic and ecological value, MAPs have been the subject of many international accords and state restrictions to ensure sustainable collecting, trading, and protection (Riaz *et al.*, 2021). These treaties and rules protect these resources. These laws' practicality is being questioned due to implementation and enforcement issues.

International frameworks

The Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) has protected endangered Medicinal and Aromatic Plants species (Mozer and Prost, 2023). The convention on International Trading in Endangered Species regulates endangered species trading. Wild Ginseng, Sandalwood, and several orchids are CITES-protected MAP species (Challender *et al.*, 2023). MAPs and the CBD (Convention on Biological Diversity) are major international agreements that promote biological resource protection and responsible usage. The CBD stresses that nations must create biodiversity plans. Protocols should be implemented for these

projects to gather MAPs sustainably. Additionally, the Nagoya Protocol to the CBD emphasises equal genetic resource benefits (Ali, 2022).

National legal frameworks

Numerous nations have enacted legislation to prevent the poaching and trade of MAPs, which threatens their endangered species and natural resources (Pathak *et al.*, 2024). The populations of many animals have been protected by various statutes. The relevant laws are now in full force across the nation. As an example, Indian legislation protects medicinal plants as well as other endangered and uncommon plant species. The Wildlife Protection Act, 1972 and the Biological Diversity Act, 2002 are two Indian legislation that fall under this category. Countries with rich biodiversity, like Brazil, China, and Nepal, have similar frameworks (Sreeram, 2025). Even in countries where these policies are in existence, their enforcement is hindered by a combination of limited resources, corruption, and a lack of local understanding. Countries with lax legal systems tend to have unregulated MAP trade and collection because of the absence of harsh penalties for violators (Pathak *et al.*, 2024).

Gaps and challenges

These national and international organisations have created a solid framework, but intergovernmental collaboration, monitoring, and enforcement are still lacking. Many worry about MAP smuggling across borders, evading legal restrictions (Silalahi *et al.*, 2023). Organised crime groups complicate this situation for law enforcement. Worse, many local harvesters violate the protections without knowing it.

Strengthening enforcement, collaborating internationally, and establishing legal frameworks are essential to ensuring MAP's sustainable use for decades and preventing its illegal trading (Pathak *et al.*, 2024).

Criminal networks and supply chains

The illegal sale of medicinal and aromatic plants is sometimes organised by national or regional criminal organisations. All trade occurs on these networks. MAPs are valuable in cosmetics, pharmaceuticals, and traditional medicine, thus criminals target them. Many countries exploit slack laws, corrupt practices, and weak enforcement to support trafficking through complex supply networks (Silalahi *et al.*, 2023).

Structure of criminal networks

MAPs criminal organisations usually have a distinct hierarchy. As most harvesting workers come from low-income families and they may not realise the legal ramifications of their acts. This is a problem since harvesting can be destructive. Commercial pressures cause MAPs harvesters to extract unsustainable numbers, reducing natural populations. Middlemen then acquire plants at a bargain to get an advantage over harvesters and profit. Therefore, larger criminal organisations can carry plants across borders since they can avoid limitations. Criminal groups that traffic narcotics, wildlife, and people also smuggle MAPs. Many nations also exchange MAPs (Silalahi *et al.*, 2023). These syndicates deliver MAPs using the same routes and networks. Organised crime groups help these networks dodge law enforcement and transport illicit MAPs worldwide.

Supply chain dynamics

Illicit MAPs trafficking begins with overharvesting plants from environmentally rich areas. Protection areas like reserves often harvest this way. This makes MAPs trafficking illegal (Pathak *et al.*, 2024). Processing centres collect, dry, package, and label plants for sale. Before selling the plants, this is done. MAPs are often disguised as real goods to hide their true nature. Thus, authorities are finding it tougher to identify criminal patterns. Cosmetic, pharmaceutical, and herbal remedy manufacturers use the plants. They reach local and global markets. Companies may not know where their plants come from

or may ignore the illegal trade for its huge profits. The space can accommodate both options.

Links to other criminal activities

The MAPs trade, drug trade, illicit logging of forests, and animal poaching are all interconnected types of organised crime. Among numerous, these are only a few instances. Criminal organisations rely on complex transportation and smuggling networks, making it difficult for law authorities to disrupt their operations. Because of corruption inside regulatory agencies and at border checkpoints, illegal MAPs are able to reach markets worldwide (Pathak *et al.*, 2024). Some of these reasons will ensure that these endeavours continue. More stringent legislative frameworks, improved law enforcement practices, and globally coordinated operations are necessary to dismantle these criminal networks. This system makes it very easy to disrupt supply networks and hold offenders accountable (Sreeram, 2025).

Criminal justice approach to curb illegal MAPs trade

Criminal law enforcement needs a comprehensive plan to stop the illegal medicinal and aromatic plant trade. This plan should include judicial system prosecution, international collaboration, and law enforcement. Deterrence and enforcement must be balanced due to the trade's complexity, which is sometimes fuelled by multinational smuggling and organised crime.

***Prosecution and judicial role**

The prosecution of MAPs traffickers and their imprisonment are essential for stopping the illegal trade. By punishing lawbreakers in court, they pay the price and set a precedent that others should avoid (Silalahi *et al.*, 2023). For the sole reason that it could inspire similar cases. There are many obstacles to prosecuting this activity. One must coordinate globally, collect evidence linking harvesters to trafficking network

executives, and prove it. MAPs and their illicit extraction's environmental impact are often unknown to judges and prosecutors. Belief is that this is one of their biggest problems (Pathak *et al.*, 2024). This enhances the risk of mishandling or mild punishment. Legal professionals must be reminded of the importance of biodiversity and MAPs enforcement. To bridge this gap and better serve the public, dedicated training programs should be provided to improve judicial responses to illicit MAPs trading.

***Challenges in law enforcement**

Many issues make it harder to pass MAPs legislation to end the illegal trade. Trouble tracing the supply chain from rural harvesters to global markets exacerbates the issue. Local collectors are routinely targeted by law enforcement due to their ignorance and low-income backgrounds (Pathak *et al.*, 2024). The difficulties of finding organised crime syndicates and traffic-coordinating middlemen prevent complete network destruction. Many illegally traded MAP species are unprotected by current laws, and it is equally important to overcome from this difficulty. Weak enforcement or soft sentences will deter future offenders even with restrictions (Silalahi *et al.*, 2023). Targeting indigenous harvesters, traffickers, and intermediates who profit from the trade is vital, because harvesting is lucrative.

***Sentencing and Penalties**

To make the trade a more effective deterrent, the punishment should match the economic loss and environmental damage it causes. China and India are among the countries that have toughened penalties for illegally collecting and selling MAPs, especially for endangered species (Silalahi *et al.*, 2023). Both the small-scale harvesters and the criminal organisations that enable the trade need to be punished by the criminal justice system for it to be effective.

***International cooperation**

The illicit MAPs trade is a worldwide problem, thus it's critical that governments work together (Silalahi *et al.*, 2023). To get over some legislative gaps in different countries, criminal organisations often use international smuggling channels. By doing so, they will be able to go around the rules as they are. Destroying these networks requires the involvement of international allies. Part of this objective is the sharing of information and the coordinating of efforts amongst law enforcement agencies. Strong lobbying organisations that promote international collaboration to combat the illicit trade of MAPs commodities include INTERPOL (The International Criminal Police Organization) and the CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora) (Challender *et al.*, 2023).

***Law Enforcement Strategies**

Law enforcement must adopt creative strategies to effectively tackle the illicit traffic in MAPs. The employment of drones and other forms of satellite tracking is one such tactic. One more example would be to step up patrols of areas known to be particularly rich in endangered species. Several Indian forest groups, for instance, use state-of-the-art equipment to keep tabs on illegal harvesting. This is a component of their effort to combat illegal harvesting (Sreeram, 2025). It is asserted that if national authorities and multinational groups cooperated, it would be easier to prosecute key figures in the illicit trade. It is possible to gather this information via establishing connections.

Role of judiciary in curbing illegal harvesting and trade of MAPs

Due to repeated cases, the courts are investigating illegal medicinal and aromatic plant (MAPs) collecting and trafficking. Each case has revealed how widespread the problem is and how the law may help. These prosecutions show that stricter laws and

punishments are needed to deter such wrongdoing.

In *United States v. Billy Joe Hurley* (2014), Great Smoky Mountains National Park Police arrested 46-year-old Billy Joe Hurley for stealing 83 American ginseng roots. Police investigated independently. Hurley was convicted of four ginseng poaching charges in two trials. Demand for American ginseng is high. The Great Smoky Mountain plant is pricey in Asia. This slow-growing plant is illegally stolen, damaging its roots. This greatly reduces the plant's long-term weather resistance. US Magistrate Judge Dennis L. sentenced Hurley to five months plus fifteen days. NPS planted despite only half of healthy roots surviving. To prevent unlawful collecting and raise awareness of poaching's damage to the species, sentences were imposed. Christopher Ian Jacobson was fined \$1,000 and imprisoned 80 days for 298 ginseng roots. An independent event. DOJ and NPS want American ginseng banned (United States Attorney Office, 2014),

In the case of *Divya Pharmacy vs. Union of India* (2018), the provisions of Biological Diversity Act, 2002 were challenged. The Act found Patanjali Ayurveda's Divya Pharmacy in contempt for not disclosing organic resource benefits. The Uttarakhand High Court has ordered that commercial enterprises like Divya Pharmacy must share revenues with local biological resource producers, backing the NBA (National Biodiversity Authority) (Law Bhoomi, 2024).

In *State Of Himachal Pradesh vs. Krishan Lal Pardhan And Others*, Supreme Court of India (1987), the Special Judge released an order in the light of the available evidence that the respondents should be held accountable for their alleged involvement in a criminal conspiracy involving unlawful tree cutting, forgery, corruption, and associated offences. The Supreme Court found the Special Judge erred in releasing the accused without proof. It was obvious before reviewing the former court's prima

facie case. The Supreme Court rejected the lower court's release of the detainee and ordered the prosecution to file charges and commence trial immediately. The court said the defendant's case circumstances didn't alter its choices. (Casemine, 1987).

In the case of Fresenius Kabi Oncology Ltd And Anr vs The State Of Maharashtra And Anr. (2023), the central issue was illegally chopping of *Mappia foetida* (narkya) trees. Chandoli National Park, Maharashtra, had these trees. Cancer drug development Alkaloid Camptothecin (CPT) plummeted. Narkya tree is vital to chemotherapy, but CPT demand threatens it. Previously, Narkya trees were in abundance. 223 violators were sued under The Indian Forest Act, 1927 and The Wildlife (Protection) Act, 1972. In January 2024, the Bombay High Court acquitted Fresenius Kabi Oncology (formerly Dabur Pharma) after a lengthy trial. The court found that the corporation bought CPT from another company without knowing it was made from unlawfully chopped narkya trees. Interestingly, the court declared CPT as non-wood. Old Supreme Court rulings supported this claim. Corporate prosecution was wrong. The unlawful trade persisted after the forest agency sued, so narkya may expand (Down to Earth, 2024).

Challenges in enforcement

Laws to stop MAPs' unlawful trade face several obstacles. These issues stem from the trade's complexity and organised crime. The wide and remote locations where MAPs are gathered lack proper monitoring resources, which must be addressed. Due to lax enforcement and monitoring, many protected areas and biodiversity hotspots allow illegal harvesting (Pathak *et al.* 2024). This is because these communities lack the resources to oversee and enforce the law. How to involve locals in harvesting is another big issue. Many humans that survive on MAP plants are unaware of their regulatory regimes. As authorities target small scale harvesters, intermediaries and traffickers will remain uncaught (Silalahi *et*

al., 2023). The divide between local police and worldwide criminal groups that manage the illicit market makes us powerless to stop it. This schism prevents clandestine market management. Ineffective enforcement is also caused by institutions that should be accountable for not functioning together. Despite worldwide MAPs trafficking, customs, police, and environmental groups do not cooperate (Silalahi *et al.*, 2023). The unlawful cross-border trade of MAPs is prevalent. Unless one cooperates, traffickers will use regulatory gaps and illegal trade ways to profit. This lets them keep conducting their business. Legal loopholes and weak sanctions reduce deterrence. Unfortunately, state and international laws do not fully protect many MAP species. The sanctions for illegal harvesting and trafficking are too light to dissuade perpetrators. Even after perpetrators are caught, economic and environmental damage requires heavier penalties. Regional corruption helps traffickers evade capture, especially at borders and in police offices (Silalahi *et al.*, 2023). Thus, corruption allows them to circumvent limitations. MAP-trafficking criminal networks are growing harder to stop. Anti-MAP trade rules are notoriously difficult to police for several reasons. Problems include corruption, inadequate agency coordination, insufficient money, and obsolete laws.

Recommendations and way forward

The illegal trade in medicinal and aromatic plants (MAPs) requires an integrated approach. The following suggestions are necessary to fix the issue:

- **Strengthening legal frameworks:** To increase MAP protections, national and international laws must be amended and expanded. Governments must comply with CBD and CITES, two international accords regulating endangered animal trade, to safeguard state residents (Challender *et al.*, 2023).
- **Capacity building for enforcement agencies:** Building the capacity of

enforcement agencies leads to better results. Law enforcement officers should be trained on the financial and environmental benefits of marine protected areas. Providing them with the knowledge and tools to monitor, investigate, and address illegal activities will strengthen enforcement efforts.

- **Community engagement and alternative livelihoods:** People must be involved in conservation efforts because MAPs collect funds at those locations. Offering alternatives to their present subsistence techniques or encouraging environmentally sound harvesting will reduce their reliance on criminal activities.
- **International cooperation:** Due to the global character of MAPs commerce, international coordination is needed immediately. Nation-states can disrupt trader related crime networks by sharing intelligence, coordinating police, and sharing information.
- **Public awareness and education:** Raising knowledge of illegal MAP trafficking's environmental impacts can reduce demand. To reduce illicit drug demand, customers, especially in traditional medicine markets, must be educated on sustainable MAPs acquisition (Silalahi *et al.*, 2023). The traditional medicine market illustrates this.
- **Technology use:** Data analytics, drones, and satellite tracking can increase remote enforcement and surveillance.

Way forward

Better government enforcement of these restrictions will reduce illicit MAP trafficking. New regulations, community involvement, better technology, and international cooperation are needed to preserve these unique plant resources for future generations (Pathak *et al.*, 2024). This is the only opportunity to achieve one's goal.

Conclusion

A growing global issue impacts local economies, ecosystems, and species. The illicit medicinal and aromatic plant (MAP) trade is complex and global. Criminal organisations profit from harassing innocent people through harvesting. Insufficient finance, international coordination, and legal gaps hinder compliance notwithstanding statutory frameworks. This problem requires greening, tougher laws, and greater enforcement. Numerous initiatives stand out. One should strengthen MAPs' legislative protections, provide enforcement personnel with specialist expertise and tools, include local populations in conservation, and promote international cooperation to combat cross-border trafficking. Public awareness and cutting-edge technologies will fight unlawful MAPs trade. Prioritising MAPs conservation and coordinating activities can save these plant species. MAPs help biodiversity, culture, medicine, and economy. Health, economic, and cultural benefits will be immense. It can protect these natural treasures and promote responsible use by working together daily.

CONFLICT OF INTEREST STATEMENT

The author declare that she has no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Review article

Diseases and traditional medicine in the Central Middle Atlas (Morocco)

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ABSTRACT

Today, despite the development of synthetic chemistry, the use of medicinal plants remains widespread due to their effectiveness in many therapeutic practices. At the same time, poverty and lack of access to modern medical care are driving a large proportion of the world's population to turn to traditional medicines to meet their primary healthcare needs. With the aim of highlighting the medicinal plants of the central Middle Atlas region, we carried out an ethnobotanical and floristic study to identify the medicinal plants used by the local population to treat various illnesses. This study has enabled us to draw up a catalog of 267 medicinal species belonging to 79 botanical families and 211 genera. Analysis of the data showed that each pathology is treated by several plants, but with different frequencies: The results of the study has been presented and discussed in the paper.

Keywords: Central Middle Atlas, disease, ethnobotanical survey, medicinal plant, Morocco.

INTRODUCTION

For a long time, natural remedies, particularly herbal remedies, were the main, if not the only, recourse in medicine. Although the rise of the pharmaceutical industry has enabled modern medicine to effectively treat many formerly fatal diseases, the use of medicinal plants and the remedies derived from them has never been completely abandoned. People continue to turn to traditional medicine, which has preserved the therapeutic traditions inherited from our ancestors. Ethno botanical studies look on how these plants' resources are used for food, medicine, fuel wood, agriculture, housing, crafts, fodder, and religious rituals (Gazala *et al.*, 2023). Today, the use of herbal medicine is experiencing a resurgence of interest in Western countries, particularly to treat imbalances brought on by modern life, from stress to weight problems and cardiovascular disorders (Nguemo Dongock *et al.*, 2018). These plants represent a vast

source of biologically active substances (Meliani *et al.*, 2023).

The Moroccan population has an ancient and rich tradition of phytotherapy, a heritage of Arab-Berber civilization largely influenced by the Islamic and Jewish religions, which makes the use of a few or several medicinal plants in the treatment of various illnesses an integral part of Moroccan culture. Thus, people have always had a traditionally rich ethnobotanical knowledge due to the cultural and ecological diversity of the environment in which they live. According to Vandebroek *et al.* (2004), this knowledge reflects the richness of the vegetation in which these indigenous peoples live. The medicinal potential of a given flora is greater when it is more diverse. Due to its special geographical location, Morocco occupies a very important place in the Mediterranean basin and is one of the first countries with the richest flora. Its terrestrial flora comprises approximately 7000 inventoried species. The central Middle

Atlas has remarkable forest potential and biodiversity. This ecological heritage is of socio-economic interest, contributing to local development by meeting the needs of the local population (Labhar and Lebaut, 2012). The multiplication of ethnobotanical studies on a national scale enables: to gather more details on medicinal plants, enhance them, and save some knowledge acquired by the local population (Dif *et al.*, 2022). With this in mind, an ethnobotanical study of medicinal plants has been carried out in the central Middle Atlas region, with the aim of compiling a written catalogue and ancestral knowledge of medicinal plants, which has been passed down by oral tradition from generation to generation.

Different illnesses treated by medicinal plants in Middle Atlas

Medicinal plants are used for their beneficial properties for human health. However, it is important to remain vigilant, as some plants can be toxic and present health risks. Medicinal and aromatic plants will continue to be potential sources of active, useful chemicals compounds that are used in the cure of various ailments (Rakesh Rathor, 2024). They can be used in a variety of ways, such as decoction, infusion or maceration. Depending on what is needed, one or more parts of these plants, such as the roots, leaves or flowers, can be used (Dutertre, 2011). In our region, they are used to treat a variety of illnesses and symptoms, the most common being digestive tract disorders, metabolic disorders and osteoarticular problems (Hachi *et al.*, 2022).

Species most commonly used in the treatment of digestive tract disorders

Origanum compactum ranks first (832 citations), a species widely used in traditional medicine thanks to its properties. The leafy stem, infused or decocted, is recommended for: digestive system problems and as a depurative. However, the very high demand for labelled raw materials (organic, wild, spontaneous, etc.), most of which are destined for export, and the poverty of the local populations, can compromise

Morocco's exceptional wealth of biodiversity. One example is rosemary, which is currently under threat from heavy exploitation and irregular climatic conditions (Fechtal, 2000); efforts are still being made to domesticate and regenerate it (Ismaili *et al.*, 2003).

Tetraclinis articulata (421 citations) is used in traditional medicine for its medicinal value against diarrhoea, gastrointestinal pain and colopathy. This species is well documented in traditional medicine for its medicinal value against diarrhoea, abdominal pain, tumours, bronchitis and indigestion (Hmamouchi, 1999; Bellakhdar, 1997).

Chenopodium ambrosioides (402 citations) decoction is used as an antipyretic and aperitif, to treat gastrointestinal disorders, diarrhoea and typhoid, both internally and externally. *Chenopodium ambrosioides* decoction, combined with *Mentha suaveolens* or *Origanum compactum*, is used as a carminative.

Other species such as *Thymus zygis*, *Aretmisia herba-alba*, *Trigonella foenum-graecum*, *Thymus vulgaris*, *Ajuga iva*, *Punica granatum*, *Pistacia lentiscus* and *Mentha suaveolens* are used to treat the digestive tract with varying frequency.

Species most commonly used in the treatment of metabolic disorders

The ethnobotanical study revealed that *Caralluma europaea* (510 citations) is the species most frequently reported by informants for the treatment of metabolic disorders, followed by *Olea europaea* (325 citations), *Artemisia herba-alba* and *Trigonella foenum-graecum* (312 citations), while *Tetraclinis articulata* was cited 216 times). Other notable species include *Nigella sativa* (209 citations), *Allium sativum* (208 citations) and *Olea europaea* var. *sylvestris* (197 citations). *Origanum compactum* and *Thymus zygis* received 115 and 113 citations respectively. Finally, *Thymus vulgaris* and *Rosmarinus officinalis* shared the same number of mentions 111, while *Alyssum spinosum* was cited 109 times.

Species most commonly used in the treatment of osteoarticular disorders

It was studied that telephium-leaved corrigiola (*Corrigiola telephiifolia*) was the most commonly used in the treatment of rheumatic diseases (182 citations), followed by wild olive (*Olea europaea* var. *sylvestris*), buttercup (*Ranunculus ballatus*), spearmint (*Mentha pulegium*), rosemary (*Rosmarinus officinalis*), olive (*Olea europaea*), round-leaved mint (*Mentha suaveolens*), false fennel (*Ferula communis*), African pyrethrum (*Anacyclus pyrethrum*) and magydaris (*Magydaris panacifolia*) (70 citations). *Corrigiola telephiifolia*, in powder form, is used to relieve joint pain and chills, while the roots are used to make fire tips for rheumatism and joint pain.

Species most commonly used in the treatment of dermal diseases

For dermal diseases, the *Origanum compactum* (89 citations) is best represented in the treatment of skin diseases, followed by *Teucrium polium* and *Allium cepa* (89 citations); *Rosmarinus officinalis*, *Caralluma europaea*, *Eugenia caryophyllata*, *Plumbago europaea* and *Agave americana* L. (45 citations).

Due to its very high frequency compared with the other species oregano (*Origanum compactum*) appears to be the medicinal species most frequently used in the treatment of skin diseases. It is a species that is very well known among the local population for its therapeutic virtues; its leaves are used in cataplasms to heal wounds and bruises, as an antiseptic and against burns, and used in lotions against hair loss and as an anti-dandruff agent.

Species most commonly used in the treatment of respiratory ailments

For respiratory treatment, *Mentha pulegium* (681 citations) is one of the species most commonly used. It is widely used by infusion, decoction and inhalation to treat colds, flu, bronchitis and coughs. *Rosmarinus officinalis*, (476 citations) *Eucalyptus*

globules 359 citations), *Marribium vulgare* (259 citations) and *Lepidium sativum* (169 citations) occupied second place with different percentages in the Middle Atlas of Morocco.

Mentha pulegium is used to treat more diseases, as an infusion, inhalation or chest poultice, for colds, sore throats, coughs, bronchitis, lung infections and colds of all kinds (Bellakhdar, 1997). It is the plant for winter illnesses.

Species most commonly used in the treatment of cardiovascular diseases

Crataegus laciniata (67 citations) is the most well-known and effective medicinal species for treating cardiovascular disease in the region studied followed by *Allium sativum* 65 citations), *Olea europaea* (56 citations) and *Olea europaea* var. *sylvestris*. (50 citations). Other notable plants include *Salvia officinalis*, which received 46 citations, and *Nigella sativa* with 45 mentions. *Origanum compactum* and *Hibiscus sabdariffa* were each cited 40 times, while *Mentha suaveolens* received 36 citations. *Mentha pulegium* and *Lavandula dentata* were mentioned 34 times, and *Apium graveolens* was cited 32 times.

Crataegus laciniata enhances myocardial contraction (Gruenwald *et al.*, 1998). Hawthorn's procyanidin extracts help to reduce cholesterol levels and reduce the size of atherosclerotic plaques. This action is the result of hawthorn's ability to maintain the integrity of the matrix (collagen) of vessel walls, making them more resistant, which can help prevent the development and progression of atherosclerotic plaque (Rose and Treadway, 1999). Oligomeric procyanidins are responsible for the cardiac actions of *Crataegus*. They increase the heart's use of oxygen and aid enzyme metabolism (Davies, 2000).

Species most commonly used to treat genitourinary disorders

For genitourinary disorders *Lavandula dentata* (452 citations) is widely used by the population as a decoction to treat ailments of the urinary tract and urogenital disorders. It is also used vaginally to treat infections of the vagina and uterus. *Herniaria hirsuta* (208 citations) is also used in powder or decoction form to remedy anuria, expel kidney stones and treat kidney lithiasis. followed by *Chenopodium ambrosioides*, with 180 citations, and *Artemisia herba-alba* with 146. *Lactuca scariola* and *Anacyclus pyrethrum* have the same number of citations, at 137. *Rosmarinus officinalis* and *Apium graveolens* also share the same total, with 135 citations each. *Crocus sativus* was cited 134 times, while *Ajuga iva* received 129 mentions. *Petroselinum sativum* and *Nigella sativa* also had the same number of citations, at 126. Finally, *Salvia officinalis* and *Melissa officinalis* both recorded 125 citations.

Species most commonly used in the treatment of neurological disorders

Lippia citriodora (270 citations) is the most widely used species, certainly because of its effectiveness against neurological diseases and its wide distribution around homes. Infusions of the plant are used to calm, sedate and induce sleep, and to treat headaches and dizziness. *Matricaria camomilla* (163 citations) is also used to treat neurological disorders. In descending order, we also find the following species: *Lavandula multifida* with 113 citations, *Melissa officinalis* with 89 mentions, and *Tetralinis articulata* 69 citations. *Salvia officinalis* follows with 60 citations, while *Ocimum basilicum* has 57. *Crataegus laciniata* was cited 56 times, and *Papaver rhoeas* earned 54 mentions.

Wild chamomile is recommended in Morocco as an anxiolytic and central nervous system balancer, of great value in cases of nervous depression (Haddad et al., 2003).

Toxic plants varies according to species

Poisoning by plants and traditional pharmacopoeia products is not negligible

throughout the world. Their occurrence is the consequence of several factors, including the popular belief that plants are not dangerous because they are natural. According to a study carried out by Morocco's Centre Antipoison et de Pharmacovigilance, intoxications by plants and traditional pharmacopoeia products accounted for 5.1% of all cases of intoxication reported between 1980 and 2008, excluding scorpionic stings and envenomations (Rhalem et al., 2022). The three main sources of plant intoxication being glue thistle (*Atractylis gummifera*) (10.1%), cannabis (*Cannabis sativa*) (4.6%) and harmel (*Peganum harmala*) (3.6%) (Kamgoui, 2004). The notion of dose is very important. Certain plants used for therapeutic purposes can present risks to human and animal health in large doses. This is the case, for example, with sage, *salvia officinalis*, mugwort and wormwood. All these plants are medicinal in low doses, but highly toxic in high doses (Belghazi and Benbaziz, 2020). The composition of a plant can vary from one specimen to another, depending on the soil, growing conditions, humidity, temperature and sunlight. Similarly, plants of dubious origin should not be used, since pollution factors, harvesting and methods of preservation and storage can alter their properties (Chen et al., 2021). Depending on the nature and the degree of toxicity, the way the remedies are prepared and the way they are administered, toxicity varies according species used. In addition, self-medication often leads to over-consumption and prolonged use, sometimes incompatible with the physiological (extreme ages, pregnancy, breast-feeding) or pathological (hepatic, renal and cardiac insufficiency, diabetes, etc.) condition of patients.

The Table 1 shows the toxic medicinal plants found in our study region:

CONCLUSION

The results of this study shows that each disease is treated by several medicinal plants, either alone or in combination with other plants. *Origanum compactum* is used for

disorders of the digestive tract, followed by *Tetralinis articulata* and *Chenopodium ambrosioides*. Metabolic disorders are treated mainly with *Caralluma europaea*, followed by *Olea europaea*. Osteoarticular disorders are treated most by *Corrigiola telephiiifolia* followed by *Olea europaea* var. *sylvestris*. For skin disorders, *Origanum compactum* is most commonly used, followed by *Teucrium polium* and *Allium cepa*. For genito-urinary conditions, *Lavandula dentata* is most frequently used, followed by *Herniaria hirsuta*. Respiratory ailments are treated with *Mentha pulegium*, followed by *Rosmarinus officinalis* and *Eucalyptus globulus*. Cardiovascular diseases are treated with *Crataegus laciniata*, followed by *Allium sativum*, *Olea europaea* and *Olea europaea* var. *sylvestris*. Finally, for neurological diseases, *Lippia citriodora* is used most often, followed by *Matricaria camomilla*.

CONFLICT OF INTEREST STATEMENT

The authors declares that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Table 1: List of toxic medicinal plants found in the Central Middle Atlas region

Scientific name	Common name Arabic/Tamazight	Toxic part	Symptoms / disorders
<i>Agave americana</i> L. ***	Sabra	Leaves	Skin
<i>Ajuga iva</i> (L.) Schreb. ***	Tûf tolba / Chendgoura	Whole plant	Neurotoxicant
<i>Ammi</i> Sp. ***		Fruit	Skin
<i>Anacyclus pyrethrum</i> L. **	Iguntas / Tagundeht	Root	Digestive, neurotoxicant
<i>Aristolochia baetica</i> L.*	Berztem / Ajrarhi	Root	Digestive, respiratory
<i>Artemisia absinthium</i> L. ***	Chiba	Whole plant	Digestive, neurotoxicant
<i>Artemisia hera-alba</i> Asso. **	Chih / Ifzi / Izri.	Whole plant	Neurotoxicant, abortive
<i>Atractylis gummifera</i> L.*	Addad.	Root	Digestive, neurotoxicant, hepatic
<i>Camelia thea</i> L. ***	Atay.	Leaves	Cardiotoxic, neurotoxicant
<i>Cannabis sativa</i> L. **	Kif	flowers, stems	Digestive, neurotoxicant
<i>Capsicum frutescens</i> L. **	Felfel Hârr /soudania	Fruit	Digestive
<i>Carum carvi</i> L. ***	Karwiya	Fruit	Hepatic, neurotoxicant
<i>Cedrus atlantica</i> (Endl.) Carr. ***	L-âr / Atgal.	Bark	Neurotoxicant
<i>Chenopodium ambrosioides</i> L. ***	Mkhinza.	Leaves	Neurotoxicant
<i>Citrullus colocynthis</i> (L.) Schrud.*	Lhdej / Tafrizite.	Fruit	Digestive
<i>Colchicum autumnale</i> L. **	Bakbuka	Whole plant	Digestive, neurotoxicant
<i>Crataegus laciniata</i> Ucr. ***	Admam	Flowers, seeds	cardiotoxic
<i>Daphne gnidium</i> L.*	Âlezzâz	Leaves, fruit	Neurotoxicant
<i>Delphinium staphysagria</i> L.*	Habbat ras	Seeds	Digestive, cardiotoxic
<i>Echinops spinosus</i> L. ***	Taskra	Root	Neurotoxicant
<i>Euphorbia</i> sp*	Tikiwt	Latex	Digestive, neurotoxicant
<i>Ephédra</i> sp***	Timitrte	aerial part	Neurotoxicant
<i>Eucalyptus globulus</i> Labill. ***	Calitous	Leaves	Neurotoxicant, renal toxicity
<i>Ferula assa-feotida</i> L.*	Hantita	Fruit	cardiotoxic
<i>Ferula communis</i> L.*	Uffal / Awli / Klakh / Lboubal.	Whole plant	Hemorrhagic
<i>Ficus carica</i> L. **	Karma / Tazarett	Leaves	Skin
<i>Foeniculum vulgare</i> P. Mill. ***	Nafaa / lbesbas / Amssa.	fruit	Digestive, cardiotoxic, neurotoxicant
<i>Globularia alypum</i> L. ***	A'yen lerneb / Taselgha	Whole plant	Digestive, cardiotoxic, neurotoxicant
<i>Glycirrizha glabra</i> L. ***	Arak sous.	Root	Digestive, cardiotoxic
<i>Juniperus oxycedrus</i> L. ***	Taqqa.	Bark	Skin
<i>Lepidium sativum</i> L. **	Hab rchad	Seeds	Digestive
<i>Linum usitatissimum</i> L.***	Zriaat al kettane	Seeds	Digestive
<i>Mandragora autumnalis</i> Bert.*	Bîd al-ghûl	Root	Digestive, cardiotoxic, neurotoxicant

<i>Marrubium vulgare</i> L. **	Mriwta / Mriwa	Whole plant	Skin
<i>Mentha pulegium</i> L. ***	Fliyou	Whole plant	neurotoxicant, abortive
<i>Mercurialis annua</i> L. **	Harryga malsa	Whole plant	Digestive
<i>Myristica fragrans</i> Houtt. **	L-gûsa	Seeds	Digestive, neurotoxicant
<i>Nerium oleander</i> L.*	Defla / Allili	Whole plant	Digestive, neurotoxicant, cardiotoxic
<i>Nicotiana tabacum</i> L. **	Tanfiha	Leaves	Respiratory, neurotoxicant, cardiotoxic
<i>Nigella sativa</i> L. **	Sanouj	Seeds	abortive
<i>Papaver rhoeas</i> L. ***	Belaamane	petals	Neurotoxicant
<i>Peganum harmala</i> L. **	Lharmal	Seeds	Digestive, neurotoxicant
<i>Pimpinella anisum</i> L. ***	Habat hlawa	Fruit	Neurotoxicant
<i>Piper cucuba</i> L.F. **	L-kubbaba	Seeds	Digestive
<i>Quercus</i> Sp***		Whole plant	Digestive
<i>Ranunculus ballatus</i> L.*	Wden l'halluf	Root	Skin, digestive
<i>Ricinus communis</i> L.*	Lkharwaa / Anguaref	Seeds	Digestive, hepatic, cardiotoxic
<i>Rosmarinus officinalis</i> L. ***	Yazir	Whole plant	Neurotoxicant
<i>Ruta montana</i> L. **	Lfijel / Iwrmi	Whole plant	Neurotoxicant, skin
<i>Salvia officinalis</i> L. ***	Salmiya	Whole plant	Neurotoxicant
<i>Satureja calamintha</i> (L.) Scheele. ***	Manta	Whole plant	Skin
<i>Taraxacum obovatum</i> DC. **	Iwjdem	Leaves, stems, flowers	Skin
<i>Taxus baccata</i> L.*	Îgen	The whole tree, especially the seeds	cardiotoxic
<i>Teucrium polium</i> L. **	Ja'ada	Whole plant	Digestive, hepatic
<i>Urginea maritima</i> (L.) Baker.*	Îkfîl / Bsel idane	Bulb	cardiotoxic
<i>Urtica dioica</i> L. **	Hurriga /Thissarkmaz	Whole plant	Skin
<i>Verbascum sinuatum</i> L.*	Aberdud n-izem	Seeds	Digestive

*: all respondents stated that these plants are toxic.

**: less than 10% of respondents stated that these plants are toxic.

***: toxicity according to literature.

Development and evaluation of *Dracaena trifasciata* hydrogel: A multifunctional approach for topical therapeutic formulations

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ABSTRACT

Dracaena trifasciata is a widely used ornamental plant that possesses an array of bioactive compounds contributing to its potent antibacterial, anti-inflammatory, antipyretic, analgesic and anti-diabetic properties. The current research involved extracting bioactive compounds from *D. trifasciata* employing ethanolic and methanolic solvents, followed by the evaluation of their phytochemical profiles and antioxidant activities. These extracts were then used to develop hydrogel formulations F1, F2, and F3, each containing ethanolic extract, methanolic extract, and a combination of both, respectively. These formulations were examined for their spreadability, pH levels, physical stability, and extrudability. The antibacterial activity of these formulations was subsequently tested against pathogens to determine their potential use as topical agent. The ethanolic and methanolic extracts of *D. trifasciata* revealed the presence of various bioactive compounds and demonstrated significant antioxidant activity. The formulations exhibited ideal physicochemical properties, including pH, spreadability, extrudability, and physical stability. They also exhibited significant antibacterial activity against *P. aeruginosa*, *S. pyogenes*, *E. coli* which validates their potential as effective and reliable topical agents.

Keywords: Antioxidant, *Dracaena trifasciata*, hydrogel, phytochemicals, topical formulation.

INTRODUCTION

Dracaena trifasciata, commonly referred to as the 'snake plant,' is a perennial evergreen herbaceous species utilized for its therapeutic properties in managing inflammatory conditions, snakebites, otalgia, edema, furuncles, and febrile illnesses (Teponno *et al.*, 2016). *Dracaena trifasciata* stands out as one of the most recommended species for enhancing indoor air quality due to its exceptional capacity to purify air by efficiently removing pollutants such as nitrogen oxides, formaldehyde, xylene, and toluene, facilitated by its Crassulacean Acid Metabolism (CAM)

adaptation (Umoh *et al.*, 2020; Kaur and Mudgal, 2021). The robust, lustrous, and lengthy natural fibers found in the leaves of this plant were traditionally utilized for crafting bowstrings and fishing lines, owing to their exceptional strength and durability, and is currently being explored for applications in the automotive industry as a reinforcement material in polyester composite manufacturing (Widyasanti *et al.*, 2020; Myint and Swe, 2019). Their phytochemical profile is characterized by a wide range of compounds, including steroidal alkaloids, sterols, flavonoids, and anthraquinones, many of which have demonstrated promising pharmacological properties supporting its traditional medicinal

application in treating various health conditions. Additionally, several studies suggest that certain attributes of snake plants may have the potential to address issues related to antimicrobial resistance (Kaur *et al.*, 2023).

Tissue damage is a common occurrence in daily life, often resulting from factors such as surgeries, burns, skin diseases, and other traumatic events. Injuries such as surgeries, burns, and skin diseases can cause significant tissue damage, resulting in the loss of essential protective mechanisms and the subsequent formation of a wound (Chi *et al.*, 2020). Wounds can be classified as acute or chronic, wherein acute wounds typically heal quickly, while the chronic wounds have a prolonged healing process, often lasting one to three months or more (Liu *et al.*, 2022). Wound healing is a complex biological phenomenon involving sequential stages of inflammation, proliferation, and remodeling, all of which are essential for the efficient repair and closure of injured tissues and wounds (Ruseva *et al.*, 2020; Gavel *et al.*, 2020). The delayed healing of chronic wounds is frequently attributed to microbial colonization within the wound bed, impeding the normal healing process. Effective management of chronic wounds is crucial to prevent complications such as amputation, sepsis, and death (Liu *et al.*, 2022). Traditional textile dressings, despite being cost-efficient, do not possess effective anti-infective properties, tend to be dry, and often adhere to wounds, thus compromising the wound healing process (Liu *et al.*, 2021).

Hydrogels are three-dimensional network structure, formed by crosslinked polymer chains through physical or covalent bonds. The unique three-dimensional network structure of hydrogels enables them to exhibit remarkable water absorption and retention properties (Liu *et al.*, 2022). The substantial water content of hydrogels effectively maintains a moist wound environment, which is crucial for reducing the risk of scar formation as well as providing a cooling effect and minimizing

tissue adherence, thereby reducing patient discomfort (Sun *et al.*, 2020; Kumar and Kaur 2020). The remarkable swelling capacity of hydrogels enables them to absorb a substantial amount of exudate, thus ensuring an effective wound management (Singh and Kumar 2020). Additionally, the high porosity of hydrogels facilitates the transmission of oxygen, promoting tissue oxygenation and enhancing wound healing (Stubbe *et al.*, 2019). Hydrogels are typically prepared from both synthetic polymers, such as carbomer (Carbopol) and carboxy methylcellulose, and natural polymers, like xanthan gum and guar gum. Synthetic polymers are preferred more often, with carbomer being the most widely used due to its excellent physical and rheological properties (Jain *et al.*, 2016).

The study involved the extraction of *Dracaena trifasciata* phytoconstituents using ethanol and methanol as solvents. The resultant crude extracts were subsequently analysed for their phytochemical composition. These extracts were then incorporated into hydrogels, with Carbopol 940 serving as the primary polymer matrix. The formulated hydrogels underwent a series of evaluations to assess their physicochemical properties and were ultimately tested for their antibacterial efficacy against wound pathogens, including *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, and *Streptococcus pyogenes*.

MATERIALS AND METHODS

Materials

Folin–Ciocalteu's reagent and 1, 1-diphenyl-2-picrylhydrazyl (DPPH) were obtained from Merck pvt ltd. Potassium dichromate, gallic acid (= GA), ascorbic acid (AA), AlCl_3 , H_2SO_4 , Nitric acid, NaOH, quercetin (QU), DMSO, EDTA, Diclofenac-Na, sodium nitrous, tween-80 and FeCl_3 were obtained from the Hi media Chemicals pvt ltd.

Sample collection and preparation

The aerial parts of *D. Trifasciata* leaves with

horizontal bands of light green with yellow margins were collected from Peelamedu region of Coimbatore district, Tamil Nadu, India. The leaves were extensively washed with distilled water to ensure the removal of all surface impurities and subsequently shade dried for 7 days. The cleaned leaves were cut into small pieces and subsequently subjected to thermal drying in a hot air oven at 120°C for 3 hrs. The dried leaves material was mechanically ground into a uniform coarse powder, ensuring homogeneity and stored in airtight containers for further use.

Sample extraction

About 450 g of the dried pulverized material was placed in an amber-colored glass container and soaked separately with 1600 mL of ethanol and methanol. The sealed containers were kept at room temperature for 7 days, with intermittent shaking and stirring. After the extraction period, the mixtures were filtered through a piece of clean white cotton, followed by Whatman No. 1 filter paper. The filtrate was allowed to evaporate at room temperature and the crude extract was reconstituted in dimethyl sulfoxide (DMSO) for subsequent analysis (Hossain *et al.*, 2016).

$$\text{Extraction efficiency (\%)} = \frac{\text{Final dry weight}}{\text{Initial dry weight}} \times 100$$

Phytochemical analysis

The extracts were subjected to a thorough phytochemical screening to identify the presence of an array of bioactive compounds such as tannins, alkaloids, saponins, flavonoids, terpenoids, anthraquinones, glycosides, steroids, reducing sugars, amino acids and phenolic compounds (Gul *et al.*, 2017; De Silva *et al.*, 2017).

Estimation of antioxidant content

phenolics content

For the quantification of total phenolics, 0.1 ml of the extract was dissolved in 0.4 ml of

methanol and then combined with 2.5 ml of Folin–Ciocalteu reagent. The solution was kept at 25 °C for 3 to 5 min to facilitate the reaction. Subsequently, 0.8 ml of sodium hydrogen carbonate solution (75 g/l) was added to the reaction mixture. The reaction mixture was incubated at 25 °C for 60 minutes to ensure, followed by absorbance measurement at 765 nm using a UV-Vis Spectrophotometer (Shimadzu). The phenolic content of the extracts was then calculated and expressed as gallic acid equivalents (GAEs) (Yuniarsih *et al.*, 2023).

Total flavonoid content

To determine the total flavonoid content 0.1 ml of the extract was mixed with 2.4 ml of methanol, 0.1 ml of a 10% aluminum chloride solution, 0.1 ml of 1M sodium carbonate, and 2.3 ml of distilled water. Following a 30min incubation at 25 °C, the solutions absorbance was measured at 432 nm using a UV-Vis spectrophotometer, with the results expressed in terms of quercetin equivalents (QEs)

Determination of antioxidant activity DPPH assay

The DPPH assay involved the preparation of a 0.1 mM DPPH solution in ethanol. 160 µL of this solution was added to 40 µL of sample solutions at various concentrations, diluted in methanol. The samples were incubated for 25 min at room temperature, and the absorbance values were subsequently measured at 517 nm. The DPPH radical scavenging activity was calculated, and the results were expressed as IC₅₀ (mg/mL), which corresponds to the concentration at which 50 % inhibition was observed.

$$\text{DPPH scavenging effect (\%)} = \frac{A_c - A_s}{A_c} \times 100$$

Wherein, A_c corresponds to the absorbance of control, while A_s denotes to the absorbance of sample. The antioxidant activity was compared using ascorbic acid as the standard reference

(Rechek *et al.*, 2021).

ABTS assay

The ABTS cation radical scavenging activity was evaluated via spectrophotometric analysis. ABTS radicals were formed by reacting 2.45 mM potassium persulfate with a 7 mM ABTS solution, which was incubated in the dark at room temperature for 12 hrs. This solution was subsequently diluted with ethanol to achieve an absorbance of 0.700 at 734 nm. For the assay, 160 μ L of the ABTS solution was added to 40 μ L of sample solutions diluted in methanol at various concentrations in a 96-well plate. The following equation was used to determine the scavenging potential of ABTS radicals

$$\text{ABTS scavenging effect (\%)} = \frac{A_c - A_s}{A_c} \times 100$$

where A_c refers to the absorbance of control; A_s refers to the absorbance of sample. Trolox served as the reference standard for comparative analysis of antioxidant activity (Rechek *et al.*, 2021).

Ferric reducing power assay

The reaction setup involved mixing 40 μ L of phosphate buffer (pH 6.6) with 50 μ L of 1% (w/v) potassium ferricyanide. Subsequently, 10 μ L of plant extract, at varying concentrations (200, 100, 50, 25, and 12.5 μ g/ml), was added. The solution was then incubated for 20 min at 50 $^{\circ}$ C to facilitate the reaction. Subsequently, the reaction mixture was treated with 50 ml of 10% trichloroacetic acid (TCA), 40 ml of deionized water, and 10 μ L of 0.1% ferric chloride (FeCl_3). The absorbance was then measured at 700 nm, where an increase in absorbance denoted enhanced ferric reducing power (FRP). The results, expressed as $A_{0.50}$, denoted the concentration necessary to attain an absorbance of 0.500. Catechin served as the standard for comparison (Rechek *et al.*, 2021).

Formulation of hydrogel

To formulate the hydrogels, was dispersed in 5 ml of sterilized water and stirred continuously at 500 rpm for 4 hours. Subsequently, 0.1 % of the *D. trifasciata* leaf crude extract was incorporated and stirred for another 2 hours. The formulation process included the addition of 0.02 g each of propyl paraben and methyl paraben. This was followed by the addition of 0.8 ml of glycerine and 0.5 ml of oleic acid. The final volume was adjusted to 10 ml with distilled water. The prepared samples were then equilibrated at room temperature for 24 hours prior to undergoing evaluation tests. Three formulations were developed: one containing ethanolic extract (F1), one with methanolic extract (F2), and a combination of both extracts (F3). A formulation lacking *D. Trifasciata* extract served as the control (Table 1) (Chirayathet *et al.*, 2019).

Evaluation of hydrogel formulation

Organoleptic analysis

The organoleptic properties of the newly formulated topical gel, encompassing its appearance, homogeneity, washability, consistency, phase separation, and odor, were systematically evaluated through visual inspection. The assessment of homogeneity and texture was conducted by positioning samples between the thumb and index finger, ensuring a comprehensive evaluation. The stability of the gel formulation was examined by subjecting it to centrifugation at 5000 rpm for 30 minutes, monitoring for any phase separation, creaming, or cracking phenomena. Concurrently, the immediate skin feel, encompassing parameters such as stiffness, grittiness, and greasiness, was assessed to ensure the formulation's overall sensory and physical integrity (Alam *et al.*, 2023).

Spreadability

The spreadability of the innovative topical

gels was quantitatively assessed by placing 0.5 g samples between glass plates, each with a predetermined weight of 100 g, and allowed to remain undisturbed for 10 min. The spreading area was measured using the formula

$$\text{Spreadability (S)} = M \times \frac{L}{T}$$

Wherein, M is the weight that is placed on the upper slide, L is the length of the glass slide and T represents the time duration (Garg *et al.*, 2002).

Physical stability

The physical stability of the novel topical gel formulation was investigated under a range of storage conditions ($40 \pm 2^\circ\text{C}$, $30 \pm 2^\circ\text{C}$, and $25 \pm 2^\circ\text{C}$) over a 28-day period. This investigation evaluated the stability and performance of the product, with careful documentation of any shifts in appearance, color, homogeneity, phase separation, and uniformity within the packaging tube to ensure comprehensive quality assessment (Alam *et al.*, 2023).

pH

The pH measurement of the gel was carried out with a digital pH meter. A 2 g gel sample was mixed in distilled water until a homogeneous suspension was formed. The suspension volume was adjusted to 40 mL, and the pH of the resulting solution was measured (Singh *et al.*, 2013).

Extrudability

The extrudability test involved filling capped collapsible aluminum tubes with the gel formulations, followed by precise weighing. The tubes were clamped between two glass slides and subjected to a 500 g weight placed on the top slide. After removing the cap, the extruded gel was collected and weighed to calculate the extrusion percentage, providing insights into the gel's behavior under mechanical stress (Mohan *et al.*, 2020).

Determination of antibacterial activity

The antibacterial efficacy of the hydrogel formulations was assessed against *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, and *Streptococcus pyogenes* using the agar well diffusion method. Bacterial cultures were grown overnight and diluted to achieve an optical density of 0.5 at 600 nm. Subsequently, 100 μL of these standardized bacterial suspensions were uniformly spread onto LB agar plates. Agar wells, 8 mm in diameter, were then created using a sterile agar well cutter to facilitate the introduction of the hydrogel formulations. Each well was loaded with 100 μL of the respective hydrogel formulations (F1, F2, F3, and Control) in 100 μL of DMSO. The plates were incubated at 37°C for 24 hours in an upright position. The zones of bacterial growth inhibition surrounding the wells were measured to determine the antimicrobial effectiveness of the hydrogel formulations (Chirayath *et al.*, 2019).

RESULTS AND DISCUSSION

Sample extraction

The extraction of *D. trifasciata* leaves using ethanol and methanol solvents yielded 15.45% and 11.12% respectively. The higher yield from methanol extraction implies that the bioactive compounds in the snake plant exhibit a polarity similar to that of methanol, demonstrating a better affinity of these phytoconstituents for methanol compared to ethanol (Marjoniet *et al.*, 2023).

Phytochemical analysis

The phytochemical screening results of the alcoholic (ethanolic) and methanolic extracts from

D. trifasciata leaves, as detailed in Table 2, confirm the presence of polyphenols, flavonoids, saponins, alkaloids, steroids, glycosides, reducing sugars, and amino acids in both extracts. Additionally, the ethanolic

extract showed the presence of tannins and anthraquinones, while the methanolic extract revealed triterpenoids. These findings are consistent with those reported in previous studies (Umoh *et al.*, 2020; Oomariyah and van Dijk, 2022; Oboho *et al.*, 2024; Sarjaniet *al.*, 2021). Phytochemicals are characterized by a wide array of potential activities that can be applied to various beneficial uses. Compounds such as cardiac glycosides, flavonoids, triterpenoids, alkaloids, phenols, and saponins imparts strong anti-inflammatory properties to these compounds (Oboho *et al.*, 2024). Additionally, the presence of alkaloids, saponins, terpenoids, steroids, glycosides, and tannins collectively enhance their antibacterial potential (Gul *et al.*, 2017). Furthermore, different flavonoid structures are associated with antifungal, antiviral, and antibacterial properties (Berame *et al.*, 2017).

Estimation of antioxidant content Total phenolics content

The quantification of total phenolic content in the ethanolic and methanolic extracts of *D. trifasciata* leaves was assessed using a gallic acid standard curve. The phenolic content was determined to be 13.84 mg GAE/g of extract for the ethanolic extract and 9.65 mg GAE/g of extract for the methanolic extract (Fig 1a). The antioxidant activity of the extracts is attributed to their phenolic components, which exhibit a well-established positive correlation with antioxidant activity due to the hydroxyl groups' scavenging ability Jain *et al.* (2016). The hydroxyl groups can donate hydrogen atoms to free radicals, thus enabling phenolic compounds to mitigate the free radical's effects (Sarjaniet *al.*, 2021).

Total flavonoid content

The total flavonoid content in the *D. trifasciata* leaf extracts was quantified, yielding 8.17 mg QE/g from the ethanol and 6.27 mg QE/g in the methanol (Fig 1b). The antioxidative activity is facilitated through the inhibition of

cyclooxygenase and lipoxygenase enzymes, which reduces prostaglandin and leukotriene levels, disrupts the arachidonic acid pathway, and decreases capillary permeability (Oboho *et al.*, 2024). Flavonoids demonstrate antibacterial activity by disrupting bacterial cell membrane integrity (Dewatisari *et al.*, 2021), in addition to exhibiting antifungal and antiviral properties (Umoh *et al.*, 2020). Moreover, flavonoids are known to possess antiseptic, anti-inflammatory, and anticancer effects (Febriani *et al.*, 2019).

Determination of antioxidant activity

A single assay is often insufficient to evaluate the total antioxidant potential, since different mechanisms play a role in the neutralization of free radicals. Consequently, the antioxidant efficacy of the *D. trifasciata* extract was subjected to three different assessment methods with the results illustrated in Fig 2.

DPPH assay

The DPPH radical scavenging assay indicated that the ethanolic extract of *D. trifasciata* achieved a 45 % antioxidant activity, while the methanolic extract displayed a 37 % antioxidant potential (Fig 2a). The data demonstrated a dose-dependent enhancement in free radical inhibition, with higher concentrations yielding greater inhibition percentages. The ethanolic extracts of *D. trifasciata* leaves demonstrated DPPH scavenging rates of 45 %, 37 %, 25 %, 16.5

%, 11 %, and 6 % at corresponding concentrations of 200, 100, 50, 25, 12.5, and 6.25 µg/ml. Meanwhile, the methanolic extracts achieved scavenging rates of 37 %, 29.5 %, 20.5 %, 12.5 %, 8.5 %, and 3.5 % at the same concentrations. The IC₅₀ values for DPPH scavenging by the ethanolic and methanolic extracts were determined to be 331.45 µg/ml and 1586.3 µg/ml, respectively. Earlier investigations have reported diverse IC₅₀ values for *D. trifasciata* leaf extracts, with results

showing 285.602 µg/ml (Lontoc *et al.*, 2018) 119.04 µg/mL (Shidhayeet *al.*, 2024), 48.77 µg/ml (Siddiqueet *al.*, 2014), and 2.19 µg/ml (Dewatisariet *al.*, 2024).

ABTS assay

The ABTS radical scavenging assay results revealed that the ethanolic extract of *D. trifasciata* exhibited a 63 % antioxidant activity, whereas the methanolic extract showed a 53.5 % antioxidant potential (Fig 2b). The data illustrated a dose-dependent increase in free radical inhibition, with higher extract concentrations resulting in higher inhibition percentages. The ethanolic extracts showed ABTS scavenging rates of 63 %, 48.5 %, 37 %, 23 %, 17 %, and 7.5

% at concentrations of 200, 100, 50, 25, 12.5, and 6.25 µg/ml, respectively. Concurrently, the methanolic extracts achieved scavenging rates of 53.5 %, 37 %, 24 %, 17.5 %, 13.5 %, and 6 % at the same concentrations. The IC₅₀ values for the ethanolic and methanolic extracts were established as 105.56 µg/ml and 176.20 µg/ml, respectively.

Ferric reducing power assay

The FRAP assay results for *D. trifasciata* leaf extracts, as shown in Fig 2c, reveal that the ethanolic extract possesses a significantly higher reducing power than the methanolic extract. The ethanolic extract had a reducing potential of 14.66 µg/ml, while the methanolic extract was at 26.71 µg/ml, with catechin as a standard showing 1.78 µg/ml at A_{0.5}. This assay evaluates the reducing power of compounds based on their ability to donate hydrogen atoms or electrons. The FRAP assay underscores the importance of hydrogen atom and electron donation capabilities in the antioxidant activity of these extracts. The high reducing power is due to the presence of hydrogen atom donors, as well as methoxy, keto, triterpenes, and acid groups in the plant extracts, which enhance their reducing potential and effectiveness as antioxidants (Shukla *et al.*,

2015).

Formulation of hydrogel

The formulation development employed pharmaceutical-grade constituents, adhering to the composition framework detailed in Table 1. The gelling agents incorporated include Carbopol 940, alongside preservatives such as Propyl paraben and Methyl paraben. The active ingredients were the ethanolic and methanolic extracts of *D. trifasciata* leaves, with oleic acid serving as a solubilizing agent and glycerin as a humectant.

Evaluation of hydrogel formulation

Organoleptic properties

The physicochemical properties of the novel topical gels were thoroughly evaluated, with specific focus on parameters such as appearance, phase separation, consistency, washability, odor, and immediate skin feel, as detailed comprehensively in Table 3. The prepared gel formulations varied in color from pale green to white for F1, F2, and F3, with the control formulation distinctly white. All formulations were grit and lump-free, exhibiting an opaque appearance. The gels applied smoothly, confirming their uniformity and washability, with no discernible odor.

Spreadability

The spreadability test results for the formulations are presented in Fig 3a. The results indicated that all the formulations fulfilled the requirements for good spreading efficiency. The evaluation revealed that formulation F3 possessed the highest spreadability at 8.86 g.cm. This was followed by formulation F1 with a spreadability of 8 g.cm, F2 with 7.18 g.cm, and the control with 7 g.cm. The ability of the newly developed gel formulation to distribute uniformly over the skin surface is a distinct characteristic, highlighting its excellent spreadability. Effective spreadability is paramount for topical formulations to ensure patient compliance. A

gel is deemed effective when it requires minimal time to disperse across the skin surface (Garg *et al.*, 2002).

Physical stability

The physical stability of the newly formulated gel was systematically evaluated at various time intervals to detect any signs of destabilization. The tubes were placed between two glass slides and were clamped on which a weight of 500 gm was placed over the slides and then the cap was removed. The results, as detailed in Table 4, confirm that the topical gel maintained its stability under the specified conditions throughout the assessment period.

pH

Ensuring an appropriate pH level is crucial for topical preparations to prevent skin irritation and scaling, with the ideal range being 4.5–6.5 (Yuniarsihet *al.*, 2023). The pH values for the developed formulations were 6.05 for F1, 6.76 for F2, 6.47 for F3, and 4.45 for the control, as depicted in Fig. 3b. These values demonstrate that all formulations comply with the required pH standards.

Extrudability

Extrudability analysis is performed to quantify the force necessary to expel the formulation from a filled tube. Gels exhibiting high consistency may resist extrusion from their tubes, whereas those with lower viscosity may flow too swiftly; hence, an appropriate consistency is necessary for optimal extrusion. Ensuring efficient extrusion of the gel from the tube is critical for ease of application and patient compliance. The values of extrudability of different formulations are 93

% for F1, 91.5 % for F2, 92 % for F3 and 87 for control, as represented in Fig. 3c. Gel formulations with extrudability rates over 90% are categorized as excellent, while those with rates at 80% are rated as good and those at 70% are considered as fair.

Determination of antibacterial activity

The hydrogel formulation F3 exhibited robust antibacterial efficacy, with inhibition zones measuring 20 mm, 12 mm, and 10 mm against *P. aeruginosa*, *S. pyogenes*, and *E. coli*, respectively (Table 5). Additionally, the F2 formulation too displayed considerable activity against *P. aeruginosa* and *E. coli*, presenting inhibition zones of 17 mm and 13 mm, respectively. In contrast, formulation F1 displayed a more selective antibacterial activity, showing significant efficacy only against *P. aeruginosa* with a 15 mm zone of inhibition. The classification of antibacterial potency based on the zone of inhibition is crucial for evaluating the efficacy of developed antimicrobial agents. A zone of inhibition ≥ 20 mm signifies a very strong level of antibacterial activity. Zones that fall within the range of 10 to 20 mm are classified as having strong activity, while those between 5 and 10 mm exhibit moderate activity (Hudzikiet *al.*, 2016).

The significant antibacterial activity observed in *D. trifasciata* extract gel formulations underscores the presence of a complex array of bioactive compounds. The broad-spectrum antimicrobial activity can be attributed to a synergistic effect of various phytochemicals present in the extract, including alkaloids, saponins, terpenoids, steroids, glycosides, tannins, acids, fats, and oils, all of which enhance the formulation's therapeutic potential (Akindele *et al.*, 2015; Andhareet *al.*, 2012). The antibacterial properties of ethanolic extracts from *D. trifasciata* leaves stem from their rich composition of bioactive molecules. These extracts show substantial potential as a natural antibacterial and antiseptic agent highlighting their potential for inclusion in natural therapeutic applications (Buyunet *al.*, 2018; Tkachenko *et al.*, 2017). The methanolic leaf extract of *D. trifasciata* demonstrates significant antibacterial activity, primarily due to its active phytochemical constituents, including saponins, phenols, and flavonoids (Febrianiet *al.*, 2019).

CONCLUSION

The phytochemical analysis of *Dracaena trifasciata* leaf extracts confirmed the presence of various secondary metabolites, including alkaloids, flavonoids, and saponins, highlighting its pharmacological relevance and supporting its traditional medicinal applications. Quantitative assessments revealed significant antioxidant activity, demonstrating the extracts' potential in mitigating oxidative stress-related diseases and promoting cellular health. The formulation of Carbopol-based hydrogels incorporating these extracts resulted in optimal homogeneity, consistency, extrudability, and spreadability. Additionally, agar plate diffusion assays validated the effective release of bioactive compounds from the hydrogels, showcasing substantial antibacterial activity against *S. pyogenes*, *E. coli*, and *P. aeruginosa*. These findings position *D. trifasciata* extracts as promising candidates for topical therapeutic applications in cosmetic science.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Table 1: Composition for hydrogel formulation with *D. trifasciata*

INGREDIENTS	F1	F2	F3	CONTROL
<i>D.trifasciata</i> Ethanolic extract	-	0.1 g	0.05 g	-
<i>D.trifasciata</i> methanolic extract	0.1 g	-	0.05 g	-
Carbopol 940	0.15 g	0.15 g	0.15 g	0.15 g
Propylparaben	0.02 g	0.02 g	0.02 g	0.02 g
Methylparaben	0.02 g	0.02 g	0.02 g	0.02 g
Glycerine	0.8 ml	0.8 ml	0.8 ml	0.8 ml
Oleic acid	0.5 ml	0.5 ml	0.5 ml	0.5 ml
Sterile H ₂ O (Make upto)	10 ml	10 ml	10 ml	10 ml

Table 2: Phytochemical investigation of *D. trifasciata* leaf extracts

S. No	Phytochemical analyzed	Ethanolic extract	Methanolic extract	S. N	Phytochemical analyzed	Ethanolic extract	Methanolic extract
1.	Polyphenols	+	+	7.	Steroids	+	+
2.	Tanins	+	-	8.	Anthraquinone	+	-
3.	Flavonoids	+	+	9.	Glycosides	+	+
4.	Saponins	+	+	10.	Reducing sugars	+	+
5.	Alkaloids	+	+	11.	Amino acids	+	+
6.	Triterpenoids	-	+				

“+” indicates presence of the compound

“-” indicates absence of the compound

Table 3: Organoleptic characterization of novel topical gel formulation

Parameters	Observation			
	F1	F2	F3	Control
Appearance	Opaque	Opaque	Opaque	Opaque
Color	Pale green to white	Pale green to white	Pale green to white	White
Homogeneity	Homogenous	Homogenous	Homogenous	Homogenous
Consistency	Good	Good	Good	Good
Phase separation	No	No	No	No
Washability	Washable	Washable	Washable	Washable
Odor	No	No	No	No
Texture	Smooth	Smooth	Smooth	Smooth
Grittiness	No	No	No	No

Table 4: Physical stability examination of the developed formulations

Day/C ondition	40 ±2°C					30 ±2°C					25 ±2°C				
	F1														
	PA	CL	PS	HM	UN	PA	CL	PS	HM	UN	PA	CL	PS	HM	UN
0	Translucent	Y/NC	No	Yes	Yes	Translucent	Y/NC	No	Yes	Yes	Translucent	Y/NC	No	Yes	Yes
7	Translucent	Y/NC	No	Yes	Yes	Translucent	Y/NC	No	Yes	Yes	Translucent	Y/NC	No	Yes	Yes
14	Translucent	Y/NC	No	Yes	Yes	Translucent	Y/NC	No	Yes	Yes	Translucent	Y/NC	No	Yes	Yes
21	Translucent	Y/NC	No	Yes	Yes	Translucent	Y/NC	No	Yes	Yes	Translucent	Y/NC	No	Yes	Yes
28	Translucent	Y/NC	No	Yes	Yes	Translucent	Y/NC	No	Yes	Yes	Translucent	Y/NC	No	Yes	Yes
	F2														
	PA	CL	PS	HM	UN	PA	CL	PS	HM	UN	PA	CL	PS	HM	UN
0	Translucent	Y/NC	No	Yes	Yes	Translucent	Y/NC	No	Yes	Yes	Translucent	Y/NC	No	Yes	Yes
7	Translucent	Y/NC	No	Yes	Yes	Translucent	Y/NC	No	Yes	Yes	Translucent	Y/NC	No	Yes	Yes
14	Translucent	Y/NC	No	Yes	Yes	Translucent	Y/NC	No	Yes	Yes	Translucent	Y/NC	No	Yes	Yes
21	Translucent	Y/NC	No	Yes	Yes	Translucent	Y/NC	No	Yes	Yes	Translucent	Y/NC	No	Yes	Yes
28	Translucent	Y/NC	No	Yes	Yes	Translucent	Y/NC	No	Yes	Yes	Translucent	Y/NC	No	Yes	Yes
	F3														
	PA	CL	PS	HM	UN	PA	CL	PS	HM	UN	PA	CL	PS	HM	UN
0	Translucent	Y/NC	No	Yes	Yes	Translucent	Y/NC	No	Yes	Yes	Translucent	Y/NC	No	Yes	Yes
7	Translucent	Y/NC	No	Yes	Yes	Translucent	Y/NC	No	Yes	Yes	Translucent	Y/NC	No	Yes	Yes
14	Translucent	Y/NC	No	Yes	Yes	Translucent	Y/NC	No	Yes	Yes	Translucent	Y/NC	No	Yes	Yes
21	Translucent	Y/NC	No	Yes	Yes	Translucent	Y/NC	No	Yes	Yes	Translucent	Y/NC	No	Yes	Yes
28	Translucent	Y/NC	No	Yes	Yes	Translucent	Y/NC	No	Yes	Yes	Translucent	Y/NC	No	Yes	Yes
	Control														
	PA	CL	PS	HM	UN	PA	CL	PS	HM	UN	PA	CL	PS	HM	UN
0	Translucent	W/NC	No	Yes	Yes	Translucent	W/NC	No	Yes	Yes	Translucent	W/NC	No	Yes	Yes
7	Translucent	W/NC	No	Yes	Yes	Translucent	W/NC	No	Yes	Yes	Translucent	W/NC	No	Yes	Yes
14	Translucent	B/C	No	Yes	Yes	Translucent	W/NC	No	Yes	Yes	Translucent	W/NC	No	Yes	Yes
21	Translucent	B/C	No	Yes	Yes	Translucent	B/C	No	Yes	Yes	Translucent	W/NC	No	Yes	Yes
28	Translucent	B/C	No	Yes	Yes	Translucent	B/C	No	Yes	Yes	Translucent	B/C	No	Yes	Yes

*PA-Physical appearance, CL–Color, PS-Phase separation, HM-Homogeneity, UN-Uniformity

**Y/NC-Yellow and No change in color, W/NC-White and change in color, B/C-Brown and change in color

Table 5: Zone of inhibition of the developed formulations

S. No	Formulation	<i>S. pyogenes</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>
		Zone of inhibition(mm)			
1.	F1	2	3	1	15
2.	F2	1	5	13	17
3.	F3	12	4	10	20
4.	Control	1	1	NA	NA

NA-No zone formation was observed

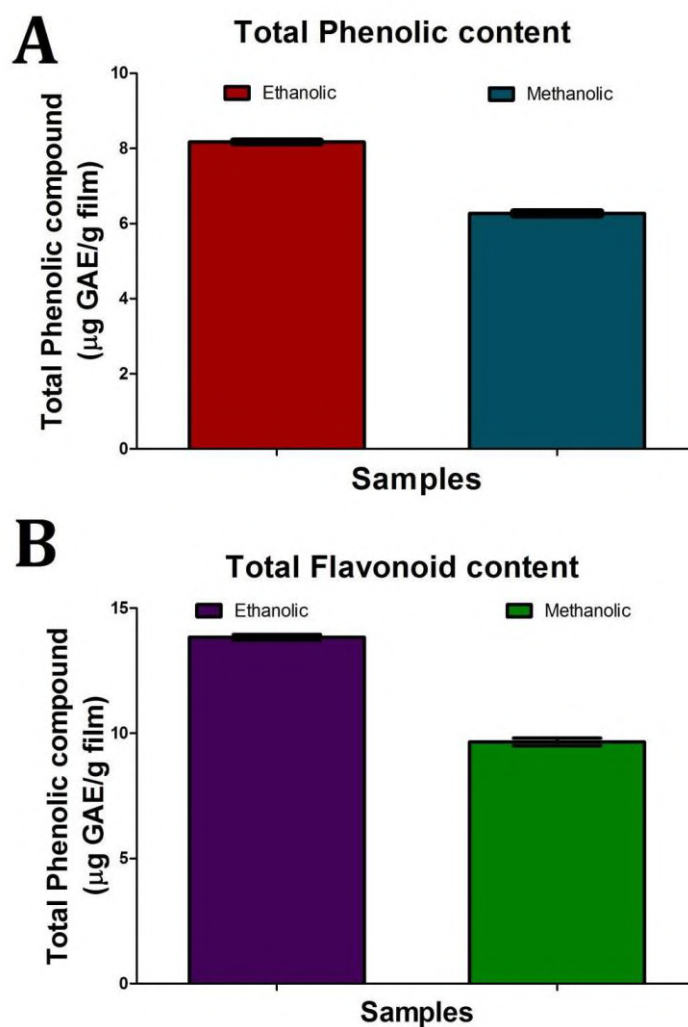


Fig 1: Estimation of antioxidant content in *D. trifasciata* leaf extracts. A. Estimation of total phenolic content in ethanolic and methanolic extracts of *D. trifasciata* leaves. **B.** Estimation of total flavonoid content in ethanolic and methanolic extracts of *D. trifasciata* leaves.

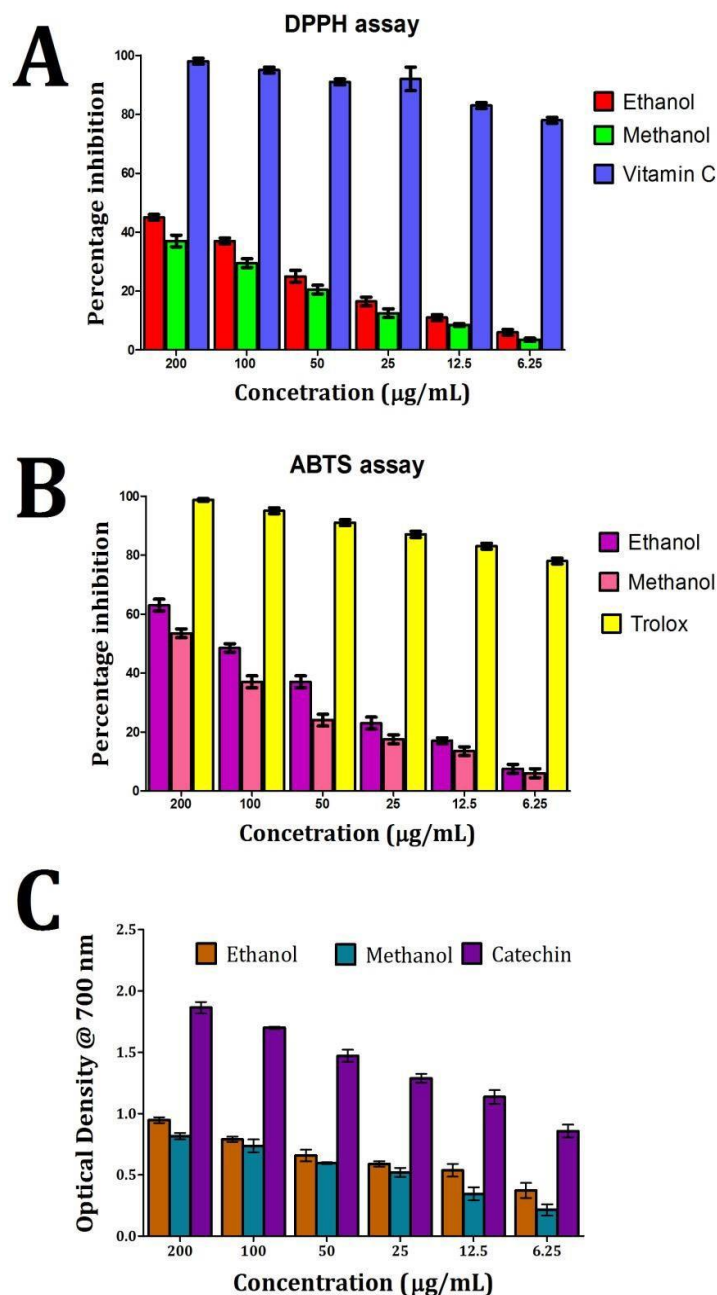


Fig 2: Assessment of antioxidant activity in *D. trifasciata* leaf extracts. **A.** Evaluation of DPPH radical scavenging activity in *D. trifasciata* extracts, **B.** Analysis of ABTS radical cation scavenging activity in *D. trifasciata* extracts and **C.** Estimation of ferric reducing power of *D. trifasciata* leaf extracts.

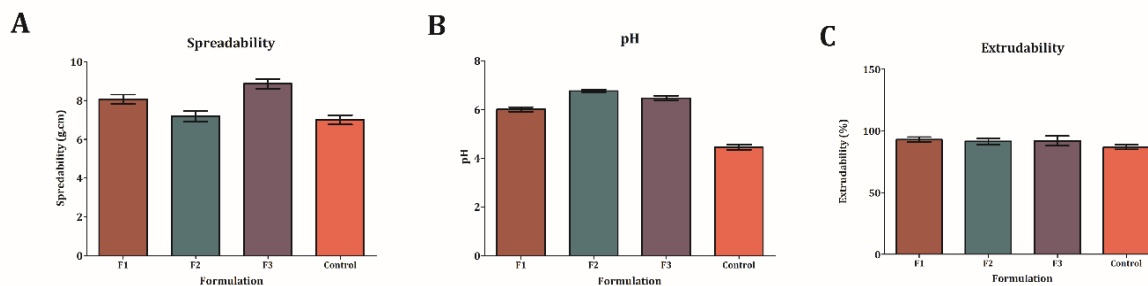


Fig 3: Characterization of hydrogels formulated with *D. trifasciata* leaf extracts. A. Assessment of spreadability of the hydrogel formulation, **B.** pH analysis of the hydrogel formulation and **C.** Evaluation of extrudability of the hydrogel formulation.

Effect of food safety knowledge among the skilled workers of food industries processing minor fruits or wild edible plants in Manipur towards food safety

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ABSTRACT

A survey work was conducted in three districts of Manipur namely Imphal East, Imphal West and Bishnupur in order to study the effect of food safety knowledge among the skilled workers of food industries, processing minor fruits or wild edible plants, towards food safety. Through this study, a total of six food industries were selected, purposive sampling were used for selecting state, district and industries. However, for selecting the respondents (100 nos.) random sampling were used. The personal variables were treated as independent variables and variables pertaining to knowledge were treated as dependent variables. The majority of skilled workers in the food industries of Manipur were found to be unaware of food safety practices. The main objectives of the study were to examine the respondents personal and socio-demographic profiles in order to assess their level of knowledge regarding food safety. From the research, it is revealed that 57% of the skilled workers had not visited any training institute and only formal training attended by them. The data pertaining to respondents visiting to training institution showed significant difference in knowledge level. ANOVA table revealed that residential area and caste of the respondents has significant differences in respondents' knowledge level. Knowledge on product safety and sanitation basic concept regarding food safety was observed in our study while respondents have less knowledge about Hazard Analysis and Critical Control Points.

Keywords: Food handlers, food safety, HACCP, knowledge, respondent

INTRODUCTION

Food provides us with energy and makes it possible for us to live a decent life, but it can also cause health hazards that could cause illness or even death. Foods are an important source for achieving livelihood security (Rajasri, 2024). Food safety pertains

how to handle, prepare and store food. Evaluating and enhancing the knowledge of food handlers on food safety has become a necessity and it is becoming a major factor in ensuring strict adherence to food safety standards. Every nation's foundation and most significant industry is the food sector

(Sadiku *et al.*, 2019). Any person who is directly involved in the production, preparation, or packaging of food commercially on food premises is referred to as a skilled food worker. Few studies have been conducted regarding the food safety knowledge among the skilled workers in food industries of Manipur. Skilled workers of food industries of Manipur were mostly unaware about the knowledge towards of food safety. An article study in Manipur by Devi and Somokanta (2016) and concluded that woman workers have less knowledge and least education so they are assigned with minimal tasks in food industries. Their study also found that male workers received managerial training as compare to females' workers. Evaluating the range of knowledge level among food industry workers can assist in identifying and comprehending the gaps in knowledge. Therefore, the current study is intended to find out the effect of knowledge about food safety among skilled workers in food industries processing minor fruits or wild edible plants of Manipur (India). The main objectives of the study were to examine the respondents personal and socio-demographic profiles and order to assess their level of knowledge regarding food safety.

MATERIALS AND METHODS

The present study was conducted from 2020 to 2022 in the Department of Pomology and Postharvest Technology, Faculty of Horticulture, Uttar Banga Krishi Vishwavidyalaya, Cooch Behar, West Bengal. However, the survey work was carried out in Manipur (India).

Research Design: Ex-post facto research is characterised as a systematic empirical investigation in which the independent variables are not directly under the researchers control since their manifestation has already occurred and they are not fundamentally manipulable. The present study was conducted in Manipur in three different districts namely Imphal East, Imphal West and Bishnupur which were

selected as per the availability of food industries. The food industries which are selected were based upon the processing of minor fruits or wild edible plants available in the state of Manipur. Thanjam Agri Industry Pvt. Ltd., Taret foods Pvt. Ltd., Meira Foods Pvt. Ltd. were the food industries selected from Imphal East district. However, only one food industry i.e. Kangleipak Spice industry was selected from Imphal West district. Similarly, from Bishnupur district, only one food industry was selected namely Kangla Foods Products Pvt. Ltd. Since each food industry vary in the number of workers a greater number of respondents were from Thanjam Agri Industry Pvt. Ltd. which is twenty-five respondents each and twenty respondents from Taret foods Pvt. Ltd. However, a smaller number of respondents were selected from the remaining industries i.e. ten each from Meira Foods Pvt. Ltd., Kangleipak Spice industry and Kangla Foods Products Pvt. Ltd. Purposive sampling was used to select state, district as well as industry. Before the data collection, a pilot study was conducted in the selected area where personal interview was taken place. A pilot study is a small-scale exploratory study carried out before any large-scale quantitative research in order to assess the possibility for a future, full-scale project. Three districts were selected purposively for pilot study. Thus, a total of 100 numbers of respondents were selected randomly for the study.

Variables and their empirical measurements

Independent variables are the variables whose values don't depend on change in other variables and there are no other factors that influence independent variables. Variable that are unaffected by the other factors in this research are gender, marital status, religion, caste, family structure, residential area, level of education, use of internet to solve problems, visit to training institute. Total of 9 independent variables were selected for the present study. In case of Gender was coded as 1 for male, 2 for

female; Marital status was assigned scores based on categories such as 1 for married, 2 for unmarried and 3 for widowed and Religion which were categorized as Hindu (1), Muslims (2), Christian (3) and others (4) for the study. Level of education was recorded for every respondent and then classified into four categories namely illiterate, upto 10th standard, 12th standard and graduate. Score assigned are 0, 1, 2 and 3 respectively. Family structure/type was classified as nuclear and joint; visit to training institute was assigned as yes and no options and taking help from internet categorized as yes and no and score of 1 and 2 were assigned. Different caste enlisted for the present study are General, OBC, SC and ST respectively with score of 1, 2, 3 and 4.

The dependent variables, which are the respondents' knowledge of food safety, were assessed using a 23-questions survey designed to evaluate product safety, sanitation and HACCP knowledge. Three possible options for answering were provided in knowledge (correct, incorrect, don't know/can't remember). The responses to the questions were scored as follows: 2 points for a correct answer, 1 point for don't know/can't remember and 0 point for an incorrect answer.

Data collection: The study was conducted on the basis of survey method through scheduled questionnaire. The questionnaires used in the study were self-administered, standardized with slight modification, after thorough consultation with experts in this field. Knowledge of food safety in food industries product safety and sanitization and Hazard Analysis Critical Control Point references were from Jubayerer *et al.*, (2020). Information was collected from respondents using an interview scheduled designed to gather data on food safety, product safety, sanitization, and Hazard Analysis Critical Control Point practices.

Statistical analysis: The data from the study were processed using Microsoft Excel and analyzed with the support of SPSS statistical

software. The statistical methods include percentage, using t-test and ANOVA were checked, including tests for normality and homogeneity of variances, where applicable as per the characteristics of data.

RESULTS AND DISCUSSION

Personal and socio-demographic profile of the respondent in our study revealed that (as shown in Table 1), most of the industrial workers were female (82%), followed by male (18%) belonging to Hindu (81%) community followed by Christian (12%). These workers were mostly unmarried (67%) and a few were married (33%) while half of the respondents belong to general category (52%) followed by OBC (38%), SC (5%) and ST (6%). Workers from nuclear family were 52% and the rest *i.e.*, 48% from joint family. However, the distribution of these industrial workers regarding to their residential area revealed that 38% were from village area followed by town (31%) and city (31%). These results were supported by Abdul-Mutalib *et al.*, (2012); Alemayehu *et al.*, (2020) and Madhwal and Sharma (2019). Additionally, Ariyawansha *et. al.* (2024) from their study, also they concluded that female workers were 86% and male were 14%.

Based on their education level, the results show that the majority of industrial workers have studied up to the 10th standard (48%), followed by 28% who are graduates, 22% who have completed the 12th standard, and 2% who are illiterate. The findings were in close proximity with Madhwal and Sharma (2019) who reported that 46.7 % were graduates, 36% of the respondents were post graduates and 17.3% of the respondents covered upto 12th standard. The distribution of respondents according to use of internet for solving their professional problems revealed that 67% of the respondent seeks the help of internet for seeking their professional problem while 33% of them doesn't use internet for any problem related to the profession. This concluded that more clearance of their doubt was by using

internet. Exposure of the respondents to various training programme were evaluated which revealed that 43% of the industry workers have visited training institute and 57% of them have not visit any training institute. This showed that respondents are more or less aware about the importance of training and the skills acquired through it. According to Malavi *et al.*(2021) their finding concluded that food safety training is an appropriate means for enhancing the knowledge and hygienic practices of food handlers.

Table 2 presents the t-test results for two qualitative categories of parameters in terms of knowledge. The data provide evidence that significant differences exist in the marital status of respondents, which correlates with their knowledge of food safety. Hence, the status of the respondents either married or unmarried has direct impact on the knowledge on food safety. On the other hand, there was also significant difference in visiting to training institute or by attending various training programme and this will enhance the knowledge level among the respondents in food safety. However, using internet to seek help regarding the profession/problem was found to be negatively significant which justified that using internet to solve the professional problem may not be the scientific approach to solve the problem as internet only gives a superficial idea hence the exact problem might have missed out. Apart from the parameters discussed above, other parameters showed non-significant differences among the variables.

Table 3 showed two categories qualitative parameters ANOVA in terms of knowledge which depict that residential area of the respondents has significant differences at 5% among the respondents. Similar results were observed in the respondents caste where a significant difference at 5% were found among the respondents belonging to different caste and also for where do they belong. However, no significant differences were found in between knowledge of the

respondent towards food safety with level of education and religion they belong. The variables that depend on others in the study were categorized as knowledge level of the respondents which differs and depends on the respective respondents.

In aspects of the product safety and sanitization (Table 4), most responses were encountered in the questionnaire leaking package causes harm to the food product shelf life and occupied 1st rank scoring 197. The second highest-ranked response (score: 192) was related to the need to inspect all raw materials immediately upon arrival at the industry before storage. The above observations suggested that respondents are aware of shelf life and safety of raw materials in regards of product storage. For this most respondents answered correct option. Other statements that are due to poor temperature control bacteria can grow on stored product also scored 192 and was in 2ndrank. However, the last rank was obtained by questionnaire necessary temperature and relative humidity must be maintained at different production areas which scored 162 marks followed by the questionnaire traffic light labelling in colour codes indicate whether the amount recommended daily intake in the serving was low (Green), medium (Yellow) and high (Red) recorded 135 marks which secured last second position. The above least rank results indicated that respondents are having modest knowledge in view of food safety which is a drawback criterion in food industries. The current finding were in confirmation with Worsfold (1993) that employee education has been found to enhance sanitary awareness and knowledge of food safety issues, as well as perhaps enhance food safety procedures. Workers in most industries have been held liable for some occurrences of food safety issues like food borne diseases and illnesses due to improper management and handling of food (Greig *et al.*, 2007 and Ansari-Lari *et al.*, 2010). Improper management and handling of food is one of the main causes of food safety

issues. Another study by Ko (2013) suggested that crucial strategy for regulating microbial development in foods by temperature management. In the food sector, there is a strong need for digitization and automated operation of several industrial operations (Hassoun *et al.*, 2023).

Among the HACCP-related knowledge questions in Table 5, the highest-ranked statement was that cleaning and disinfection are part of a prerequisite program. This was correctly acknowledged by 89 respondents, with a total score of 188. Among the remaining respondents, 10 answered 'Don't know' or 'Can't remember' and one responded incorrect. The above obtained results represented that respondents have good knowledge in terms of prerequisite programs in food industries. Another statements *i.e.*, falsifying records was a criminal offense rank 2nd which was responded by 87 respondents scoring 187 in which no one has given an incorrect choice. Metal detection just before the packaging was a CCP (Critical Control Point) which scored 133 marks responded least by the respondents placing itself in the second last position. Second-last ranked statement was 'HACCP must be implemented in the plant to ensure food safety,' which scored 134 points. Respondents in food industries in Manipur have less knowledge in terms of Critical Control Points and HACCP which is an important step in preventing hazard in food industry. According to a study by Jubayer *et al.* (2020) in food industry, staff members had a thorough comprehension of food manufacturing process, employees were aware of the physical risks, the requirement for calibration, maintaining checklists, record keeping, cleanliness, and disinfection as a preparatory programme and some of the respondents were unaware that falsifying records is a crime and the HACCP system is poorly understood by the workers which was in line with our study. An assertion on cleaning disinfection as a prerequisite programme and remedial action in the work area of the study seem to be understood by

all respondents. *Ocimum* species essential oil may be used in food industries to successfully manage phytopathogenic fungus (Guragai *et al.*, 2022). According to Walker *et al.* (2003) poor temperature comprehension may be a significant barrier to efficient HACCP implementation because temperature treatment is typically the crucial control point in a production process would be a justification with our findings where we have emphasized that HACCP must be implemented implant to ensure food safety. Additionally, industrial food safety training prioritizes hygiene over generic food safety knowledge as stated by Worsfold and Griffith, 2003. Another study by Mortimore and Wallace (2013) reported that falsifying record is a criminal offense is known to most of the respondents which supported our study as 87 respondents agreed. To ensure correct food handling and to set rules for food hygiene and safety, food safety culture is necessary as stated by de Andrade *et al.* (2020) and Nyarugwe *et al.* (2018). Jubayer *et al.*, (2020) pointed out that the respondents were satisfactory when dealt with product safety and hygiene. In our present study we also emphasize that the management should be strongly advised to provide ethical training for their staff regarding CCP and HACCP.

CONCLUSIONS

Food safety knowledge among skilled workers in the food industry is essential, as they are the ones ultimately handling products from raw materials to their final form. Thus, the personal and socio-demographic condition of the skilled workers of food industries will definitely have a direct impact on their knowledge and food safety. Most of the workers were female so male should be encouraged to work in food industry. These workers mainly belong to Hindu community under the general category of approximately 50% from nuclear family. The personal and socio-demographic factors of respondents had a significant impact on their food safety knowledge, as evidenced by their frequent use of the internet to address

work-related issues. Attending training institutes or participating in more training programs further enhanced their understanding of food safety. Thus, working experience of respondents could be increased and visiting to training institute could improve their knowledge on food safety. Knowledge of the respondents needs to be highlighted more regarding CCP of food products which can be acquired attending various training programme. However, quality standard before dispatch, storage of food, temperature monitoring, traffic light labelling in colour codes and implementation of HACCP was less in their knowledge of respondents regarding food safety. So, the managing team can conduct training and awareness program regarding food safety and the respondents themselves can learn from online source also.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Table 1: Personal and socio-demographic profile of the skilled workers

Sl.No.	Statements	Parameters	Percentage (%)
1.	Gender	Male	18
		Female	82
2.	Religion	Hindu	81
		Muslim	0
		Christian	7
		Others	12
3.	Marital status	Married	33
		Unmarried	67
		Widowed	0
4.	Caste	General	51
		OBC	38
		SC	5
		ST	6
5.	Family structure	Nuclear	52
		Joint	48
6.	Residential area	Village	38
		Town	31
		City	31
7.	Level of education	Illiterate	2
		Upto10 th	48
		12 th	22
		Graduate	28
8.	Help from Internet	Yes	67
		No	33
9.	Visit to any training institute	Yes	43
		No	57

Table 2: t-test for two categories qualitative parameters on knowledge of the skilled workers

Variables	Dependent (Variable knowledge)	Variables	Mean	t-value	Probability
Gender	Knowledge	Male	76.500	-1.953	0.054
		Female	80.148		
Marital status	Knowledge	Married	82.545	3.038*	0.003
		Unmarried	78.044		
Family Structure	Knowledge	Nuclear	80.470	1.407	0.163
		Joint	78.404		
Visit to training Institute	Knowledge	Yes	81.186	2.015*	0.047
		No	78.280		
Use of internet	Knowledge	Yes	78.209	-2.677*	0.009
		No	82.212		

*Significance Level: $p < 0.05$ indicates a statistically significant difference (2-tailed test)

Table 3: ANOVA for more than two categories qualitative parameters on knowledge of the skilled workers

Variables	Knowledge			
	Mean	SD Error	F value	Probability
Level of education				
Illiterate	77.500	5.132	0.910	0.439
<10 th	79.375	1.048		
12 th	81.591	1.547		
Graduate	78.321	1.372		
Residential area				
Village	76.289 ^b	1.108	7.231	0.001*
Town	80.806 ^a	1.227		
City	82.226 ^a	1.227		
Caste				
General	80.039 ^a	0.907	3.955	0.010*
OBC	80.789 ^a	1.168		
SC	73.400 ^b	2.675		
ST	72.333 ^b	3.946		
Religion				
Hindu	79.962	0.774	2.02	0.138
Christian	74.285	3.455		
Others	79.666	2.133		

Significance Level: $p < 0.05$ indicates a statistically significant difference (2-tailed test)

Table 4: Distribution of skilled workers towards product safety and sanitation in food industries (Total number of respondents=100)

Sl. No .	Questions	Correct (2)	Incorrect (0)	Don't know/remember (1)	Total Weighted Score	Weighted Mean Score	Weighted Mean Rank
1.	Storing conditions of food can have possible effects on human health	90	1	9	189	1.89	III
2.	It was required to clean the food thermometers at a regular interval	86	0	14	186	1.86	V
3.	Environment pollution condition can affect the product	83	0	17	183	1.83	VII
4.	Necessary temperature and relative humidity must be maintained at different production areas	62	0	38	162	1.62	IX
5.	Raw materials should not be stored with finished goods	88	0	12	188	1.88	IV
6.	Leaking package causes harm to the food product shelf life	97	0	3	197	1.97	I
7.	When a shipment of raw materials arrives, it was required to inspect all right away before storing	93	1	6	192	1.92	II
8.	Before and after production, cleaning of processing area and equipment was a must to ensure food safety	85	0	15	185	1.85	VI
9.	Traffic light labelling in colour codes indicate whether the amount recommended daily intake in the serving was low (Green), medium (Yellow) and high (Red)	43	8	49	135	1.35	X
10.	Use Class II preservatives have maximum permissible limits of their use as per law	77	4	19	173	1.73	VIII
11.	Due to poor temperature control bacteria can grow on stored product	92	0	8	192	1.92	II

Table 5: Respondents Knowledge of HACCP (Hazard Analysis and Critical Control Points) Related to Product Safety and Sanitation in the Food Industry (Total number of respondents=100)

Sl. No.	Questions	Correct (2)	Incorrect Responses (0)	Don't know/ Can't Remember (1)	Total Score (Weighted)	Weighted Mean	Rank based on Weighted Mean
1.	Dirt, broken glass and staples from packaging are classified as physical hazards	90	6	4	184	1.84	III
2.	Metal detection just before the packaging was a CCP (Critical Control Point)	34	1	65	133	1.33	XI
3.	All checklists at every point of production floor must be updated at 30 min interval	74	2	24	172	1.72	VIII
4.	Falsifying records was a criminal offense	87	0	13	187	1.87	II
5.	The first line of defence was the workers in processing facility	84	6	10	178	1.78	VI
6.	Cleaning and disinfection was a type of pre-requisite program	89	1	10	188	1.88	I
7.	Calibration of equipment such as balances, measuring instruments, temperature meters etc./was compulsory in due time	75	1	24	174	1.74	VII
8.	Document the deviation was a step in the corrective action process	76	5	19	171	1.71	IX
9.	It was necessary to construct flowchart of individual processes and display it in relevant area	83	2	15	181	1.81	IV
10.	During record keeping, you should always sign and date as necessary	82	2	16	180	1.8	V
11.	Critical control point prevent the occurrence of food hazard	75	3	22	172	1.72	VIII
12.	HACCP must be implemented in plant to ensure food safety	40	6	54	134	1.34	X

Effect of sowing time on growth, yield and nutritional properties of cabbage Microgreens grown in soilless culture

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ABSTRACT

An experiment was conducted to assess the impact of sowing time (January, March, May, July, September and October) on different parameters of cabbage microgreens grown under artificial light and soilless media. The cabbage seeds were sown in HDPE tray consist of a combination of cocopeat, perlite and vermiculite in 3:1:1 ratio. Significant variation was noted for growth (seedling height, hypocotyl length, root length, cotyledon area, fresh weight and dry weight), yield and biochemical (moisture, chlorophyll a, chlorophyll b, total chlorophyll, total phenol, ascorbic acid, beta carotene, flavonoid, antioxidant capacity, acidity, total sugar and reducing sugar) parameters. Peak growth was noted during May and March. However, the highest yield (1325.899 g/m²) was observed during January. Seed sown during November, September and January observed the maximum total chlorophyll content i.e. 0.955, 0.884 and 0.851 mg/g microgreen FW respectively. Biochemical parameters such as total phenol, ascorbic acid, DPPH Assay were recorded highest in May followed by March. Total sugar content was seen in greater amount during September, March and May than the rest months. The results revealed that growing of cabbage microgreens during summer months (March and May) performed better with respect to nutrition. But to achieve good production January is the best time for sowing.

Key words: Ascorbic acid, cabbage, DPPH, Microgreens, phenol, soilless, yield,

INTRODUCTION

Microgreens are celebrated for their brilliant colour, delicate texture and high nutrient density. These are considered an essential component of health-conscious diets worldwide. These young greens are collected at cotyledon stage with the presence or absence of true leaves which have been considered to possess the higher amount of nutrients (vitamins, minerals and antioxidants) than their mature ones. They are considered as superfood due to the presence of several bioactive compounds, secondary metabolites

and nutrients (Pratap *et al.*, 2023). The unique combination of vibrant flavours, appealing aesthetics and high nutritional content has significantly increased consumer interest in microgreens.

Planting time is one of the considerable factors which influence the growth, yield and nutrient composition. Several climatic factors such as temperature, humidity, light availability etc. affect the growth and productivity of the microgreens (Samuoliene *et al.*, 2019; Dubey *et al.*, 2024; Abaajeh *et al.*, 2023). The

growing of microgreens has been practiced in indoors as well as in controlled environment as they have short growing cycle (Budavari *et al.*, 2024). The practice of growing indoors offers control of the environment. The peak nutritional development of microgreens is achieved at specific stage which is influenced by planting time as well as harvesting time (Ortiz *et al.*, 2024, Yanez Medelo *et al.*, 2025).

Brassicaceae microgreens have been considered as an excellent source of various pigments, anthocyanin, flavonoid, vitamins, phenolic acid, isothiocyanates and glucosinolates (Marchioni *et al.*, 2021; Dereje *et al.*, 2023). The presence of these phytochemicals has different biological activities which help to fight against severe human diseases. They act as anti-inflammatory, anti-diabetics, anti-cancer and antioxidant (Dereje *et al.*, 2023). Presence of more complex polyphenols is observed in microgreens than their counter part in *Brassica* crops (Sun *et al.*, 2013). This provides a gateway to explore cabbage, a *Brassica* crop, as microgreen.

The rapid increase of urbanization and shortage of agricultural land demands a challenge to feed the population. Microgreens being compact growth and short growing period are suitable for urban farming. This offers a sustainable way to provide high value crop with low minimal input. Given the increasing demand for microgreens and their potential to address urban farming challenges, it is crucial to understand the influence of sowing time on their growth, yield and nutritional quality. Therefore, the present study aims to evaluate the effect of sowing time on these parameters in cabbage microgreens.

MATERIALS AND METHODS

The study was carried out in Horticulture Farm, Sriniketan. The room consists of several racks which contains shelves with a dimension

of 97 cm x 34 cm. To provide light, LEDs were used with a light intensity of 1900 lux \pm in each shelf. To grow microgreens, HDPE trays of 1.5 ft x 2 ft having 5cm depth with drainage facilities were used. These trays were filled with soilless media which is a combination of three media *i.e.*, cocopeat: perlite: vermiculite in 3:1:1 ratio. To assess the performance of microgreens, the seeds of cabbage were sown manually in six different months (first week) *i.e.* January, March, May, July, September and November. Complete randomized design was used with three replications. After sowing, the seeds were watered and covered with another tray for etiolation which enhances germination. After germination, the covered trays were removed and the germinated seeds were watered by the feel method depending upon the requirement. Microgreens were harvested when the two cotyledon leaves have fully emerged with a true leaf. Some morphological parameters (seedling height, hypocotyl length and cotyledon area) were collected before harvesting and some were noted after harvesting (root length, fresh weight of 10 seedlings and yield). After harvesting the microgreens were assessed for biochemical parameters. For proximate analysis moisture content (%) and dry weight (g/10 microgreens) were taken into consideration. Pigments such as Chlorophyll a, Chlorophyll b and total chlorophyll were assessed by adopting the method described by Hiscox and Israelstam (1979) using Dimethyl sulfoxide with the following formula.

$$\text{Chlorophyll a} = \frac{[12.7(D_{663}) - 2.69(D_{645})] \times 1000 \times W}{V}$$

$$\text{Chlorophyll b} = \frac{[22.9(D_{645}) - 4.68(D_{663})] \times 1000 \times W}{V}$$

$$\text{Total chlorophyll} = \text{Chlorophyll a} + \text{Chlorophyll b}$$

Method described by Deb *et al.* (2024) was used to work out the total phenol content using

Folin-Ciocalteu reagent and measuring absorbance at 760 nm using UV-VIS Spectrophotometer. Standard curve was plotted and value was expressed as mg GAE/ g FW (GAE represent Gallic acid Equivalent & FW represent Fresh weight). Ascorbic acid was determined by the method described by AOAC (1990) using titration by metaphosphoric acid. Beta-carotene was worked out as per the method of Davis (1976). The absorbance was noted at 452 nm using UV-VIS spectrophotometer and calibration curve was prepared to calculate the concentration. Total flavonoid was estimated using the method described by Zhishen *et al.* (1999). Aluminium chloride and potassium acetate was used to extract sample. The absorbance was noted using UV-VIS spectrophotometer at 415 nm and expressed as mg QE/g FW (QE represent Quercetin). DPPH Assay was determined to calculate the antioxidant capacity of the microgreens using the method described by Brand-Williams *et al.* (1995). For this 2,2-diphenyl 1-picrylhydrazyl was used and absorbance was noted at 517 nm using UV-VIS Spectrophotometer. Titratable acidity was calculated using the method described by Sadasivam and Manickam (1996). It was expressed as %.

Total Sugar was estimated spectrophotometrically using Phenol-Sulphuric Method (Thimmaiah; 2021). Here, the extract was treated with 5% Phenol and 98% Sulfuric acid and kept in dark. The absorbance was noted at 485 nm and result was expressed as mg glucose/g FW. Reducing sugar was estimated using Dinitrosalicylic (DNS) Acid Method (Thimmaiah; 2021). Aliquot of the sample was allowed to mix with 3 ml DNS reagent. Then was allowed to stand for some time and absorbance was noted at 575 nm in UV-VIS spectrophotometer. Graph was plotted to estimate the amount of reducing sugar and expressed as mg glucose/g FW.

Statistical analysis was carried out using IBM SPSS Software v.25. The mean

value have been presented in the table and graph. The difference in mean value for different traits among the treatments has been assessed through the Duncan Multiple Range Test.

RESULTS AND DISCUSSION

The result of different morphological parameters (seedling height, hypocotyl length, root length, cotyledon area, fresh weight of 10 seedlings & yield) has been displayed in Table 1 which shows significant variation. Microgreens grown during March, May and September exhibited significantly higher seedling height. Mean hypocotyl length of 4.68 cm was noted. Root length ranged from 3.310 to 3.594 cm. The maximum cotyledon area (1.474 cm²) was noted in May sowing and was statistically comparable to that of March, July and September sowing. Significant differences were noted for the biomass accumulation. Peak fresh weight of cabbage microgreens was recorded for May sowing. Mean yield of 1297.324 g/m² was obtained. The maximum yield obtained was 1328.651 g/m² for September sowing, which was statistically at par (*p* value>0.05) with January, March, November and May. Variation in biometric parameters of microgreens were influenced by several environmental factors such as temperature, humidity etc. (Dubey *et al.*, 2024).

Figure 1 & Figure 2 illustrates the proximate analysis (moisture content and dry weight respectively) across sowing months. Statistically significant differences were noted among the treatments. Mean moisture content of 94.0 % was recorded. May sowing result the highest moisture content i.e. 95.480 %. Mean dry weight of 0.05 g was observed.

Pigment content of cabbage microgreens have been illustrated in Figure 3. The results showed significant differences in chlorophyll content (chlorophyll a, chlorophyll b and total

chlorophyll). A good amount of total chlorophyll was noted for sowing during September, November and January as compared to the other sowing time.

Statistically significant differences were noted for the different biochemical parameters under this study which has been shown in Table 2. Sowing of cabbage microgreens during May showed the maximum presence of total phenol (305.507 mg GAE/100 g FW) and ascorbic acid (87.825 mg/100 g FW) as compared to the other planting time; whereas the least were observed during January sowing. Beta carotene content was observed maximum in March followed by May sowing. Sowing of cabbage microgreens during May and March observed the presence of higher amount of flavonoid as compared to other sowing time. Similar range of flavonoid content was found in cabbage microgreens under soilless media (Gunjal *et al.*, 2024). Flavonoid content has been related to various therapeutic treatments, like the anticancer action, antioxidant activity, helps in stroke prevention, antiviral activities, antibacterial action, etc. (Ullah *et al.*, 2020). The highest antioxidant capacity was observed in May sowing i.e. 4.052 $\mu\text{mol TE/g FW}$. Antioxidant property has been associated with the presence of vitamin C, phenolic compound, chlorophyll and carotenoid in plants (Podsdek *et al.*, 2006 and Singh *et al.*, 2006). Titratable acidity ranged from 0.291-0.365%. Maximum total sugar content was found in September sowing (11.985 mg glucose/g FW), which was at par with March, May and July sowing (at 95% CI).

CONCLUSION

The findings from this present study revealed that proper sowing time coordinates with the favourable environmental conditions not only contribute to growth but also economic viability of microgreen production. From the present research, it was found that the cabbage microgreens sown in March and May resulted

in good growth and presence of nutritional composition; whereas pigment content was found maximum during November and January. The maximum yield was achieved during September. Microgreens being delicate in nature have to be grown in specific time to harness the maximum benefit. The findings of this study will help producers to grow according to their requirement based on yield and nutritional importance and feed the fresh, nutrient dense cabbage microgreens to consumer.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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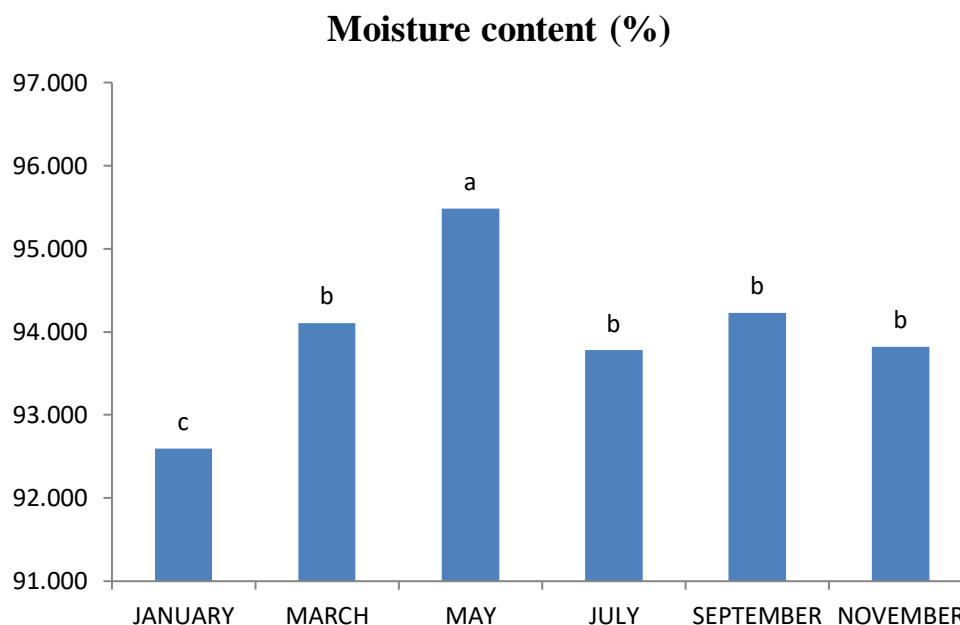
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Table 1: Vegetative parameters of cabbage microgreens across different sowing time

Treatment	Seedling height (cm)	Hypocotyl length (cm)	Root length (cm)	Cotyledon area (cm ²)	Fresh weight of 10 seedlings (g)	Yield (g/m ²)
January	5.322 ^b	4.488 ^c	3.310 ^c	1.274 ^{bc}	0.824 ^b	1325.899 ^a
March	5.634 ^a	4.909 ^a	3.586 ^a	1.435 ^{ab}	0.937 ^b	1297.324 ^a
May	5.676 ^a	4.974 ^a	3.594 ^a	1.474 ^a	1.123 ^a	1237.580 ^a
July	5.405 ^b	4.704 ^b	3.492 ^{ab}	1.399 ^{abc}	0.879 ^b	1114.121 ^b
September	5.630 ^a	4.590 ^{bc}	3.585 ^a	1.396 ^{abc}	0.896 ^b	1328.651 ^a
November	5.323 ^b	4.438 ^c	3.377 ^{bc}	1.227 ^c	0.863 ^b	1260.568 ^a
Grand mean	5.50	4.68	3.49	1.37	0.92	1260.69
SE(m)±	0.05	0.06	0.05	0.05	0.05	39.73
CD(0.05)	0.14	0.18	0.16	0.16	0.15	122.41
CV(%)	1.43	2.12	2.58	6.75	9.13	5.46

Note: letters within the treatment indicates the difference derived through Duncan Multiple Range Test.

**Figure 1: Moisture content (%) of cabbage microgreens across different sowing time times**

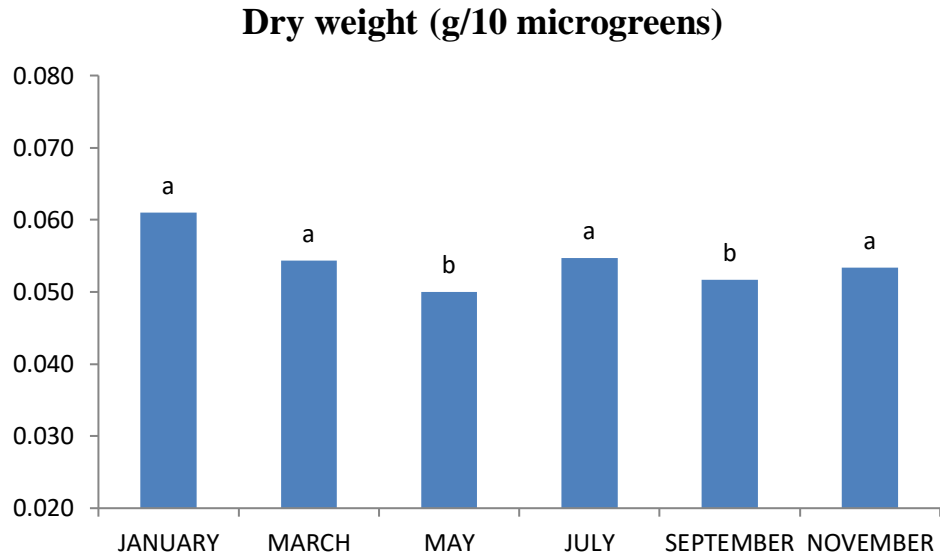


Figure 2: Dry weight (g/10 microgreens) of cabbage microgreens across different sowing time

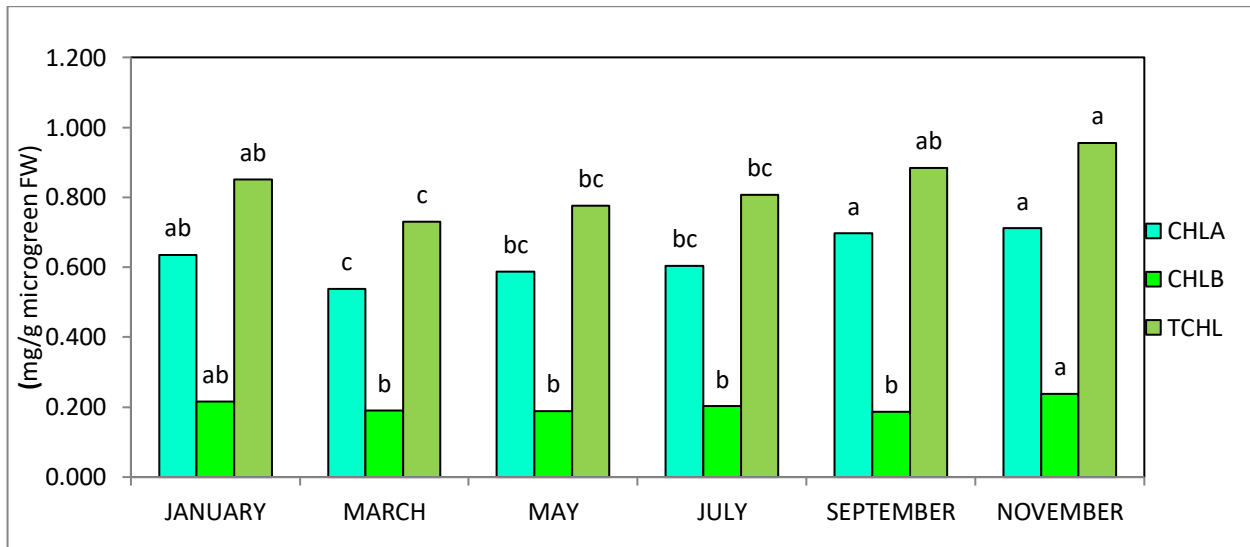


Figure 3: Pigment content of cabbage microgreens across different sowing time

Note: CHL A- Chlorophyll a, CHL B-Chlorophyll b, TCHL-Total Chlorophyll, Letters indicate the difference among the treatments

Table 2. Biochemical parameters of cabbage microgreens across different sowing time

Treatment	Total Phenol (mg GAE/100 g FW)	Ascorbic acid (mg/100 g FW)	Beta carotene (mg/100 g FW)	Flavonoid (mg QE/g FW)	DPPH (μmol TE/g FW)	Acidity (%)	Total sugar (mg glucose/g FW)	Reducing sugar (mg glucose/g FW)
January	228.531 ^c	72.360 ^c	3.945 ^c	0.512 ^c	3.229 ^c	0.308 ^{bc}	9.213 ^c	3.505 ^c
March	283.407 ^b	79.977 ^b	4.437 ^a	0.765 ^a	3.779 ^b	0.352 ^{ab}	11.620 ^a	4.866 ^a
May	305.507 ^a	87.825 ^a	4.210 ^b	0.807 ^a	4.052 ^a	0.365 ^a	11.133 ^a	4.991 ^a
July	268.284 ^b	81.513 ^b	3.968 ^c	0.639 ^b	3.715 ^b	0.345 ^{ab}	10.896 ^{ab}	4.377 ^b
September	278.247 ^b	81.560 ^b	3.837 ^c	0.754 ^a	3.396 ^c	0.341 ^{ab}	11.985 ^a	4.759 ^{ab}
November	240.447 ^c	73.641 ^c	3.830 ^c	0.461 ^c	3.251 ^c	0.291 ^c	9.900 ^{bc}	3.735 ^c
Grand mean	267.40	79.48	4.04	0.66	3.57	0.33	10.79	4.37
SE(m) \pm	4.94	1.16	0.07	0.02	0.05	0.02	0.38	0.14
CD(0.05)	15.23	3.57	0.22	0.07	0.16	0.05	1.18	0.42
CV(%)	3.20	2.52	3.02	5.79	2.57	7.85	6.14	5.36

Note: Letters within the treatment indicates the difference derived through Duncan Multiple Range Test, FW-Fresh weight, GAE-gallic acid equivalent, QE-Quercetin, TE-Toluene

Uttar Sugandhi: High yield potential aromatic rice developed through pure line selection

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ABSTRACT

Uttar Sugandhi was developed through pure line selection from Kalonunia. The variety was released by state variety release committee for cultivation in West Bengal. It showed 16.15% yield advantage over Kalonunia in West Bengal, and it had 96.98% and 112.09% yield advantage against national check (Badshabhog) and regional check (Kalanamak) respectively in Maharashtra. It also showed 52.86% yield advantage over regional check (Kalanamak) in Odisha. Apart from yield advantage, it bears many desirable grain characters: the milling, hulling and head rice recovery are 81.85%, 70.8% and 67.0% respectively; it has medium slender kernel, 22.25% amylose content, 63.50 mm gel consistency and 14.83 g test weight. It has strong grain aroma. DNA finger printing was done along with the check varieties to characterize the variety at molecular level.

Keywords: Aromatic rice, high yield potential, medium slender, Uttar Sugandhi,

INTRODUCTION

In terms of worldwide food grain production, rice (*Oryza sativa* L.) is ranked second only to wheat as a key food crop and it is farmed everywhere. The USDA predicts that worldwide rice production will reach 513.5 million tonnes in 2023-2024, still the greatest amount ever. West Bengal is known as the 'bowl of rice', containing around 450 rice landraces (Deb, 2005; Chatterjee *et al.*, 2008). Rice is grown here on more than 65 percent of the agricultural land (Adhikari *et al.*, 2012) in three separate seasons: Aus (autumn rice), Aman (winter rice), and Boro (summer rice). West Bengal's rice landraces and cultivars are

known for their distinct scent, taste and resistance to disease.

Indian aromatic rice in general and 'Basmati' in particular has dominated the domestic and international market for aroma. More than 100 compounds have been identified (Buttery *et al.*, 1988; Grimm *et al.*, 2001) for aroma of rice. Among those, 2-acetyl-1 pyrroline (Buttery *et al.*, 1983; Niu *et al.*, 2008) is the principal aroma compound in all the aromatic rice cultivars. Aromatic rice is an important commodity worldwide and command premium price over non-scented varieties. The Indian subcontinent has the *Natural Gift* of basmati and much other non-

Basmati aromatic rice. In addition to Basmati, many local landraces are grown traditionally, which excel in aroma, grain quality and cooking quality. Most of these genotypes are having short bold to medium bold grain. However, most of the non-Basmati aromatic rice has poor yield potential. Exploration of collected germplasm can be used to identify new sources of genes that can be used to breed improved varieties (Atta *et al.*, 2023). With the objective of yield increment of the non-Basmati aromatic rice, pure line selection was followed to develop Uttar Sugandhi.

MATERIALS AND METHODS

Material

Uttar Sugandhi IET 24616) was developed through pure line selection from Kalonunia. Kalonunia is a popular aromatic landrace of Cooch Behar, Jalpaiguri, Alipurduar districts and northern part of Uttar Dinajpur district of West Bengal. Kalonunia has been registered under Geographical Indication, bearing the Registration No: 743. It is highly photoperiod-sensitive, tall, lodging susceptible and long duration, and grown during *Kharif* season. Kalonunia has low yield potential ranging from 1.5 to 2.0 t/ha.

Uttar Sugandhi is also highly photoperiod-sensitive, long duration and lodging susceptible. However, it has high yield potential as compared to Kalonunia and distinct from Kalonunia by some DUS characters. Uttar Sugandhi has been assigned national identity number IC 633329 by National Bureau of Plant Genetics Resources, New Delhi.

Yield trials

Initially for two consecutive *Kharif* (May to November) seasons (2012 and 2013), the variety was tested at University Research Farm, Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar, West Bengal. On satisfactory performance in the Station Yield Trials, the variety was sent for testing under

All India Coordinated Rice Improvement Project. It was tested for two consecutive *Kharif* seasons under Initial Varietal Trial-Aromatic Short Grain (IVT-ASG, 2014) and Advanced Varietal Trial-1-Aromatic Short Grain (AVT-1-ASG, 2015) with three checks, namely Badshabhog (National Check), Kalanamak (Regional Check) and Kalonunia (Local Check). Based on the better performance in two years of trials in All India Coordinated Rice Improvement Project (AICRIP), the variety was further tested under multi-location trial in West Bengal. The variety was also tested under adaptive trial in the farmers' field.

Grain yield and yield attributing data were obtained from AICRP trials (AICRIP, 2014 & 2015). Trials were also conducted in two locations of northern part of West Bengal, namely Regional Research Sub Station (Terai Zone), UBKV, Kharibari, Darjeeling district and University Research Farm, UBKV, Pundibari, Cooch Behar district.

DNA finger printing

DNA was isolated using standard CTAB method from rice leaves (Xu *et al.*, 2005). Thirty-two SSR markers were used for the DNA finger printing of KNS-2-1 and KNS-3-1 (Uttar Sugandhi) with standard varieties, namely Gobindabhog and Kalonunia. This study was conducted at Department of Biochemistry, Uttar Banga Krishi Viswavidyalaya.

RESULTS AND DISCUSSION

Performance of Uttar Sugandhi under AICRIP trials

The results (Table 1) revealed that the variety, Uttar Sugandhi (IET 24616) recorded higher grain yield of 2215 and 4147 kg/ha in West Bengal and Odisha respectively accounting to about 16.15% increase in yield over the local check variety Kalonunia (1907 kg/ha) in West Bengal and 52.86% increase in yield over regional check variety Kalanamak (2713

kg/ha) under the Coordinated Trial, IVT-ASG conducted during *Kharif*, 2014 (AICRIP, 2014). The variety also recorded higher grain yield of 4507 and 2952 kg/ha in Maharashtra and Uttar Pradesh respectively accounting to about 96.98 and 12.70% increase in yield over the national check variety Badshabhog (2288 and 2619 kg/ha respectively). However, Uttar Sugandhi showed mean grain yield of 3455.25 kg/ha while yield estimation was done over four locations in the Coordinated Trial, IVT-ASG conducted during *Kharif*, 2014 (AICRIP, 2014).

Performance of Uttar Sugandhi under yield trials in West Bengal

The yield trial was also conducted in two different locations in northern part of West Bengal. The results (Table 2) revealed that Uttar Sugandhi recorded maximum yield at Kharibari (5083 kg/ha). Its mean grain yield over two locations was 4492 kg/ha accounting to about 52.57% more over local check variety Kalonunia (1907.00 kg/ha) under trials conducted in northern part of West Bengal during *Kharif*, 2018.

Yield Attributing Characters

It could be revealed through Table 3 that Uttar Sugandhi showed plant height of 141.5 cm, days to 50% flowering of 120.5 days and number of panicle per m² of 256.5 while average was done over two years. All these characters of Uttar Sugandhi were comparable to that of Badshabhog.

Quality Parameters

The milling, hulling and head rice recovery of Uttar Sugandhi were recorded as 81.85%, 70.8% and 67.0% (Table 4) respectively, while average was done over two years. Based on kernel length, breadth and L:B ratio, Uttar Sugandhi showed its grains as medium slender. Chalkiness of the Uttar Sugandhi was absent, alkali spreading value was 5.0, amylose content was 22.25%, gel consistency

was 63.50 mm and test weight was 14.83 g. Amylose content is an important rice grain quality parameter in respect of consumer preference. In India, consumers prefer medium amylose content (20- 25%) in the endosperm and this is an important parameter for promotion of rice entries in All India Rice Improvement Project (Anonymous, 2017; Roy et al., 2020; Roy et al., 2021). Starch content (amylose) of rice is very important factors in grain yield, processing and palatability. Uttar Sugandhi possesses strong aroma.

Some Distinctiveness, Uniformity and Satiability (DUS) Characters of Uttar Sugandhi

The DUS characterization was done following the guidelines as outlined by PPV&FRA (2007) for rice. The detail of the DUS characterization of Uttar Sugandhi has been presented in Table 5. The main desirable grain features of this variety are medium slender (Fig. 1D&F), strong aromatic and having medium range of amylose (22.50%). Grain awns are small and distributed only in the upper part of the panicle (Fig. 1C&E). It possesses some undesirable features also plants are tall (116 cm excluding panicle; Fig. 1A&B), lodging susceptible and highly photoperiod sensitive. However, this variety has remarkable higher yield potentiality as compared to Kalonunia.

DNA fingerprinting

Total 32 SSR markers were used for the DNA fingerprinting of KNS-2-1 and KNS-3-1 with standard varieties, namely *Gobindabhog* and *Kalonunia* (Table 6; Fig. 2). Out of which, 12 markers (37.5%) were found polymorphic and 20 markers were monomorphic (62.5%). Out of 12 polymorphic markers, 5 markers (RM114, RM1, RM6250, RM165 and RM294) showed size based allelic polymorphism; whereas, remaining 7 markers (RM16655, RM10022, RM23, RM288, RM172, RM159, RM38) showed

polymorphism based on presence and absence of alleles.

CONCLUSION

Uttar Sugandhiis aromatic rice developed through pure line selection from Kalonunia. It had been released by state variety release committee for cultivation in West Bengal. It is highly photoperiod-sensitive and can be cultivated only during *Khari* season. It had 16.15% yield advantage over Kalonunia in West Bengal, and it had 96.98% and 112.09% yield advantage against national check (Badshahbhog) and regional check (Kalanamak) respectively, in Maharashtra. It also showed 52.86% yield advantage over regional check (Kalanamak) in Odisha. Apart from yield advantage, it bears many desirable grain characters: the milling, hulling and head rice recovery is 81.85%, 70.8% and 67.0%, respectively; it has medium slender kernel, 22.25% amylose content. It has strong grain aroma. DNA finger printing was done along with the check varieties to characterize the variety at molecular level.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Table 1: Performance of Uttar Sugandhi (IET 24616) in respect of grain yield under the AICRIP trials (IVT-ASG) conducted during *Kharif*, 2014

The Test Entry of Rice and Check Varieties	West Bengal		Maharashtra		Uttar Pradesh		Odisha		Mean grain yield over four locations (kg/ha)
	Yield (kg/ha)	% increase in yield in IET 24616 over checks	Yield (kg/ha)	% increase in yield in IET 24616 over checks	Yield (kg/ha)	% increase in yield in IET 24616 over checks	Yield (kg/ha)	% increase in yield in IET 24616 over checks	
<i>Uttar Sugandhi</i>	2215	-	4507	-	2952	-	4147	-	3455.25
<i>Badshabhog</i> (NC)	2205	0.45	2288	96.98	2619	12.70	4561	-	-
<i>Kalanamak</i> (RC)	2158	2.64	2125	112.09	2944	0.27	2713	52.86	-
<i>Kalonunia</i>	1907	16.15	-	-	-	-	-	-	-

NC: National Check, RC: Regional check

Table 2: Performance of Uttar Sugandhi in respect of its grain yield in West Bengal during *Kharif*, 2018

Rice Genotypes	Yield (Kg/ha)		Mean yield	Yield advantage over Kalonunia
	RRSS, Kharibari	RRS, Pundibari		
UBKVR-111	6750	5934	6342	-
UBKVR-124	7250	6220	6735	-
UBKVRA-1	3833	3096	3465	-
Uttar Sugandhi	5083	3901	4492	52.57
Nabin (LC)	-	4368	4368	-
Kalonunia (LC)	-	1907	1907	-

Source: Annual Report- Institutional Rice Project, 2018-19, Directorate of Research, UBKV, Pundibari, Cooch Behar 736165, West Bengal. p. 6.

Table 3: Plant height, Days to 50% flowering & No. of Panicles/m² of the test entry, Uttar Sugandhi under Coordinated Varietal Trials (*Kharif*, 2014 & 2015)

Characters	<i>Kharif</i> , 2014		<i>Kharif</i> , 2015		Mean of Uttar Sugandhi
	Uttar Sugandhi	Badshabhog	Uttar Sugandhi	Badshabhog	
Plant height (cm)	149	150	134	135	141.5
Days to 50% flowering	118	115	123	114	120.5
No. of Panicles/m ²	277	261	236	254	256.5

Table 4 Quality characteristics of the proposed entry, Uttar Sugandhi in Coordinated Varietal Trials (IVT-ASG Kharif, 2014 and AVT-ASG Kharif, 2015).

Characters	IET 24616	Shohini (NC)	Badshabhog	CR Sugandhdhan907	Dubraj (QC)
Hulling (%)	81.85		78.25	78.3	78.1
Milling (%)	70.8		69.1	66.9	63.5
Head Rice Recovery (%)	67.0		65.1	62.6	56.0
Kernel Length (mm)	5.04		3.77	5.26	5.27
Kernel Breadth(mm)	1.87		1.94	1.97	2.23
Length and Breadth Ratio	2.69		1.94	2.67	2.36
Grain type	MS	SS	SB	MS	SB
Grain Chalkiness	A	VOC	VOC	A	VOC
Alkali Spreading Value	5.0		4.5	4.0	4.0
Amylose Content (%)	22.25		20.87	25.75	26.04
Gel Consistency (mm)	63.5		51	23	43
Aroma	Strong	Mild	Strong	Mild Scent	Mild
Test weight	14.83 g	-	-	-	-

SB: Short bold; **MS:** Medium slender; **SS:** Short Slender; **VOC:** Very Occasionally Chalkiness; **A:** Absent; **NC:** National Check; **QC:** Quality check

Table 5: Some morphological and qualitative characters of test entry, Uttar Sugandhi based on the “Guidelines for the Conduct of Test for Distinctiveness, Uniformity and Stability (DUS) on Rice (*Oryza sativa* L.)” of PPV & FRA (2007)

Sl. No.	Characters	Classification
1.	Coleoptiles: Colour	Colourless
2.	Basal leaf: sheath colour	Green
3.	Leaf: Intensity of green colour	Medium
4.	Leaf: Anthocyanin colouration	Absent
5.	Leaf sheath: anthocyanin colouration	Absent
6.	Leaf: Pubescence of blade surface	Strong
7.	Leaf: Auricle	Present
8.	Leaf: Anthocyanin colouration of auricle	Absent
9.	Leaf: Collar	Present
10.	Leaf: Anthocyanin colouration of collar	Absent
11.	Leaf: Ligule	Present
12.	Leaf: Shape of ligule	Split
13.	Leaf: Colour of ligule	White
14.	Culm: attitude	Semi-erect
15.	Time of heading (days to 50% flowering)	Late (120 days, when sown in third week of June)
16.	Days to Maturity*	Late (150 days, when sown in third week of June)
17.	Flag leaf: Attitude of blade (early observation)	Drooping
18.	Male sterility	Absent
19.	Lemma: Anthocyanin colouration of keel	Absent

20.	Lemma: Anthocyanin colouration of area below apex	Weak
21.	Lemma: Anthocyanin colouration of apex	Medium
22.	Spikelet: Colour of stigma	White
23.	Stem: Length (excluding panicle length)	Medium (116 cm)
24.	Stem: Thickness	Medium (0.5 cm)
25.	Stem: Anthocyanin colouration of nodes	Absent
26.	Stem: Anthocyanin colouration of internodes	Absent
27.	Panicle: Length of main axis	Medium (25 cm)
28.	Panicle: Curvature of main axis	Deflex
29.	Panicle: Number per plant	Medium (17/panicle)
30.	Spikelet: Colour of tip of lemma	Purple
31.	Lemma and Palea: Colour	Purple spots/furrows on straw
32.	Panicle: Awns	Present
33.	Panicle: Exertion	Well exerted
34.	Sterile lemma: Colour	Strew
35.	Decorticated grain: Colour	White
36.	Decorticated grain: Length	Medium (5.04 cm)
37.	Decorticated grain: Width	Medium(1.87 cm)
38.	Decorticated grain: Shape	Medium slender
39.	Endosperm: content of amylose	Medium (22.50%)
40.	Decorticated grain: Aroma	Present (Strong)



Fig. 1: Some important characters of Uttar Sugandhi. A) Field view; B) Panicle; C) undehusked grains; D) Dehusked grains; E) Undehusked grains length and breadth on graph paper; F) Dehusked grains length and breadth on graph paper.

Table 6: DNA fingerprinting pattern and amplifications of bands/alleles of proposed variety, KNS-3-1 (Uttar Sugandhi) and KNS-2-1 with standard varieties *Gobindabhog* and *Kalonunia*

Sl. No.	Primers	Expected size (bp)	Observed alleles	Varieties			
				<i>Gobindabhog</i>	<i>Kalonunia</i>	KNS-2-1	<i>Uttar Sugandhi</i> (KNS-3-1)
1.	RM114-a	200	2	0	1	1	1
	RM114-b			1	1	0	0
2.	RM108	80	1	1	1	1	1
3.	RM16655	200	1	0	1	1	1
4.	RM10022	200	1	0	1	1	1
5.	RM23	150	1	1	0	1	0
6.	RM327	200	1	1	1	1	1
7.	RM3872	150	1	0	0	0	0
8.	RM288	170	1	0	0	1	1
9.	RM1-a	100-120	2	0	1	0	0
	RM1-b			0	0	1	1
10.	RM256	65	0	0	0	0	0
11.	RM6250-a	180	2	1	0	1	0
	RM6250-b			0	1	0	0
12.	RM172	180	1	0	0	1	1
13.	RM159	150	1	1	1	0	1
14.	RM165-a	200-300	4	1	1	0	0
	RM165-b			1	1	1	1
	RM165-c			1	1	1	1
	RM165-d			1	1	1	1
15.	RM250	200	1	1	1	1	1
16.	RM23835	200	1	1	1	1	1
17.	RM321	200	1	1	1	1	1
18.	RM314	170	1	1	1	1	1
19.	RM291	200	1	1	1	1	1
20.	RM342	180	1	1	1	1	1
21.	RM7376	200	1	1	1	1	1
22.	RM460	300	1	1	1	1	1
23.	RM434	185	1	1	1	1	1
24.	RM3134	185	1	1	1	1	1
25.	RM332	180	1	1	1	1	1
26.	RM469	85	1	1	1	1	1
27.	RM285	150-200	0	0	0	0	0
28.	RM169	200	1	1	1	1	1
29.	RM195	300	1	1	1	1	1
30.	RM311	300	0	0	0	0	0
31.	RM38	900	1	1	1	0	1
32.	RM294-a	180-200	3	1	1	1	1
	RM294-b			0	1	0	0
	RM294-c			1	1	1	1

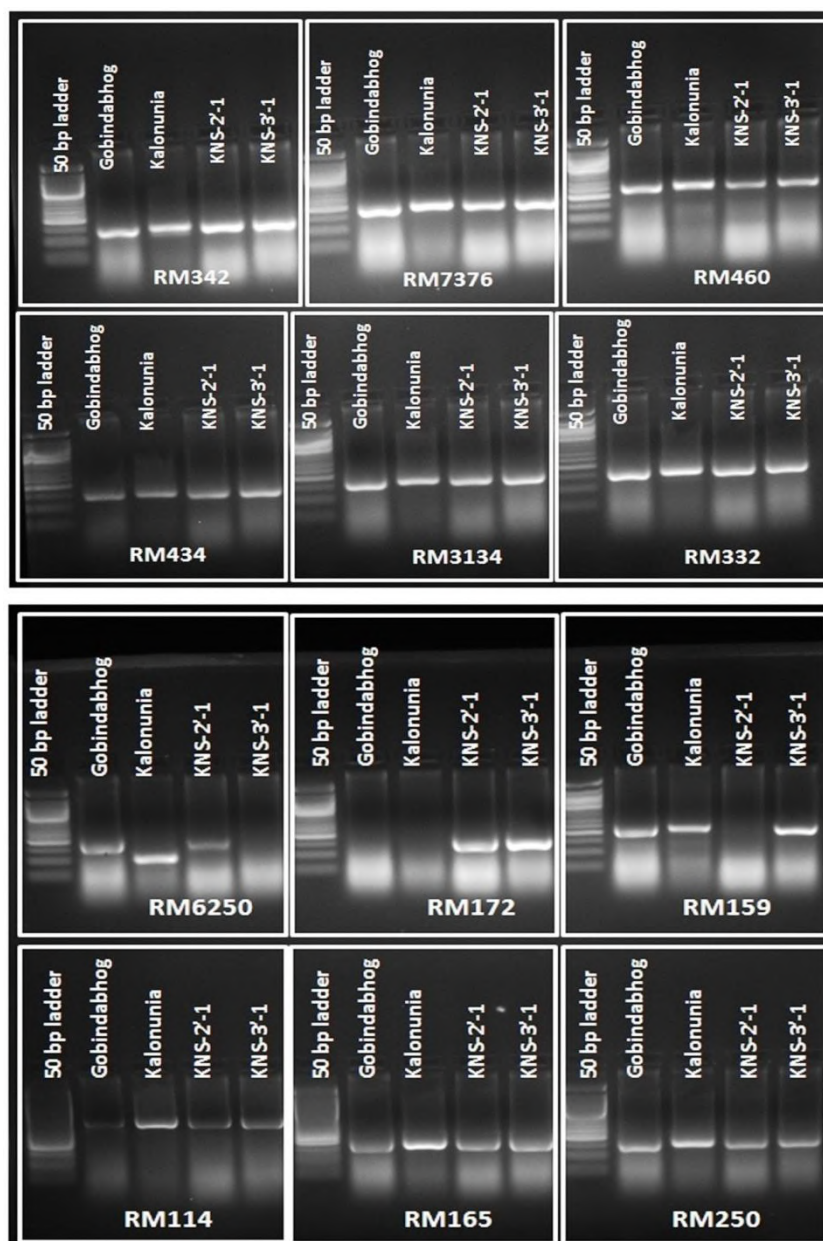


Fig. 2: DNA profile of *Gobindabhog*, *Kalonunia*, KNS-2-1 and KNS-3-1 (Uttar Sugandhi) with primers RM 342, RM 7376, RM 460, RM 434, RM 3134, RM 332, RM 6250, RM 172, RM 159, RM 114, RM 165 and RM 250; L= 50 bp ladder.

Influence of anthropogenic and natural factors on the enzymatic activity of soils pistachio and walnut forests of Kyrgyzstan

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ABSTRACT

The article presents the results of a study of the influence of anthropogenic and natural factors on the enzymatic activity of soils pistachio and walnut forests. The situation of production and immobilization of the enzymatic pool of microbiological activity of soils under various conditions of economic use of typical sierozem soils of the Fergana Range and the valley of the same name is a genetic diagnostic indicator of fertility and is used in planning agrotechnological measures to increase soil fertility. Among the soils studied, the enzymatic activity of mountain-forest black-brown soils is very high. This is related to the soil organic matter content because forest litter plays a key role as a precursor for the synthesis and stabilization of enzymes. The enzyme activity indicators presented in the article can be used as diagnostic indicators of soil fertility in this territory.

Key words: walnut-fruit forest, pistachio forest, sierozem soils, mountain-forest black-brown soils, humus, enzymes

INTRODUCTION

In Kyrgyzstan, walnut-fruit forest and pistachio fields are mainly found in the Jalal-Abad region. The pistachio tree is one of the oldest fruit-bearing plants in human history. It reaches up to 10 meters in height and can live up to 400 years. Pistachios contain a large number of useful substances, which has a beneficial effect on human health (Bolotov *et al.*, 2005). Due to its exceptional drought resistance and unpretentiousness to unfavorable soil conditions, pistachio is of great importance for forest reclamation – the development of currently treeless, eroded to varying degrees, low-productivity rained lands (Hemery, 1998).

Our walnut-fruit forests have enormous ecological and landscape (water protection, water regulation, soil protection),

commercial (fruits, berries, medicinal plants, valuable timber, etc.), economic (sales of environmentally friendly nuts, fruits, berries, honey, medicinal herbs) and recreational (ecotourism, sanatoriums, resorts) significance. Walnut-fruit forests are distinguished by the uniqueness of the components of the biosphere, where on tens of thousands of hectares grow the most valuable species of trees and shrubs, medicinal and honey plants, forage herbaceous plants and the soils are represented by highly fertile black-brown soils. In addition, the mountain forest black-brown soils of the walnut-fruit forests serve as a fertility standard for the soils of Central Asia in terms of fertility indicators (Karabaev *et al.*, 2022, Gryza *et al.*, 2008).

Anthropogenic and natural factors of soil formation have a significant impact on microbiological activity, and especially on the work of soil enzymes. Indeed, enzymes are actively involved in the biological processes of transformation of soil organic matter, on which the direction and intensity of synthesis, the release of nutrients, the breakdown of humus, as well as the hydrolysis of organic compounds and the redox regime depend. Nutrients, as a result of enzymatic processes, are transferred from difficult-to-digest compounds into easily accessible forms for microorganisms and plants, i.e. the higher the activity of the enzyme pool, the better the supply of living organisms.

To identify various types of anthropogenic load on the soils of the agroecosystem of the vertical zonality of the mountains, different types of economic impact were studied. The object of our study is to investigate the influence of anthropogenic and natural factors on the enzymatic activity of soils in pistachio and walnut forests.

MATERIALS AND METHODS

The study was conducted in the pistachio forestry of the Suzak district (elevation -853 m., latitude -40°54'58.41"N, longitude - 72°56'15.16"E) and the Kara-Alma walnut-fruit forest (elevation – 1801m., latitude -41°12'54.66"N, longitude - 73°23'00.05"E) of the Jalal-Abad region of Kyrgyzstan. The research work was carried out in 2019-2023 at the Jalal-Abad State University named after B. Osmonov.

To study the fertility of pistachio and walnut-fruit forests, soil samples were taken from genetic horizons. At the same time, soil sections were described based on the morphological characteristics of the genetic horizons of the soil profile. Laboratory analyzes were done according to generally accepted methods adopted in the Kyrgyz Republic, in the Republican laboratory of soil-agrochemical station of Kyrgyzstan (Arinushkina, 1963). The enzymatic activity of soils was determined in the laboratory of the University of Texas, USA, by Tabatabai method (1994).

RESULTS AND DISCUSSION

As can be seen from Table 1, natural plant communities and agrocenoses of irrigated agriculture are a powerful factor in the life of microorganisms, incl. enzymatic activity. The biological characteristics of plants in pastures, pistachio woodlands and in irrigated agriculture leave a certain imprint on the activity of glucosidase enzymes. Plants create and form microbial cenoses and enzymes, influencing the microbial population of the root system and crop residues.

Natural plant biocenoses activate the soil enzyme pool, with the highest β -glucosidase activity observed in the 0-2 cm soil layer of pistachio woodlands (809.5 mg p-nitrophenol kg⁻¹ soil h⁻¹), and then sharply decreases in the lower soil horizons (Table 1). This means that in the soils of pistachio woodlands, enzymatic activity is concentrated in the thin upper (0-2 cm) layer of soil, i.e. the bulk of *Streptomyces* microorganisms are located here and a rhizosphere is formed in close proximity to the daytime soil surface, favorable for the development of enzymatic activity. With such indicators, the probability of replenishing soil humus reserves with products of cellulose decomposition is high, where in the process of mineralization there is a synthesis of specific organic compounds - humus, specific mineral compounds - clay minerals, as well as the release of simple inorganic compounds (Sakbaeva *et al.*, 2013). As a result of such processes, the upper horizon of sierozem soils of pistachio open forests acquires erosion resistance, and here, as a result of enzymatic processes, nutrients are transferred from difficult-to-digest compounds into easily accessible forms for microorganisms and plants. These processes form the basis of soil fertility and lead to the redistribution of chemical elements in the landscape we study.

On pasture lands of sierozem soils, β -glucosidase in the A₀ horizon is 428.7 mg p-nitrophenol kg⁻¹ soil hour⁻¹, (almost two times lower than in pistachio open forest), and in the A₁ layer β -glucosaminidase is 43.3 mg p-nitrophenol kg⁻¹ soil hour⁻¹. In contrast to the soils of pistachio woodlands in layer A₁ (2-13

cm), there is no sharp decrease in β - glucosidase (209.3 mg p-nitrophenol kg⁻¹ soil hour⁻¹) and β - glucosaminidase (34.5 mg p-nitrophenol kg⁻¹ soil hour⁻¹). As can be seen in natural phytocenoses of pastures, enzymatic activity is observed in the 0-13 cm layer of soil, where the largest mass of the plant root system is concentrated. The release of extracellular enzymes in roots during the metabolic process, manifesting a rhizosphere effect on soil microflora, activates their vital activity with root exudates (Sakbaeva *et al.*, 2021).

Here, for comparison, we can give the activity content of glucosidase enzymes in the mountain-forest black-brown soils of the walnut-fruit forests of the Kara-Alma basin of the Kok-Art river. As can be seen from Table 1, high enzymatic activity of the soil is noted for β -glucosidase and ranges from 11.1 to 1235.9 mg p-nitrophenol kg⁻¹ soil h⁻¹ in mountain-forest black-brown soil. Of the glucosidase enzymes, β -glucosidase is dominant over β -glucosaminidase. In general, soil enzymatic activity decreases with increasing depth. The decrease in enzyme activity with depth can be explained by a decrease in the biological activity of soils down the profile. A decrease in enzyme activity and microbial biomass with soil depth was noted in studies by Acosta-Martinez *et al.* (2007), Kizilkaya and DengIz, (2010). The enzymatic activity of soil depends on many natural conditions of soil formation, such as physical, chemical properties, geomorphological conditions of distribution of the studied soils, as well as the influence of anthropogenic factors (Mirkin and Naumova, 2015). Anthropogenic factors have different effects on the enzymatic activity of soil.

On irrigated arable lands of sierozem soils, there are serious changes in the enzymatic activity of soils. Here, in the arable horizon of the arable land, where the roots are concentrated and the soil is subject to anthropogenic influence (mechanical treatment, fertilizer, watering, etc.), the greatest enzymatic activity is observed. However, the intensity of enzymatic processes in arable soils of agroecosystems is significantly lower than in soils of natural landscapes. On the arable horizon of irrigated

typical sierozem soil, where cotton is grown) contains 69.8 mg p-nitrophenol kg⁻¹ soil h⁻¹ β -glucosidase and 11.9 mg p-nitrophenol kg⁻¹ soil h⁻¹ β -glucosaminidase. Here, due to the low content of soil organic matter (spread of the root system of the agroecosis, accumulation of humus and plant residues), immobilizers of humic substances enzymes, weak supply of enzymes with organic matter of plant origin and small secretions of plants and microflora, low glucosidase activity is observed on irrigated arable lands of sierozem soils.

Differences in the enzymatic activity of the studied soils of anthropogenic and natural ecosystems cover the entire soil profile. The high intensity of biological processes in natural soils is characteristic mainly of the upper root-saturated layer of the soil profile, and in natural biogeocenoses enzymatic activity is not distributed to significant depths than in arable soils. If on irrigated arable lands an additional impulse is given for the supply of phytomass (green manure, cultivation of alfalfa, leaving a lot of plant residues by-products), then better conditions are created for increasing the enzymatic activity of arable land. This means that the entire range of agricultural technology for agricultural crops should be aimed at increasing the supply of plant residues and creating optimal air, water, nutrient and thermal regimes for arable land.

Table 2 shows the activity of phosphatase enzymes in sierozems. Genetic characteristics, plant composition, level of agricultural culture and physicochemical properties are the determining components of phosphatase activity. Soils containing acid phosphatases have an acidic reaction; in soils with a slightly alkaline reaction, alkaline phosphatases predominate, which is confirmed by our research materials. In the soils of pistachio woodlands, alkaline phosphatase (1021.5 and 67.3 mg p-nitrophenol kg⁻¹ soil h⁻¹ in the upper layers of soil) is much higher than acid phosphatase (286.4 and 22.9), this indicates the provision of phosphorus regime Alkaline phosphatase enzymes play a major role in the nutrition of these soils. These indicators are very

important for diagnosing the genetic characteristics of sierozem soils of pistachio open forests. Table 2 shows that sierozem soils of pastures contain much less alkaline phosphatase (594.3 and 285.5 mg p-nitrophenol kg⁻¹ soil h⁻¹) than in soils of pistachio woodlands and they dominate over acid phosphatase (176.2 and 81.8). Enzymes that provide the most important metabolic processes in the soil are more significant, and among the hydrolytic enzymes, the most informative are: invertase and phosphatase (Khaziev, 1982).

As can be seen in Table 2, mountain sierozem soils contain from 1.3 to 176.2 mg nitrophenol kg⁻¹ soil hour⁻¹ acid phosphatase, from 9.6 to 594.3 mg nitrophenol kg⁻¹ soil hour⁻¹ alkaline phosphatase, from 5.3 to 303.6 mg nitrophenol kg⁻¹ soil h⁻¹ phosphodiesterase. The amount of enzymes sharply decreases in the subarable soil horizon, which directly correlates with the humus content of the arable land (Sakbaeva *et al.*, 2012). In the upper horizons of mountain gray soils, an increased content of enzymes is observed. This is due to the abundance of organic matter and soil microorganisms on the surface horizons of mountain sierozems (Sakbaeva *et al.*, 2013). The pH of the sierozem soils in the Kok-Art river basin fluctuates at 7.9-8.3, which contributes to an increase in the content of alkaline phosphatases. The highest amount of acid and alkaline phosphatases and phosphodiesterase accumulates in the upper soil horizon of pistachio woodland (1868.8 mg p-nitrophenol kg⁻¹ soil hour⁻¹). Enzyme activity decreases sharply down the soil profile. This picture of the distribution of enzymes confirms that it is necessary to protect the surface horizons of these soils from erosion and to guard against excessive grazing pressure. The content of acid phosphatase in irrigated sierozem soil is 228.9 mg nitrophenol kg⁻¹ soil hour⁻¹, alkaline phosphatase - 162.1, phosphodiesterase - 83.4. In cotton fields, the increased content of acid phosphatase is explained by the influence of applied nitrogen and phosphorus fertilizers, which have an acidic pH environment and an optimal temperature regime (+25-35C°), against the background of good regular

watering. These anthropogenic factors contribute to the active supply of mineral phosphorus to the roots and improve the phosphorus nutrition of cotton. Many works have noted that mountain forest black-brown soils are characterized by high natural fertility. The soils of walnut-fruit forests have large amounts of phosphorus in the form of organic compounds, which comes with the dying remains of plants, animals and microorganisms that accumulate in humus. As shown in Table 2, the enzymatic activity of acid phosphatase in mountain forest black-brown soil ranges from 18.0 to 712.7 mg nitrophenol kg⁻¹ soil h⁻¹, the amount of alkaline phosphatase ranges from 8.1 to 1809.8 mg nitrophenol kg⁻¹ soil hour⁻¹, while alkaline phosphatase dominates over acid phosphatase and phosphodiesterase (Sakbaeva *et al.*, 2012).

Arylsulfatases play an important role in the sulfur cycle, i.e. hydrolysis of sulfate esters in the soil and they play a positive role in improving the quality of agricultural products. The content of organic matter, total nitrogen and activity of the enzyme arylsulfatase in the sierozem soils of the Kok-Art river basin are given in Table 3.

The content of the arylsulfatase enzyme on the surface soil horizon of pistachio open forests is 115.4 mg p-nitrophenol kg⁻¹ soil hour⁻¹ and pastures - 81.3, which indicates that the activity of the arylsulfatase enzyme in sierozem soils is directly correlated with organic matter and the rhizosphere of natural plant communities. Their quantity on irrigated arable land (cotton) is much lower than the above indicators for similar soils (23.4 mg p-nitrophenol kg⁻¹ soil hour⁻¹). Enzymes are fixed in the soil, maintaining their activity and becoming protected from the action of microorganism proteases. In this case, the inhibitory effect of humic acids on enzyme activity appears (Shcherbkova, 1983). As can be seen, in comparison with other types of soils studied, the sierozem soils of the Kok-Art river basin were formed under conditions dominated by an arid climate with a deficiency of precipitation and high temperatures, which affects the activity of the enzyme arylsulfatase. Among sierozem soils,

the lowest activity of the arylsulfatase enzyme was observed in arable lands where cotton was grown for many years in a row. Aryl sulfatase activity in these areas ranges from 9.1 to 23.4 mg p-nitrophenol kg⁻¹ soil h⁻¹. This is probably due to the low content of organic matter and the small supply of post-harvest plant mass on cotton plantations, when, according to existing technology, all above-ground mass is alienated for economic needs. As can be seen from Table 3, a low amount of organic matter (OM) and total nitrogen (N) is contained in irrigated sierozem soils, which is correlated with the content of the enzyme arylsulfatase. In mountain-forest black-brown soils of walnut-fruit forests, the content of organic substances was at a high level, i.e. 12-16% in A₀ and 9-12% in A₁ horizons. In accordance with this, the activity of arylsulfatase was also at a high level, which was contained within 498.9 mg p-nitrophenol kg⁻¹ soil h⁻¹ in the A₀ horizon and 439.2 mg p-nitrophenol kg⁻¹ soil h⁻¹ in A₁ horizons.

CONCLUSIONS

The enzymatic activity of sierozem soils of various uses is a complex of natural and anthropogenic components, combined into three groups: biological (quantitative and qualitative composition of phytomass, content and composition of microflora, enzymatic activity, intensity of CO₂ release and cellulose decomposition in the soil); agrochemical (humus, pH, indicators of the soil-absorbing complex, content and forms of plant nutrients); agrophysical (mechanical composition, structure and structure of the arable layer, thickness of the humus horizon, density, porosity, reserves of productive moisture, air, thermal properties and their regimes). And of these, biological activity and enzyme activity works to improve the nutritional regime of soils and is one of the diagnostic indicators of fertility.

The soils of pistachio forests and pastures of sierozem soils, compared to irrigated arable land, are characterized by increased enzymatic activity, which indicates the provision of nitrogen and phosphorus nutrition and to increase the enzymatic pool of arable land, an additional supply of organic substances is required.

Among the studied soils, the enzymatic activity of mountain-forest black-brown soils of walnut forests is very high and it depends on the gross content of humus and organic phosphorus, which are the food substrate for the enzyme. The enzyme of mountain forest black-brown soils is involved in the decomposition of plant, animal and microbial residues, as well as the synthesis of humus. Forested areas tend to contain higher microbial biomass compared to pastures and croplands, which can be explained by the higher levels of enzymes found in forested areas.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Table 1: Activity of glucosidase enzymes in sierozem and mountain-forest black-brown soils of Kok-Art river basin of Jalalabat region (mg p-nitrophenol kg⁻¹ soil hour⁻¹)

Location	Soil types	Horizon	Depth (cm)	β -glucosidase	β -glucosaminidase
Pastures	Foothill typical sierozem soils	A ₀	0-2	428,7	43,3
		A ₁	2-13	209,3	34,5
		AB	13-44	25,7	11,2
		B	44-86	7,4	6,2
		C	86-170	2,0	2,9
Pistachio forest	Typical sierozem soils	A ₀	0-2	809,5	51,0
		A ₁	2-14	38,3	8,8
		B ₁	14-52	16,0	10,6
		B _k	52-105	7,6	7,5
		C	105-165	5,3	5,6
Arable land (cotton)	Irrigated sierozem soils	A	0-14	69,8	11,9
		A ₁	14-30	22,8	5,8
		B	30-50	3,3	4,2
Walnut-fruit forest	Mountain forest black-brown soils	A ₀	0-4	1235,9	298,5
		A ₁	4-18	546,9	106,0
		A ₂	18-57	74,6	7,3
		AB	57-91	17,8	10,9
		B	91-130	14,7	1,7
		C	130-185	11,1	14,4

Table 2: Activity of phosphatase enzymes in sierozem and mountain-forest black-brown soils of Kok-Art river basin of Jalalabat region (mg p-nitrophenol kg⁻¹ soil hour⁻¹)

Location	Soil types	Depth (cm)	Acid phosphatase	Alkaline phosphatase	Phosphodiesterase
Pasture	Foothill typical sierozem soils	0-2	176,2	594,3	303,6
		2-13	81,8	285,5	177,7
		13-44	21,0	72,7	53,4
		44-86	2,4	20,5	11,5
		86-170	1,3	9,6	5,3
Pistachio woodland,	Typical sierozem soils	0-2	286,4	1021,5	560,9
		2-14	22,9	67,3	17,6
		14-52	17,9	59,0	33,7
		52-105	12,0	36,0	26,0
		105-165	2,8	6,6	8,0
Arable land (cotton), Suzak	Irrigated sierozem soils	0-14	228,9	162,1	83,4
		14-30	42,7	84,5	53,9
		30-50	31,5	80,4	67,7
Walnut-fruit forests	Mountain forest black-brown soils	0-4	712,7	1809,8	714,2
		4-18	897,7	757,8	754,5
		18-57	272,5	283,9	225,9
		57-91	59,8	27,7	41,7
		91-130	27,7	18,7	11,9
		130-185	18,0	8,1	15,4

Table 3: Activity of the enzyme arylsulfatase in sierozem and mountain-forest black-brown soils of Kok-Art river basin of Jalalabat region(mg p-nitrophenol kg⁻¹ soil hour⁻¹)

Land use	Soil types	Horizon	Depth, (cm)	Organic substance, %	Total Nitrogen, %	Arylsulfatase
Pasture	Foothill typical sierozem soils	A ₀	0-2	2,13	0,15	81,3
		A ₁	2-13	1,49	0,12	34,9
		AB	13-44	0,60	0,10	18,4
		B	44-86	0,23	0,04	3,5
		C	86-170	0,21	0,04	2,4
Pistachio woodland,	Typical sierozem soils	A ₀	0-2	3,55	0,46	115,4
		A ₁	2-14	0,96	0,08	5,3
		B ₁	14-52	0,74	0,08	8,7
		B _k	52-105	0,42	0,05	8,1
		C	105-165	0,22	0,04	1,7
Arable land (cotton), Suzak	Irrigated sierozem soils	A	0-14	0,79	0,07	23,4
		A ₁	14-30	0,64	0,06	14,0
		B	30-50	0,52	0,14	9,1
Walnut-fruit forests	Mountain forest black-brown soils	A ₀	0-4	16,13	1,88	498,9
		A ₁	4-18	12,5	0,84	439,2
		A ₂	18-57	5,51	0,38	158,3
		AB	57-91	2,06	0,13	15,3
		B	91-130	1,37	0,14	4,8
		C	130-185	1,23	0,08	2,2

Study of fruit drop pattern in date palm (*Phoenix dactylifera* L.)

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ABSTRACT

Fruit drop is a common phenomenon in date palm which may significantly affect yield and commercial viability. To understand the pattern of fruit-drop the study was done in three date palm genotypes: Barhee, MDP-20, and MDP-21, under the Gujarat North West Agro-Climatic Zone (Zone-V) at the Date Palm Research Station, Mundra-Kachchh. The research was conducted over three years, with periodic observations of fruit drop at different growth stages. The results reveal that the highest fruit drop occurs between 30-45 days after pollination, followed by the initial 0–30day period. Understanding fruit drop dynamics is crucial for development of effective management strategies, including optimized irrigation, nutrient application, and climatic interventions. This will also serve as a base information to improve fruit retention and ultimately enhancing date palm productivity and economic returns for farmers.

Keywords: Date palm, fruit drop, fruit retention, *Phoenix dactylifera*

INTRODUCTION

Date palm (*Phoenix dactylifera* L.) is one of the most important fruit crops cultivated in arid and semi-arid regions worldwide. It plays a crucial role in the economy and food security of many countries, particularly in the Middle East, North Africa, and parts of South Asia (Khan *et al.*, 2022). The fruit is highly valued for its nutritional content, being rich in carbohydrates, fibre, and essential minerals. Due to its high adaptability to harsh climatic conditions and saline soils, date palm cultivation has been expanding to new regions. In India, particularly in Gujarat, date

palm cultivation has gained prominence, with Kachchh being a major production hub (Muralidharan *et al.*, 2022; Baidiyavadra *et al.*, 2019). Unlike other parts of the world, In India the focus is one the cultivation of fresh dates (Khalal stage) rather than ripened stage (tamar stage) due to climatic compulsions (Sharma *et al.*, 2019, 2022).

Fruit drop in date palm occurs at different growth stages and is influenced by a combination of genetic, environmental, and physiological factors. It can be categorized into post-pollination drop, pre-maturity drop, and maturity drop, each linked to specific physiological mechanisms such as

fertilization failure, environmental stress, nutrient deficiency, and hormonal imbalances (Khan *et al.*, 2022). Studies have shown that fruit drop rates vary among cultivars and environmental conditions, necessitating region-specific studies to develop mitigation strategies (Abbas *et al.*, 2000). While natural fruit drop is a physiological process, excessive loss of fruits can adversely impact economic returns for growers. Various factors, including genetic characteristics, environmental conditions, and cultural practices, influence fruit drop. The timing and intensity of fruit drop vary among cultivars, making it essential to study their specific patterns. By understanding the fruit drop dynamics in different cultivars, farmers can optimize their management practices and enhance productivity. The maturity of the fruits are also genotype specific and may need different heat units (Sharma *et al.*, 2022), and similarly the fruit drop pattern may vary from genotype to genotype. Moreover, the fruit drop may be influenced by the environment, a local study of the fruit drop is needful. Understanding the patterns of fruit drop in this agroclimatic zone will aid in developing region-specific management strategies to enhance fruit retention and improve overall yield.

MATERIAL AND METHODS

The experiment was conducted at the Date Palm Research Station, Mundra-Kachchh, which falls under the Gujarat North West Agro-Climatic Zone (Zone-V). The study involved three date palm cultivars, namely Barhee, MDP-20, and MDP-21, planted under similar agronomic conditions in the year 2012. This region is characterized by extreme climatic conditions, including high temperatures, low rainfall, and arid soils. The location is ideal for date palm research as it closely resembles the natural habitat of the species. Furthermore, Kachchh is a leading producer of date palm in India, making it a critical area for studying factors affecting productivity (Muralidharan *et al.*,

2022). The experiment was done using Randomized Block Design (RBD) with each genotype serving as a separate treatment during 2020-2022. Each treatment had eight replications, with one bunch per replication selected from different plants to ensure variability in the data. Random five strands were tagged per bunch at the time of pollination which were used for observations on number of fruits.

Observations for the number of flowers per strand were recorded at the time of pollination (0 days). From the 30th day onwards, the number of retained fruits per bunch was recorded at intervals of every 15 days. The fruit count data collection continued until 105 days after pollination. Fruit drop percentage was calculated based on the initial flower count and subsequent fruit counts at each recorded interval using the following formula.

$$\text{Fruit drop percentage} = \frac{(\text{Number of fruits at T1 day} - \text{Number of fruits at T2 days}) \times 100}{\text{Number of fruits at 0 days}}$$

Standard agronomic practices, including pruning of dry leaves, dethorning, pollination, inflorescence covering, and tying of bunches, were followed uniformly for all plants under study. Drip irrigation was installed, with an average of 300 litres of water applied per palm per day to maintain optimal moisture levels.

The collected data were subjected to statistical analysis using ANOVA, and the means were compared using the critical difference (CD) at a 5% significance level to determine the statistical significance of fruit drop variations among cultivars. The visualization is made using R programming with ggplot package.

RESULTS AND DISCUSSION

The study revealed distinct fruit drop patterns in the three date palm genotypes (Barhee, MDP-20 and MDP-21) over a period of 105 days after pollination (Figure

1). The data, collected over three consecutive years, showed a progressive decline in the number of fruits per strand, with the most decline in the initial 45 days after pollination followed by a partial stability. This early phase of fruit drop was the most critical period, aligning with previous finding due to fertilization failure, resource competition and environmental stress (Shalom *et al.*, 2024; Khan *et al.*, 2022). The stabilization of fruit count after 75 DAP suggests that the remaining fruits have a higher likelihood of reaching maturity.

Among the genotypes, Barhee exhibited the highest initial fruit count per strand but experienced a steep decline between 0–45 DAP, followed by a slower fruit drop rate in the later stages. The pooled data for Barhee closely followed individual year-wise trends, indicating a consistently high fruit drop rate in the early phase. This is consistent with studies suggesting that genotypic factors contribute significantly to fruit retention (Khan *et al.*, 2022). In contrast, MDP-20 had a lower initial fruit count and a gradual fruit drop over time, with a notable decline around 30–60 DAP. The greater variation in fruit drop across years for this genotype suggests a strong influence of environmental conditions, such as temperature fluctuations, irrigation levels, and nutrient availability (Abbas *et al.*, 2000; Saengpook *et al.*, 2007).

The percentage fruit drop among the different genotypes is presented in Table 1, while the comparative analysis of fruit drop in different duration is presented in Table 2. The highest fruit drop was observed in the early growth stages, particularly between 0–30 days after pollination (DAP) and 30–45 DAP, confirming that the initial fruit development phase is the most vulnerable to fruit shedding. Among the genotypes, MDP-20 exhibited the highest fruit drop in the first 30 days (21.33%), while MDP-21 had the highest drop between 30–45 DAP (25.60%), suggesting that these two genotypes experience greater fruit loss in the critical

early stage. Barhee, in contrast, showed a more gradual decline, indicating relatively better early-stage fruit retention.

As fruit development progressed, the mid-stage fruit drop (45–75 DAP) showed a declining trend, with MDP-20 still exhibiting a relatively high drop rate (20.65% between 45–60 DAP), whereas Barhee and MDP-21 showed moderate declines (15.59% and 18.57%, respectively). The gradual stabilization of fruit retention after 45 DAP aligns with findings in previous studies, which indicate that nutrient availability, environmental conditions, and irrigation management significantly influence mid-stage fruit abscission (Khan *et al.*, 2022). The lower fruit drop observed during this period suggests that improved carbohydrate allocation and stronger pedicel attachment might contribute to fruit retention.

In the late-stage fruit drop (75–105 DAP), the rate of fruit drop decreased further across all genotypes, with Barhee and MDP-21 exhibiting the lowest fruit drop percentages ($\leq 5\%$), while MDP-20 still experienced slightly higher losses (9.99% between 90–105 DAP). This suggests that MDP-20 may have a weaker fruit retention capacity in the later growth stages, potentially due to physiological limitations or environmental factors such as temperature fluctuations and water stress (Saengpook *et al.*, 2007; Hagemann *et al.*, 2014). The stabilization of fruit count after 75 DAP supports the hypothesis that retained fruits have a higher likelihood of reaching full maturity.

The similarity in pooled and yearly data suggests that fruit drop in this genotype follows a relatively predictable trend, reinforcing previous observations that fruit drop patterns are genotype-dependent (Lordan *et al.*, 2021). The total fruit drop percentage across all stages was highest in MDP-20 (55.58%), followed by MDP-21 (48.69%) and Barhee (47.92%), with a pooled mean fruit drop of 50.74%. These

values confirm that approximately half of the total fruit set is lost due to natural abscission, reinforcing the need for strategic interventions to enhance fruit retention.

The findings suggest that targeted management strategies should focus on minimizing fruit drop during the first 45 days after pollination, as this is the most critical period for fruit retention. Nutrient management, particularly potassium (K) and urea applications, has been shown to significantly reduce fruit drop in date palms (Khan *et al.*, 2022). Additionally, optimized irrigation management during the early fruit development stage may help reduce fruit abscission, as water stress is a known trigger for increased ethylene production, which accelerates fruit shedding (Shalom *et al.*, 2024). Furthermore, the application of auxins such as 2,4-D has been reported to delay fruit drop by inhibiting abscission zone formation, making it a potential strategy for improving fruit retention in date palms (Shalom *et al.*, 2024).

Based on the results the fruit drop in date palm follows three distinct phases: early fruit drop (0–45 DAP), mid-stage fruit drop (45–75 DAP), and late-stage fruit drop (75–105 DAP). The early phase is the most critical, with the highest fruit loss which might be due to fertilization failure, resource competition, and hormonal imbalances. In the mid-stage, fruit drop declines and it might be influenced by nutrient uptake, irrigation efficiency, and hormonal balance. The late-stage drop stabilizes and the retained fruits are likely to reach maturity.

Understanding these patterns allows for targeted interventions such as optimizing irrigation, foliar nutrient application, and shading techniques to minimize fruit drop losses. Future studies should focus on identifying physiological markers for early detection of fruit drop-prone bunches, allowing for timely interventions and improved yield stability.

CONCLUSION

This study confirms that fruit drop in date palm is a significant challenge, with around 50% fruit loss occurring during the fruiting period. The highest fruit drop was recorded between 30-45 days after pollination, followed by 0-30 days and 45-60 days. The findings highlight genotypic variations in fruit drop trends, with MDP-20 experiencing higher losses, particularly in the early and mid-stages. These variations indicate the importance of customized agronomic practices based on genotype-specific responses. Future research should focus on climatic correlations with fruit drop patterns, allowing for the development of predictive models and adaptive management strategies to improve date palm productivity.

CONFLICT OF INTEREST STATEMENT

The author declare that he has no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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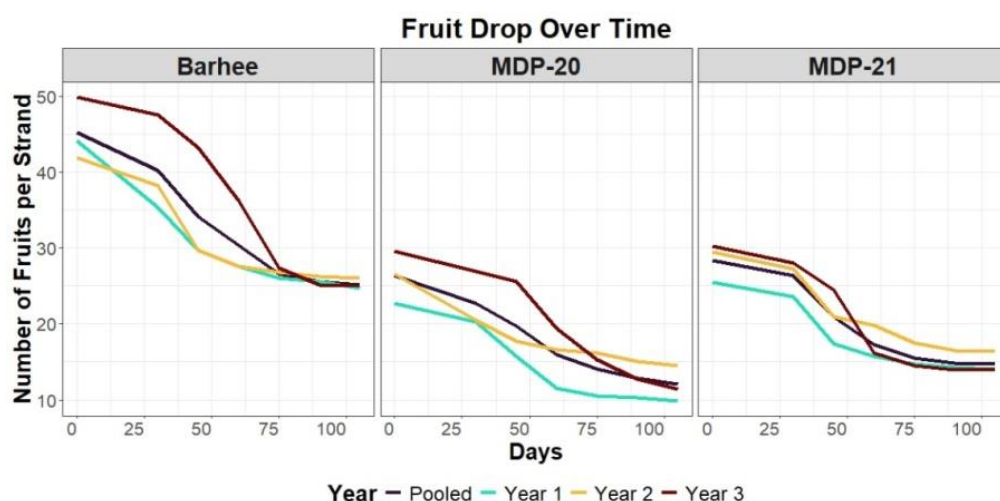


Figure 1: Fruit drop in date palm over time

Table 1: Fruit drop percentage in date palm (pooled for three years)

Genotype	Fruit drop percentage between the days (pooled for three years)*					
	0 th to 30 th day	30 th to 45 th day	45 th to 60 th day	60 th to 75 th day	75 th to 90 th day	90 th to 105 th day
Barhee	18.57 (11.14)	21.21 (13.86)	15.59 (7.98)	13.44 (7.81)	12.35 (6.05)	4.88 (1.08)
MDP-20	21.33 (14.16)	18.90 (11.67)	20.65 (14.51)	12.82 (6.41)	11.42 (5.27)	9.99 (3.56)
MDP-21	14.72 (7.21)	25.60 (19.27)	18.57 (12.22)	12.71 (5.94)	8.01 (2.94)	4.82 (1.12)
Mean	18.21 (10.84)	21.91 (14.94)	18.27 (11.57)	12.99 (6.72)	10.59 (4.75)	6.56 (1.92)
SEm ±	0.89	0.81	1.20	1.53	1.03	0.67
C.D. @ 5 %	2.52	2.29	3.41	NS	2.92	1.91
C.V. %	23.81	17.97	32.03	57.38	47.32	50.04

* Value are arc-sin transformed, value in parenthesis are original value

Table 2: Fruit drop percentage distribution in different date palm genotypes (pooled for three years)

Days	Fruit drop percentage (pooled for three years)*			
	Barhee	MDP-20	MDP-21	Mean
0 th to 30 th day	18.57 (11.14)	21.33 (14.16)	14.72 (7.21)	18.96 (10.84)
30 th to 45 th day	21.21 (13.86)	18.90 (11.67)	25.60 (19.27)	22.31 (14.94)
45 th to 60 th day	15.59 (7.98)	20.65 (14.51)	18.57 (12.22)	18.95 (11.57)
60 th to 75 th day	13.44 (7.81)	12.65 (6.42)	12.70 (5.94)	14.08 (6.72)
75 th to 90 th day	12.35 (6.05)	12.81 (5.26)	8.01 (2.93)	11.93 (4.75)
90 th to 105 th day	4.88 (1.08)	11.41 (3.56)	4.82 (1.12)	7.46 (1.92)
Mean	14.34 (7.99)	15.85 (9.26)	14.07 (8.12)	15.62 (8.46)
Total fruit drop (%)	(47.92)	(55.58)	(48.69)	(50.74)
SEm ±	1.04	1.15	1.08	0.58
C.D. @ 5 %	2.95	3.25	3.06	1.65
C.V. %	35.99	35.83	38.06	18.52

* Value are arc-sin transformed, value in parenthesis are original value

Phytochemical and antioxidant studies on dried leaves of *Crotalaria gajureliana* Gholave, Madhav & Gosavi

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ABSTRACT

Crotalaria gajureliana is a new plant species and its biological activity and phytochemical composition are unknown. Leaves are a renewable plant part, meaning they can be harvested without harming the plant or threatening its survival. The current study is an evaluation of the phytochemical profile, total phenolic content (TPC), total flavonoid content (TFC), along with antioxidant activity of dried leaves of *Crotalaria gajureliana* by successive solvent extraction. Analysis began with standard qualitative tests to confirm the presence of these phytoconstituents. The phytochemical analysis revealed that the extracts contained flavonoids, alkaloids, tannins, glycosides, and phenolic compounds. The methanol extract exhibited the highest levels of total phenolic and flavonoid content, followed by the aqueous extract, and then the ethanol extract. DPPH antioxidant activity here shows significant free radicals scavenging, where aqueous extract showed the highest inhibition percentage which can be explained by the higher levels of total phenolic and Flavonoid compounds.

Keywords: Antioxidant activity, *Crotalaria gajureliana*, phytochemicals, successive extraction, total flavonoid content, total phenolic content

INTRODUCTION

Many plants or plant-based materials are getting a lot of attention in the field of modern medicine for the development and extraction of possible therapeutic candidates for the treatment of many diseases (Mirihaigalla & Fernando, 2021). *Crotalaria gajureliana* Gholave, Madhav & Gosavi, *Synonym: Phatakadi*, is a recently discovered plant of the genus *Crotalaria* in the Fabaceae plant family (subfamily: Papilionoideae). The plant is so far known to occur in two locations in Maharashtra, India: Nandur-Madhyameshwar and Chamar Leni (Gholave *et al.*, 2021). This herb, thrives in open grassland areas (Khot *et al.*, 2023), whose phytochemical and pharmacological properties have not yet been fully investigated. *Crotalaria gajureliana* is a plant that has never been documented in the

literature, and the primary goal of this effort is the first scientific analysis of this plant.

For herbal medication compositions to be safe, effective, and of high quality, medicinal plant standardization is essential. Extraction of bioactive chemicals varies by successive extraction using solvents with different polarities, whereas first phytochemical screening provides information about the chemical components that may have pharmacological effects. *Crotalaria gajureliana* leaves will be subjected to DPPH (1, 1-Diphenyl-2, Picryl-Hydrazyl) free radicals to assess their free radical scavenging activity, TPC, TFC, in addition to successive extraction yield. This will help *Crotalaria gajureliana* 's scientific validity as well as its possible uses in herbal medicine. Future pharmacological and phytochemical studies on this newly

discovered plant species will be built upon the results.

MATERIALS AND METHODS

The study was made at Pharmacognosy PG Laboratory, Department of Pharmacognosy, Mahatma Gandhi Vidyamandir's Pharmacy College, Nashik, Maharashtra, India 402003. The leaves of *Crotalaria gajureliana* was collected from Chamar Leni, Nashik District, India, in August 2024. The plant was identified by Dr. Avinash Gholave, a botanist at Department of Botany, K.V.N. Naik Arts, Commerce and Science College, Nashik, India. The voucher sample (SRV-02) was held as a future reference in the specimen section of the Department Museum. After collection, the leaves were dried under shade and used for further research (Figure 1).

Every chemical, solvent, and reagent employed in the investigation was of analytical grade. The Soxhlet apparatus, water bath, electronic balance and UV-Spectrophotometer were among the equipment utilised. Using a Soxhlet apparatus with polarity-increasing solvents pet ether to be followed by ethyl acetate, methanol, ethanol, followed by water, along with dried leaves of *Crotalaria gajureliana* were extracted one after the other. The effective extraction of various plant phytoconstituents based on their solubility in various solvents was guaranteed by this approach. Upon completion, the extract was concentrated by solvent evaporation and the dried residue was weighed to determine the extraction values. The % yield of extract was evaluated by employing following formula: $\text{Weight of Extract (g)} \times \text{Weight of Leaf Powder (g)} \times 100 = \text{Percentage Yield (\%)}$. The standard procedure outlined by Khandelwal was used to conduct the preliminary screening (Khandelwal, 2016).

Total phenol content: *Crotalaria gajureliana*'s total phenol content was assessed by employing modified version of the (Dewanto *et al.* 2002) technique.

0.25mL of Folin Ciocalteu reagent was mixed with a diluted extract in aliquots of 0.5, 1, 1.5, 2, and 2.5ml at concentrations of 1mg/ml. Elucidation was shaken thoroughly after distilled water was added to reduce its final amount to 3ml. A produced blank was compared to the solution's 765 nm reading following incubation and dark storage. Plant part's TPC had been expressed in milligrammes of gallic acid equivalents per gramme of dry weight. Complete sample was analysed in 3 replicates.

Total Flavonoid Content: Using an aluminium chloride colorimetric technique, the flavonoid content of the *Crotalaria gajureliana* extract was estimated as a percentage (Mervat *et al.*, 2009). After adding 3 ml of methanol to 0.5 ml of an extract with different concentrations (0.5, 1, 1.5, 2, and 2.5ml of 1mg/ml), the mixture was shaken vigorously. Next, 2.8ml of distilled water was added, along with 0.1ml of potassium acetate, and then 0.1ml of 10% AlCl₃ was added to test solution while it was being shaken. After the solution remained for half an hour, absorbance was examined at 415nm. Flavonoid concentration in the test samples was determined and reported as equivalent to quercetin (QE) per gram of sample. All samples were analyzed in three separate trials.

Free radical scavenging activity: The inhibition percentage of test substance was assessed for DPPH free radical scavenging activity. Test tubes were set up containing 1 ml of each concentration: 20, 40, 60, 80, and 100 µg/ml. After combining 1.5ml of each concentration with 1.5ml of 0.1% methanolic DPPH, mixture was kept in dark for 30min. Following this period, the samples were examined for color changes from purple to yellow, and absorbance was recorded at 510nm by employing colorimeter. Additionally, each test was performed in triplicates (Baliyan *et al.*, 2022). The radical scavenging activity was evaluated by employing given formula: $\text{DPPH radical scavenging activity (\%)} \times 100 = (\text{Absorbance})$

of control-Absorbance of test sample)/ (Absorbance of control). Each test sample's IC₅₀ value was determined.

RESULTS AND DISCUSSION

Yield of Extraction

Different yields were obtained by successively extracting the dried leaves using solvents with increasing polarity. The lowest yield, 0.1%, was obtained from the ethyl acetate extract, whereas 1.6% was obtained from the petroleum ether extract. 1.5% and 4.2% were obtained from the methanol and ethanol extracts, respectively (Table 1). The aqueous extract, which made up 20.8% of the total extracts, produced the highest yield. The significant yield in the water extract indicates a larger concentration of polar components in the plant material, according to these results.

Preliminary phytochemical screening

Preliminary phytochemical screening of various solvent extracts of *Crotalaria gajureliana* leaves showed the presence of various plant constituents (Table 2). Petroleum ether extract showed the presence of fixed oils and oils. Ethyl acetate extract was shown to contain fixed oils, fats, steroids and acidic compounds. Methanol extract was shown to contain flavonoids (flavanes), tannins, phenolic compounds and alkaloids. It was found that the ethanol extract contained alkaloids, tannins, phenolic chemicals, and flavonoids (chalcones, aurones, and flavanes). It was discovered that the aqueous extracts contained alkaloids, tannins, phenolic compounds, flavonoids (flavanes), and cardiac glycosides (cardenolides and deoxysugars). These findings show the various phytochemical components in various solvent extracts, suggesting potential pharmacological action. Many of the bioactive properties of plant extracts are due to phytochemicals, which are secondary plant metabolites. (MacDonald *et al.*, 2022).

Total phenolic content

The linear calibration curve of gallic acid, whose equation is $y = 0.3448x + 0.5064$ $R^2 = 0.9911$, was used to determine the phenolic content (Figure 2). The obtained results showed that the solvent employed for extraction affects the amount of phenolic content in the dried leaves of *Crotalaria gajureliana*. At 26.46 mg/g, the methanolic extract had the highest phenolic content, while the aqueous extract came in second with 20.65 mg/g. The phenolic concentration of the ethanolic extract was 11.14 mg/g, which was a rather low amount. According to these results, the best solvent to extract the phenolic compounds from *Crotalaria gajureliana*'s dried leaves is methanol. As phenolic compounds have been known for being antioxidants, they can scavenge the harmful free radicals that are produced within cells by oxidising the substance's phenolic group. Since harming free radicals and non-communicable diseases (NCDs) are strongly correlated, this feature is attributed to the potential to fight against NCDs (Bulugahapitiya *et al.*, 2020).

Total flavonoid content

The linear calibration curve of quercetin, whose equation is $y = 0.3448x + 0.5064$ $R^2 = 0.9911$, was used to determine the flavonoid content (Figure 3). Total phenolic and flavonoid content has been presented in Table 3. According to the results, the content of flavonoids in the dried leaves of *Crotalaria gajureliana*, measured in quercetin equivalents (QE/g of extract), varies depending on the solvent used. With 36.51 QE/g, the methanolic extract had the highest flavonoid content, followed by the aqueous extract (12.44 QE/g), while the ethanolic extract (7.24 QE/g) had the lowest. According to these results, the best solvent to extract the flavonoid compounds from plant material is methanol. Various aromatic and medicinal plants are abundant in phytochemicals, which are known for their antioxidant properties which include phenolic compounds, flavonoids, sterols, tannins, and essential oils (Almi *et al.*, 2022).

Free radical scavenging activity

The antioxidant profile of compounds Methanol, Ethanol along with aqueous extracts was assessed by evaluating percent of inhibition against DPPH reagent via test tube method. The compound Methanol, Ethanol and Aqueous extracts exhibited good antioxidant activity against DPPH scavenging reagent and however concentration rises, antioxidant activity of compound also rises as compared to the standard ascorbic acid. Using methanol, ethanol, and aqueous solvents, the DPPH free radical scavenging activity of leaf extracts from *Crotalaria gajureliana* was assessed at different doses (20–100 µg/ml). The findings show that the antioxidant activity of all extracts increases in a concentration-dependent manner. Following the methanolic extract at 55.95% and the ethanolic extract at 51.81%, the aqueous extract demonstrated the highest scavenging capability among them, reaching 62.17% at 100 µg/ml. This finding is supported by the IC₅₀ values, which reveal that the aqueous extract has the highest antioxidant activity with the lowest IC₅₀ value of 67.92 µg/ml. In addition, the IC₅₀ values of the methanolic and ethanolic extracts were 80.84 µg/ml and 98.06 µg/ml, respectively. According to these results, *Crotalaria gajureliana* leaf aqueous extract has the strongest free radical scavenging ability among all extracts. The results shown in (Figure 4) indicate the Percentage inhibition of DPPH for different extracts of *Crotalaria gajureliana* Gholave, Madhav & Gosavi leaves. Table No. 4 shows the results for Free radical scavenging activity and Table No. 5 shows the results for IC 50 value of different extracts. Phenolics and flavonoids, directly contribute to the antioxidant capacity of plants, according to (Abou Zeid *et al.*, 2014; Ali *et al.*, 2023)

CONCLUSION

The extractability of bioactive compounds varies as per the polarity of the solvent used for extraction. Significant bioactive components, including flavonoids, tannins,

alkaloids, glycosides, and phenolics, were detected by phytochemical screening. Due to highest antioxidant activity in DPPH analysis, highest TPC and TFC of the solvents were in methanol extract. Ethanol and aqueous extracts also exhibited principal bioactive features. These results guarantee that the leaves of *Crotalaria gajureliana* can be an effective natural source of antioxidants, validating the potential of pharmacological investigations. The function of these bioactive substances in scavenging free radicals is demonstrated by the link seen between antioxidant activity and phenolic and flavonoid levels.

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CONFLICT OF INTEREST STATEMENT

The authors affirm that none of their known financial conflicts or personal connections might have influenced the research presented in this paper.

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Table 1: Extractive Values by successive extraction of leaves of *Crotalaria gajureliana*

Sr. No.	Solvent	Percentage yield
1	Petroleum ether	1.6%
2	Ethyl acetate	0.1%
3	Methanol	1.5%
4	Ethanol	4.2%
5	Water	20.8%

Table 2: Preliminary phytochemical screening of different extracts of *Crotalaria gajureliana*

Sr. No.	Test	Extracts				
		Petroleum Ether	Ethyl acetate	Methanol	Ethanol	Water
1	Carbohydrates	-	-	-	-	-
2	Proteins	-	-	-	-	-
4	Fats & Oils	+	+	+	+	-
5	Terpenoids	-	-	-	-	-
6	Steroids	-	-	-	-	-
7	Triterpenoids	-	+	-	-	-
8	Glycosides	-	-	-	-	+
9	Cardiac glycosides	-	-	-	-	+
10	Saponins	-	-	-	-	-
11	Flavonoids	-	-	+	+	+
12	Tannins & Phenolic Compounds	-	-	+	+	+
13	Alkaloids			+	+	+

Table 3: Phenol and Flavonoid content of Methanolic, Ethanolic and Aqueous extracts of *Crotalaria gajureliana*

Sr. no.	Sample	Total phenolic content of <i>Crotalaria gajureliana</i> leaves (mg GAE/g of extract)	Total flavonoid content of <i>Crotalaria gajureliana</i> leaves (QE/g of extract)
1	Methanolic extract	26.46	36.51
2	Ethanolic Extract	11.14	12.44
3	Aqueous extract	20.65	07.24

Table 4: *Crotalaria gajureliana* leaves DPPH scavenging activity in various solvent extracts.

Concentration (µg/ml)	Methanol	Ethanol	Aqueous
20	20.72%	10.88%	16.58%
40	31.60%	20.72%	23.31%
60	37.30%	30.56%	45.07%
80	49.74%	33.16%	57.51%
100	55.95%	51.81%	62.17%

Table 5: IC 50 value of different extracts.

Extract	IC 50 value (µg/ml)
Methanol	80.84
Ethanol	98.06
Aqueous	67.92



Figure 1: Dried leaves of *Crotalaria gajureliana* Gholave, Madhav & Gosavi

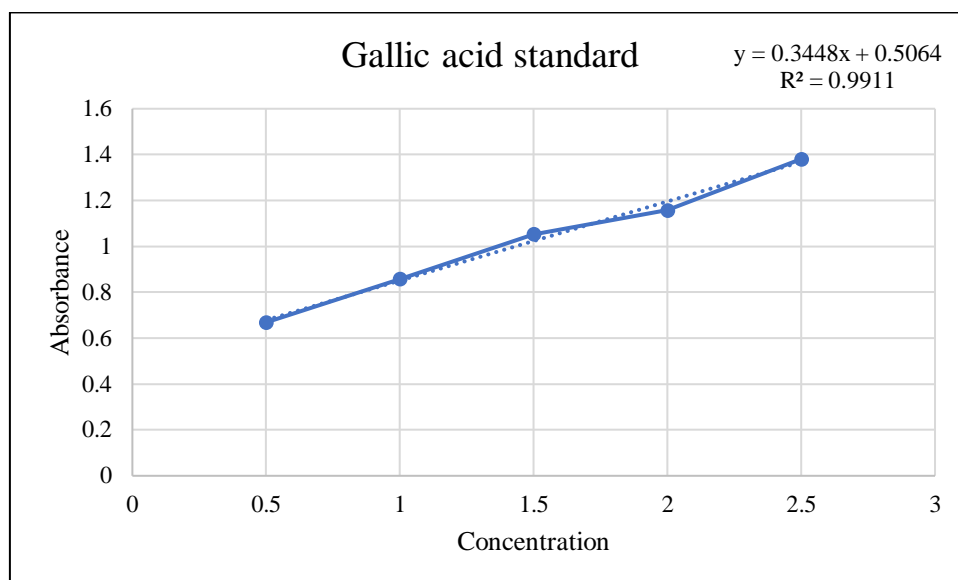


Figure 2: Evaluation curve of standard Gallic acid against absorbance measured at 765nm

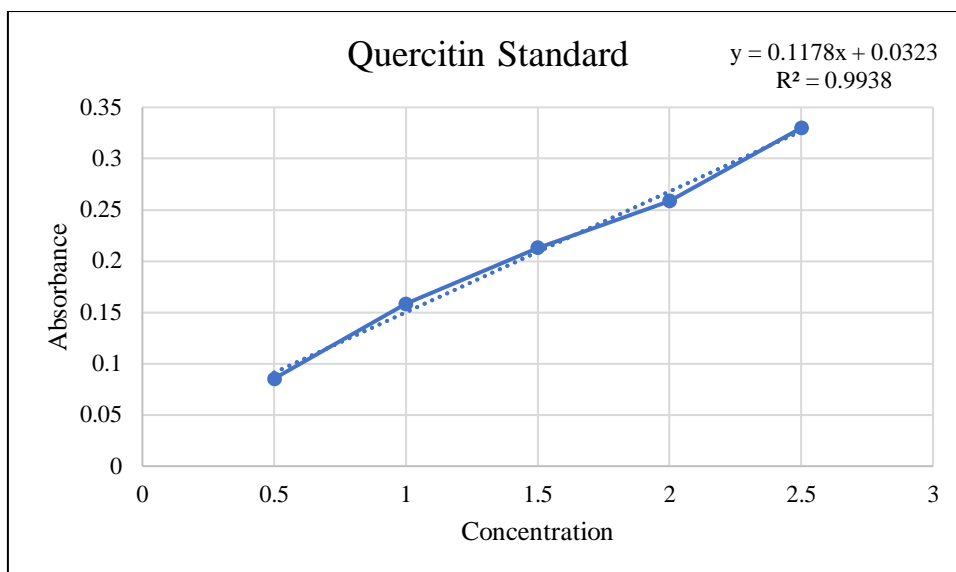


Figure 3: Evaluation curve of standard Quercetin against absorbance measured at 510nm

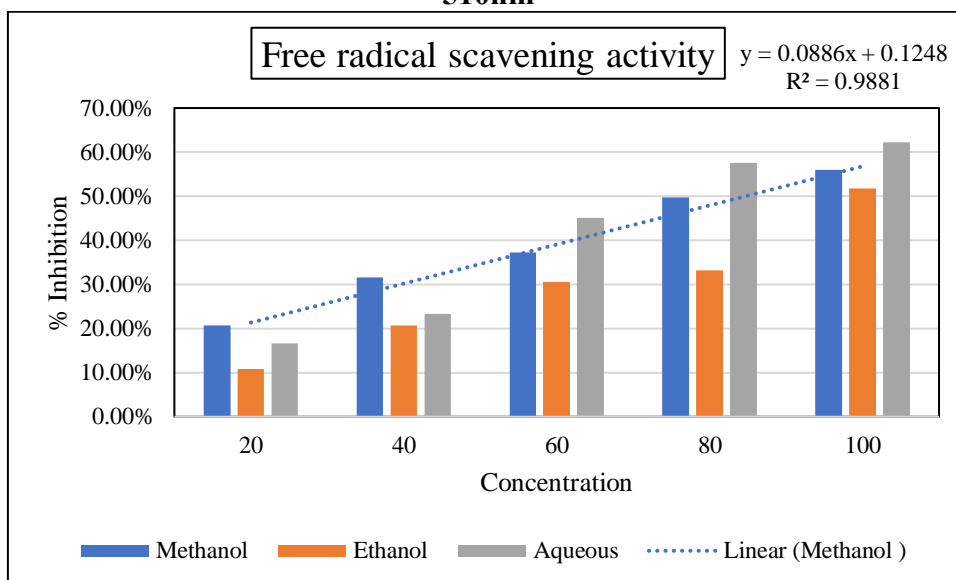


Figure 4: Percentage inhibition of DPPH for different extracts of *Crotalaria gajureliana* Gholave, Madhav & Gosavi leaves

Sennoside variations due to environmental changes in Sonamukhi: An Indian herb

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ABSTRACT

Sonamukhi, Senna, (*Cassia angustifolia*) used in traditional formulations for the treatment of various disease conditions. The physicochemical properties fluctuate with the season and in response to stress. The present study aim to evaluate the physiochemical fluctuations in the leaves of Sonamukhi and sennoside, an alkaloidal constituent in these leaves. Plant leaves were collected in every month of a year at different time and places. The leaves were evaluated for proximate phytochemical analysis, extractive values in petroleum ether, chloroform, ethyl acetate, ethanol, water, and determination of the concentration of component sennoside. Phytochemical composition was same in all season; however the levels of extractive values fluctuated in response to seasonal variations. To get herbal medicine with good effectiveness it is important to collect it from source at appropriate conditions. Ethanolic plant concentrate gives sennoside proportion in high range at spring, at highest altitude place and at early day.

Keywords: Effectiveness, environmental condition, sennoside, seena, traditional medicine

INTRODUCTION

Sonamukhi, Senna (*Cassia angustifolia*) is a popular herbal medicine due to presence of active compounds in it. Senna is rich in a constituent sennoside that helps to lower the constipation. Different symptoms of constipation involve gastrointestinal tract related disorders such as bowel movement, hardness of stools and feeling of uneasiness. Currently available medication has limitations to cure the disease due to its multifactorial causes of constipation and treatment in single range (Gallagher and Mahony, 2009). Senna is among the most well-known laxative ingredients in teas that contains glycosides

that imparts laxative action by stimulating bowel movement of stomach. To cure of GIT disorders, Senna act as a popular safe and effective due to its laxative action in stomach and so used commonly (Satish *et al.*, 2021).

Collection of genuine raw materials is one of the necessary steps during preparation of a quality product. Season has impact on active principles in medicinal plants. In ancient ayurvedic texts, Charaka and Susrutha mentioned time and seasons for collection of medicinal plant parts used for the medicine preparation. In Ashtangahridaya, the factors affecting quality of the herbs have been stated as (i) period of harvesting, (ii) age, (iii) soil, (iv)

altitude, (v) post-harvest conditions. There is no general rule for the harvesting time for better yield of specific secondary metabolites. The seasonal variation is associated with the vegetative and reproductive stages of the plant, it has direct influence with the variation in chemical constituents of the plants (Jayanthi, 2013). Appropriate period of collection of plant part was mentioned in Charaka samhita (Kalpam), where it was mentioned that the roots (Ashwagandha, Ginger) should be collected only after the completion of seed shedding and in the case of fruits (Pepper, Ficus), time should be near the ripening period i.e., full grown but unripe (Sharma, 2007). Phytochemical changes due to various seasons were reported by Palshikar and Shanmugapandiyan (2023). The purpose of the study is to evaluate specific season, time and place (altitude) for collection of particular herbal raw material like sonamukhi, senna, (*Cassia angustifolia*) so that the active component like sennoside content will be in higher proportion and the effect it will create is to improve potency of pharmaceutical product.

MATERIALS AND METHODS

The plant material was collected from places nearby Pune city, Maharashtra, India in every month of the year 2023-24, i.e., in the rainy season (June, July, August, September), winter (October, November, December, January) and summer (February, March, April, May), from places of different altitude i.e. low (560 meters-Pune city), medium (920 meters-Atkarwadi village) and high (1,412 meters-Sinhgad fort) at morning 6 am. Authentication was done by Taxonomist of the Botanical Survey of India, Pune. A voucher specimen (No.BSI/WRC/100-1/Tech./2023/07) was deposited in the Herbarium of Botanical Survey of India, Pune. Experimentation and evaluation was performed at laboratory of Genba Sopanrao Moze College of Pharmacy, Wagholi, Pune.

Phytochemical screening: The method was followed as detailed by Mahire and Patel (2020). 1000 gm coarse powder of Sonamukhi leaves was taken and extracted by continuous hot extraction method using soxhlet apparatus (Borosil, India) with different solvents of increasing polarity such Petroleum ether, Chloroform, Ethyl acetate (Reachem laboratory chemicals Pvt. Ltd., India), Ethanol (95 %) (Jiang Su Huaxi International Trade Co. Ltd., China) respectively. Each time before extracting with the next solvent, the material was dried. All the extracts were concentrated by distilling the solvent and the extracts were dried on water bath. Then consistency, color, appearance of the extracts and their percentage yield were noted. The extracts obtained from successive solvent extraction were then subjected to various qualitative chemical tests to determine the presence of various phytoconstituents like alkaloids, glycosides, carbohydrates, phenolics and tannins, proteins and amino acids, saponins and phytosterols using reported methods.

HPTLC Analysis: HPTLC of Ethanol extracts (high yield) was performed for sennoside. Weighed accurately 20 mg of each extracts individually into volumetric flask and to it add 10 ml methanol. Dissolved and filtered it with whatman filter paper no. 1 and used for HPTLC analysis.

Standard preparation: Weighed accurately 10 mg of each standard individually into volumetric flask and to it add 10 ml methanol. Dissolved and filtered it with whatman filter paper no. 1 and used.

Procedure: The procedure adopted as per method detailed by Nicoletti (2011). Development of HPTLC plates was performed by using the automatic and reproducibly developing chamber, saturated with the mobile phase for 20 minute at 25⁰ C. The developing solvents were carefully studied before the analysis. The length of the chromatogram run was 70 mm from the point of application. The developed layers were allowed to dry at 100⁰ C for 5 min and then derivatised with a selected solution. The

plate is heated at 100⁰ C for 2- 3 min and then dipped into anisaldehyde sulphuric acid. Finally, the plates are dried for 5 min at 120⁰ C before inspection. All treated plates were then inspected under a UV light at 254 nm under reflectance at a CAMAG TLC visualizer, before and after derivatisation with standard AUC 2650 and using Pet. ether: Ethyl acetate (6:4) mobile phase. Win CATS software 1.4.4 was used for the documentation of derivatised plates.

RESULTS AND DISCUSSION

Analysis of sonamukhi leaves ethanolic extract in various seasons of the year *i.e.*, from January to December in low, medium and high altitude places, Sennoside alkaloidal content vary and its percentage obtained as 6.36 % w/w in April (Summer), 6.12 % w/w in December (Winter) and 6.75% w/w in August (Rainy) season (Table 1). It shows that, alkaloidal content percentage is significantly variable in rainy season *i.e.*, in August, at high altitude place as compared to medium and low altitude place.

HPTLC analytical method was used to confirm the availability of sennoside in ethanolic plant extract with its yield obtained as 3.44 mg/gm in April (summer), 4.36 mg/gm in August (rainy) and 3.32 mg/gm in December (winter) in high altitude. It shows more yield of active compound sennoside in August month *i.e.*, rainy season of the year (Table 2).

It was reported that analysis of Sonamukhi leaves extracts shows presence of alkaloids, sennoside, oils, lipids, glycosides etc. (Junaid, 2020). Results stated that, HPTLC analytical pattern gets vary according to external environmental conditions (Palshikar *et al.*, 2023) Sennoside content is significantly variable in rainy season *i.e.* in August, at morning time and high altitude (Fig. 1, 2). Current research work can be useful for selection of month, place and time of harvesting crude drugs.

Tavhare *et al.* (2016) proved the effect of seasonal variations on the phytoconstituents of *Asvagandha* in relation to lunar cycles. Environmental factors like climate, altitude, rainfall etc. may affect growth as well as quality of bioactive constituents present in it even when it is produced in the same region (Kokate *et al.*, 2004 and Geetha, 2014). Seasonal changes shows variation in yield depends on which seasons receive water additional or less. Increases in spring precipitation led to growth reductions, where as increases in summer precipitation led to increases in growth (Santos *et al.*, 2012).

CONCLUSION

From the Current research work it was observed that, chemical components present in plant material get vary according to external conditions related with the season, time and place of collection of the plant. In month of August sennoside content found to be more at morning and at 638-meter-high altitude.

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CONFLICT OF INTEREST STATEMENT

The author declare that he has no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Table 1: Monthly Variation in sennoside alkaloids with altitude n= 3 P< 0.05.

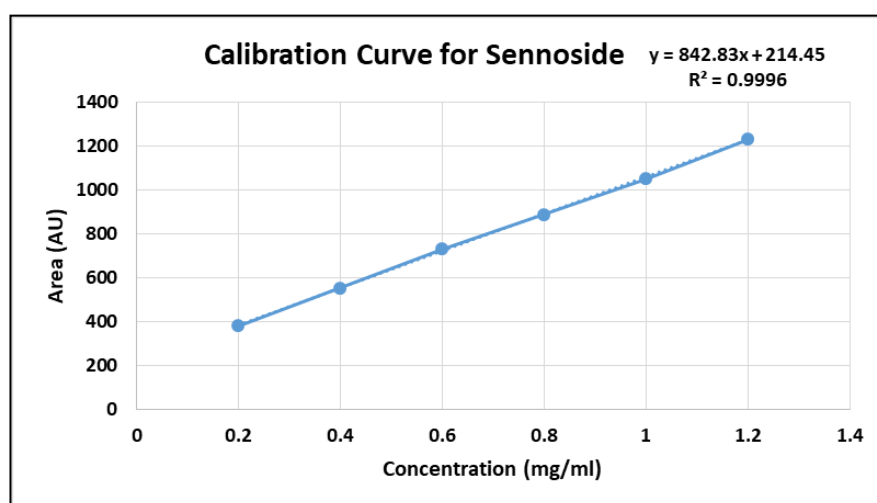
A.	Month											
	1	2	3	4	5	6	7	8	9	10	11	12
L.	5.45 ±0.57	6.10 ±1.00	6.25 ±1.00	6.50 ±1.00	6.60 ±1.00	6.05 ±1.00	6.33 ±1.52	6.37 ±1.52	6.32 ±1.15	6.50 ±1.00	5.27 ±1.15	5.78 ±1.15
M.	5.74 ±0.57	6.23 ±1.00	6.15 ±1.00	6.23 ±1.00	6.13 ±1.00	6.06 ±1.00	6.03 ±1.52	6.33 ±1.52	6.46 ±1.15	6.04 ±1.00	5.72 ±1.15	5.35 ±1.15
H.	5.46 ±0.57	6.14 ±0.57	6.24 ±0.57	6.36 ±0.57	6.39 ±0.57	6.34 ±0.57	6.07 ±1.15	6.75 ±1.15	6.73 ±0.57	6.39 ±0.57	6.05 ±1.00	6.12 ±1.00

A= Altitude, L= Low, M= Medium, H= High

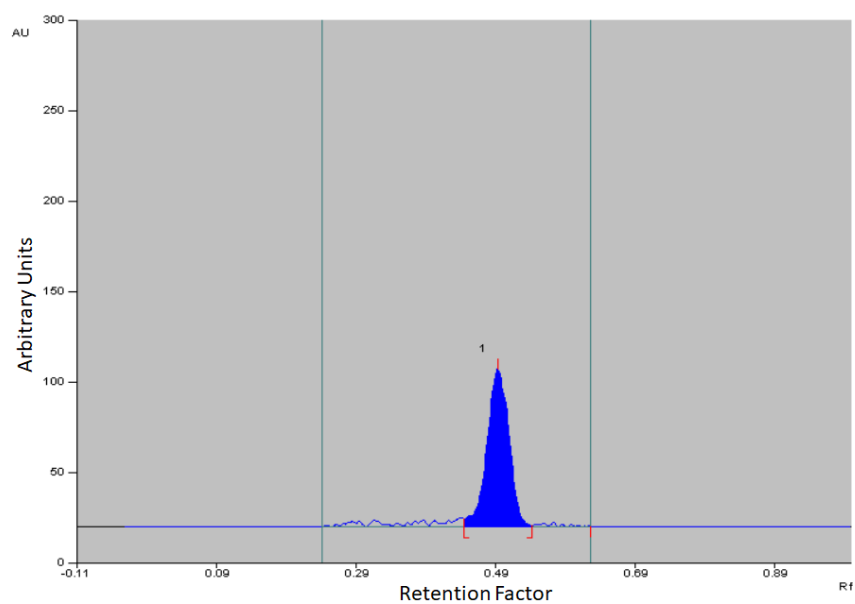
Table 2: Monthly Variation in Sennoside alkaloidal HPTLC Yield mg/g with altitude n= 3 P< 0.05.

A.	Month											
	1	2	3	4	5	6	7	8	9	10	11	12
L.	2.10 ±0.20	2.12 ±0.26	2.23 ±0.55	2.43 ±0.11	2.82 ±0.05	3.10 ±0.05	3.27 ±0.05	3.34 ±0.05	3.23 ±0.11	2.42 ±0.05	2.25 ±0.10	2.62 ±0.60
M.	2.52 ±0.20	2.58 ±0.32	2.84 ±0.65	3.03 ±0.10	3.46 ±0.11	3.78 ±0.05	3.84 ±0.05	3.93 ±0.05	3.82 ±0.15	2.97 ±0.10	2.72 ±0.10	3.15 ±0.60
H.	3.24 ±0.58	2.85 ±0.05	3.25 ±0.60	3.44 ±0.11	3.83 ±0.05	4.17 ±0.05	4.22 ±0.05	4.36 ±0.05	4.24 ±0.11	3.49 ±0.05	3.23 ±0.10	3.32 ±0.05

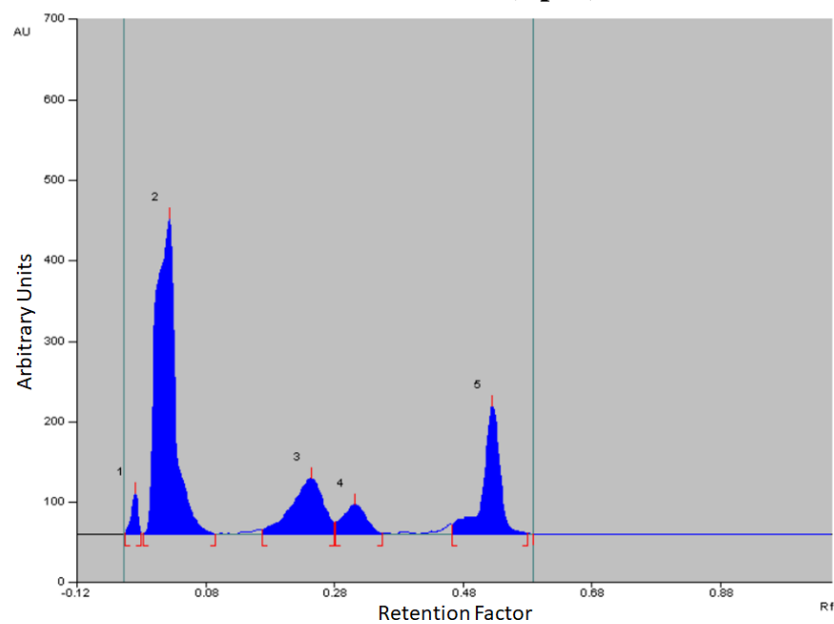
A= Altitude, L= Low, M= Medium, H= High

**Figure: 1 Calibration curve of Sennoside**

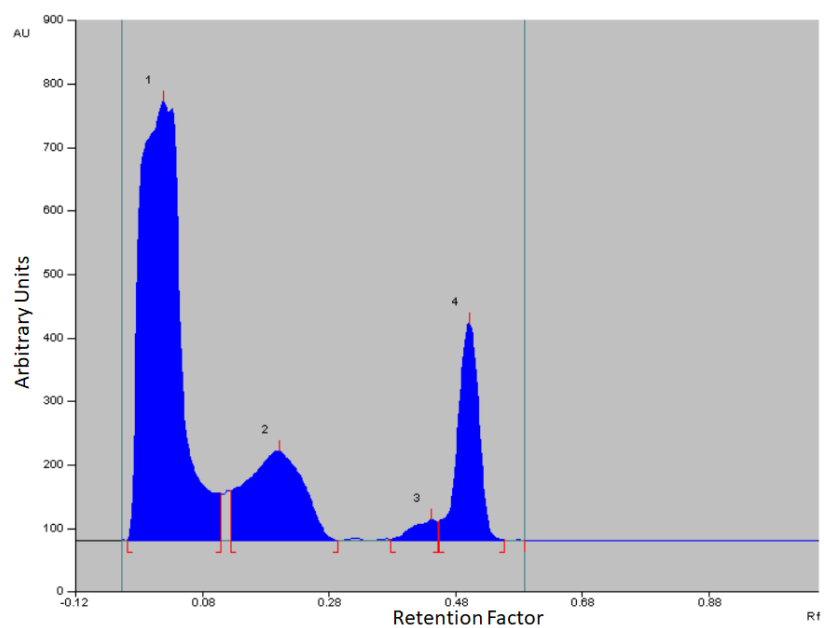
Standard Sennoside



Ethanolic extract (April)



Ethanolic extract (August)



Ethanollic extract (December)

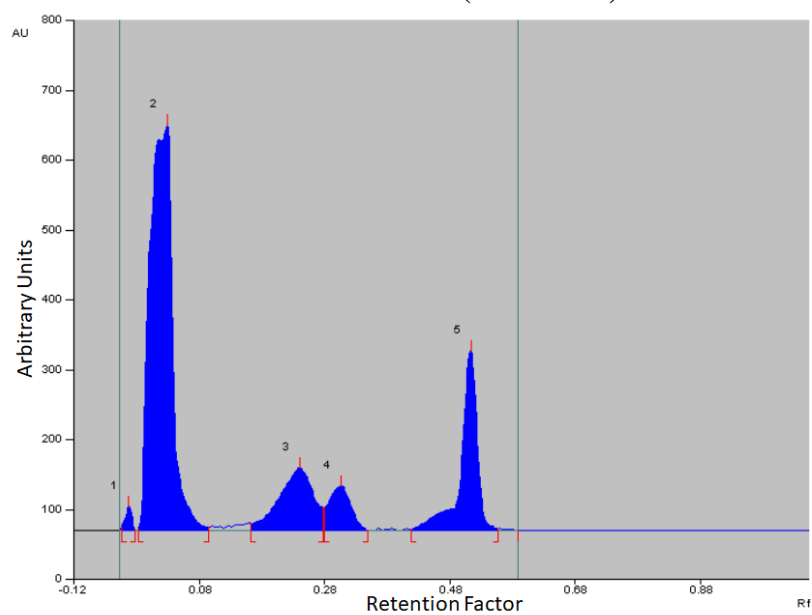


Figure: 2 Chromatogram of Sennoside

Ripening associated physico-chemical changes in star gooseberry [*Phyllanthus acidus* (L.) Skeels], an underutilized fruit of North-East Himalayan region.

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ABSTRACT

Star gooseberry [*Phyllanthus acidus* (L.) Skeels] is a small berry type fruit, found to grow in North-east Himalayan states of India; yellow in colour, having ethnomedicinal uses by the ethnic tribes living here and used for preparation of syrup, juice, jelly, pickle etc. The physico-biochemical changes of the fruit, from their set to harvest is hitherto unknown, which should be considered as prime factor for considering the stage of harvest for its targeted utilization. Therefore, a research attempt was made to evaluate the ripening associated physico-biochemical changes of star gooseberry fruits, grown at Mizoram. Results of the physical parameters revealed that at 2 days after fruit set (DAFS) fruit length (4.40 ± 0.55 mm), diameter (4.60 ± 0.89 mm), weight (0.09 ± 0.01 g); seed length (0.80 ± 0.45 mm) and seed weight (0.01 ± 0.00 g) was low; which got increased and recorded maximum at 24 DAFS [fruit length (15.20 ± 0.84 mm), diameter (20.80 ± 1.30 mm), weight (4.64 ± 0.22 g); seed length (5.20 ± 0.84 mm) and seed weight (0.37 ± 0.07 g)]. However, data of the mentioned parameters clearly indicated an initial period incremental growth, followed by a slow growth as lag phase and subsequently a rapid growth phase, signified double sigmoid growth in star gooseberry fruits. While biochemical parameters like total soluble solids (TSS), TSS:acid ratio, sugars and ascorbic acid content had marked increment and scored highest, whereas titratable acidity and total phenol content was minimum at 24 DAFS. Based on physico-biochemical parameters, it can be concluded that star gooseberry fruits are of optimum maturity for harvesting after 22-24 days from fruit set for further utilization.

Keywords: Ascorbic acid, double sigmoid, firmness, peel colour, pulp recovery, total phenol

INTRODUCTION

Star gooseberry [*Phyllanthus acidus* (L.) Skeels] is a sour yellowish berry fruit of Phyllanthaceae family which is thought to be originated from tropical Madagascar. Within India, apart from southern part; in north-east Himalayan region, which falls under Indo-Myanmar hotspot; this fruit tree is commonly found in states like Mizoram, Manipur, Tripura, Nagaland and Arunachal Pradesh,

where it is either found in home stead gardens or in forest land. Fruits are generally sold in weekly market and consumed raw or with adding salt. Ethnic tribes inhabited here use the unripe and ripe fruit for their health wellness and different ethno-medicinal preparations. The tree bears fruits in cluster during October-November and ripe fruits are available in November-December, during winter months. Ripe fruits are preserved into

sugar syrup and consumed later by the local people. Fruits can be utilized for making syrup, juice, jelly, chutney, sweet preserve, pickle, vinegar etc. (Mazumdar, 2004). Apart from leaves which is commonly reported to have immense medicinal uses like anti-diabetic, hepatoprotective, antimicrobial, analgesic, laxative, antibilious, anti-diarrhoea and diaphoretic properties; fruits also have medicinal uses as liver tonic, stomachic, blood purifier, purgative and as digestive stimulant (Lemmens *et al.*, 1999; Banik *et al.*, 2010).

The fruit is reported to have multiple health benefits and potential post-harvest uses. However, only a negligible quantity is utilized compared to other commercial fruits, perhaps due to its lack of systematic orcharding, leaving it under-utilized. Though the fruits are with immense potentiality of processing and value addition apart from its medicinal uses, still is commercially underutilized in this region. Moreover, there is no scientific report on its ripening behaviour and associated physico-chemical changes, which is quite important for commercial and therapeutic utilization of the fruit. Stages of ripening with its maximum pulp recovery, TSS: acid ratio, ascorbic acid and phenolic content may help to decide the stage of maturity and subsequent use for processing or ayurvedic formulations. So, research attempt was made to evaluate the physico-chemical changes in star gooseberry fruits at different stages after fruit set.

MATERIALS AND METHODS

The experiment was carried out during November-December, 2022 at the Research Laboratory, Department of Horticulture, Aromatic and Medicinal Plants, School of Earth Sciences and Natural Resources Management, Mizoram University situated at Tanhril, Aizawl, Mizoram, India. Fruits, which were used as samples were collected from Chawnpui, Aizawl, India. Initially in all directions of trees *viz.*, north, south, east and

west, two branches were tagged with ribbons during commencement of flowering. Date of fruit set was calculated based on the 50% fruit set on the tagged branch. Subsequently, fruit samples were taken from tagged branches after every 2-day interval from fruit set *i.e.*, pin-head stage of the fruit till it reaches maturity and used for fruit physical and biochemical analysis at laboratory condition.

Various physical characteristics of the fruit were recorded which includes parameters such as fruit length, diameter and weight; seed length and weight; pulp recovery and pulp: seed ratio, fruit firmness and colour. Physical parameters of 5 fruits randomly selected from the harvested lot of each stage of maturity (at 2 days interval) was measured. Fruit length, diameter and seed length was measured using digital vernier caliper (Starrett, USA) and expressed in mm. Fruit weight and seed weight was measured using digital weighing balance (Sartorius AG) and expressed in g. Pulp recovery was calculated by using the below mentioned formula at each stage:

$$\text{Pulp recovery (\%)} = \frac{(\text{Fruit weight} - \text{Seed weight})}{\text{Fruit Weight}} \times 100$$

Pulp: seed ratio was calculated by dividing the fruit weight (g) with seed weight (g) from each sample and done in five samples for each stage and expressed as number. Fruit firmness was measured using digital fruit penetrometer (PCE Instruments, UK) and expressed as Ncm⁻². Fruit peel colour was determined at different stage of maturity using portable colorimeter (Konica Minolta, Singapore) and expressed in L,a,b. Colour chart was developed with corresponding L,a,b value using NIX Color Sensor software.

Fruits were prepared for analysis by cutting and macerating the pulp with mortar and pestle and strained with clean muslin cloth. Analysis was carried out for the following constituents in triplicate. Digital

handheld refractometer (Mettler Toledo, USA) was used for determination of TSS. Total and reducing sugars were estimated with standard procedure (AOAC, 1990) using Fehling's A and Fehling's B reagents and methylene blue as an indicator. Titratable acidity was determined by titrating the extracted juice against N/10 NaOH using phenolphthalein as an indicator (AOAC, 1990). TSS: acid ratio was calculated by dividing the TSS content value with acidity content and expressed in number. Ascorbic acid content was determined using 2,6 Dichlorophenol indophenol dye titration method (Rangana, 1986) and expressed in $\text{mg}100\text{g}^{-1}$ fruit weight. Total phenol content was estimated using folin-ciocalteu reagent and catechol as standard and expressed as mg phenols g^{-1} of fruit (Sadasivam and Manickam, 2005).

Data were analyzed for statistical inference following the statistical method for One-Way Analysis of Variance (ANOVA) described by Sahu (2017). Data were presented as mean \pm standard deviation (SD) of determinations made. Further, Duncan's multiple range test ($P < 0.05$) was done to compare the means.

RESULTS AND DISCUSSION

Temporal changes in fruit dimensions

Results showed that the length and diameter of the star gooseberry fruit consistently increased from 2 days after fruit set (DAFS) to 24 DAFS. Fruit length at 2 DAFS was 4.40 ± 0.55 mm and that increased to maximum (15.20 ± 0.84 mm) at 24 DAFS (Table 1, Fig. 1). Whereas, the minimum diameter (4.60 ± 0.89 mm) was recorded at 2 DAFS, which reached the maximum (20.80 ± 1.30 mm) at 24 DAFS. The continuous development in terms of length and diameter in developing fruit marked the growth of the fruit from fruit set to maturity. Interestingly, a lag phase with minimal changes in both fruit length and diameter was observed from 8 to 12 DAFS. A similar increase in length and diameter was observed in *Phyllanthus*

emblica fruits (Devi *et al.* 2020). However, during development of fruit, Indian gooseberry had initial period of lag phase with slow growth followed by rapid growth (Kishore, 2017).

Development in fruit weight

Fruit weight of star gooseberry had increased throughout the period of growth from fruit set to maturity. At 2 DAFS, weight was the minimum ($0.09 \pm 0.01\text{g}$), which increased and recorded the maximum value (4.64 ± 0.22 g) at 24 DAFS (Table 1). Fruit weight gain was relatively fast at initial period (2-6 DAFS) followed by a period with comparatively slow (8-16 DAFS) and further subsequent acceleration till maturity (18-24 DAFS). Indian gooseberry fruits were reported to have an initial rapid increment in fruit weight followed by a relatively slow growth and a comparatively rapid phase of fruit weight increment at last stage, signified a double sigmoid growth pattern (Singh *et al.*, 2006). Increment in hormonal activity of auxin, gibberellins and cytokinin was thought to be the reason for rapid growth increment (Mariotti *et al.*, 2011; Sosnowski *et al.*, 2023).

Progressive changes in seed length and weight

Length of the seed was found very short (0.80 ± 0.45 mm) at 2 DAFS, and it remained reasonably low till 12 DAFS ($< 3.60 \pm 0.55$ mm). From 14 days after fruit set, seed length was increased rapidly and attained the maximum (5.20 ± 0.84 mm) at 24 DAFS. In parity with the rate of development in seed length, seed weight also accelerated after 12 DAFS. Initially (up to 4 DAFS) seed weight was found negligible (0.01 ± 0.00 g) whereas, at 12 DAFS it was recorded $0.14 \pm 0.01\text{g}$ and reached the maximum ($0.37 \pm 0.07\text{g}$) at 24 DAFS. Seed weight was reported to have significant increment with advancement of fruit growth in Indian gooseberry (Bakshi *et al.*, 2018) and longan (Mukherjee *et al.*, 2023). Due to promotion of growth it was noticed that seed weight increased with the

increasing fruit weight (Drvodelic *et al.*, 2018).

Temporal trends in pulp recovery percentage and pulp: seed ratio

The developing star gooseberry fruit was found quite unique in terms of pulp recovery percentage and pulp: seed ratio. It was observed that recovery percentage of fruit pulp was initially high (ranged between 93.28 ± 1.53 to 94.74 ± 1.11 %) at 2 to 4 DAFS, followed by a consistent dip from 6 to 12 DAFS (ranged from 94.73 ± 0.78 to 82.30 ± 1.71 %) and subsequent increment (ranged between 83.08 ± 1.35 to 92.07 ± 1.48 %) from 14 DAFS to 24 DAFS. Having a close similarity with it, pulp: seed ratio too recorded initially high (ranged between 92.07 ± 1.48 to 18.00 ± 5.23) at 2 to 4 DAFS, followed by significant reduction (from 17.96 ± 2.48 to 4.65 ± 0.56) at 6 to 12 DAFS and further increment (from 4.91 ± 0.43 to 11.61 ± 2.98) at 14 to 24 DAFS. Initially pulp recovery and pulp: seed ratio was high as the seed weight was very low compared to fruit weight; which was followed by reasonable gain in seed weight that may have reduced the pulp recovery and pulp: seed ration, however, as there were significant gains in fruit weight at later stages which significantly impacted higher pulp recovery and pulp: seed ration in star gooseberry. Small seed size has resulted higher flesh recovery in developing litchi fruit (Wang *et al.*, 2017). Pulp recovery which was recorded low, had drastic increment at final stage of fruit growth in developing red fleshed dragon fruit (Lalduhsangi and Mandal, 2023).

Dynamic changes in Fruit firmness

Developing star gooseberry fruits had consistent increment in fruit firmness from 2 DAFS (11.98 ± 1.26 N cm⁻²) to 12 DAFS (27.78 ± 0.68 N cm⁻²). However, from 14 DAFS (24.96 ± 3.37 N cm⁻²) to 24 DAFS (19.67 ± 2.70 N cm⁻²) fruit firmness had reasonably reduced with advent of maturity of the fruit. Fruit maturity and ripening had decreased fruit firmness (Bron and Jacomino, 2006). Ripening of fruit increased the

ethylene production, which impacted the activities of pectic enzyme and caused the loss of firmness (Jeong *et al.*, 2002).

Evolution of fruit colour

Perusal of the data presented in Table 4 and Fig. 2 corresponding colour, it was found that external colour of the fruit peel changed from bright green (L:45.44, a:-19.87, b: 42.60; at 2DAFS) to light green (L:58.02, a:-11.19, b: 55.55; at 18DAFS), to greenish yellow (L:54.24, a:0.65, b: 47.62; at 20DAFS) and finally to yellowish (L:70.69, a:0.57, b: 52.27; at 24 DAFS) at maturity. During fruit maturity, peel colour of carambola also found to change from green to yellow (Martins *et al.*, 2006). Change in fruit colour during ripening is reported to be controlled by growth hormone, gene, transcription factors, enzymes related to biosynthetic pathway of pigments and environmental factors (Wang *et al.*, 2020; Kapoor *et al.*, 2022).

TSS, titratable acidity and TSS: acid ratio

Star gooseberry fruits gained significantly in total soluble solids (TSS) content during its period of fruit growth and development. TSS content which was recorded minimum (2.13 ± 0.12 °Brix) at 2 DAFS increased consistently and become maximum (8.20 ± 0.40 °Brix) at 24 DAFS. However, fruit acidity lowered with advent to ripening. Titratable acidity of the star gooseberry fruit was found highest (3.42 ± 0.15 %) at 2 DAFS and subsequently it reduced throughout the developmental period and scored lowest (2.75 ± 0.24 %) at 24 DAFS. TSS: acid ratio got significant change through the period of fruit growth. It was minimum at 2 DAFS (0.62 ± 0.06) and considerably increased in parity with advancement of fruit growth and development and scored maximum (2.98 ± 0.37) at 24 DAFS. Increment in TSS content while decreasing acidity is the most common biochemical changes reported in both climacteric fruits like tomato, mango and non-climacteric fruits like passion fruit, Kinnow mandarin *etc.* during ripening, which resulted in higher TSS: acid ratio (Moneruzzaman *et al.*, 2008; Goldenberg *et*

al., 2012; Nordey *et al.*, 2016; Nawaz *et al.*, 2020). Sugar content and metabolism of organic acid in ripening fruits are dependent on climacteric and factors responsible for senescence (Obando-Ulloa *et al.*, 2009). Accumulation of sugar in the later stages of fruit development has caused higher TSS and with dropping acidity resulted in high TSS: acid ratio (Ladaniya and Mahalle, 2011). Enzymatic hydrolysis of starch to sugar is responsible for increment in sugar and TSS content in ripened fruit (Bashir *et al.*, 2003) while malic and citric acid, the major players in fruit acidity use to decrease at ripening as malate used as respiratory substrate and citric acid due to catabolism of citrate (Batista-Silva *et al.*, 2018).

Changes in total sugars and reducing sugars contents

Both total sugars and reducing sugars content of the fruit had significant increment during the period of growth. It was observed that total sugar content was lowest ($1.62 \pm 0.02\%$) at 2 DAFS and it consistently increased and attained highest ($5.94 \pm 0.70\%$) at 24 DAFS. Fully matured aonla fruits was reported to have 5-6 % total sugar content (Datta *et al.*, 2024). Likewise, reducing sugar content of the developing fruit was recorded minimum ($1.05 \pm 0.05\%$) at 2 DAFS contrasting with the value at 24 DAFS ($4.73 \pm 0.55\%$), where it was found maximum. With maturation of fruit and advent of ripening sugar generally use to accumulate which was observed in ripening of apples (Li *et al.*, 2012), loquat (Cai *et al.*, 2019), tomato (Moneruzzaman *et al.*, 2008), banana (Li *et al.*, 2011), mango (Nordey *et al.*, 2016), litchi (Fan *et al.*, 2021), grapes (Castellarin *et al.*, 2011) etc. both in climacteric and non-climacteric fruit.

Accumulation of sugar in ripening fruit is related to breakdown of the starch, import of sugar from other plant part, fruit metabolic changes, sugar signaling and hormonal influence (Duran-Soria *et al.*, 2020). Unlike endogenous ethylene, which is prevalent in fruit ripening and sugar increment in

developing climacteric fruit, ABA plays the crucial role and found to have positively correlated with sugar accumulation in maturation of non-climacteric fruit by suppressing the activity of GA and IAA (Alferez *et al.*, 2021).

Accumulation of ascorbic acid and total phenol content over time

The ascorbic acid content of star gooseberry fruit increased during its growth and development. From 2 to 6 days after fruit set (DAFS), the ascorbic acid content was relatively low, ranging from 13.33 ± 2.89 to 18.33 ± 2.89 mg 100 g⁻¹. This was followed by a steady increase from 8 to 14 DAFS (22.42 ± 3.44 to 32.78 ± 2.68 mg 100 g⁻¹) and a rapid rise from 16 to 24 DAFS (36.67 ± 1.67 to 48.75 ± 2.17 mg 100 g⁻¹), reaching a maximum increase of approximately 12 mg 100 g⁻¹ over this period. Similar increases in ascorbic acid content during ripening have been observed in other fruits, such as tomatoes (Yahia *et al.*, 2001), strawberries (Cruz-Rus *et al.*, 2011), and grapes (Cruz-Rus *et al.*, 2010). Biosynthetic enzymes, including D-galacturonate reductase, monodehydroascorbate reductase, and myo-inositol oxygenase, have been positively correlated with ascorbic acid accumulation during fruit ripening (Cruz-Rus *et al.*, 2011), suggesting similar mechanisms may contribute to the observed trends in star gooseberry.

Star gooseberry fruits are rich in total phenolic constituents at immature stage. Besides, the total phenol content of the fruits significantly decreased with the advancement of growth. It was found that the total phenol content of the fruit was the maximum (120.31 ± 2.34 mg CE g⁻¹) at 2 DAFS, which got decreased and become minimum (66.56 ± 1.12 mg CE g⁻¹) at 24 DAFS. Total phenol content of fruit like aonla (Devi *et al.*, 2020), guava (Bashir *et al.*, 2003), peach (Li *et al.*, 2023) etc. reduced drastically with fruit maturation and ripening. Changes in total phenol content have relationship with

the fruit enzymatic activities. It was reported that increased activity of polyphenol oxidase with decreased activity of phenylalanine ammonia lyase, superoxide dismutase, guaiacol peroxidase and catalase are responsible for decrease in total phenol content with fruit maturity and ripening (Zainudin *et al.*, 2014).

CONCLUSION

This study revealed that during the growth and development of star gooseberry fruit, physical parameters, including fruit length, diameter, weight, seed length, and seed weight, increased and reached their maximum at 24 days after fruit set (DAFS; Table 1). These parameters exhibited a double sigmoid growth pattern, characterized by initial rapid growth (2–6 DAFS), a lag phase with slow growth (8–12 DAFS), and final rapid growth (13–24 DAFS). In contrast, pulp recovery and pulp: seed ratio were high from 2 to 10 DAFS, decreased from 12 to 16 DAFS, and increased again from 18 to 24 DAFS. Fruit firmness was low at 2–4 DAFS, peaked from 6 to 14 DAFS, and then consistently declined until 24 DAFS. The fruit skin color, initially green, transitioned to yellow at full maturity (22–24 DAFS). Biochemical parameters, including total soluble solids (TSS), TSS: acid ratio, total and reducing sugars, and ascorbic acid content, increased throughout development, while titratable acidity consistently decreased. Total phenol contents decreased during development but remained relatively high in fully ripened fruit. Therefore, star gooseberry fruits are suitable for harvest at 22–24 DAFS, when they are fully mature and ripened, exhibiting optimal physical and biochemical qualities for utilization.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Fig. 1. Star gooseberry fruits at different stages of development

Table 1: Changes in fruit length, diameter and weight of star gooseberry during fruit growth and development

Stage of Fruit Growth (DAFS)	Fruit Length (mm)	Fruit Diameter (mm)	Fruit Weight (g)
2 DAFS	4.40±0.55 a	4.60±0.89 a	0.09±0.01 a
4 DAFS	5.50±0.79 ab	5.20±0.45 ab	0.19±0.01 ab
6 DAFS	6.40±0.55 b	6.20±0.84 b	0.30±0.01 b
8 DAFS	7.70±0.67 c	9.20±1.10 c	0.48±0.01 c
10 DAFS	7.80±0.84 cd	9.24±0.43 c	0.61±0.03 c
12 DAFS	8.80±0.84 cde	10.40±0.89 cd	0.79±0.02 d
14 DAFS	9.00±0.71 de	11.60±1.14 de	1.01±0.03 e
16 DAFS	9.80±0.45 e	12.60±0.55 ef	1.26±0.04 f
18 DAFS	11.40±0.55 f	13.60±0.55 f	1.85±0.04 g
20 DAFS	12.20±0.84 f	15.20±0.45 g	2.49±0.16 h
22 DAFS	14.60±0.55 g	17.40±0.55 h	3.76±0.13 i
24DAFS	15.20±0.84 g	20.80±1.30 i	4.64±0.22 j

*DAFS=Days after fruit set. The values are mean ± SD of determinations made in five times. Mean values followed by different letters within same column differ significantly ($p < 0.05$).

Table 2: Changes in seed length and seed weight of star gooseberry during fruit growth and development

Stage of Fruit Growth (DAFS)	Seed Length (mm)	Seed Weight (g)
2 DAFS	0.80±0.45 a	0.01±0.00 a
4 DAFS	1.80±0.45 b	0.01±0.00 a
6 DAFS	2.40±0.55 bc	0.02±0.00 a
8 DAFS	3.20±0.45 cd	0.03±0.01 a
10 DAFS	3.40±0.55 cde	0.05±0.01 a
12 DAFS	3.60±0.55 def	0.14±0.01 b
14 DAFS	4.20±0.45 defg	0.17±0.01 bc
16 DAFS	4.40±0.55 efg	0.18±0.01 bc
18 DAFS	4.60±0.55 fg	0.21±0.04 c
20 DAFS	4.80±0.84 g	0.22±0.04 c
22 DAFS	5.00±0.71 g	0.31±0.02 d
24DAFS	5.20±0.84 g	0.37±0.07 e

*DAFS=Days after fruit set. The values are mean ± SD of determinations made in five times. Mean values followed by different letters within same column differ significantly (p < 0.05).

Table 3: Changes in pulp recovery percentage, pulp: seed ratio and fruit firmness of star gooseberry during fruit growth and development

Stage of Fruit Growth (DAFS)	Pulp recovery (%)	Pulp: seed ratio	Fruit firmness (N cm ⁻²)
2 DAFS	93.28±1.53 de	13.89±2.69 cd	11.98±1.26 a
4 DAFS	94.74±1.11 e	18.00±5.23 d	17.55±1.65 b
6 DAFS	94.73±0.78 e	17.96±2.48 d	26.13±1.93 bc
8 DAFS	92.80±1.97 de	12.88±3.25 c	26.79±2.92 cd
10 DAFS	91.43±1.95 d	10.67±2.17 bc	27.03±2.63 de
12 DAFS	82.30±1.71 a	4.65±0.56 a	27.78±0.68 de
14 DAFS	83.08±1.35 a	4.91±0.43 a	24.96±3.37 de
16 DAFS	85.82±0.81 b	6.05±0.43 a	24.67±2.84 de
18 DAFS	88.60±1.84 c	7.77±1.26 ab	24.49±1.25 de
20 DAFS	91.24±1.07 d	10.42±1.25 bc	23.82±0.46 e
22 DAFS	91.75±0.74 d	11.12±1.17 bc	22.45±1.10 e
24DAFS	92.07±1.48 de	11.61±2.98 bc	19.67±2.70 e

*DAFS=Days after fruit set. The values are mean ± SD of determinations made in five times. Mean values followed by different letters within same column differ significantly (p < 0.05).

Table 4: Changes in external colour of star gooseberry fruit during fruit growth and development

Stage of Fruit Growth (DAFS)	L	A	b
2 DAFS	45.44	-19.87	42.6
4 DAFS	44.65	-14.99	36.07
6 DAFS	49.87	-12.78	43.32
8 DAFS	49.41	-10.96	39.83
10 DAFS	41.58	-9.87	41.12
12 DAFS	50.12	-13.48	41.41
14 DAFS	41.54	-12.11	35.51
16 DAFS	51.84	-6.93	49.16
18 DAFS	58.02	-11.19	55.55
20 DAFS	54.24	0.65	47.62
22 DAFS	63.28	3.11	43.59
24DAFS	70.69	0.57	52.27

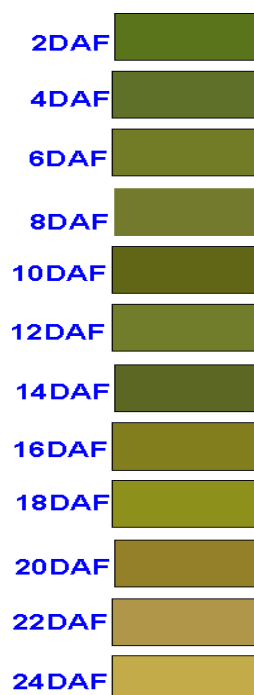
**Fig. 2. Peel colour of star gooseberry at fruit developmental stages**

Table 5: Changes in total soluble solids (TSS), titratable acidity and TSS: acid ratio of star gooseberry during fruit growth and development

Stage of Fruit Growth (DAFS)	TSS (^o Brix)	Titratable Acidity (%)	TSS: acid ratio
2 DAFS	2.13±0.12 a	3.42±0.15 d	0.62±0.06 a
4 DAFS	2.73±0.31 ab	3.37±0.20 cd	0.81±0.14 ab
6 DAFS	3.13±0.50 bc	3.22±0.35 bcd	0.97±0.28 abc
8 DAFS	3.80±0.40 cd	3.12±0.31 abcd	1.22±0.30 bcd
10 DAFS	3.93±0.42 cde	3.10±0.15 abcd	1.27±0.12 cd
12 DAFS	4.40±0.53 de	3.05±0.20 abcd	1.44±0.30 d
14 DAFS	4.67±0.42 e	2.95±0.26 abc	1.58±0.17 d
16 DAFS	4.73±0.50 e	2.88±0.18 ab	1.64±0.27 d
18 DAFS	6.53±0.70 f	2.86±0.30 ab	2.28±0.27 e
20 DAFS	7.27±0.31 fg	2.82±0.17 ab	2.58±0.38 ef
22 DAFS	7.87±0.70 gh	2.78±0.25 ab	2.83±0.15 f
24DAFS	8.20±0.40 h	2.75±0.24 a	2.98±0.37 f

*DAFS=Days after fruit set. The values are mean ± SD of determinations made in five times. Mean values followed by different letters within same column differ significantly (p < 0.05).

Table 6: Changes in total sugar and reducing sugar content of star gooseberry during fruit growth and development

Stage of fruit growth (DAFS)	Total sugar (%)	Reducing sugar (%)
2 DAFS	1.62±0.02 a	1.05±0.05 a
4 DAFS	2.17±0.07 ab	1.23±0.07 a
6 DAFS	3.08±0.43 bc	1.78±0.38 ab
8 DAFS	3.65±0.43 cd	2.36±0.25 bc
10 DAFS	3.75±0.80 cde	2.65±0.26 cd
12 DAFS	3.81±0.66 cde	2.89±0.17 cde
14 DAFS	3.96±0.23 cdef	3.16±0.69 def
16 DAFS	4.23±0.44 cdef	3.28±0.57 def
18 DAFS	4.55±0.71 def	3.42±0.23 def
20 DAFS	4.87±1.02 efg	3.68±0.59 ef
22 DAFS	5.06±0.86 fg	3.84±0.61 f
24DAFS	5.94±0.70 g	4.73±0.55 g

*DAFS=Days after fruit set. The values are mean ± SD of determinations made in five times. Mean values followed by different letters within same column differ significantly (p < 0.05).

Table 7: Changes in ascorbic acid and total phenol content of star gooseberry during fruit growth and development

Stage of Fruit Growth (DAFS)	Ascorbic Acid (mg 100g ⁻¹)	Total Phenol (CE mg g ⁻¹)
2 DAFS	13.33±2.89 a	120.31±2.34 j
4 DAFS	15.56±2.55 ab	118.72±2.44 i
6 DAFS	18.33±2.89 bc	107.48±2.49 h
8 DAFS	22.42±3.44 cd	97.92±2.14 g
10 DAFS	24.24±2.78 de	95.92±2.25 fg
12 DAFS	28.33±2.52 e	93.28±1.28 f
14 DAFS	32.78±2.68 f	89.62±1.73 e
16 DAFS	36.67±1.67 fg	83.95±1.49 d
18 DAFS	39.72±2.10 gh	79.84±2.87 c
20 DAFS	42.22±1.73 hi	74.63±1.55 b
22 DAFS	45.42±1.91 ij	69.74±1.32 a
24DAFS	48.75±2.17 j	66.56±1.12 a

*DAFS=Days after fruit set. The values are mean ± SD of determinations made in five times. Mean values followed by different letters within same column differ significantly (p < 0.05).

Oregano aromatherapy as support management in alleviation of depression: A prospective-interventional study

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ABSTRACT

*Managing stress and having a positive uplifting of mood and promoting relaxation makes aromatherapy a great alternative treatment for depression. It has also been shown to improve sleep quality, decrease stress levels, and aid in pain relief. Using essential oils is not meant to replace medical attention, but oils paired with therapy, medicine, or lifestyle changes can greatly help mental wellness. One of the biologically active medicinal herbs is *Origanum vulgare* L. (*O.vulgare*) of the Lamiaceae family, which is reported to have multiple biological activities. Recent studies have shown in- vivo using oregano essential oil showed increased vigor and lowered stress levels in patients suffering from anxiety and depression. In this study, the objective was to evaluate the potency of inhalation intervention of volatile oil of *O. vulgare* in human depression using the conventional PHQ-9 ("Patient Health Questionnaire-9") and PROMIS depression scale. Aromatherapy study results suggest that essential oils help in treating depression in over 136 days. With these observed outcomes, aromatherapy was proven to be effective as a supplementary treatment throughout this study.*

Keywords: Aromatherapy, depression, Lamiaceae, Oregano, *Origanum vulgare*

INTRODUCTION

One of the main causes of disability is depression, which affects 4.4% of young people in the United States (USA) and 57 million people in India, which makes up 18% of the world's population. For clinical depression, Western medicine often recommends psychotherapy and selective serotonin reuptake inhibitors (SSRIs); however, when these approaches don't work because of side effects or patient noncompliance, other medications are

frequently employed (Saeed *et al.*, 2019; Haller *et al.*, 2019). One possible non-pharmacologic approach that has received a lot more attention recently is aromatherapy. Aromatherapy is the application of essential oils for medicinal purposes. When essential oils are inhaled, the aroma molecules reach the brain straight from the olfactory nerves, particularly affecting the brain's emotional center, the amygdala. In general, essential oils are thought to have mood-altering, relaxation-enhancing, anxiety-reducing, and

stress-reduction properties that may help cure depression (Tan *et al.*, 2023). The essential oil content of *Origanum vulgare* L. (*O. vulgare*), a significant medicinal herb in the Lamiaceae family, has been shown to exhibit a range of biological effects (Marrelli *et al.*, 2016). In traditional phytotherapy, *O. vulgare*, a fragrant Mediterranean herb, is extremely beneficial. Its medicinal significance stems from its historical use in the treatment of many ailments such as headaches, depression, pruritus, menstruation complications, cold, whooping and convulsive cough and gastrointestinal disorder (Fikry *et al.*, 2019; Lombrea *et al.*; 2020; Singh *et al.* 2024). Most of the research on this species of plant has focused on its antibacterial, antifungal, and anti-inflammatory qualities instead of how it directly affects mood. Over the past years, few *in-vivo* studies have assessed the effects of oregano essential oil in anxiety and depression and has been reported to elevate vitality and decrease stress (Amiresmaeili *et al.*, 2018; Capatina *et al.*, 2021). This study analyses the efficiency of volatile oil of *O. vulgare* inhalation in treating depression in test subjects using the often utilized PHQ-9 ("Patient Health Questionnaire-9") and the PROMIS (Patient-Reported Outcomes Measurement Information System) depression scale (Kroenke *et al.*, 2021).

MATERIALS AND METHODS

Ethical approval

All the protocols used in current study were carried out in compliance with the Helsinki Declaration and its subsequent revisions. The study was approved by the Institutional Ethics Committee of Chitkara University, Faculty of Pharmacy (Protocol no. EC/NEW/INST/2023/531/254A).

Study Design

This prospective-interventional study was conducted in 2024 at different rural and urban regions of Patiala district of Punjab including of Rajpura, Banur, Kalomajra,

Ramnagar and Chitkara University, Punjab, India. The focus was to study the effect of *O. vulgare* aroma therapy on stress (personal and professional related stress) and depression. The study population comprised of general population (Housewives, shop owners, workers on daily wages) and some faculty members of different schools and colleges of Chitkara University, Punjab, India, who met the inclusion and exclusion criteria for the study. Informed consent was taken from the subjects before participation in the study. (In English, Hindi and Native Language). The sample size was calculated by epi info, as (n) 207 with 95% Confidence Interval of the study and 5 % margin of error. The alpha level of the study was considered as $p = 0.05$.

Inclusion and Exclusion criteria

The housewives, shop owners, workers on daily wages and working professionals of Chitkara University, Punjab who were aged 18 years to 55 years (male and female) and were willing to participate in the study were included. They were screened for the stress and depression symptoms in accordance with PROMIS and PHQ Rating scale. The pregnant women employees, individuals with co-morbidities associated with brain, skin and mood disorders as well as individuals involved in the participation in another interventional study, within past 3 months were excluded from the study.

Intervention

Aromatherapy was administered for a total duration of 4.5 months, using *O. vulgare* essential oil, known for their stress-relieving properties. Participants received aromatherapy sessions under standardized conditions, ensuring consistency in exposure. Initially during first 3 sessions of aromatherapy, 2 to 3 drops of oil were applied on forehead and was gradually increased to 4 to 5 drops during the rest 7 sessions of the therapy. The participant underwent a total of 10 intervention

sessions, with the overall duration segmented into distinct phases. Initially, the intervention was divided into two primary phases, Phase I and Phase II, to determine which phase produced a more significant effect on the participant's health. Subsequently, the results of the transition from Phase I to Phase II were evaluated, followed by an analysis of the effects from Phase II to Phase III. Finally, to assess the overall impact, the cumulative effect from Phase I to Phase III was statistically analysed, with all findings detailed in the results section.

Measuring Tools

Depression scales

To reach a diagnosis, a thorough clinical evaluation that includes a detailed interview, symptom assessment, and consideration of other criteria is required; hence, a single scale or questionnaire is often insufficient. Therefore, the level of depression in the study participants was assessed using two commonly used published questionnaires: PROMIS Emotional Distress– Depression – Short Form (Level -2 Depression) and Patient Health Questionnaire -9 (PHQ-9).

Patient Health Questionnaire -9 (PHQ-9)

In order to evaluate the severity of depression, it is often useful to employ self-administered questionnaires like the Patient Health Questionnaire 9 (PHQ-9). This specific Questionnaire consists of a set of nine questions regarding the details of suicidal thoughts, levels of exhaustion, disturbances of sleep, depressed moods, and a lack of interest in activities once enjoyed. When factoring in all the items, the total score can range between 0 to 27, categorizing depression as none, mild, moderate, and severe. The scoring methodology of each item ranges from “not at all” to “almost every day” which is a range from zero to three. Because of its high accuracy, simple practicality, and capability for longitudinal analysis, it is

popular in both clinical and investigative environments (Ford *et al.*, 2020).

PROMIS Emotional Distress – Depression – Short Form (Level -2 Depression)

The NIH created the PROMIS Depression Scale to determine depressive symptoms in multiple populations. It does not measure grade depression, instead measures factors of emotional pain such as sadness, social withdrawal, and feelings of worthlessness. PROMIS is useful in evaluating the results of research studies as well as longitudinal changes in symptoms over time. It's systematic scoring system improves the reliability of research and clinical assessments which boosts the effectiveness of evaluations of treatment outcomes even for aromatherapy (Kroenke *et al.*, 2021).

Statistical Analysis

Both analysis and description are provided for the results of the current research. The sample population's data was revealed to be normally distributed so a parametric test could be opted. To assess the difference in means owing to the effect of *O. vulgare* essential oil through aromatherapy on depression, a paired T-test was carried out.

RESULTS AND DISCUSSION

Phase I

The duration of this phase extended over sixty-one days from 15-August 2024 to 15-October 2024. The PHQ-9 and PROMIS questionnaire for assessing depression severity was provided to all the study participants pre and post aromatherapy. The significant reduction ($p = 0.001$) was seen in the PHQ-9 scores of participants post aromatherapy ($M = 15.4$, $SD = 3.43$) in comparison to pre-aromatherapy ($M = 22.3$, $SD = 2.28$) scores. Similarly, there was a significant drop $t(199) = 54.61$. ($p = 0.001$) in PROMIS Questionnaire scores of study participants post aromatherapy ($M = 18.02$, $SD = 3.52$) in contrast to the pre

aromatherapy scores ($M = 26.49$, $SD = 3.56$). the results were found significant as $t(199) = 56.02$, $p = 0.001$. The analysis of PROMIS Questionnaire for the study participants for Phase I has been presented in table 1 and graphically in Figure 1.

Phase II

The duration of this phase extended over 75 days from 15th October, 2024 to 29th December, 2024. The PHQ-9 and PROMIS questionnaire for assessing depression severity was provided to all the study participants pre and post aromatherapy. The PHQ-9 questionnaire result exhibited significant difference in the scores for the second phase of aromatherapy ($M = 5.60$, $SD = 3.14$) and the first phase of aromatherapy ($M = 15.4$, $SD = 3.43$) conditions; $t(199) = 59.61$, $p = 0.001$. Similarly, PROMIS Questionnaire Result indicated there was a significant difference in the scores for the second phase of aromatherapy ($M = 7.25$, $SD = 3.09$) and the first phase of aromatherapy ($M = 18.02$, $SD = 3.52$) conditions; $t(199) = 66.26$, $p = 0.001$. The analysis of PROMIS Questionnaire for the study participants for Phase II has been presented in Table 2 and Figure 2.

Phase I to III

The total duration of this study extended over 136 days from 15th August, 2024 to 29th December, 2024. The PHQ-9 questionnaire result indicated significant difference in the scores for post aromatherapy ($M = 5.60$, $SD = 3.14$) and pre-aromatherapy ($M = 22.32$, $SD = 2.28$) conditions; $t(199) = 88.26$, $p = 0.001$. The PROMIS questionnaire result showed significant difference in the scores for post aromatherapy ($M = 7.25$, $SD = 3.09$) and pre-aromatherapy ($M = 26.49$, $SD = 3.56$) conditions; $t(199) = 88.0$, $p = 0.001$. The analysis of PROMIS Questionnaire *via* t-Score for Phase I to III of this study and the interpretation of t-scores for PROMIS

Scale has been represented in Table 3 and Table 4 respectively. Post oregano aromatherapy, the significant shift in the t-score values were noted with most subjects having t-score of < 55 . The pre and post therapy scores reduction *via* PHQ-9 scale and PROMIS scale has been represented in Figure 3 & Figure 4 respectively. Both the scales showed reduction in the mean values. Additionally, figure 5 denotes study population with their t-score in PROMIS Scale for Phase I to III of study. These results from the scores from both the questionnaires indicate the effectiveness of essential oil in the management of depression.

Results showed that depression scores significantly decreased after the intervention, implying that *O. vulgare* may be effective in reducing emotional discomfort. Since both Phase I and Phase II transitions showed significant symptom reduction, data also indicated that benefits were different between stages, supporting the idea that exposure to aromatherapy over time intensifies therapeutic effects. Numerous chemical moieties belonging to the terpenes and terpenoidal groups have previously been documented in published literature for their effectiveness against depression, having been found in the volatile oil of *O. vulgare*. Among the examples are sesquiterpenoids (β -caryophyllene), bicyclic monoterpenes (α -pinene, borneol), acyclic monoterpenes (geraniol, linalool), and monocyclic monoterpenes (thymol, carvacrol) (Singh *et al.* 2024). Together these might perform neurotransmitter-modulating, anti-inflammatory, and antioxidant activities to fight depression. According to research, carvacrol is beneficial in the central nervous system (CNS), and that could be the reason why it has potential antidepressant properties. It's also been proved that carvacrol modulates neurotransmitter systems, such as serotonin, which is essential to mood regulation (Imran *et al.*,

2022). Thymol has been mentioned to be neuroprotective and can act in GABAergic modulation, which could account for calming effects on the nervous system (Banerjee *et al.*, 2022). Para-cymene reportedly attenuates the symptoms of anxiety and depression through modulation of receptor signaling pathway, neuron projection, and neuroactive ligand-receptor interaction (Khanh *et al.*, 2024). Widely known for its relaxant, anti-anxiety, and mood elevating capabilities, linalool has direct effects on the GABAergic system, which helps with relaxation, great contribution towards anxiety and depression alleviation (De Lucena *et al.*, 2020). Also, gamma-terpinene, which is a monoterpene antioxidant, is believed to play a role in elevating the mood through oregano oil. It may improve mental health indirectly, including depression, through reducing oxidative stress (Amiresmaeili *et al.*, 2018). These elements working together may be the cause of improved mood and reduction of depression symptoms. More research is needed, as many of the ingredients incorporated in the essential oils or whole plant extracts have not been tested adequately in depression models. For example, they found that terpene carvone and glycosylated flavonoid linarin had a pretty good antidepressant principle; however, the actual mechanism of action is still not known (Guzmán-Gutiérrez *et al.*, 2023; Fonseca *et al.*, 2023). Gamma-terpinene, believed to be a monoterpene with antioxidant qualities, is also considered a contributor to the ability of oregano oil to elevate mood. It may indirectly improve mental health, including depression, by reducing oxidative stress. These elements working together may be the cause of improved mood and reduction of depression symptoms. There is room for more research because many of the ingredients included in the essential oils or whole plant extracts have not been thoroughly tested in depression models. For

example, terpene carvone and glycosylated flavonoid linarin were revealed to have a good antidepressant principle, however their exact mechanism of action is yet unknown. The effectiveness of hispidulin (a flavonoid) and its associated processes in depressive disorders can be investigated further. It has been shown to improve social withdrawal behavior in mice (Mouri *et al.*, 2020).

Limitations

The population being studied in this study is diverse in terms of age, gender, and ethnicity. As a result, various populations and environmental condition may produce different results. *O. vulgare* is a rather diverse species. Geographical locations, environmental conditions, and climate all have a considerable impact on the makeup of its essential oils. For a meaningful link with its diverse biological activities, it is essential to standardize its volatile oil content and extracts unique to each place based on primary components.

CONCLUSION

The overall findings of this study are in favor of using aromatherapy as a supplemental treatment for depression. Given the notable decline in depression scores, *O. vulgare* aromatherapy may be a safe, all-natural way to enhance mental health, especially in high-stress settings like corporate offices, educational institutions, and many more.

CONFLICT OF INTEREST STATEMENT

The author declare that he has no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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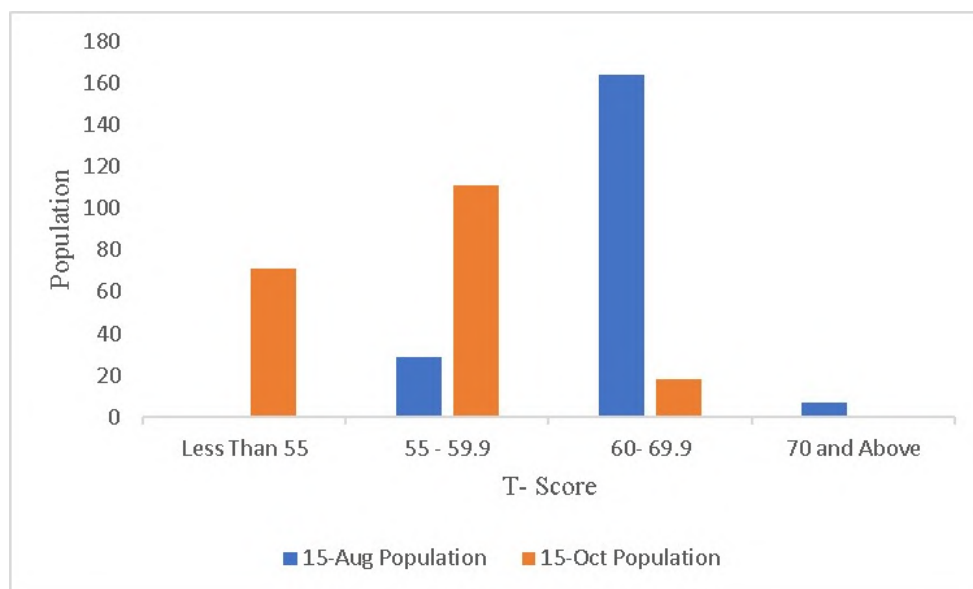


Figure 1: Graphical representation of population with their t-Score in PROMIS Scale for Phase I to II of study.

Table 1: Analysis of PROMIS Questionnaire *via* t-Score for Phase I

t- score	15 th August, 2024	15 th October, 2024
	Population	Population
Less Than 55	0	71
55 - 59.9	29	111
60 - 69.9	164	18
70 and above	7	0

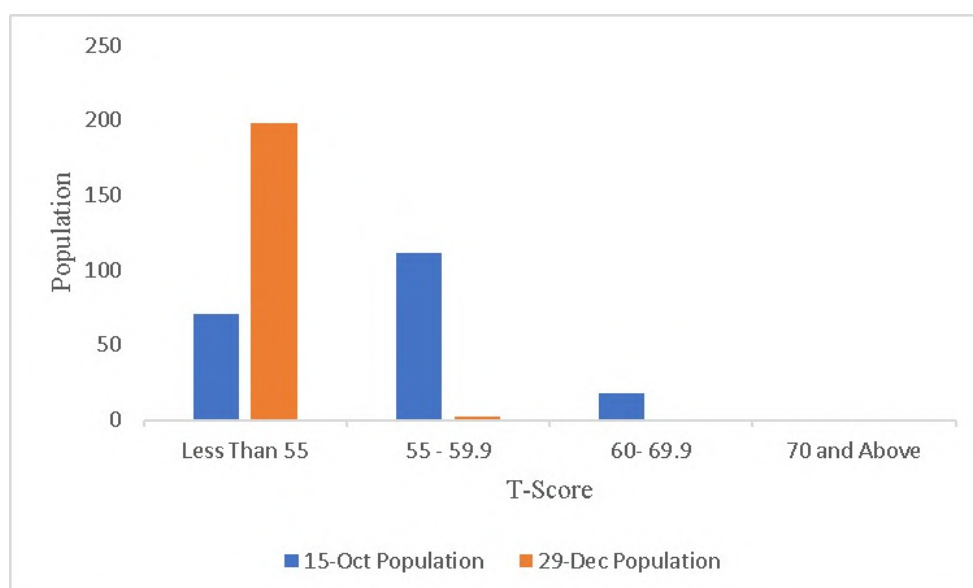


Figure 2: Graphical representation of population with their T-Score in PROMIS Scale for Phase II to III of study.

Table 2. Analysis of PROMIS Questionnaire via t-Score for Phase II:

t- score	15 th October, 2024	29 th December, 2024
	Population	Population
Less Than 55	71	198
55 - 59.9	111	2
60 - 69.9	18	0
70 and above	0	0

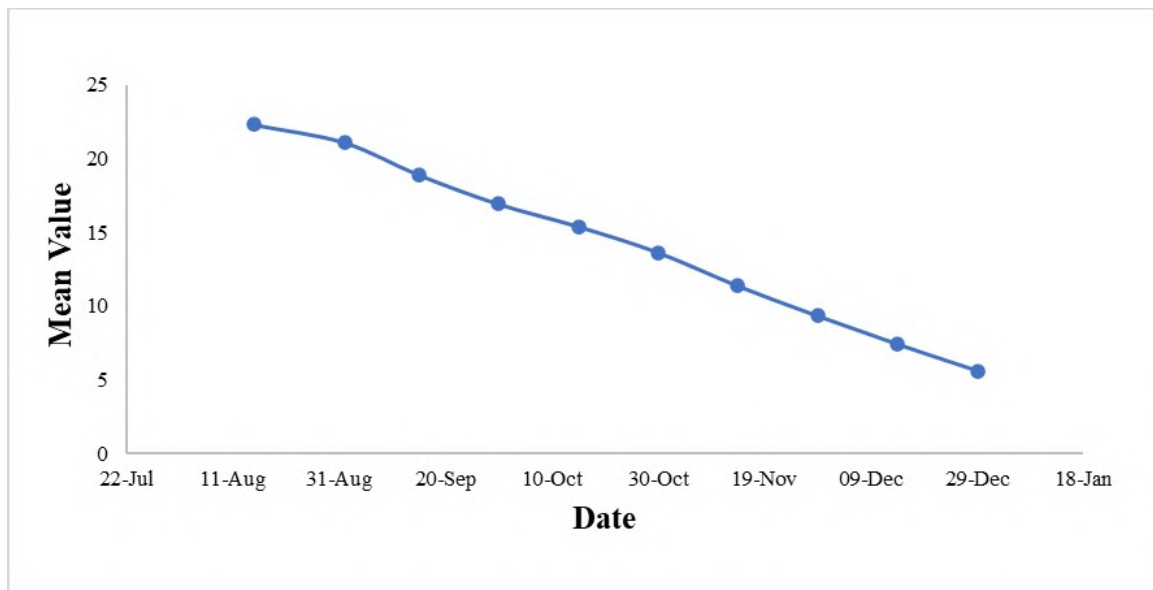
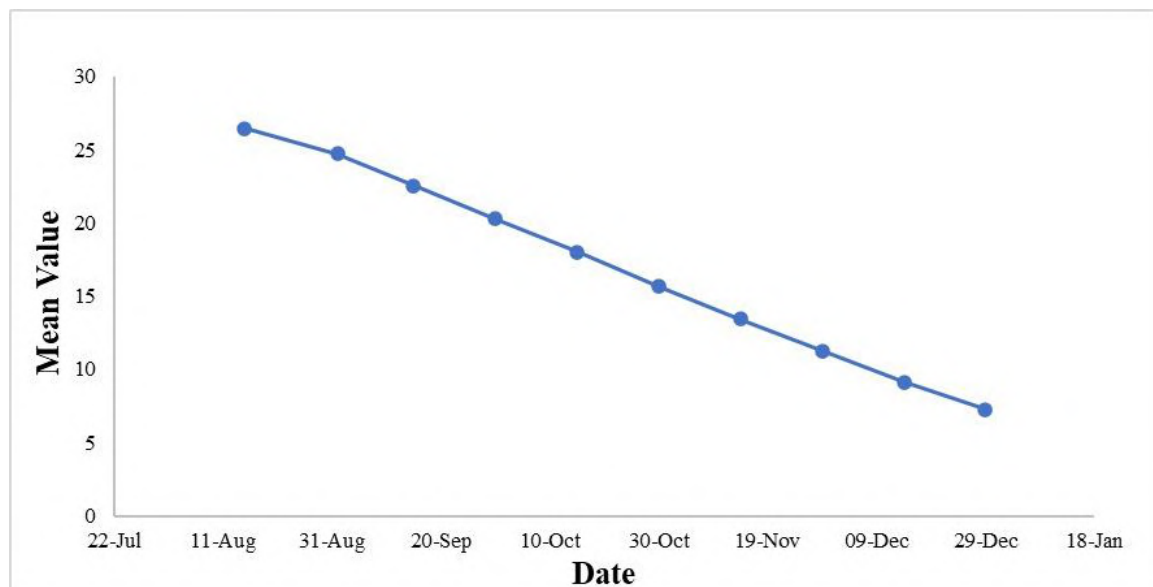
**Figure 3: Graphical representation for pre and post therapy scores reduction via PHQ Scale.****Figure 4: Graphical representation for pre and post therapy scores reduction via PROMIS Scale.**

Table 3: Analysis of PROMIS Questionnaire *via* t-Score for Phase I to III

t- score	15 th August, 2024	29 th December, 2024
	Population	Population
Less Than 55	0	198
55 - 59.9	29	2
60 - 69.9	164	0
70 and above	7	0

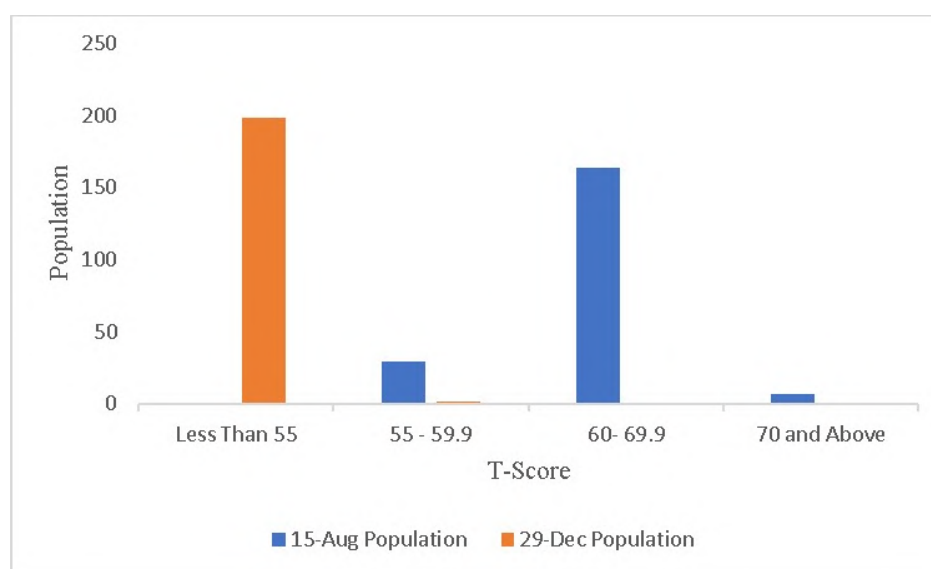


Figure 5: Graphical representation of study population with their t-score in PROMIS Scale for Phase I to III of study.

Table 4: Interpretation of t- scores for PROMIS Scale

T-Score	Interpretation
Less than 55	None to Slight Depression
55.0 – 59.9	Mild Depression
60.0 - 69.9	Moderate Depression
70 and above	Severe Depression

Zinc deficiency and toxicity-induced alterations of chloroplast pigments in acid lime [*Citrus aurantiifolia* (Christm.) Swingle]

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ABSTRACT

Zinc is an essential micronutrient involved in various physiological processes in plants, particularly in chloroplast development and pigment biosynthesis. Although application of zinc have been widely studied in citrus crops, limited information exists on the chloroplast pigment response of acid lime to graded zinc levels applied through substrate under controlled greenhouse conditions. This study aimed to fill that gap by evaluating how zinc deficiency and toxicity affect chlorophyll a, chlorophyll b, total chlorophyll, chlorophyll a/b ratio, and carotenoid contents over a two-year period. A pot experiment was conducted using acid lime seedlings grown in acid-washed river sand under greenhouse conditions. Seven zinc concentrations (0.0 to 15.0 mM/l ZnSO₄) were applied at regular intervals, and pigment contents were quantified at six growth stages using spectrophotometry. The results demonstrated a biphasic zinc response, where moderate zinc application (particularly at 7.5 mM/l) significantly enhanced chlorophyll a, b, total chlorophyll, and maintained a balanced chlorophyll a/b ratio, indicative of optimal chloroplast function. Both zinc deficiency (0.0 mM/l) and toxicity (≥ 12.5 mM/l) led to pigment degradation, reduced photosynthetic efficiency, and signs of physiological stress. Carotenoid accumulation was elevated under Zinc-deficient and Zinc-toxic treatments, suggesting a photoprotective response against oxidative damage. In conclusion, the study identifies 7.5 mM/l zinc as the optimal concentration for maximizing chloroplast pigment stability and minimizing stress-induced degradation in acid lime.

Keywords: carotenoids, chloroplast pigments, *Citrus aurantiifolia*, deficiency, nutrient stress, toxicity, zinc,

INTRODUCTION:

Acid lime [*Citrus aurantiifolia* (Christm.) Swingle], commonly known as key lime or Mexican lime, holds significant economic importance, particularly for small and marginal farmers, because of its year-round flowering and fruiting habit, which ensures regular income (Ladaniya *et al.*, 2020). The fruits are rich in vitamin C, organic acids, flavonoids, and essential oils, which confer antimicrobial, antioxidant, and anti-

inflammatory properties (Abirami *et al.*, 2022) like as other citrus (Deb *et al.*, 2024, Deb *et al.*, 2025). These attributes make acid lime a valuable crop not only in the fresh market but also in processed products like beverages, pickles, and nutraceuticals. Furthermore, with the increasing global demand for natural, functional foods and the growing emphasis on climate-resilient crops, acid lime contributes meaningfully to nutritional security, agro-industry

diversification, and sustainable horticulture (Kumar and Jain, 2018).

Micronutrients play a fundamental role in plant growth, and among them, zinc is especially crucial for acid lime cultivation. Zinc is involved in numerous physiological functions, including enzyme activation, protein metabolism, and hormone regulation, all of which are essential for overall plant development and fruit production (Fageria *et al.*, 2002). Zinc also plays a direct role in the synthesis of chlorophyll and maintenance of chloroplast structure, thereby influencing the efficiency of photosynthesis (Cakmak, 2000). In many tropical fruit-growing regions, zinc deficiency is common due to calcareous soils or high pH conditions, which limit zinc availability to roots (Alloway, 2008). Deficiency symptoms in acid lime include Interveinal chlorosis, small and narrow leaves, shortened internodes, and poor flowering and fruit set (Mousavi *et al.*, 2013). These symptoms lead to a noticeable decline in tree vigour, productivity, and fruit quality (Tisdale *et al.*, 1993). On the other hand, excessive application of zinc can cause toxicity, which is expressed as leaf necrosis, chlorosis, and restricted root and shoot growth, ultimately inhibiting photosynthetic function and plant development (Zhao and McGrath, 2009).

A well-balanced zinc supply supports better chlorophyll retention, enhances photosystem activity, and improves plant resilience under environmental stress (Zhao and McGrath, 2009). While numerous studies have examined the impact of foliar-applied zinc on citrus crops, there remains a clear knowledge gap in understanding how different concentrations of media-applied zinc under controlled (greenhouse) conditions affect chloroplast pigment dynamics in acid lime. Most existing research focuses on field applications or nutrient deficiencies, with limited emphasis on the physiological pigment responses to graded zinc levels in a protected environment. Furthermore, the relationship

between zinc concentration and its threshold for toxicity, particularly its inhibitory effects on carotenoid accumulation has not been sufficiently explored. In this context, the present study was conducted to investigate how varying concentrations of zinc applied through the growing medium affect the biosynthesis of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids in acid lime under greenhouse conditions. The objective was to determine the optimal zinc concentration that maximizes photosynthetic pigment content without triggering toxicity symptoms, and to assess whether pigment trends are consistent across seasonal growth cycles and years.

MATERIALS AND METHODS:

The present investigation was conducted at the Instructional Farm of the Department of Horticulture & Postharvest Technology, Institute of Agriculture, Visva-Bharati, Sriniketan, West Bengal over two consecutive years (2022–2023 and 2023–2024) in a controlled greenhouse environment to evaluate the effect of varying levels of zinc application through the substrate (acid washed river sand) on chloroplast pigment synthesis in acid lime seedlings. The experiment was designed as a pot (20cm diameter) trial using seedlings grown of seeds collected from uniform sized fresh ripe fruits. Only the nuclear seedlings were considered for the present experiment. The each pot as fed with Modified Hoagland solution (without zinc) @ 100ml in every week and the watering was done with distilled water @ 500 ml per pot in every 5 days interval. Standard plant protection measures were followed throughout the period of experimentation. The zinc treatments were applied as zinc sulfate ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$) mixed thoroughly into the potting medium at seven concentrations (T₁: Control or 0.0 mM/l (milli mole per litre), T₂: 2.5 mM/l, T₃: 5.0 mM/l, T₄: 7.5 mM/l, T₅: 10.0 mM/l, T₆: 12.5 mM/l and T₇: 15.0 mM/l) at 15 days interval starting from 3 months age of the plants @ 100 ml per pot.

These treatments were arranged in a Completely Randomized Design (CRD) with three replications per treatment and keeping 10 plants under each replication. Uniform irrigation and cultural practices were maintained across all treatments.

To assess the influence of zinc on chloroplast pigment synthesis, the following pigments like chlorophyll a, chlorophyll b, total chlorophyll (sum of a + b) and carotenoids were quantified at six growth stages viz. 3, 6, 9, 12, 15, and 24 months after planting, spanning the full experimental period. Fully expanded, healthy leaves were sampled from each treatment and replication at each interval. For consistency, the same relative leaf position was maintained across plants and time points. Pigments were estimated using the method as described by Lichtenthaler and Wellburn (1983), which remains a widely accepted protocol for chlorophyll and carotenoid quantification. For this, 0.5 g of fresh leaf tissue was homogenized in 80% acetone and centrifuged. The clear supernatant was collected, and absorbance readings were taken using a UV-Visible spectrophotometer at specific wavelengths (663 nm for chlorophyll a; 645 nm for chlorophyll b and 480 nm for Carotenoids). The equations used for quantification are as follows:

$$\text{Chlorophyll } a = 12.7(A_{663}) - 2.69(A_{645})$$

$$\text{Chlorophyll } b = 22.9(A_{645}) - 4.68(A_{663})$$

$$\text{Total chlorophyll} = 20.2(A_{645}) + 8.02(A_{663})$$

$$\text{Carotenoids} = (1000 \times A_{470} - 1.82 \times \text{Chl } a - 85.02 \times \text{Chl } b) / 198$$

The experiment followed a Completely Randomized Design (CRD) with seven treatments and three replications. The pooled data from two years were statistically analyzed using Analysis of Variance (ANOVA) as per standard procedure. Treatment means were compared using the Critical difference (CD) test at 5% level of significance. Standard error of the Mean (SEm \pm) was also calculated for each parameter. Statistical analyses were carried

out using IBM SPSS Statistics (Version 27.0). Prior to conducting ANOVA, assumptions such as normal distribution and homogeneity of variance were verified.

RESULTS AND DISCUSSION:

To assess the impact of zinc on pigment composition, the greenhouse pot experiment was conducted to evaluate how zinc deficiency and toxicity influence chloroplast pigments in acid lime, focusing on chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, and the chlorophyll a/b ratio over a 24-month period.

Chlorophyll a content: The chlorophyll a (Chl a) content of acid lime leaves responded significantly to varying concentrations of zinc over time under greenhouse conditions (Table 1). At 3 months after treatments, Chl a content ranged from 0.94 mg/g FW in T₇ (15.0 mM/l zinc) to 1.08 mg/g FW in T₄ (7.5 mM/l zinc). Although differences at 3 months were not statistically significant, a trend emerged indicating enhanced Chl a in plants treated with moderate zinc doses. By 6 months and onwards, treatment differences became statistically significant ($p < 0.05$), confirming the role of zinc concentration on Chl a dynamics. At 6 months, maximum Chl a (1.12 mg/g) was observed in T₄, while the lowest (0.94 mg/g) occurred in T₆ (12.5 mM/l zinc). Similarly, at 9 months and 12 months, T₄ consistently maintained the highest Chl a levels (1.18 and 1.21 mg/g, respectively), whereas T₇ declined sharply (0.79 and 0.71 mg/g). This pattern persisted through 15 and 24 months, with T₄ showing optimal Chl a content (1.23 and 1.28 mg/g), suggesting long-term enhancement of chlorophyll biosynthesis under moderate zinc application. In contrast, both zinc deficiency (T₁, 0.0 mM/l) and toxicity (T₆, 12.5 mM/l; T₇, 15.0 mM/l) significantly reduced Chl a content over time, with T₇ recording the lowest levels at 24 month (0.63 mg/g). This highlights a biphasic zinc response, wherein chlorophyll synthesis is promoted by adequate zinc levels but

inhibited under both deficient and toxic concentrations.

Zinc is a vital cofactor in chlorophyll biosynthesis, involved in the structure and activation of enzymes such as carbonic anhydrase and RNA polymerase (Marschner, 2012). Moderate zinc supplementation likely facilitated enzyme activity and protein synthesis necessary for chlorophyll formation, thus explaining the higher Chl a in T₃ and T₄ treatments. Moreover, zinc plays a structural role in maintaining membrane integrity, which is crucial for thylakoid function and chlorophyll stabilization (Alloway, 2008). Zinc deficiency, as observed in T₁, likely impaired the synthesis of δ -aminolevulinic acid, a precursor of chlorophyll, thus reducing Chl a concentration. Deficient zinc also leads to increased oxidative stress due to impaired antioxidant enzyme systems, causing chlorophyll degradation (Broadley *et al.*, 2007). Conversely, excessive zinc in T₆ and T₇ may have induced phytotoxic effects by generating reactive oxygen species (ROS), leading to lipid peroxidation and chloroplast damage, ultimately lowering chlorophyll content (Cakmak, 2000). The temporary increase in Chl a observed in T₇ at 6 months (1.05 mg/g) followed by a sharp decline suggests an initial physiological acclimation phase before toxicity symptoms manifested. This aligns with earlier findings where high zinc doses initially stimulated pigment production before causing oxidative damage (Prasad *et al.*, 1999). Overall, the data suggests that zinc at 7.5 mM/l (T₄) optimally supports chlorophyll a biosynthesis in acid lime, while both deficiency and excess zinc result in deleterious effects on chloroplast pigment levels.

Chlorophyll b content: The chlorophyll b (Chl b) content in acid lime showed variable responses to different zinc concentrations over time under greenhouse conditions (Table 2). Although treatment differences during the initial growth phases (3, 6, and 9 months after treatment) were statistically

non-significant, a clear pattern emerged from 12 months onward, where significant variations in Chl b were observed due to the contrasting zinc treatments. At 3 months after treatment, Chl b content ranged narrowly from 0.34 mg/g fresh weight (FW) in T₇ (15.0 mM/l zinc) to 0.45 mg/g in T₄ (7.5 mM/l zinc), indicating early mild responsiveness of pigment synthesis to Zinc. By 9 months, plants under T₄ maintained the highest Chl b (0.58 mg/g), suggesting that moderate zinc supplementation promoted chlorophyll accumulation. From 12 months onwards, significant treatment effects were evident (CD at 5% = 0.11 at 12 months). The maximum Chl b was recorded under T₄ (0.60 mg/g), followed by T₃ (5.0 mM/l zinc, 0.43 mg/g), while zinc-deficient (T₁) and zinc-toxic treatments (T₆ and T₇) consistently showed lower values. At 24 months, Chl b content in T₄ remained relatively high (0.48 mg/g), whereas, T₆ (12.5 mM/l) and T₇ (15.0 mM/l) recorded the lowest values of 0.36 and 0.40 mg/g, respectively. Interestingly, while T₃ (5.0 mM/l zinc) showed a late increase to 0.53 mg/g, it was still slightly lower than the earlier peak seen under T₄, indicating a sustained but plateaued effect under moderately optimal Zinc conditions.

Chlorophyll b, primarily associated with the light-harvesting complex II (LHCII), depends on adequate zinc levels for structural integrity of thylakoid membranes and efficient protein synthesis (Barickman *et al.*, 2014). Moderate zinc (T₃ and T₄) thus promoted pigment accumulation by facilitating biosynthetic pathways and reducing oxidative damage. Deficient zinc levels (T₁, 0.0 mM/l) likely disrupted protein synthesis and impaired the formation of δ -aminolevulinic acid, a precursor of chlorophyll molecules, leading to reduced Chl b (Broadley *et al.*, 2012). On the other hand, elevated zinc concentrations (T₆ and T₇) induced toxicity, possibly through enhanced generation of reactive oxygen species (ROS), causing peroxidation of lipids and degradation of pigments (Feng *et al.*, 2010). The observed late-stage reduction in

Chl b under zinc toxicity may also be attributed to antagonistic effects on nutrient uptake, particularly magnesium and iron—key elements in chlorophyll synthesis (Kabata-Pendias, 2010). The transient increase in Chl b at 9 month in T₇ (0.51 mg/g) followed by a subsequent decline indicates a short-term adaptive response, after which toxic effects overwhelmed the plant's metabolic balance. Similar biphasic responses to micronutrients have been observed in citrus and leafy crops exposed to varying metal stress conditions (Ranjbar and Bahmaniar, 2007). In conclusion, chlorophyll b synthesis in acid lime is maximally promoted under moderate zinc application (especially 7.5 mM/l), while deficiency and excessive zinc concentrations reduce pigment levels due to biosynthetic limitations or toxicity-induced degradation.

Total chlorophyll content: The total chlorophyll content in acid lime plants varied considerably with different zinc concentrations across the growth period (Table 3). While early intervals (3 and 6 months after treatment) exhibited non-significant differences among treatments, clear and statistically significant trends emerged from 9 months onward, indicating the progressive impact of zinc on chloroplast pigment synthesis under greenhouse conditions. At 3 month, total chlorophyll ranged from 1.49 mg/g fresh weight (FW) in T₇ (15.0 mM/l zinc) to 1.59 mg/g in T₄ (7.5 mM/l zinc), with all values being statistically non-significant. A similar trend was observed at 6 month, where zinc levels had not yet exerted a strong differentiating effect on pigment development. However, as the experiment progressed, substantial differences in chlorophyll content were recorded. By 9 months, T₄ (7.5 mM/l zinc) showed the highest total chlorophyll content (1.76 mg/g FW), followed by T₃ (5.0 mM/l zinc) at 1.62 mg/g, both significantly higher than zinc-deficient (T₁, 1.40 mg/g) and zinc-toxic treatments (T₆ and T₇ at 1.36 and 1.30 mg/g, respectively). The same pattern persisted through 12, 15, and 24 months. At

24 months, T₄ maintained the highest chlorophyll level (1.86 mg/g FW), while T₇ (zinc toxicity) recorded the lowest (1.08 mg/g).

This data clearly indicates that moderate zinc supplementation (particularly at 7.5 mM/l) enhances chlorophyll synthesis and retention, whereas, both zinc deficiency and excess lead to progressive pigment degradation. Zinc plays a crucial role in chlorophyll biosynthesis and structural stabilization of chloroplast membranes (Rehman *et al.*, 2012). It is a cofactor for carbonic anhydrase and other enzymes involved in chlorophyll formation and photosynthetic carbon fixation (Yusuf *et al.*, 2011). Under zinc-deficient conditions (T₁), limited enzyme activity and impaired protein synthesis likely hampered chlorophyll production and led to premature senescence. Zinc deficiency also induces oxidative stress, which degrades chlorophyll molecules due to the accumulation of reactive oxygen species (ROS) (Dang *et al.*, 2024). This explains the consistent decline in chlorophyll content in T₁ across all intervals. On the other hand, zinc toxicity at high concentrations (T₆ and T₇) likely disrupted cellular homeostasis, leading to chloroplast damage, reduced pigment biosynthesis, and enhanced chlorophyll degradation. High zinc concentrations can interfere with iron (Fe) and magnesium (Mg) uptake—both essential for chlorophyll synthesis—resulting in impaired pigment metabolism (Kalayci *et al.*, 2008). Moreover, excessive zinc is known to displace essential metals from enzymatic sites, destabilize protein structures, and generate oxidative stress in plant tissues (Roosta *et al.*, 2018). The optimal performance under T₄ and T₃ treatments supports the idea that zinc enhances photosynthetic efficiency when applied at proper doses. A similar improvement in total chlorophyll content under moderate zinc levels has been reported in citrus and other crops (Sharma *et al.*, 2010).

Chlorophyll a/b Ratio: The chlorophyll a to b (Chl a/b) ratio is a crucial physiological

indicator reflecting the balance between light-harvesting complex proteins and core photosynthetic pigments. In this study, the Chl a/b ratio of acid lime showed significant variation under different zinc concentrations at various growth stages, especially from 6 months after treatment onwards (Table 4). At 3 months, the Chl a/b ratio ranged from 2.41 (T₄) to 2.76 (T₇), but the differences were statistically non-significant. This suggests that early-stage photosynthetic pigment balance was not yet strongly influenced by zinc treatments. However, at 6 months, notable shifts began to emerge. The ratio was highest in T₇ (2.69) and lowest in T₁ (1.79), with T₄, T₃, and T₅ showing intermediate and more stable ratios (2.32-2.38). From 9 months onward, significant differences became more evident. The Chl a/b ratio in the optimal zinc range (T₃ and T₄: 5.0–7.5 mM/l) remained relatively balanced, maintaining values around 2.01–2.74, suggesting a harmonious development of both chlorophyll forms. The T₄ treatment at 12 months showed a ratio of 2.01, indicating efficient chlorophyll b synthesis relative to chlorophyll a. Meanwhile, plants under zinc deficiency (T₁: 0.0 mM/l) or toxicity (T₆ and T₇: 12.5 and 15.0 mM/l, respectively) showed irregular patterns. Notably, T₇ exhibited a sharp drop in the ratio at 9 months (1.55), which continued to decline to 1.57 at 24 month indicating a disproportionate degradation of chlorophyll a or increased accumulation of chlorophyll b.

These findings suggest that both zinc deficiency and excess disrupt the balance of chlorophyll synthesis. Zinc is known to be vital for protein synthesis, enzyme activation, and membrane integrity factors that collectively influence chloroplast structure and function (Broadley *et al.*, 2007). Disruption in these processes can lead to oxidative degradation or impaired synthesis of chlorophyll a, skewing the Chl a/b ratio. Higher Chl a/b ratios generally indicate dominance of reaction centre chlorophylls, while lower ratios suggest expansion of light-harvesting complexes

(LHCs), often as an acclimation response to stress (Anderson *et al.*, 1995). In zinc-deficient and zinc-toxic treatments, the observed fluctuations may result from stress-induced overexpression or degradation of LHC proteins, affecting chlorophyll b content and consequently altering the ratio. The optimal balance found in T₃ and T₄ treatments supports previous research where moderate zinc application enhanced pigment biosynthesis, maintained chloroplast ultrastructure, and supported balanced growth (Fathiet *al.*, 2009). Conversely, extreme zinc concentrations either too low or too high likely led to reduced enzymatic activity for chlorophyll biosynthesis, disrupted plastid development, and oxidative damage to photosynthetic proteins (Shao *et al.*, 2008). The elevated Chl a/b ratio in zinc-toxic treatments (T₇ and T₆) during early stages might reflect an initial suppression of chlorophyll b synthesis or an adaptive thinning of LHCs to limit light absorption under oxidative stress. Over time, however, such treatments led to pigment degradation and structural damage, explaining the final low ratios at 24 month.

Carotenoid content: Carotenoids are essential accessory pigments that play a crucial role in light harvesting and photo-protection by quenching excess energy and reactive oxygen species (ROS). In this study, carotenoid content in acid lime exhibited significant variation in response to different zinc concentrations across six time intervals under greenhouse conditions (Table 5). At the early stage (3 months after treatment), carotenoid content was highest in T₆ (12.5 mM/l zinc; 0.69 mg/g FW) and T₁ (0.0 mM/l zinc; 0.67 mg/g FW), while the lowest values were observed in T₄ (7.5 mM/l; 0.187 mg/g FW). Similar trends continued at 6 month, with carotenoid levels declining across the optimal zinc treatments (T₃ and T₄) and remaining elevated under zinc deficiency (T₁) and zinc toxicity (T₆). As time progressed, carotenoid content declined gradually in T₁ and T₆, reaching 0.39 and 0.41 mg/g FW respectively by 24 month.

Meanwhile, T₄ and T₅ treatments (7.5 and 10.0 mM/l zinc) showed a gradual and consistent increase in carotenoid content from 3 month to 24 month, ending with values of 0.28 and 0.35 mg/g FW respectively. This progressive increase may indicate the activation of carotenoid biosynthesis as a photoprotective response under moderately favourable zinc concentrations. Interestingly, T₂ (2.5 mM/l zinc) showed a steady increase in carotenoids over time, peaking at 0.38 mg/g FW at 24 month, which surpassed some of the higher zinc levels. On the other hand, T₃ (5.0 mM/l zinc), which showed optimal chlorophyll content in earlier observations, had relatively moderate carotenoid levels, peaking at 0.447 mg/g FW at 3 month and declining to 0.26 mg/g FW at 24 month.

These trends suggest a complex interaction between zinc nutrition and carotenoid metabolism. High carotenoid content under zinc deficiency and toxicity conditions (T₁ and T₆) might represent a defensive strategy to counteract oxidative stress caused by impaired chloroplast function and excess ROS generation (Singh and Prasad, 2014). Zinc plays a pivotal role in maintaining membrane stability and activating antioxidant enzymes. In its absence or excess, ROS accumulation may trigger carotenoid accumulation as part of the plant's adaptive stress response (Hasanuzzaman *et al.*, 2020). Moreover, the moderate zinc levels in T₄ and T₅ may have provided optimal conditions for maintaining balanced growth, improving photosystem stability, and enhancing non-photochemical quenching, reflected by a consistent rise in carotenoids (Cakmak, 2000). This aligns with findings where carotenoid biosynthesis was enhanced under conditions of improved zinc-mediated stress mitigation. Conversely, a decline in carotenoids over time in T₃ and T₆ may suggest pigment degradation due to long-term zinc imbalance, despite initial adaptation. The early high values in these treatments could indicate an initial oxidative response, followed by pigment breakdown

due to prolonged stress, especially under zinc toxicity (Rout and Das, 2003). Overall, the results indicate that carotenoid content in acid lime is not linearly related to zinc concentration, but rather modulated by the plant's oxidative and metabolic status. Treatments with moderate zinc concentrations (especially T₄ and T₅) favoured a healthy and sustained accumulation of carotenoids, likely supporting both light harvesting and photo-protection under greenhouse conditions.

CONCLUSION

Zinc application significantly influenced chloroplast pigment dynamics in acid lime under greenhouse conditions. Moderate zinc levels, particularly at 7.5 mM/l (T₄), consistently enhanced chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid contents across all time points, supporting optimal pigment biosynthesis and chloroplast function. In contrast, both zinc deficiency (0.0 mM/l) and toxicity (≥ 12.5 mM/l) reduced pigment concentrations, likely due to impaired enzymatic activity, oxidative stress, and disrupted nutrient uptake. The chlorophyll a/b ratio remained balanced under moderate zinc but deviated under stress conditions, indicating pigment imbalance and structural damage. Carotenoid trends revealed a defensive role under zinc-induced stress, with accumulation under both deficiency and toxicity. Overall, the results underscore a biphasic zinc response, where only moderate concentrations sustain photosynthetic pigment stability and promote physiological resilience in acid lime.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Table 1: Effect of varied doses of zinc on changes in chlorophyll a content of acid lime seedlings:

Treatments (zinc conc.) milli mole per litre (mM/l)	3 month	6 month	9 month	12 month	15 month	24 month
T ₁ (0.0 mM/l/l)	1.01	0.97	0.95	0.93	0.86	0.79
T ₂ (2.5mM/l/l)	1.03	1.07	1.01	0.99	0.93	0.87
T ₃ (5.0 mM/l/l)	1.06	1.10	1.13	1.18	1.20	1.23
T ₄ (7.5mM/l/l)	1.08	1.12	1.18	1.21	1.23	1.28
T ₅ (10.0 mM/l/l)	1.04	1.09	1.10	0.97	1.01	1.03
T ₆ (12.5mM/l/l)	0.99	0.94	0.90	0.85	0.80	0.75
T ₇ (15.0 mM/l/l)	0.94	1.05	0.79	0.71	0.67	0.63
SE±m	NS	0.04	0.03	0.03	0.02	0.04
CD (0.05)	NS	0.13	0.09	0.09	0.08	0.13

Table 2: Effect of varied doses of zinc on changes in chlorophyll b content of acid lime seedlings:

Treatments (zinc conc.)	3 month	6 month	9 month	12 month	15 month	24 month
T ₁ (0.0 mM/l/l)	0.40	0.54	0.45	0.41	0.39	0.38
T ₂ (2.5mM/l/l)	0.41	0.46	0.41	0.37	0.41	0.43
T ₃ (5.0 mM/l/l)	0.43	0.47	0.49	0.43	0.48	0.53
T ₄ (7.5mM/l/l)	0.45	0.47	0.58	0.6	0.54	0.48
T ₅ (10.0 mM/l/l)	0.42	0.47	0.4	0.39	0.42	0.45
T ₆ (12.5mM/l/l)	0.38	0.43	0.46	0.42	0.39	0.36
T ₇ (15.0 mM/l/l)	0.34	0.39	0.51	0.36	0.38	0.4
SE±m	NS	NS	NS	0.03	0.03	0.02
CD (0.05)	NS	NS	NS	0.11	0.09	0.08

Table 3: Effect of varied doses of zinc on changes in total chlorophyll a content of acid lime seedlings:

Treatments (Zinc conc.)	3 month	6 month	9 month	12 month	15 month	24 month
T ₁ (0.0 mM/l/l)	1.51	1.49	1.40	1.34	1.26	1.18
T ₂ (2.5mM/l/l)	1.53	1.51	1.42	1.27	1.35	1.43
T ₃ (5.0 mM/l/l)	1.57	1.58	1.62	1.54	1.59	1.66
T ₄ (7.5mM/l/l)	1.59	1.64	1.76	1.70	1.78	1.86
T ₅ (10.0 mM/l/l)	1.55	1.52	1.50	1.49	1.41	1.37
T ₆ (12.5mM/l/l)	1.50	1.46	1.36	1.28	1.22	1.14
T ₇ (15.0 mM/l/l)	1.49	1.44	1.30	1.18	1.11	1.08
SE±m	NS	NS	0.04	0.04	0.03	0.06
CD (0.05)	NS	NS	0.12	0.13	0.10	0.15

Table 4: Effect of varied doses of zinc on changes in the ratio of chlorophyll a and chlorophyll b content of acid lime seedlings:

Treatments (Zinc conc.)	3 month	6 month	9 month	12 month	15 month	24 month
T ₁ (0.0 mM/l/l)	2.52	1.79	2.11	2.27	2.20	2.08
T ₂ (2.5mM/l/l)	2.51	2.32	2.46	2.67	2.26	2.02
T ₃ (5.0 mM/l/l)	2.46	2.34	2.30	2.74	2.50	2.32
T ₄ (7.5mM/l/l)	2.41	2.38	2.03	2.01	2.27	2.67
T ₅ (10.0 mM/l/l)	2.47	2.32	2.75	2.48	2.40	2.29
T ₆ (12.5mM/l/l)	2.60	2.18	1.95	2.02	2.05	2.08
T ₇ (15.0 mM/l/l)	2.76	2.69	1.55	1.97	1.76	1.57
SE±m	NS	0.06	0.07	0.06	0.05	0.06
CD(0.05)	NS	0.18	0.21	0.18	0.16	0.19

Table 5: Effect of varied doses of zinc on changes in carotenoid content of acid lime seedlings:

Treatments (zinc conc.)	3 month	6 month	9 month	12 month	15 month	24 month
T ₁ (0.0 mM/l/l)	0.67	0.63	0.59	0.55	0.51	0.39
T ₂ (2.5mM/l/l)	0.287	0.30	0.313	0.327	0.34	0.38
T ₃ (5.0 mM/l/l)	0.447	0.42	0.393	0.367	0.34	0.26
T ₄ (7.5mM/l/l)	0.187	0.20	0.213	0.227	0.24	0.28
T ₅ (10.0 mM/l/l)	0.21	0.23	0.25	0.27	0.29	0.35
T ₆ (12.5mM/l/l)	0.69	0.65	0.61	0.57	0.53	0.41
T ₇ (15.0 mM/l/l)	0.38	0.40	0.42	0.44	0.46	0.52
SE±m	NS	0.02	0.02	0.02	0.02	0.02
CD (0.05)	NS	0.06	0.05	0.06	0.05	0.05

Influence of Agriphotovoltaics on performance of turmeric (*Curcuma longa* L)

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ABSTRACT

An experiment was conducted to integrate turmeric crop production under the GM-APV system, so as to analyse the performance and also elaborate the feasibility of the concept. The experiment was conducted using randomized block design with five treatments as growing conditions of the turmeric crop having three replications. The details of treatments were T_1 -Sole 3.75 m panel, T_2 -Sole 1.75 m panel, T_3 – Turmeric below 3.75 m panel, T_4 -Turmeric between 3.75 m panel, T_5 -Turmeric below 1.75 m panel, T_6 - Turmeric between 1.75 m panel and T_7 Open conditions (Sole Turmeric). Results revealed that different GM APVs influenced the growth and yield parameters of turmeric cultivar Salem significantly, except emergence count at 60 DAP which differed non significantly. The treatment T_3 where turmeric planted below 3.75 m recorded significantly more number of tillers clump⁻¹ (3.96), pseudostem height clump⁻¹ (32.50 cm), number of leaves clump⁻¹ (22) and leaf area clump⁻¹ (72.60 cm²), Chlorophyll content (35.40 Spad), crop duration (306.48 days), rhizome yield plant⁻¹ (0.65 kg), fresh rhizome yield ha⁻¹ (40.70 t) and dry rhizome yield ha⁻¹ (8.75 t).

Keywords: Ground mounted agrivoltaic systems, growth, turmeric, yield.

INTRODUCTION

India has started the energy generation from renewable resources and set the goal of generating half of its electricity from renewables by 2030 and has established 18 Gigawatt (GW) of solar photovoltaics in 2022. Energy generation from coal will be surpassed by Solar photovoltaics power capacity by 2027 and it will become the largest energy generating capacity in the world (Anamalagundam *et al.*, 2023). However, the biggest challenge in large scale adaption is requirement of large area for these ground mounted solar panels *i.e.*, usage with approximately 1.2-1.7 hectare per installed mega watt power (MW_p) (Anas *et al.*, 2023; Axel *et al.*, 2019; Max *et al.*, 2019). According

to the Japanese energy policy, crop yield under Agriphotovoltaics should not be less than 80% of those grown in open-field (OF) conditions to ensure food security (Elborg, 2017). Thus, achieving crop yield, and the arrangement of solar panels that allows cultivation, has become important area of research.

Turmeric is shade loving and has adaptation to low light saturation hence intensive cultivation in the form of inter-cropping with castor crop is regular practice in the region (Thombre, 2022). The APV system is an emerging concept and very few studies highlighting the crop response to AVP systems was carried out by the earlier workers (AL-Agele *et al.*, 2021; Moon *et al.*, 2022; Ko *et al.*,

2023). Therefore, a wide range of investigation is needed to be initiated in order to develop effective management strategies for the horticulture crops so as to fully exploit the potential benefits of GM-APV system that offers. Very scanty information is available of growing turmeric crop under solar panels is seen. Therefore, present study on turmeric performance under GM- APV has been conducted.

MATERIALS AND METHODS

The field study was performed at Manvat Dist. Parbhani (19°18'0N76°30'0'E). The ground mounted (GM) Photovoltaic panels (PVPs) have been arranged in north-south oriented strips with installation capacity of 1.4 MW_{DC} spread over area of 4.2 acres having five sections (Four APV and one open section). Panel specification comprises of 540 kWp bifacial panels with 20% efficiency having tilt of 11° and ground clearance of 3.75m and 1.74 m and pitch distance of 5.4 m and 10 m, respectively having approximate cost of Rs 2 crores per MW. The site soil is clay loam with bulk density of in range of 1.35-1.40 g/cc, top soil pH ranging from 7.95 to 8.14, EC in the range of 0.24 to 0.37 dS/m, CaCO₃ content of 7.2 % and 0.47 to 0.79 % range of organic matter content. Parbhani district comes under assured rainfall region with semi-arid climate. The average annual precipitation of the district is 844 mm and the region has been categorized as an assured rainfall agro-climatic zone. The region experiences hot dry summer (March - May), cold dry winter (October - February) and wet humidity with medium rainfall in monsoon season (June - September). Rainfall received during experimental year (2023-24) was 890.8 mm and distributed in 49 rainy days. During the period of experiment the mean maximum temperature ranged from 28.2 to 44.6° C whereas, mean minimum temperature ranged from 8.1 to 0.1° C while relative humidity was seen 48 to 97 per cent in

morning period and 14 to 82 per cent during afternoon hours.

The experiment with five treatments was laid out in a Randomized Block Design (RBD) with three replications. Turmeric variety Salem was planted on raised beds at spacing of 60 x 25 cm on 3rd June 2023. The details of treatments were T₁– Turmeric below 3.75 m panel, T₂ -Turmeric between 3.75 m panel, T₃ -Turmeric below 1.75 m panel, T₄- Turmeric between 1.75 m panel and T₅ Open conditions (Sole Turmeric). The observations regarding growth parameters viz. emergence count, No. of tillers clump⁻¹, *pseudo* stem height (cm) clump⁻¹ 210 DAP, number of leaves clump⁻¹ 210 DAP, leaf area (cm²) clump⁻¹ 210 DAP, chlorophyll content (Spad units), rhizome yield (kg/plant), rhizome yield (tha⁻¹), dry rhizome yield (tha⁻¹) were recorded at the time of harvest i.e on 24th of April 2024 . The data collected was subjected to statistical analysis of variance as suggested by Panse and Sukhatme (1985).

RESULTS AND DISCUSSION

Perusal of data presented in Table 1 clearly revealed that different GM - APVs influenced the growth and yield parameters of turmeric cultivar Salem significantly except, emergence count at 60 DAP which differed non significantly. Emergence count taken at 60 DAP showed that turmeric planted in open field showed more emergence count (84.15 %) as compared to the treatments of turmeric planted in ground mounted agriphotovoltaics (GM- APVs). Less emergence count (83.67 %) was recorded in the treatment T₁ where turmeric was planted below 3.75 m panel having pitch of 5.4 m. However, the emergence count differed non significantly among all treatments under study. It might be due to external factors affecting emergence i.e., diurnal changes during day and night period do not vary too much as it was July and August period of monsoon season.

The data regarding number of tillers clump⁻¹ of turmeric differed significantly in different treatments under study and ranged from 3.03 to 3.96. The treatment T₁ where turmeric planted below 3.75 m recorded more number of tillers clump⁻¹ (3.96) followed by the treatment T₅(3.70) and T₂ (3.4) and where found statistically at par with each other. Minimum number of clump⁻¹ was recorded in a treatment T₄ (3.03). *Pseudostem* height clump⁻¹ of turmeric recorded at 210 DAP ranged from 22.3 cm to 32.50 cm in different treatments under investigation. Significantly more pseudostem height clump⁻¹ (32.50 cm) of turmeric planted below 3.75 m panel was recorded and was followed by the treatment T₂ (29.4) and was found at par with each other. While less pseudostem height clump⁻¹ (22.3 cm) was recorded in turmeric planted under open conditions.

It was evident from the data presented regarding the number of leaves clump⁻¹ and leaf area clump⁻¹ recorded at 210 days DAP clearly showed that turmeric planted below 3.75 m panel recorded more number of leaves (22.24) and leaf area (72.16 cm²) and was closely followed by the treatment T₂ (20.82 and 68.64 cm² respectively) and were found at par with each other. While minimum the number of leaves clump⁻¹ (19.22) and leaf area clump⁻¹ (60.26 cm²) was recorded in the treatment where turmeric was grown in open field. Chlorophyll content was recorded more in the treatment T₁ (35.40 Spad units) where turmeric was planted below 3.75 m panel and was followed by the treatment T₂, T₃ and T₄ and where found at par with each other. While, minimum chlorophyll content was recorded in the treatment T₅ (28.84 Spad units) i.e in open field conditions. (Trommsdorff *et al.*, 2022; Max *et al.*, 2019) found that soil temperature at night under the modules was lower than that of soil temperatures under full sunny morning hours. Further evapotranspiration reduced the temperature of the surrounding atmosphere

under the APV system by around 1 to 1.5°C than ambient temperature. Valle *et al.*, 2022 reported a decline of about 14% to 29% of evapotranspiration. Hence, moisture levels near soil and air would be higher beneath PV system. APV panels lessen the impact of heavy rainfall, frost, hail storms and high temperatures on crops grown underneath of it. Kostik *et al.*, (2020) and Williams *et al.*, (2023) suggested good plant growth can be anticipated in the regions of hot windy, and turbulent conditions as they act like windbreaks and this could help to minimize wind erosion.

The data regarding crop duration of turmeric cv. Salem also revealed significantly difference as influenced by the growing conditions of APVs. Relatively more crop duration (306.48 days) was taken by turmeric grown below 3.75 m panel in comparison with rest of growing conditions, except treatment T₂ and T₃ which were found at par with each other. Turmeric crop grown in open conditions was earlier to be harvested (272.44 days) among all treatments studied. Crop duration depends upon total heat that accumulated in the field over time which can be directly related to growing degree days. As field accumulated crop experience accelerated growth leading to earlier maturity. In this investigation the crop required more number of days to reach maturity under GM-APVs conditions for want of more field heat to be accumulated in the crop in comparison to open field conditions.

It was clear from the data presented in Table 2 regarding yield parameters of turmeric as influenced by different growing conditions of APVs varied significantly. Turmeric planted below 3.75 m panel recorded significantly more rhizome yield plant⁻¹ (0.65 kg), fresh rhizome yield ha⁻¹ (40.70 t) and dry rhizome yield ha⁻¹ (8.75 t). The next best treatment in this regard was the treatment where turmeric was planted between the panels of 3.75 m (0.58 kg plant⁻¹, 36.32.70 t

ha⁻¹ and 6.9 t ha⁻¹, respectively). Turmeric planted in open condition recorded minimum rhizome yield plant⁻¹ (0.4 kg), fresh rhizome yield ha⁻¹ (25.04 t) and dry rhizome yield ha⁻¹ (4.5 t). Horticulture PV system provides shading which may lead to crop growth increase or decrease depending on crop. Shading also improves protection against heat waves and strong precipitation such as hail as reported by Guerin (2019); Hiebsch and McCollum (1987). Studies on yield impact showed wide variations in results. Leafy vegetables and legumes increase yield, while crops like rice and wheat showed a significant reduction in yields, and most other crops revealed mixed results as suggested by Homma *et al.*, 2016; Gonocruz *et al.*, 2021; Weselek *et al.*, 2019; Zang *et al.*, 2025). It is clear from the above literature reviews that the performance of the crop is subject to factors like type of crop selected, growing agroclimatic conditions and erected panel elevations.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Table 1: Influence of different GM-APVs on growth performance of turmeric cv. Salem

Treatment details	Growth parameters of turmeric						
	Emergence count (%) at 60 DAP	No. of tillers clump ⁻¹	Pseudo stem height (cm) clump ⁻¹ 210 DAP	Number of leaves clump ⁻¹ 210 DAP	Leaf area (cm ²) clump ⁻¹ 210 DAP	Chlorophyll content (Spad units)	Crop duration (Days)
T ₁ - Turmeric below 3.75 m panel	83.67 (97.02)*	3.96	32.50	22.24	72.16	35.40	306.48
T ₂ - Turmeric between 3.75 m panel	83.85 (97.10)	3.40	29.403	20.82	68.64	35.20	302.6
T ₃ -Turmeric below 1.75 m panel	83.90 (97.60)	3.10	27.48	20.10	65.26	33.40	298.34
T ₄ - Turmeric between 1.75 m panel	84.05 (97.88)	3.03	23.15	19.80	62.60	29.30	289.26
T ₅ - Open conditions (Sole Turmeric)	84.15 (97.90)	3.70	22.35	19.22	60.26	28.84	272.44
SE ±	0.65	0.22	1.61	1.21	2.07	2.56	6.72
CD at 5%	NS	0.66	4.83	3.63	6.221	7.68	11.16

* Figures in the brackets are angular transformed value of %.

Table 2: Influence of different GM-APVs on yield performance of turmeric cv. Salem

Treatment details	Yield parameters of turmeric		
	Rhizome yield (kg/plant)	Fresh Rhizome yield (tha ⁻¹)	Dry Rhizome yield (tha ⁻¹)
T ₁ - Turmeric below 3.75 m panel	0.65	40.70	8.75
T ₂ - Turmeric between 3.75 m panel	0.58	36.32	6.9
T ₃ -Turmeric below 1.75 m panel	0.52	32.56	5.86
T ₄ - Turmeric between 1.75 m panel	0.47	29.43	5.21
T ₅ - Open conditions (Sole Turmeric)	0.40	25.04	4.5
SE ±	0.02	1.11	1.21
CD at 5%	0.06	3.33	3.63

***In vivo* and *in silico* approaches for exploring the hypoglycemic potential of *Moringa oleifera* Lam. flowers' extract**

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ABSTRACT

Moringa oleifera, also known as shajina, is rich in bioactive phytochemicals, including flavonoids, alkaloids, and phenolics, which contribute to its therapeutic potential. Various parts, including leaves, barks, and flowers exhibit antioxidant, anti-inflammatory, and antimicrobial properties. The present study was designed to explore the antidiabetic potential of the flower extract through the *in vivo* and *in silico* study. The hypoglycemic activity of the flower extract was evaluated in Wistar rat using an oral glucose tolerance test (OGTT), while molecular docking identified key phytochemicals targeting diabetic pathway proteins. The *in vivo* study revealed significant glucose-lowering effects of flower extract fractions, particularly n-hexane (NHF) and chloroform. At 200 mg/kg, both n-Hexane and chloroform fractions reduced blood glucose by 30.22% and 33.83%, respectively, increasing to 37.01% and 49.86% at 400 mg/kg, nearing the standard hypoglycemic drug miglitol's 53.92% efficacy. *In silico* analysis showed strong binding affinity of kaempferol, quercetin, and ar-turmerone to pancreatic alpha-amylase (5E0F) with binding energies of -8.8, -9.0, and -6.9 kcal/mol, respectively. ADMET analysis confirmed their favorable pharmacokinetics, including good solubility, non-toxicity, and non-carcinogenicity. The outcomes of the study assist in concluding the presence of some bioactive substances with promising hypoglycemic activity.

Keywords: Bioactive Phytochemicals, diabetics, hypoglycemic activity, molecular docking, *Moringa oleifera*,

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from impaired insulin secretion, insulin action, or both. Type 2 diabetes mellitus accounts for over 90% of all diabetes cases and is associated with severe complications such as cardiovascular diseases, nephropathy, neuropathy, and retinopathy (Faselis *et al.*, 2020). The increasing global prevalence of diabetes necessitates the

continuous search for novel therapeutic agents with minimal side effects. Natural products derived from medicinal plants have long been explored as alternative treatments for diabetes due to their bioactive phytoconstituents with hypoglycemic potential (Ríos *et al.*, 2015, Jugran *et al.*, 2021). On the other hand, the use of synthetic drugs poses a significant global health risk and increases the likelihood of conditions such as cancer, diabetes, and neurodegenerative diseases. To address this

issue, it is essential to focus on developing medicines derived from natural herbs (Karim *et al.*, 2025a).

Moringa oleifera (Family: Moringaceae), commonly known as the drumstick tree, is a nutritionally and medicinally important plant widely used in traditional medicine for its diverse pharmacological activities. Although the leaves and seeds have been extensively studied for their medicinal benefits, the therapeutic potential of the flowers remains underexplored (Karim *et al.*, 2025b). Recent phytochemical investigations indicated that *M. oleifera* flowers contain a rich profile of bioactive compounds, including flavonoids, alkaloids, and terpenoids, which may contribute to their pharmacological action like anti-asthmatic, anti-diabetic, hepatoprotective, anti-inflammatory, anti-fertility, anti-cancer, anti-microbial, anti-oxidant, cardiovascular, anti-ulcer, CNS activity, anti-allergic, wound healing, analgesic, and antipyretic activity (Paikra *et al.*, 2017).

This study aims to evaluate the hypoglycemic potential of *M. oleifera* flower extract through both *in vivo* and *in silico* approaches. The *in vivo* study investigates the glucose-lowering effects of different solvent fractions of the extract using the oral glucose tolerance test in Wistar rats (Kifle *et al.*, 2020, Goyal and Jeyabalan, 2021). The *in silico* study involves molecular docking analysis to identify key phytochemicals responsible for modulating diabetes-related proteins (Ajiboye *et al.*, 2022). Additionally, pharmacokinetic and ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) predictions were performed to assess the drug-likeness properties of the identified compounds (Bitew *et al.*, 2021, Sucharitha *et al.*, 2022).

MATERIALS AND METHODS

Collection and preparation of the plant sample

The fresh flower of *M. oleifera* was collected from Kushtia, Bangladesh, in March 2024, and the sample's authenticity was verified by Jahangirnagar University Herbarium, Bangladesh (JUH- 10271). After being washed, freshly picked flowers were dried for a few days at ambient temperature (25°C-30°C) and relative humidity (60%-70%). Then, the dried sample was ground into powder and stored in a closed container.

Extraction and fractionation

The dried powder sample of flower was extracted in methanol using the cold extraction procedure. In a clean beaker, 300 gm of the powder was soaked in about 1L of methanol for 15 days with occasional stirring. The filtrate was collected using a cotton-plugged funnel and then completely evaporated using a rotary evaporator set to a fixed temperature and pressure, condensing the filtrate into a dry crude extract. Around 5.0 gm of the crude extract was dissolved in 10 % aqueous methanol and the fractionation was carried out using n-hexane (NHF), chloroform (CF), ethyl acetate (EAF), and aqueous (AQF) depending upon the polarity applying the modified Kupchan partitioning protocol (Van Wagenen *et al.*, 1993) to separate phytochemicals in different solvents based on their polarity index. The fractionated extracts were collected by evaporation in a Rotary Evaporator in a different quantity; n-hexane soluble fraction (1.7gm), Chloroform soluble fraction (1.4gm), Ethyl acetate soluble fraction (0.8gm), Aqueous fraction AQF (1.1gm).

Drugs and reagents

All reagents involved in the study were of analytical grade. Methanol (100%), n-hexane, chloroform, ethyl acetate, dimethyl sulfoxide (DMSO), Tween-80, and glucose were purchased from BDH Chemicals. Saline

water (from Popular Pharmaceuticals Ltd.) and miglitol (provided by Incepta Pharmaceuticals Ltd.) were also used.

Experimental animal

Wistar rats (standard outbred Wistar strain) were collected from the International Centre for Diarrheal Diseases and Research, Bangladesh (ICDDR'B), and were used to conduct the study. These rats were 8-10 weeks old and weighed 280-340 g. The weight variation may be due to the biological, age, diet or environmental factors. To provide them with a suitable housing duration, the recommended temperature of $24 \pm 2^\circ\text{C}$, relative humidity is 60-70% and other stipulated circumstances were met. The rodent food and water provided to the experimental rats were prepared by ICDDR'B. The Animal Ethics Committee at the Faculty of Biological Science, University of Dhaka conducted a panoramic assessment of the ethical guidelines and protocols of the investigation and generated their systematic review and approval (Ref. No. 270/Biol. Sci.). Then, the investigation was performed according to the ARRIVE guideline 2.0 (Percie du Sert *et al.*, 2020).

In-vivo study

Hypoglycemic activity. The hypoglycemic property of the various fractions of the methanolic extract of *M. oleifera* flower was assessed using a slightly modified form of the oral glucose tolerance test (OGTT) (Bogdanet *et al.*, 2020). The OGTT is a widely accepted method to evaluate glucose homeostasis, insulin sensitivity, and possible antidiabetic properties of test compounds. Initially, six rats in each group (Negative control, positive control, and test groups) had their blood glucose levels measured using a glucometer by drawing blood from the tail vein (Arifin and Zahiruddin, 2017). In this study, miglitol (10 mg/kg) was used as a positive control, and a 1% Tween 80 saline

solution (10 mL/kg) as a negative control, with all rats initially given a 10% glucose solution (2 g/kg) to induce hyperglycemia. The test group received oral doses of plant fractions (200 and 400 mg/kg), and blood glucose levels were measured at 30, 60, 120, and 180 minutes to evaluate the antihyperglycemic effect compared to the synthetic drug, highlighting the impact of miglitol in reducing elevated glucose levels. To evaluate the activity, the test sample's percent reduction in blood glucose level relative to the standard was calculated using the following equation:

$$\% \text{ Reduction} = \frac{(T_n - T_s)}{T_n} * 100$$

Where, T_n represents the mean of Blood glucose level in the control group, while T_s represents the mean blood glucose level in the sample treatment groups after 30 minutes of oral administration.

Statistical analysis. The data processing and graph construction from the in vivo data were conducted using MS Excel (version 10.0) and GraphPad software. To accurately represent the results of the in vivo evaluations, the mean \pm SEM was used to convey the average values and their corresponding standard errors of the mean. The p-values of the assays were obtained using the student t-test calculator (unpair t-test) and any data with p-values < 0.05 was considered as statistically significant.

In-silico molecular modeling studies

Phytochemicals selection and preparation:

In this study, 72 compounds from *Moringa oleifera* flowers were identified through literature and the IMPPAT database (Mohanraj *et al.*, 2018). Ligand 3D conformers were retrieved in SDF format from the PubChem database and processed using Open Babel to compile a ligand library. Energy minimization was performed with PyRx 0.8 and Open Babel 2.3.1, employing the MMFF94 force field (Kim *et al.*, 2016).

Finally, AutoDock Tools was used to convert the ligands into pdbqt format for further analysis.

Selection and preparation of proteins: To perform docking studies, the crystal structure of the human pancreatic Alpha-Amylase complexed with Mini-Montbretin A (PDB ID: 5E0F) was retrieved from the Protein Data Bank. The protein, containing a single chain, was prepared using PyMOL (v2.3) (<https://pymol.org/2/>) and cleaned by removing unwanted ligands, heteroatoms, and water molecules (Akash *et al.*, 2023). The structure was then imported into AutoDock Tools for conversion into PDBQT format following standard protocols (Chatterjee *et al.*, 2018). Energy minimization of the receptor was conducted using Swiss PDB Viewer before saving the refined structure as a PDB file for further analysis (Kaplan and Littlejohn, 2001).

Methods of molecular docking: To ensure proper binding of the drug to the target receptor, a clean receptor site free from interference by water or other molecules is essential. In the docking study, the grid box parameters were set to X=-8.4005, Y=21.6258, and Z=-18.9668, with an exhaustiveness value of 8 to optimize the protein-ligand binding conformation. Using PyRx, ligands were transformed into pdbqt format, and proteins developed as macromolecules. Molecular docking was performed using Auto Dock Vina (Dallakyan and Olson, 2014). The docked complexes were visualized in PyMOL, and further analysis of key amino acid residues and interaction sites was conducted using BIOVIA Discovery Studio Visualizer.

Lipinski rule and drug-likeness properties analysis: The pharmacokinetics and Lipinski's Rule of Five for the selected drugs were analyzed using the Swiss ADME online tool (<http://www.swissadme.ch>) (Azzam, 2023). These parameters evaluate structural and

chemical properties to determine a molecule's similarity to existing drugs. Key factors include hydrophobicity, drug-likeness, hydrogen bonding, molecular weight, size, bioavailability, and other relevant characteristics (Ji *et al.*, 2020).

Prediction of ADMET profile: Insufficient pharmacokinetic and safety profiles are major factors in the failure of drug development. Computational methods can help address these challenges. Among these, pkCSM offers a promising alternative for predicting pharmacokinetic properties and ADMET (absorption, distribution, metabolism, excretion, and toxicity) features. Research indicates that ADMET predictions are valuable for assessing the pharmacokinetics of biomolecules prior to clinical and preclinical trials (Avram *et al.*, 2020, Sun *et al.*, 2022). The pkCSM web tools (<https://biosig.lab.uq.edu.au/pkcsm/>) (Azzam, 2023) and <http://lmmd.ecust.edu.cn/admet2/result/?tid=742441> were used to assess and analyze the ADMET feature.

RESULTS AND DISCUSSIONS

Hypoglycemic Activity

The effects of various extracts on glucose levels were assessed over a period of 180 minutes following oral glucose administration. The data are presented in Table 1. In the control group, glucose levels peaked at 30 minutes (10.63 ± 0.28 mmol/L) and declined to 8.33 ± 0.15 mmol/L by 180 minutes. The standard drug (STD) significantly reduced glucose levels at all time points ($***p < 0.001$), confirming the experimental setup. Both NHF-400 and CF-400 extracts showed strong antihyperglycemic effects, significantly lowering glucose at 60, 120, and 180 minutes, with CF-400 being more effective. Lower doses (NHF-200, CF-200) showed moderate activity, while EAF-200 and EAF-400 exhibited no significant

effects. AQF-400 and AQF-200 showed slight, non-significant glucose reductions, indicating weak activity.

The findings of the investigation confirmed that both the dose (200 mg/kg and 400 mg/kg of body weight) of n-hexane fraction (NHF) and chloroform fraction (CF) exhibited statistically significant ($p < 0.05$) hypoglycemic effect up to three hours from the administration of glucose solution, compared to the control group. The 200 mg/kg body weight dose of NHF and CF expressed a 30.22% and 33.83% reduction of blood glucose level after 180 minutes, respectively and the 400 mg/kg body weight dose of NHF and CF exhibited 37.01% and 49.86% reduction of blood glucose levels which was very much comparable to the percent reduction value (53.92%) of the positive control drug miglitol (Figure 1).

The *in vivo* findings suggest that *Moringa oleifera* flower extract has notable hypoglycemic effects, likely mimicking the mechanisms of synthetic antidiabetic drugs, such as enhancing insulin release, reducing glucose production, inhibiting glucose absorption, or activating PPARs (Subramoniam, 2016).

***In Silico* Studies**

Molecular docking analysis: To justify the *in vivo* results, molecular docking analysis is widely performed for determining the ligand-protein interactions. It offers a detailed understanding of the binding sites of the proteins, binding style, and probable mechanism of action among the existing pathways. H bonding as well as hydrophobic bonding are the main reasons for docking scores because protein-ligand interaction is crucial in structurally oriented drug design. If the docking score is more than -6.00 kcal/mol, the drug is considered standard (Cosconati *et al.*, 2010). The identification of the ligand-receptor complex structure is the main

objective of molecular docking. This can be achieved in two interrelated steps: first, by sampling ligand arrangements on protein active sites, and second, by organizing the distortions utilizing a score function.

Blind docking analysis revealed that all compounds exhibited strong binding affinities with human pancreatic alpha-amylase (PDB: 5E0F), with binding energies ranging from -6.9 to -9.0 kcal/mol Table 2. Notably, kaempferol, quercetin, and ar-turmerone showed the highest affinities, indicating potential hypoglycemic effects of *M. oleifera* phytochemicals against type 2 diabetes (Ponnusamy *et al.*, 2015).

Protein-ligand Interaction: Pymol application software and the BIOVIA Discovery Studio were utilized to produce the interaction diagrams for drug-protein configurations, hydrogen bonds, and molecular docking pockets. Protein and ligand interactions have been studied about hydrogen bond donor and donor-acceptor interactions, hydrophobic interactions (such as pi-sigma, alkyl, and pi-alkyl interactions), and hydrogen bond interactions (including conventional and non-conventional H bonds). Hydrophobic and hydrogen bond interactions are important in drug activity. Various involvement and binding activities between the medication and the intended target protein are shown in Figure 2. In hydrogen bonding, the acceptor region is described as red-green, and the receptor region as violet. Furthermore, the two-dimensional image of active amino acid residues shows that A: GLN63 (2.49893), HIS305 (2.42039), ASP197 (2.506.9), ASP300 (2.9675), ASP300 (2.26897), TRP59 (4.83897), TRP59 (4.16462), TRP59 (5.90182), TRP59 (4.33158), and TYR62 (4.94086) are generated for Human pancreatic alpha-amylase (PDB ID 5E0F) with

Lipinski rule analysis for oral medication: The Lipinski rule suggests that orally active drugs should be modest due to

their pharmacological or biological activity, as they possess the necessary molecular and physical properties for oral consumption by mammals. According to the Lipinski Rule, a suitable oral drug must have a topological polar surface area between 17.07 and 131.36 and a molecular weight between 286.24 and 306.24. Additionally, all drugs have greater bioavailability ratings (0.55) (Table 3). The GI absorption rate is another crucial metric that shows how effectively the drugs are absorbed in the digestive system (Table 3).

ADMET profile prediction: The computational inspection techniques may be used in drug development to detect ADMET parameters, which have a substantial influence on therapeutic absorption, distribution, metabolism, solubility, and oral bioavailability (Daoud *et al.*, 2021). Each of the ADMET features given has a different water solubility value. Since their actual water solubility values vary from -4 to -6, the ligands LM02 and LM03 are extremely soluble in water, with a range of -2.925 to -4.454. It is implied that the remaining substances are highly soluble in fatty substances or lipids since they are only weakly soluble in water. A thorough summary of medication distribution, including volume distribution and blood-brain barrier permeability, is given in Table 4. Another factor that prevents undesirable substances from accessing the brain and CNS is the blood-brain barrier or BBB (Cosconati *et al.*, 2010). In our findings, the BBB permeability range was -1.098 to 0.512 (Table 4).

Since highly water-soluble oral medications provide superior oral bioavailability and maximal absorption capacities, water solubility is essential for contemporary drug research. The development of quick, accurate, structure-based strategies for determining an active drug candidate's solubility in water is highly desired (Wang *et al.*, 2018). Theoretical results showed that quercetin (LM02) and ar-turmerone (LM03)

are highly water-soluble, with low volume of distribution (VD), suggesting higher plasma concentration and limited tissue penetration; most ligands exhibited low VD and BBB permeability ranged from -1.098 to 0.512. All ligands satisfied Lipinski's rule, with suitable topological polar surface area (17.07–131.36 Å²), molecular weight (286.24–306.24 g/mol), and bioavailability scores (0.55), indicating good oral drug potential. (Khan *et al.*, 2019).

CONCLUSION

The present study explored the hypoglycemic potential of *Moringa oleifera* flower extract through *in vivo* and *in silico* approaches. The n-hexane and chloroform fractions demonstrated significant glucose-lowering activity in OGTT assays. Molecular docking analysis identified kaempferol, quercetin, and ar-turmerone as potential inhibitors of pancreatic alpha-amylase, with favorable binding affinities and pharmacokinetic properties. These findings suggest that *Moringa oleifera* flowers contain bioactive compounds with antidiabetic potential. Further experimental validation, including studies on diabetic animal models and mechanistic investigations, is necessary to confirm their therapeutic efficacy.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Table 1: Average glucose level (mmol/L) after loading the glucose sample

Group	Average glucose level (mmol/L) after loading the glucose sample				
	0 minutes	30 minutes	60 minutes	120 minutes	180 minutes
CTL	5.68±0.08	10.63±0.28	10.02±0.23	9.58±0.11	8.83±0.15
STD	5.45±0.11	10.98±0.24	7.82±0.13***	6.20±0.16***	3.95±0.06***
NHF- 200	7.55±0.20	10.15±0.24	9.45±0.16	8.03±0.16**	7.08±0.15***
NHF -400	6.80±0.14	9.05±0.23	7.20±0.25***	6.32±0.26***	5.70±0.20***
CF -200	5.32±0.17	10.78±0.26	9.33±0.12*	8.95±0.13**	7.13±0.18***
CF -400	6.02±0.16	9.73±0.38	6.17±0.25***	5.46±0.24***	4.88±0.24***
EAF -200	5.48±0.11	11.32±0.26	10.01±0.28	9.58±0.28	8.80±0.21
EAF -400	5.70±0.07	10.69±0.29	9.87±0.23	9.30±0.14	8.80±0.14
AQF-200	5.50±0.07	9.82±0.36	9.43±0.28	9.07±0.13	8.58±0.10
AQF-400	6.15±0.15	10.90±0.21	9.50±0.18	9.18±0.21	8.37±0.16

Note: Data are mentioned as mean ± SEM, n = 6. *p<0.05; **p<0.01; ***p < 0.001 versus negative control.

CTL=Control group; NHF=N-Hexane Fraction; CF=Chloroform Fraction; EAF=Ethyl Acetate Fraction; AQF=Aqueous Fraction

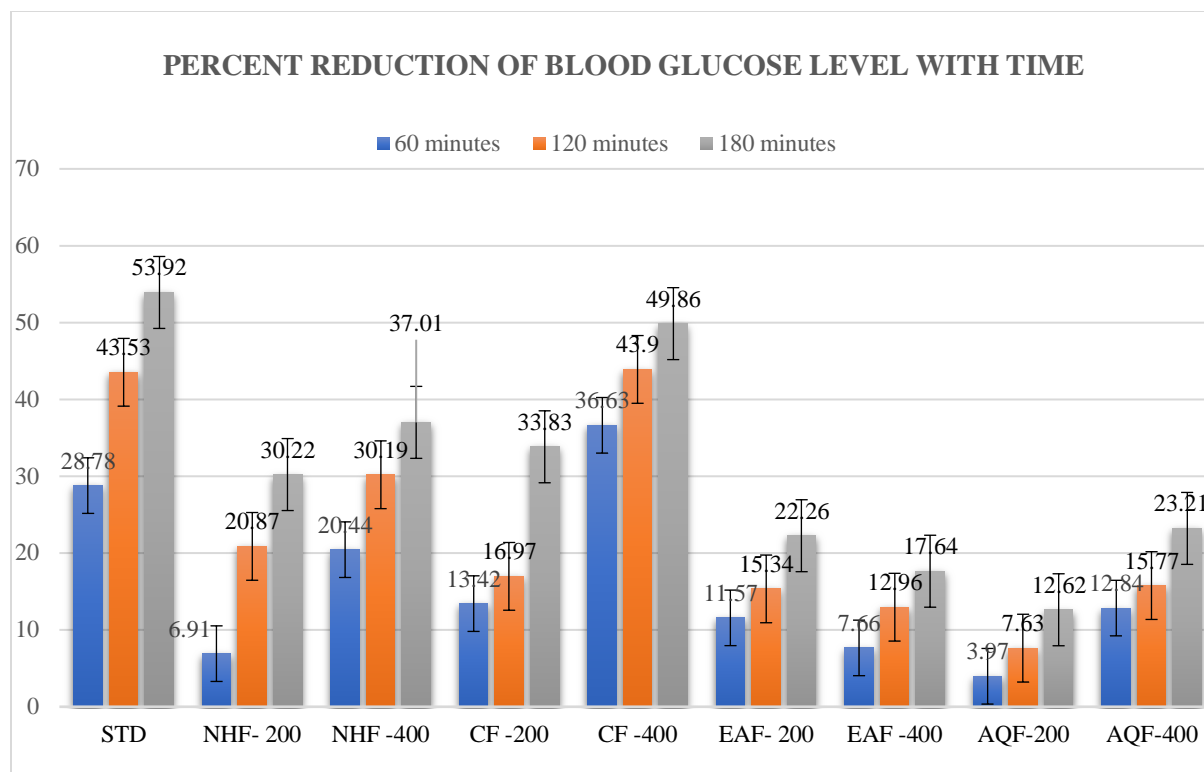
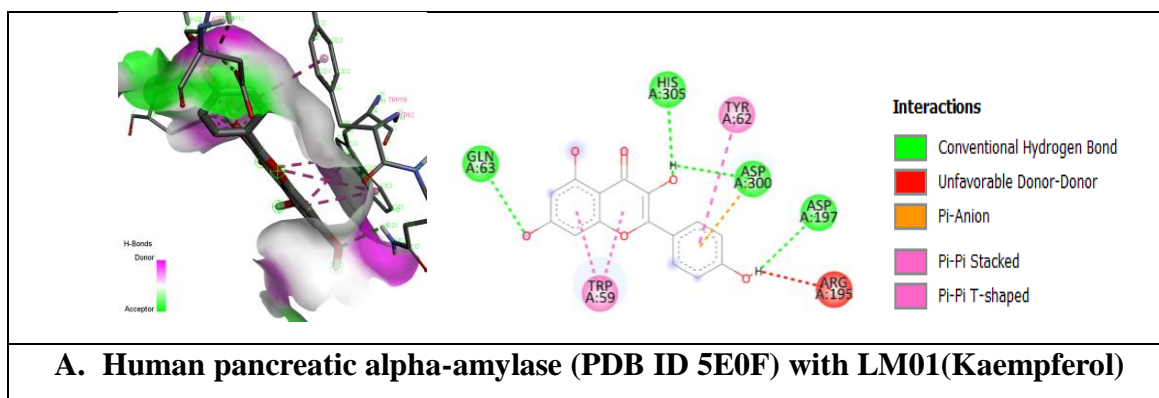


Figure 1. Percent glucose level reduction (vertical unit) for different fractions *Moringa oleifera* flower with time.

Table 2: Data on binding energy and the name of the interacted ligand for human pancreatic alpha-amylase (PDB: 5E0F).

Ligand	Binding Affinity (kcal/mol)	No of H bonds	No of Hydrophobic bonds	Others	Total bonds
Kaempferol	-8.8	5	4	1	10
Quercetin	-9	4	5	1	10
ar-Turmerone	-6.9	0	7	0	7
Standard (miglitol)	-5.9	3	3	0	6



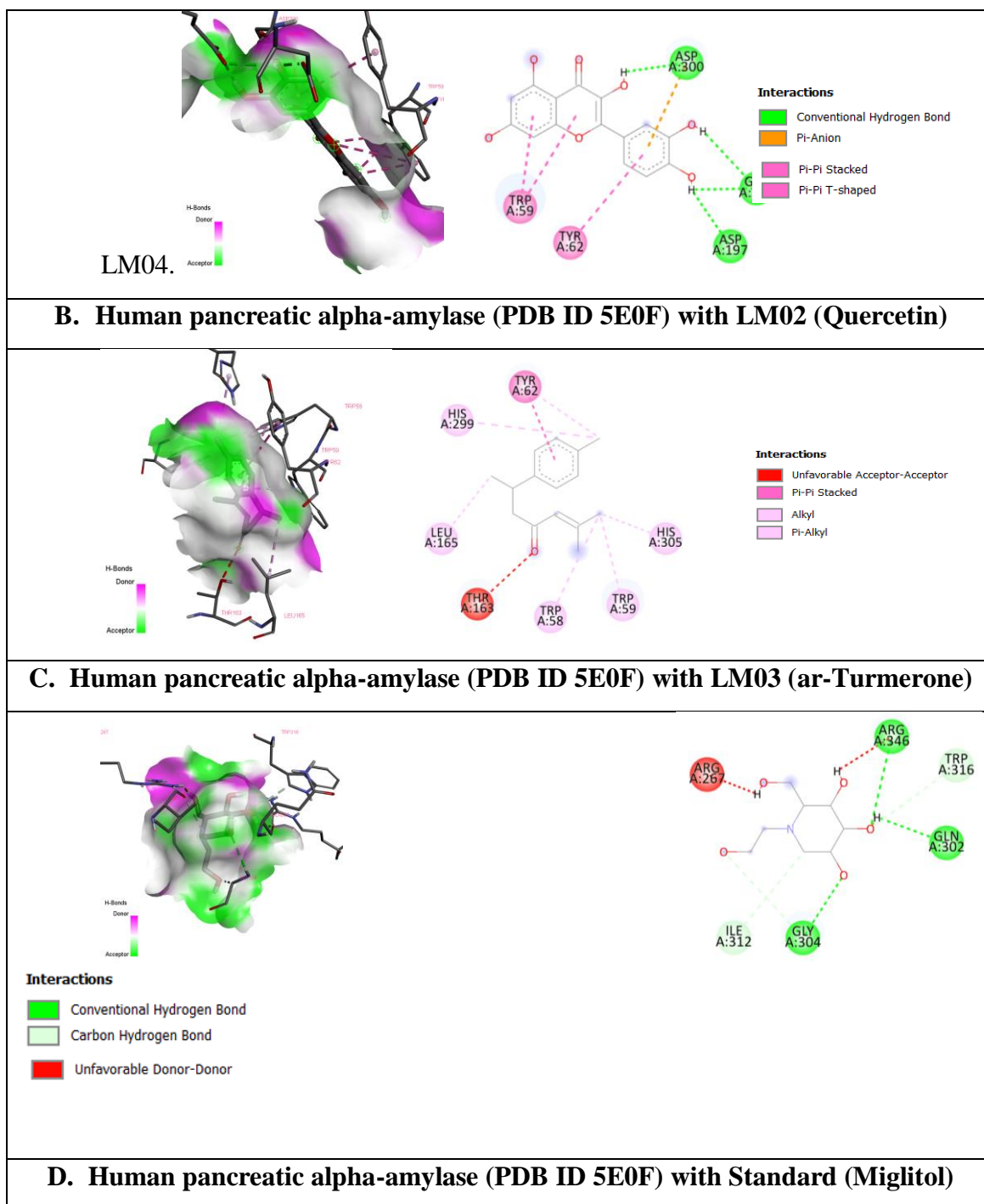


Figure 2. Molecular docking experiments reveal the interactions between proteins and substances.

Table 3: Data of Lipinski rule, pharmacokinetics, and drug likeness

CID	Molecular weight g/mol	H-bond acceptor	H-bond donor	Molar Refractivity	Topological polar surface area(Å ²)	Consensus Log Po/w	Lipinski rule		Bioavailability
							Result	violation	
5280863 (Kaempferol)	286.24	6	4	76.01	111.13	1.58	Yes	0	0.55
5280343 (Quercetin)	302.24	7	5	78.03	131.36	1.23	Yes	0	0.55
160512 (ar-Turmerone)	216.32	1	0	69.75	17.07	3.84	Yes	0	0.55
441314 Miglitol	207.22	6	5	51.08	104.39	-1.94	Yes	0	0.55

Table 4. Computational ADMET Data Prediction

I No	CID	Absorption		Distribution		Metabolism		Excretion		Toxicity			
		Water solubility (Log mol/L)	Human Intestinal Absorption (%)	VDs (log L/kg)	BBB Permeability (log BB)	CYP450 1A2 Inhibitor	CYP450 2D6 Substrate	Total Clearance (log ml/min/kg)	Renal OCT2 substrate	Max. tolerated dose (log mg/kg/day)	Skin Sensitization	Hepatotoxicity	AMES toxicity
01	5280863 (Kaempferol)	-3.04	74.29	1.274	-0.939	Yes	No	0.477	No	0.531	No	No	No
02	5280343 (Quercetin)	-2.925	77.207	1.559	-1.098	Yes	No	0.407	No	0.499	No	No	No
03	160512 (ar-Turmerone)	-4.454	94.489	0.621	0.512	Yes	No	0.295	No	0.846	Yes	No	No
04	441314	1.229	41.462	-0.607	-1.501	No	No	0.815	No	2.239	No	No	No

Effect of harvesting time and washing treatment on post-harvest quality of mango

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ABSTRACT

To study the quality of mango fruit as influenced by sap burn injury during harvesting time and washing treatment a field research was done in farmer field at KanchanRupa municipality, Saptari and laboratory work at National Citrus Research Program, Dhankuta from 13th June, 2023 to 28th June, 2023. The research was laid out in two factorial completely randomized designs with three replications. The first factor consists of harvesting time (09-11 am, morning time and 02-04 pm, day time). The second factor, washing treatment done in field consists of (No washing, Normal tap water, Sodium chloride - 1%, Potassium metabisulphate- 1%, Detergent- 1%, and Calcium hydroxide- 0.5%). The physiochemical quality, shelf-life, sap burn and physiological loss in weight (PLW) were studied. The highest total soluble solids (TSS) was obtained in No washing (16.63 °Brix) and the lowest in Calcium hydroxide (14.03 °Brix) at 10 days after harvesting. Similarly, the lowest titratable acidity of fruit was obtained in washing treatment with Sodium chloride (0.62%) and highest in both Normal tap water and Calcium hydroxide (0.79%). Among different washing treatments, the longest shelf-life of mango was obtained in Calcium hydroxide (12.10 days) washed fruit followed by Detergent (10.17 days). The shortest shelf-life was obtained in washing treatment No washing (8.25 days). The sap burn injury was recorded more in day harvest (2.03) than morning harvest (1.94) which are statistically not different apart. The PLW of fruit was observed lowest in Calcium hydroxide (7.62%) treated fruit upto 13th days of storage. Overall, Calcium hydroxide was found as best washing treatment for better post-harvest life of mango than rest of the treatments.

Key Words: Mango, physiological property, sap burn, washing treatment,

INTRODUCTION

Nepal is blessed with diverse agro-ecological conditions that favor the production of different horticultural produce. Mango (*Mangifera indica* L.) is a popular fruit in the Nepalese market due to presence of excellent flavor, fragrance, beautiful colour, taste and nutritional properties. Mango is an abundant source of vitamin A, C, E and crucial antioxidants that enhance immunity and

nourish skin (Deb *et al.*, 2024). In context of fruit, mango is grown in Nepal in an area of 42,773 ha with production 5,13,055 mt, productivity of 11.99 mt/ha that is lower than productivity of Saptari district 14.84 mt/ha (MoALD, 2022/23). Mango fruit besides local consumption, is an exchange earning commodity in Saptari. The Sap burn injury is considered as most serious problem in fruit quality of harvested mango. The stem

(pedicel) of mango fruit exudes sap which spreads over fruit peel that makes skin damage. Significant volume of mangoes (about 50 %) undergoes sap contamination due to poor harvesting methods (Mazhar *et al.*, 2010). Sab *et al.* (2017) also reported 34.49 % post-harvest loss in mango from harvesting to consumption. Abu *et al.* (2021) reported that latex flow in harvested mango occurred even after physiological maturity at decreasing rate which persisted upto packaging. Amwoka (2021) concluded that 5 % solution of lime Ca (OH)₂ was best for postharvest loss management in mango rather than that of 10 %, 15 % & 20 % solution. Krishnapillai *et al.* (2016) indicated for the simple, inexpensive, and environmentally friendly method that could be used to reduce sap burn injury in mango. Nowadays growers are interested in production of high-quality fruit due to increase in demand of quality produce. The research was assigned to know the effect of different washing treatment and harvest time on the post-harvest quality of mango fruits.

MATERIALS AND METHODS

The field research was done in farmer orchard at Saptari district (26° 38' 12 '' N; 86° 54' 31 '' E), Madhesh Province, Nepal. The Saptari district has tropical climate, located at 200 m above mean sea level (masl). The laboratory work was done in National Citrus Research Program, Paripatle (27° 00' 02 '' N; 87 ° 18 ' 30 '' E), Dhankuta, Nepal located at 1385 masl.

Each mango tree was applied with 300 gm urea, 400 gm DAP, 300 gm Potash fertilizer in Ring-basin method at the end of Rainy season. Mid-season cultivar Maldah was selected for the research. Mango fruits were harvested at physiological maturity stage with (5–8) cm pedicel attached on the fruit. Then pedicel base was maintained by cutting one cm from the fruit surface. All the harvested fruit was washed in washing treatment for 3-5 minutes

in plastic bucket except No washing (Control). Again, sample fruit that has gone under washing treatment was rinsed in normal tap water. The fruit sample was washed in washing treatment within one hour of a harvest. All field work was carried out in an ambient temperature. The sample fruit was laid out in concrete floor with cartoon paper. The washed fruit sample was transported in Bolero Pickup Van from field to Lab in 3 hour and 40-minute time. The temperature and Relative humidity of Lab ranges from 22.7-23.5 °C and 90-99 % in an ambient room condition.

Experimental details

The experiment consisted of two factors (Time of harvest) and (Washing treatment). The Completely Randomized Design (CRD) along with three replication was done. Each experimental unit / treatment unit consisted 15 fruit sample. Altogether 270 (15*6 *3) mango fruit was washed in morning time. The same quantity of fruit sample was washed in day time at research site.

Time of harvesting (Factor A)

Harvest time (H₁) – Morning time (09-11 am)

Harvest time (H₂)- Daytime (02-04 pm)

Washing treatment (Factor B)

WT₁= Control (No washing)

WT₂= Normal tap water

WT₃ = Sodium chloride (1% Solution)

WT₄= Potassium metabisulphite (1% Solution)

WT₅= Detergent (1%)

WT₆= Calcium hydroxide (0.5% solution)

The data were recorded on 1st, 4th, 7th, 10th and 13th days after harvesting (DAH) of mango. The fruit sample were categorized under destructive and non-destructive parameter. Parameter recorded under destructive sample was total soluble solids (TSS) and titratable

acidity (TA). Similarly, under non-destructive sample the parameter recorded were shelf-life, sap burn injury and physiological loss in weight (PLW).

Total soluble solids (TSS) was measured with the help of digital refractometer. Titratable acidity was measured with the help of PAL acid-brix meter. The shelf life of fruits was determined from the days of harvesting to marketable stage till the 50 % of fruit are easily transportable to nearby market. The sap burn injury of mango was assigned by score level (0-4) as given by (Maqbool *et al.*, 2007). Physiological loss in weight (PLW) of fruit was determined using the formula:

Physiological loss in weight = [(Initial weight – Final weight)/Initial weight] x 100(%)

The collected raw data was entered and tabulated into MS-Excel (2010), analyzed by statistical software package R studio (Version 4.3.1) by using ANOVA table. Mean values were considered at 5 % significance level ($p < 0.005$)

RESULTS AND DISCUSSION

Total soluble solids (TSS) of mango:

The effect of harvesting time and washing treatments on TSS of mango was analyzed (Table 1). There were no significant differences on TSS at different harvesting time. Similarly, washing treatment at 1 DAH and 4 DAH doesn't have significant difference. At 7 DAH, significantly the highest TSS of 14.54 °Brix was recorded in Normal water than other treatments. At 10 DAH, the highest TSS of 16.63 °Brix was found in No washing treatment which was statistically similar with Potassium metabisulphite and Normal water ($p < 0.005$). Amin *et al.* (2008) also reported that control treatment has highest TSS and lowest in lime treated mango fruit. The increase in TSS might be the outcome of conversion of carbohydrate into simple sugar by complex mechanism during the storage

which increases with storage period and temperature.

Titrateable acidity of mango:

The effect of harvesting time and washing treatments on titrateable acidity of mango was analyzed (Table 2). The significant difference of TA with respect to harvesting time was found at 1, 4 and 10 DAH. There was no significant difference at 7 DAH with respect to harvesting time. The washing treatment has significant effect on TA content of fruit in 1, 7 and 10 DAH. At 10 DAH, TA content of fruit was highest in No washing, Normal water, potassium metabisulphite and calcium hydroxide than rest of the treatment. Mounika *et al.* (2017) reported that highest titrateable acidity (0.70) % in mango cv. Amrapali treated with 2 % calcium nitrate, and lowest titrateable acidity (0.30) % in control. The decrease in acidity of mango is due to conversion of citric acid into sugars and its utilization in metabolic process of fruits.

Shelf-life of mango fruit

The shelf life of mango was recorded based on harvesting time and washing treatments (Table 3). There was no significant difference in shelf-life with respect to harvesting time. The mango fruit treated with washing treatment has significant difference in shelf life ($p < 0.001$). The shelf life of mango was observed highest in washing treatment with calcium hydroxide (12.17 days) than rest of the treatments. Mounika *et al.* (2017) reported prolong storage life in Amrapali variety of mango with calcium treated fruit after harvest. Calcium hydroxide or lime helps to maintain membrane integrity, stability in reducing weight loss and anthracnose incidence (Kirby & Pilbeam, 1984).

Sap burn injury in mango

The sap burn injury with respect to harvesting time was scored (Table 4). There is no significant difference in sap burn injury with

respect to harvesting time (Morning time and Day time). Bayogan *et al.* (2021) reported that the Carabao mango cultivar harvested in the morning time showed higher sap injury resulting in lower quality of fruit. Barman *et al.* (2015) reported that mango harvested in the morning (7:00-9:30 am) has lowest sap injury than harvested in the afternoon (12:00-2:00 pm) despite the higher sap volume. The contradiction in finding may be due to growth of different cultivar of mango in different climatic condition.

Physiological loss in weight (PLW %)

Physiological loss in weight generally increased as the storage period advanced, which is slow at initial days and more rapidly after fourth days of harvest (Table 5). At 13th days of storage, the physiological loss in weight was minimum in calcium hydroxide treated fruit (2.54 %) which is not significantly different than Normal water and potassium metabisulphite. Thokchom and Mandal (2018) reported gradual increase in weight loss with storage period in Aonla. Calcium hydroxide treated fruit exhibited minimum PLW in different storage days which might be due to delay of senescence of harvested crops caused by decrease in respiration and desiccation. It has been stated that calcium salts interferes with ethylene link and acts as powerful tool for postharvest management of climacteric fruit (Mounika *et al.*, 2017).

CONCLUSION

The results show that washing treatment helps in improvement of fruit quality by prolonging shelf-life, level of TSS and decrease in physiological loss in weight. There is positive interaction between harvesting time and washing treatment. The anti-sap chemical particularly calcium hydroxide yielded satisfactory result. The washing treatment has improved cosmetic look of the fruit.

CONFLICT OF INTEREST STATEMENT

The authors declares that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Table 1: Effect of harvesting time and washing treatments on TSS content of mango cv. Maldah

Treatments	Total soluble solids (°Brix)			
	1 DAH	4 DAH	7 DAH	10 DAH
Harvesting time(Factor A)				
Morning time (09 -11 am)	5.48	7.23	12.18	15.36
Day time (02-04pm)	5.38	7.56	12.58	15.77
SEm (\pm)	0.08	0.23	0.22	0.15
LSD _{0.05}	0.23	0.68	0.65	0.43
F-test	ns	ns	ns	ns
Washing treatments (Factor B)				
No washing	5.57	7.96	12.78 ^b	16.63 ^a
Normal tap water	5.59	7.93	14.54 ^a	15.96 ^{ab}
Sodium chloride (1% solution)	5.55	7.56	12.54 ^b	15.64 ^c
Potassium metabisulphite (1% solution)	5.17	7.28	11.88 ^b	16.25 ^{ab}
Detergent (1% solution)	5.35	7.14	12.02 ^b	14.88 ^c
Calcium hydroxide (0.5% solution)	5.35	6.58	10.52 ^c	14.03 ^d
SEm (\pm)	0.13	0.40	0.38	0.25
LSD _{0.05}	0.39	1.18	1.13	0.75
F-test	ns	ns	**	***
CV, (%)	5.99	13.29	7.61	3.47
Grand mean	5.43	7.41	12.38	15.57

Notes: DAH: Days after harvest. ns: Non significant at 5% level; ***Significant at 0.1% level; **Significant at 1% level ;SEm: Standard error of mean; Values with same letter(s) in a column are not significantly different at 5% level by DMRT

Table 2: Effect of harvesting times and washing treatments on titratable acidity of mango cv. Maldah

Treatments	Titratable acidity(TA)%			
	1 DAH	4 DAH	7 DAH	10 DAH
Harvesting times (Factor A)				
Morning time (09 -11 am)	1.09 ^b	1.14 ^b	0.83	0.77 ^a
Day time (02-04pm)	1.26 ^a	1.5 ^a	0.80	0.71 ^b
SEm (\pm)	0.03	0.04	0.02	0.01
LSD _{0.05}	0.07	0.12	0.05	0.04
F test	***	***	ns	*
Washing treatments (Factor B)				
No washing	1.02 ^c	1.27	0.75 ^a	0.72 ^{ab}
Normal tap water	1.34 ^a	1.21	0.86 ^{ab}	0.79 ^a
Sodium chloride (1% solution)	1.32 ^a	1.4	0.76 ^{bc}	0.68 ^b
Potassium metabisulphite (1% solution)	1.21 ^{ab}	1.24	0.83 ^{abc}	0.76 ^a
Detergent (1% solution)	1.07 ^{bc}	1.18	0.90 ^a	0.74 ^b
Calcium hydroxide (0.5% solution)	1.08 ^{bc}	1.63	0.80 ^{bc}	0.79 ^a
SEm (\pm)	0.05	0.07	0.03	0.02
LSD _{0.05}	0.13	0.21	0.078	0.07
F-test	***	ns	*	*
CV, (%)	9.50	9.03	8.75	7.58
Grand mean	1.17	1.33	0.82	0.74

Notes: DAH: Days after harvest. ns-Non significant at 5% level; ***Significant at 0.1% level; * Significant at 5% level; SEm: Standard error of mean; Values with same letter(s) in a column are not significantly different at 5% level by DMRT

Table 3: Effect of harvesting times and washing treatments on shelf-life of o mango cv. Maldah

Treatments	Shelf-life (Days)
Harvesting time	
Morning time (09 -11 am)	8.89
Day time(02-04pm)	9.39
SEm (\pm)	0.26
LSD _{0.05}	0.77
F-test	ns
Washing treatments	
No washing	8.25 ^c
Normal tap water	8.33 ^c
Sodium chloride (1% solution)	7.33 ^c
Potassium metabisulphite (1% solution)	8.5 ^c
Detergent (1 % solution)	10.17 ^b
Calcium hydroxide (0.5 % solution)	12.17 ^a
SEm (\pm)	0.45
LSD _{0.05}	1.33
F-test	***
CV, (%)	12.14
Grand mean	9.13

Notes: ns: Non significant at 5% level;***Significant at 0.1% level; SEm: Standard error of mean; Values with same letter(s) in a column are not significantly different at 5% level by DMRT

Table 4: Effect of harvesting times on sap burn injury of mango cv. Maldah at 7 DAH

Treatments	Sap burn injury fruit
Harvesting time	
Morning time (09 -11am))	4 (1.94)
Day time (02-04pm)	4 (2.03)
SEm (\pm)	0.39
LSD _{0.05}	1.29 (0.29)
F-test	ns
Injury (Score)	
No injury	6.54 (2.57) ^a
Very mild	5.83 (2.51) ^a
Mild	5.16 (2.31) ^a
Moderate	1.66 (1.43) ^b
Severe	0.83 (1.12) ^b
SEm (\pm)	0.68
LSD _{0.05}	2.04(0.466)
F-test	**
CV, (%)	41.67(19.34)
Grand mean	4(1.99)

Notes: **Significant at 1 % level; SEm: Standard error of mean; Values with same letter(s) in a column are not significantly different at 5% level by DMRT. Figure in parenthesis indicate $\sqrt{x + 0.5}$ transformation

Table 5: Effect of harvesting times and washing treatments on (PLW %) of mango cv. Maldah

Treatments	Physiological loss in weight (PLW %)				
	Storage period (Days)				
Harvesting time	1	4	7	10	13
Morning time (09-11 am)	0	1.88 ^a	2.51 ^a	1.99	2.96
Day time (02-04 pm)	0	1.23 ^b	2.00 ^b	2.19	2.97
SEm(±)		0.09	0.06	0.08	0.06
LSD _{0.05}		0.27	0.18	0.25	0.18
F-test		***	***	ns	ns
Washing treatments					
No washing	0	1.42	2.34 ^{ab}	2.31 ^a	3.10 ^{ab}
Normal tap water	0	1.40	2.09 ^{bc}	2.13 ^{ab}	2.81 ^{bc}
Sodium chloride (1% solution)	0	1.90	2.35 ^{ab}	2.28 ^a	3.26 ^a
Potassium metabisulphite (1% solution)	0	1.70	2.31 ^{ab}	1.68 ^b	2.84 ^{bc}
Detergent (1% solution)	0	1.65	2.53 ^a	2.27 ^a	3.23 ^a
Calcium hydroxide (0.5% solution)	0	1.28	1.94 ^c	1.86 ^{ab}	2.54 ^c
SEm (±)		0.16	0.11	0.15	0.11
LSD _{0.05}		0.67	0.32	0.43	0.32
F-test		ns	*	*	**
CV, (%)		25.55	12.02	17.58	9.15
Grand mean		1.56	2.26	2.09	2.96

Notes: ***Significant at 0.1% level; ** significant at 1 % level; * Significant at 5% level; SEm: Standard error of mean; Values with same letter(s) in a column are not significantly different at 5% level by DMRT

SHORT COMMUNICATION

Impact of the market price information system on the sustainable provision of non-timber forest products in Western Nepal

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ABSTRACT

The present study was carried out in Dolpa, Salyan, and Banke districts of Nepal to determine the function of MPIS in NTFP distribution among usufructs and long-term sustainable NTFP management. Vegetables, Fruits, fish, nuts, medicinal herbs, gum, lac, resins, essences, and a variety of barks and fibers including Rattans, Bamboo and other grasses and palms are all considered NTFP. Because they serve various purposes, people gather and sell these items. Usufructs must be aware of the market and price of NTFPs in order to conserve the forest and cultivate and harvest non-timber forest products on time. The exploratory research design and cross-sectional descriptive served as the foundation for the investigation. Data from 466 respondents was gathered using a mixed strategy. The respondents were chosen by straightforward random selection procedure, as well as the data was gathered using a structured questionnaire survey. According to the analysis, MPIS performed terribly when it came to benefit sharing and NTFP sustainable management. Since the Market Price Information System (MPIS) provides usufructs with information on the price and market of NTFP, the study's goal is to determine how well MPIS works in Nepal for benefit sharing and sustainable NTFP management. Fewer people were urged to participate in the NTFPs sustainable management, and even fewer profited from learning about their prices. As a result, this study has created and suggests the MPIS process for sustainable NTFP management in order to significantly enhance the current market price information system and inform buyers, usufructs, and other stakeholders about expanding their access to formal channels for NTFP management.

Keywords: Market-Price Information System (MPIS), NTFP (Non-timber forest Products), Sustainable Management, Usufructs, CFUGs (Community Forest User Group)

Plants or plant parts that are deemed valuable enough for consumption or commercial purposes to motivate their removal from the forests are known as Non-timber Forest products, or NTFPs. Plants and their derivatives are utilized for fuel, food, fodder and storage, biochemical and medicinal purposes. Additionally, animals such as fish, birds, and reptiles are utilized for their feathers and food (Adepoju and Salau, 2007). NTFPs can be produced in forest plantations and agroforestry projects, or they can be collected from the trees outside of forests or in the wild (Carr *et al.*, 2008). Because of its high cultural and economic value in developing nations, Non-timber forest products or NTFPs are becoming more and more important (Inglehart and Baker, 2001). When it came to the effect of industry growth on regional sustainability, researchers discovered that non-timber forest products were significantly reducing household poverty and improving the livelihoods and food security of locals (Schunko *et al.*, 2019; Thanh *et al.*, 2020; Taghouti, *et al.*, 2021). For rural residents, NTFPs are significant sources of employment and income, and some are even traded internationally (Akinnifesi *et al.*, 2005).

NTFPs include nuts, vegetables, medicinal plants, fish and game, essences, resins, and a variety of barks and fibers like rattans, bamboo, and a host of other grasses and palms that are used for various purposes. Consequently, people collect and sell them (Rawal and Kumar, 2020). The gathering of NTFPs is a significant economic activity in almost every tropical nations. NTFP processing and collecting provide excellent work opportunities for millions of individuals throughout Asia Pacific. In India, over 7.5 million people work part-time as collectors of Tendu (*Diospyrus melanoxylon*) leaves, with another 3 million processing the leaves to Bidi. NTFPs have a large market in Nepal, India, and other nations. In Nepal, there are around 700 plant species of medicinal value, of which 238 are utilized actively and 100 are marketed. The Government of Nepal has

prioritized 30 species, 12 of which are for commercial production and market promotion (Banjade and Paudel, 2008). NTFPs are becoming increasingly popular in national and worldwide markets since they are key constituents in a variety of herbal cosmetics, herbal teas, foods, and medications. According to a 1995 survey of NTFP producers, traders, and processors working from the country's eastern border to the mid-western town of Nepalgunj, 100 entrepreneurs handled 42 thousand tons of over 100 distinct NTFP commodities, amounting to USD 26 million (Banjade and Paudel, 2008). The management of NTFP has received growing attention in recent years. NTFPs are given a high priority in Nepal's forest policy and overall development plans. In general, intermediaries have control over price information. The mistrust and non-transparent nature of the pricing reporting process, controlled by a few dominant purchasers in pricing and marketing, and the production of irregularity in demand from the ultimate buyers/processors are impeding the utilization of NTFP resources (Bhatta, and Rawal, 2001). The current unsustainable collecting practices, which are generally unregulated, are the result of not just the collectors' lack of awareness of the plants and their environment, but also of inadequate price information transmission. The primary goal of NTFP collectors is to obtain accurate market and pricing information. The Interim Plan of Government of Nepal also recognizes that Nepal doesn't have sufficient knowledge of NTFPs to use them as a significant means for reducing poverty. As a result, action research plans are chosen for programs to get more knowledge about conservation, advancement of cultivation technology, promotion and marketing of high-value NTFPs (Banjade and Paudel, 2008). To overcome this gap, the current study recommends the most effective use of the Market Price Information System (MPIS) for long-term NTFP management in the western Nepali districts of Dolpa, Salyan, and Banke. The current study aims to assess the MPIS's strengths, shortcomings, opportunities, and

threats. The study advised revisions and adjustments for replicating and implementing the current MPIS in additional Nepalese districts.

The study was carried out in Banke, Salyan, and Dolpa districts (Nepal), which represent the three geographical layers: hill, mountain, and plain region in year 2017-18 & 2018-19 during the research from Mewar University, Gangrar, Chittorgarh, Rajasthan, India. During the investigation, 466 respondents were picked for the survey using a simple random sample procedure. The study looked at usufructs' awareness and access to MPIS of NTFPs, present MPIS practice, MPIS efficacy, and the difficulties and opportunities for equitable distribution of NTFPs across usufructs. The cross-sectional study design was used, and the data was collected and analyzed quantitatively. The questionnaire was meant to identify important obstacles and impediments to fair usufruct distribution, and some observation and discussion were conducted in parallel to learn about the real practice of MPIS and its usefulness in the practice of usufructs in sustainable NTFP management.

Evaluate users' knowledge and accessibility to MPIS

Market price information system (MPIS) is more technical issue; basically, developed in online system. In this context, it is not so easy to access on MPIS and its use for the people who are living in the rural areas. There is need of internet facility and adequate knowledge of internet search among the usufructs to collect the information regarding MPIS. In relations to the MPIS of NTFP, the study was measured the knowledge and accessibility of usufructs on MPIS in Nepalese context.

Name of NTFPs that was produced or sold by Usufructs

This research was conducted in three districts, Dolpa, Salyan and Banke where all 27 NTFPs is being produced and traded. Among these NTFPs Timur is being produced and traded by highest proportion of the respondents (50.9%) followed by Mentha (34.3%), Chamomile (10.1%), Aduwa

(8.4%), Rittha (6.2%), Lemon Grass (3.9%) and other listed NTFPs is also being produced and traded but in less amount.

Timur (85.9%), Aduwa (13%) and Rittha (10.7%) are the most produced and traded NTFPs in Salyan while in case of Banke, Mentha (88.3%), Chamomile (26.1%) and Lemon Grass (10%) are the most produced and traded NTFPs. In case of Dolpa, 68.8 produced Gucchi Chyau followed by 56.3% produced Kunti. 50% of Dolpa also reported that they produced Jatamasi and 43.8% produced Kurilo. 14 out of 15 retailers reportedly said they trade Aduwa and 12 out of 15 retailers were found to trade Timur (Table 1). Sundriyal *et al.* (2004) reported that *Spondias axillaris* was sold in highest quantity and more retailers were also involved in business of species *Machilus edulis*, *Diplazium esculentum*, *Eleagnus latifolia*, *Dendrocalamus hamiltonii*, and *Baccaurea sapida* as edible wild plant. Saha and Sundriy (2012) also concluded that the 76 NTFPs species sold in the market in the form of plant parts and processed product in the highest quantity. Few other species such as *Juglans regia* (135 kg @ Rs. 10–15 kg⁻¹), *Diplanzium esculentum* (124 kg @ Rs. 5 bundle⁻¹) were sold commonly in Bomdila market.

We are living in the age of communication, so people can obtain information quickly from one location to another. People use internet media to get information quickly, yet not everyone has equal access to online media or communication opportunities. In such cases, they contact their friends, neighbours, or any relevant office and personally visit to obtain the information.

In regard to NTFP information, forest user groups were questioned on information sources. All kinds of producers and consumers have the same issue of not knowing the true cost of a commodity or service. The actual farmers and producers are not receiving the true price for their commodities due to misinformation about the market pricing. According to statistics in

Table 2, 63.3% of people in total learned about the NTFP MPIS from a friend. A small percentage of respondents (10.1%) mentioned the name of a government office as a source of information, while 56.9% obtained information from the CFUG.

MPIS encourages participation in NTFPs' sustainable initiatives

From the standpoint of protecting NTFP, particularly herbal plants, and increasing usufruct revenue, sustainable management of NTFP is crucial. Herbal plants are one of the many natural resources found in Nepal. The introduction of MPIS promotes responders to handle the NTFPs sustainably, as shown in Table 3. 20.2 per cent of respondents were encouraged to direct NTFP seeding, followed by "encourage me to establish nursery of NTFPs" (12.7%), "encourage me to conservation/enrichment of natural seedlings and plants" (5.6%), "encourage me to conservation/enrichment of NTFPs and other tree species" (3.3%), "encourage me to plant the seedlings in appropriate places of the community forest" (4.7%), "encourage me to protect the NTFPs during the community forest cleaning operation" (1.8%), "encourage me to use appropriate technology during harvesting" (1.8%), and "provides information on the right time of the harvesting adopted (different parts of the day or season)" (2.2%), encouraged me to use acceptable harvesting equipment (.7%), "provided proper drying techniques (not under direct sun or fire)" (1.3%), and "encouraged me to use appropriate and safe place of storing" (1.1%). The majority of respondents who stated that the MPIS's launch had inspired them in some ways were CFUG members and those from Banke. Nobody feels that MPIS is effective in NTFP administration and benefit sharing in the Dolpa district. The results of the finding were supported by Rawal and Kumar (2020), who observed and recommended the need to establish the effective MPIS for sustainable management of NTFPs.

MPIS made it simple to obtain NTFP pricing information

Table 4 shows the respondents' opinions regarding whether the MPIS's launch has made it easier for them to obtain NTFPS pricing information. Of the total respondents, only 2.6% responded "Yes." Regarding Banke, Salyan, and Dolpa, 93.9 per cent, 99.6 per cent, and 100 per cent of the respondents, respectively, stated that the MPIS's launch makes it difficult for them to obtain NTFP pricing information, while no retailer feels that the MPIS's introduction has made it simple for them. A similar result was reported by Rawal and Kumar (2020) also revealed that MPIS is useful for getting the information for judicious prices of NTFP.

The necessity of NTFP's MPIS sustainability

For rural residents who are more reliant on the forest for their income, NTFPs have proved essential. The significance of NTFP for people's livelihoods has also been demonstrated in earlier literature. NTFPs, including as fuel wood, medicinal plants, wild edible crops, building materials, etc., are essential to daily livelihood activities, particularly for tribal people, according to Sarmah *et al.* (2006). Forests, including non-timber forest products (NTFPs), are an important source of food, economic, social, ecological, and cultural values for those who depend on them in rural tropical areas (Sardeshpande and Shackleton, 2019; Kazungu *et al.*, 2020; Shackleton and de Vos, 2022; Chervier *et al.*, 2024). According to the current analysis, one of the consistent revenue streams for forest users is NTFP. While there is no efficient MPIS for NTFP, the income from NTFP is used to handle the basic requirements of rural communities. In general, residents of high-lying areas like Dolpa are far removed from MPIS and other trustworthy information sources. Because buyers, particularly middlemen, set the price, they were receiving a very low price for their NTFP. Thus, in the Nepalese context, the study has recognized the necessity of maintaining the MPIS of NTFP. According to a study by Shit and Pati (2012), NTFPs—a type of forest resource—are crucial for preserving the socioeconomic and ecological

safety net of forest inhabitants. According to their research, practically every forest inhabitant depends in one way or another on forest products other than lumber. It is also noted that 63% of people who live in forests rely on them for their financial well-being.

By increasing MPIS's efficacy, the current study has demonstrated the necessity of NTFP management that is sustainable. Usufructs must have a thorough understanding of NTFP cultivation, harvesting, and distribution in order to manage the crop effectively and maintain it over time. In order to effectively administer the NTFPs, the usufructs have reported a few social and technological issues. They discussed some of the obstacles to the sustainable management of NTFPs, including the absence of a market system for setting prices, the dominance of middlemen and traders, the inaccessibility of large markets, the lack of awareness of NTFP prices, the lack of appropriate knowledge of NTFPs, the inaccessibility of MPIS, water scarcity, low market prices, etc. Therefore, the Nepali government should concentrate on finding solutions to these issues. Every participant in the key informant interview and questionnaire survey acknowledged the significance and impact of NTFP on rural residents' socioeconomic standing. Respondents thought that the information provided by several groups, like the Asian

Network on Sustainable Agriculture and Bio-resources (ANSAB) and the Jadibuti Association of Nepal (JABAN), was insufficient and untrustworthy. When the MPIS was introduced, 91.2% of respondents claimed that market price information was opaque. For the sustainable management of NTFPs and to boost the access of buyers, usufructs, and other stakeholders to formal channels, the current market price information system needs to be greatly improved.

Based on analysis of quantitative and qualitative data, it found that current practice of MPIS is not effective. Still, there was negotiation process to determine the price of NTFPs. Basically, in the rural setting where people are yet to have facilities and infrastructure, communication and transportation. Especially in Dolpa district, the access to information, similarly, information system was weak than the Salyan and Banke districts because of its difficult geographical setting. Thus, the results show that the existing MPIS for NTFPs have not been so effective to provide the market price information to usufruct. Moreover, it has also not been a reliable source of information because very few usufructs believed on price list published in the website of existing MPIS. Thus, this study has developed the process of sustainable management of NTFP through MPIS as follows:

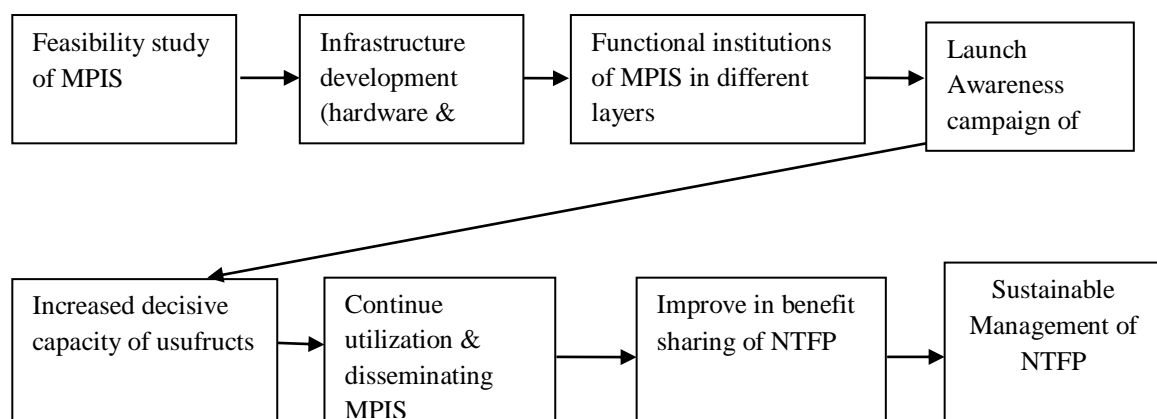


Figure: Process of sustainable management of NTFP through MPIS

One powerful tool of modern technology that may quickly and effectively gather and distribute pricing and market information for

specific items is the market price information system. For rural residents, non-timber forest products constitute a reliable source of

income over the long term. The majority of the High Mountain and hill inhabitants rely on forest products as a source of income. For them, the forest products are valuable since they may be utilized both as food for everyday consumption and as medicine to treat a variety of illnesses. Therefore, it is vitally crucial to manage, distribute, and sustain them properly. The study examined the function of MPIS in long-term sustainable management of NTFP and benefit sharing of usufructs. Geographic context and variety must be taken into account in this case. The essential infrastructure, including the hardware and software, should be constructed based on the findings of the feasibility study. From an economic and technological standpoint, this is a crucial time for the country, thus it requires a sufficient budget as well as experienced personnel to carry out the task efficiently and on schedule.

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CONFLICT OF INTEREST STATEMENT

The author declare that he has no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Table 1: Name of NTFPs that was produced or sold by Usufructs

Types of NTFPs	Total		District						Type of Respondent				Gender			
			Banke		Salyan		Dolpa		CFUG Member		Retailer		Female		Male	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
A. Please tell me the name of NTFPs that you produce or sell																
<i>Zanthoxylum piperitum</i> (Timur)	237	50.9	5	2.8	232	85.9	0	0	225	50.0	12	75.0	70	42.4	167	55.5
<i>Mentha piperita</i> (Mentha)	160	34.3	159	88.3	1	.4	0	0	156	34.7	4	25.0	68	41.2	92	30.6
<i>Matricaria chamomilla</i> L. (Camomole)	47	10.1	47	26.1	0	0	0	0	43	9.6	4	25.0	19	11.5	28	9.3
<i>Zingiber officinale Roscoe</i> (Aaduwa)	39	8.4	4	2.2	35	13.0	0	0	25	5.6	14	87.5	4	2.4	35	11.6
<i>Sapindus mukorossi</i> (Ritha)	29	6.2	0	0	29	10.7	0	0	28	6.2	1	6.3	9	5.5	20	6.6
<i>Asparagus racemosus</i> (Kurilo)	18	3.9	9	5.0	2	.7	7	43.8	11	2.4	7	43.8	6	3.6	12	4.0
<i>Cymbopogon flexuosus</i> (Lemon Grass)	18	3.9	18	10.0	0	0	0	0	14	3.1	4	25.0	10	6.1	8	2.7
<i>Aconitum heterophyllum</i> (Attish)	14	3.0	6	3.3	5	1.9	3	18.8	2	.4	12	75.0	4	2.4	10	3.3
<i>Aconitum heterophyllum</i> (Jatamasi)	14	3.0	4	2.2	2	.7	8	50.0	8	1.8	6	37.5	5	3.0	9	3.0
<i>Emblica officinalis</i> (Amala)	13	2.8	7	3.9	6	2.2	0	0	4	.9	9	56.3	1	.6	12	4.0
<i>Morchella esculenta</i> (L.) (Gucchi Chyau)	12	2.6	1	.6	0	0	11	68.8	10	2.2	2	12.5	7	4.2	5	1.7
<i>Picorrhiza kurroa</i> (Kutni)	11	2.4	1	.6	1	.4	9	56.3	8	1.8	3	18.8	5	3.0	6	2.0
<i>Sapindus trifolatus</i> (Sutho)	11	2.4	0	0	11	4.1	0	0	3	.7	8	50.0	1	.6	10	3.3
<i>Swertia Chirayita</i> (Chiraita)	9	1.9	0	0	3	1.1	6	37.5	7	1.6	2	12.5	3	1.8	6	2.0
<i>Thymus himalayicus</i> (Sithaila)	9	1.9	1	.6	3	1.1	5	31.3	8	1.8	1	6.3	5	3.0	4	1.3
<i>Rheum australe</i> Padam (Chal)	7	1.5	1	.6	1	.4	5	31.3	4	.9	3	18.8	2	1.2	5	1.7
<i>Cymbopogon martini</i> (Palmonora)	7	1.5	0	0	1	.4	6	37.5	6	1.3	1	6.3	6	3.6	1	.3
<i>Acorus calamus</i> (Bhojho)	6	1.3	2	1.1	3	1.1	1	6.3	1	.2	5	31.3	2	1.2	4	1.3
<i>Cinnamomum glaucescens</i> (Sughandha Kokila)	6	1.3	1	.6	2	.7	3	18.8	4	.9	2	12.5	1	.6	5	1.7
<i>Cinnamomum zeylanicum</i> (Dalchini)	5	1.1	1	.6	4	1.5	0	0	0	0	5	31.3	1	.6	4	1.3
<i>Senegalia rugata</i> (Sikakai)	5	1.1	1	.6	0	0	4	25.0	4	.9	1	6.3	0	0	5	1.7
<i>Parnassia nubicola</i> (Nirmashi)	4	.9	0	0	0	0	4	25.0	3	.7	1	6.3	4	2.4	0	0
<i>Bergenia ligulata</i> (Pakhan Bed)	4	.9	2	1.1	2	.7	0	0	0	0	4	25.0	0	0	4	1.3
<i>Berberis aristata</i> (Daruhaladi)	3	.6	0	0	3	1.1	0	0	0	0	3	18.8	1	.6	2	.7
<i>Machilus odoratissima</i> (Kaulo cover)	3	.6	0	0	0	0	3	18.8	3	.7	0	0	3	1.8	0	0
<i>Paris polyphylla</i> (Satuwa)	3	.6	0	0	0	0	3	18.8	3	.7	0	0	3	1.8	0	0

<i>Butea monosperma</i> (Paltis)	3	.6	0	0	0	0	3	18.8	3	.7	0	0	3	1.8	0	0
<i>Pistacia integerrima</i> (Kakarsinghi)	2	.4	0	0	2	.7	0	0	0	0	2	12.5	1	.6	1	.3
<i>Ficus religiosa</i> (Pipal)	2	.4	2	1.1	0	0	0	0	0	0	2	12.5	0	0	2	.7
<i>Asparagus racemosus</i> (SatuwaJara)	2	.4	0	0	1	.4	1	6.3	0	0	2	12.5	1	.6	1	.3
<i>Valeriana jatamansi</i> Jones (Sugandhawal)	2	.4	0	0	0	0	2	12.5	2	.4	0	0	1	.6	1	.3
<i>Ophiocordyceps sinensis</i> (Yarsagumba)	2	.4	0	0	0	0	2	12.5	2	.4	0	0	2	1.2	0	0
<i>Dactylorhiza hatagirea</i> (Panchaunle)	2	.4	0	0	0	0	2	12.5	2	.4	0	0	2	1.2	0	0
<i>Garcinia pedunculata</i> (Amalbed)	1	.2	1	.6	0	0	-	-	1	.2	0	0	0	0	1	.3
<i>Ephedra gerardiana</i> (Somlata)	1	.2	0	0	0	0	1	6.3	1	.2	0	0	0	0	1	.3
<i>Mimosa rubicaulis</i> (Sajaige)	1	.2	0	0	0	0	1	6.3	1	.2	0	0	1	.6	0	0

Source: Field Survey, 2017-18 N = Number of respondents

Source of NTFP MPIS information

Table 2: Information Source for MPIS of NTFPs

Source of Information of MPIS NTFPs	Total		District						Type of Respondent				Gender			
			Banke		Salyan		Dolpa		CFUG Member		Retailer		Female		Male	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
Friends	295	63.3	60	33.3	223	82.6	12	75.0	283	62.9	12	75.0	95	57.6	200	66.4
Community Forest User Groups (CFUG)	265	56.9	153	85.0	107	39.6	5	31.3	264	58.7	1	6.3	107	64.8	158	52.5
Government office	47	10.1	1	.6	43	15.9	3	18.8	43	9.6	4	25.0	11	6.7	36	12.0
NGO staff	31	6.7	15	8.3	15	5.6	1	6.3	29	6.4	2	12.5	10	6.1	21	7.0
Newspapers/magazines	20	4.3	15	8.3	5	1.9	0	0	16	3.6	4	25.0	7	4.2	13	4.3
Radio	16	3.4	9	5.0	6	2.2	1	6.3	5	1.1	11	68.8	3	1.8	13	4.3
Wholesale business man	16	3.4	3	1.7	10	3.7	3	18.8	8	1.8	8	50.0	2	1.2	14	4.7
From CFUG president	7	1.5	4	2.2	0	0	3	18.8	7	1.6	0	0	3	1.8	4	1.3
Internet/ website	3	.6	1	.6	2	.7	0	0	0	0	3	18.8	0	0	3	1.0
From India	2	.4	2	1.1	0	0	0	0	1	.2	1	6.3	0	0	2	.7
Posters/Brochures/Factsheets	1	.2	1	.6	0	0	0	0	1	.2	0	0	1	.6	0	0

Source: Field Survey, 2017-18 N = Number of respondents

Table 3: Encouraged to participate in NTFPs' sustainable activities by MPIS

Encouraged by MPIS to involve in sustainable activities of NTFPs	Total		District						Type of Respondent				Gender			
			Banke		Salyan		Dolpa		CFUG Member		Retailer		Female		Male	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
Encourage me to direct seeding of the NTFP	92	20.2	88	48.9	4	1.1	0	0	90	20.5	2	13.3	42	26.9	50	16.7
Encourage me to establish Nursery of NTFP	57	12.7	57	31.7	0	0	0	0	55	12.6	2	13.3	24	15.4	33	11.2
Encourage me to conservation/enrichment of natural seedlings and plants	25	5.6	25	13.9	0	0	0	0	25	5.7	0	0	11	7.1	14	4.8
Encourage me to conservation/enrichment of NTFPs and other tree species	15	3.3	15	8.3	0	0	0	0	15	3.4	0	0	6	3.8	9	3.1
Encourage me to plantation of the seedlings in appropriate places of the community forest	21	4.7	21	11.7	0	0	0	0	21	4.8	0	0	11	7.1	10	3.4
Encourage me to safe guard the NTFPs during the cleaning operation of community forest	8	1.8	8	4.4	0	0	0	0	8	1.8	0	0	5	3.2	3	1.0
Encourage me to used appropriate technology during harvesting	8	1.8	8	4.4	0	0	0	0	8	1.8	0	0	3	1.9	5	1.7
Provides information on right time of the harvesting adopted (different parts of the day or season)	10	2.2	10	5.6	0	0	0	0	10	2.3	0	0	5	3.2	5	1.7
Provided right techniques of drying (not under direct sun or fire)	6	1.3	6	3.3	0	0	0	0	6	1.4	0	0	2	1.3	4	1.4
Encourage me to use appropriate tools of harvesting were used	3	.7	3	1.7	0	0	0	0	3	.7	0	0	1	.6	2	.7
Encourage me to use appropriate and safe place of storing	5	1.1	5	2.8	0	0	0	0	5	1.2	0	0	2	1.3	3	1.0

Source: Field Survey, 2017-18 N = Number of respondents

Table 4. MPIS made it simple to obtain NTFP pricing information.

Made easiness to get the price information of NTFPs by MPIS		District			Type of Respondent		Gender		Total
		Banke	Salyan	Dolpa	CFUG Member	Retailer	Female	Male	
Yes	N	11	1	0	12	0	7	5	12
	%	6.1%	.4%	0.0%	2.7%	0.0%	4.2%	1.7%	2.6%
No	N	169	269	16	438	16	158	296	454
	%	93.9%	99.6%	100.0%	97.3%	100.0%	95.8%	98.3%	97.4%
Total	N	180	270	16	450	16	165	301	466
	%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%

Source: Field Survey, 2017-18 N = Number of respondents

SHORT COMMUNICATION

Optimization of number of offshoots in date palm (*Phoenix dactylifera* L.)

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ABSTRACT

Date palm (*Phoenix dactylifera* L.) is a one of the important fruit crop in arid and semi-arid regions of the world. The major method of the propagating date palm vegetatively is through offshoots and tissue culture. However, the retention of excessive offshoots on a mother plant can impact growth, fruit yield, and economic returns due to resource competition. The objective of the current study was determining the optimal number of offshoots per plant for maximizing productivity while maintaining sufficient offshoot for propagation. The study was conducted during 2019-2023 using the high offshoot-bearing genotype MDP-29 and examined the impact of offshoot retention (0, 2, 4, 6, and 8 per plant) on growth and yield. Results showed that increasing the number of offshoots reduced plant growth, canopy spread, and yield, with fruit production declining by almost forty percent. The optimum number of offshoots for optimized fruit yield is four per palm.

Keywords: Date palm, offshoot retention, fruit yield, resource allocation, propagation

Date palm (*Phoenix dactylifera* L.) is one of the important fruit crop in arid and semi-arid regions of the world. Worldwide, the date palm cultivation is ongoing since last 5000 years (Gros-Balthazard and Flowers, 2021) but in India the crop is estimated to be of around 450 years in the coastal belt of Kachchh district of Gujarat (Sharma *et al.*, 2019a). Majority of the old plantation are of seedling origin, thus bears huge variability among themselves. Vegetatively, the date palm is propagated either through offshoot or tissue culture plants. Although tissue culture plants are a method for mass propagation, the usage of offshoot is still demanding as the number of genotypes having tissue culture protocol is limited. Generally, the number of offshoots borne by any plant varies from four to ten in its life time, but for few of the genotypes may go higher (Sharma *et al.*, 2019b, 2022a). In the past three decades the

farmers are now focusing to produce and plant offshoots to improve with elite genotypes to improve farm yield which are early maturing and can avoid peak rainfall (Sharma *et al.*, 2022b). Among these the offshoots of the elite genotypes are very high and thus farmers often try to keep and produce as much as offshoots possible, however, at the end of the offshoot bearing period, they also wish to keep the fruit harvest. The number of offshoots retained on a mother plant influences nutrient allocation, thereby affecting growth and fruit yield and may also increase in parthenocarpic fruits (Chao and Krueger, 2007; Qaddoury and Amssa, 2003). Higher number of offshoot may reduce fruit size or yield (Ali khani-Koupaei and Aghdam, 2022). Thus, there is a need to optimize the number of offshoots to maximize plant growth and yield and ascertain sufficient offshoot for propagation.

This study aimed to determine the optimal number of offshoots that should be retained per plant to achieve a balance between growth, yield, and economic returns.

The experiment was conducted at the Date Palm Research Station, Sardarkrushinagar Dantiwada Agricultural University, Mundra-Kachchh, Gujarat from 2019 to 2023 under North West Gujarat Agro-Climatic Zone-V conditions. The plant was planted in the year 2016 and the numbers of offshoots were maintained since the year 2019 onwards. The yield was calculated from year 2021 to year 2023 (three years). The date palm variety used was MDP-29, an indigenous genotype which is a high offshoot bearing and the number of offshoots may go as high as thirty-two per plant. The experiment followed a Randomized Block Design (RBD) with five treatments (0, 2, 4, 6, and 8 offshoots per plant) and five replications, with one palm per replication. A common agronomic practice was followed in all the treatments. Drip irrigation was applied at approximately 200 L/day per plant. The fertilization regime included 50 kg FYM along with 1:1:1 kg N:P₂O₅:K₂O per palm. Pollination was done during February within two days of spathe cracking using male strands. All the pollination in the treated plants was done using the same male plant. The fruits were harvested between third week of June to first week of July. Regular observation for pests and diseases were conducted to avoid any damage to the plant. Data on various growth parameters, yield, and economic returns were recorded and analysed statistically.

The study revealed significant differences among treatments presented in Table 1. The results indicated that as the number of offshoots increases, there is a gradual decline in various growth and yield parameters. The palms with zero offshoots exhibited the highest stem girth (150.00 cm), plant height (530.26 cm), and canopy spread (E-W: 599.80 cm, N-S: 630.86 cm), whereas the palms with those with eight offshoots

showed the lowest values for these parameters (137.86 , 482.80 , 497.33 , and 500.13 cm, respectively).

The decline in plant vigour with increasing number of offshoots is in line with the concept of source-sink competition, where excessive offshoot retention creates strong competing sinks that divert assimilates away from main plant growth and reproductive structures (Alikhani-Koupaei and Aghdam, 2022). Alikhani-Koupaei and Aghdam (2022) also found that date palms with a higher number of suckers had reduced stem girth, lower plant height, and weaker canopy development. Similarly, the presence of multiple offshoots might have led to competition for nutrients, water, and photosynthates, which limits overall vegetative growth. In date palms, the reduction in canopy spread with increasing number of offshoots might have increased competition for light and nutrients, which may have affected overall vegetative growth and efficiency in carbon assimilation. Additionally, under a secondary observation it was noted that the plants with higher number of offshoots, the main trunk was not easily visible and induction of the spathe was also lower.

The number of bunches per palm followed a declining trend, where the palms with zero-offshoot producing the highest number of bunches (6.13), while those with eight offshoots produced the least (3.93). Leaf production also declined with increasing offshoot numbers, confirming that excess offshoots may act as strong sinks that reduce resource allocation to other parts.

Among the various observation, yield performance is an important trait, where the palms with zero-offshoot recorded the highest yield (37.73 kg/palm), followed by two offshoots (33.86 kg), four offshoots (32.80 kg), which were at-par with the zero-offshoot plant and the lowest yield in eight-offshoot palms (21.26 kg/palm) on averaging the yield of three years. This confirms the

trend on the negative impact of excessive offshoot retention on fruit development. It might be due to the source-sink relationship which was explored in other similar studies. Dorel *et al.* (2016) demonstrated that in banana, removing all suckers until the mother plant was harvested led to increased fruit diameter, higher bunch weight, and improved overall yield, while retaining higher number of suckers impacts negatively. Similar mechanism might be applicable to date palms, where limiting offshoot retention allows for better fruit set and gives higher yield. It was confirmed by Alikhani-Koupaei and Aghdam (2022), who noted that when too many offshoots are present, they act as competing sinks, diverting carbohydrates and nutrients away from developing fruits.

Additionally, as a secondary observation of the plants, when all the matured offshoots were removed, the trunk of the plants bearing eight offshoots or higher, the plant becomes weak and narrow at the base and was very prone to damage by high wind velocity during cyclone. After the completion of the experiment, after the harvest, the plants were impacted by high wind velocity due to the cyclone *Biporjoy* in the year 2023 and cyclone *Asan* in the year 2024. The damage causes the plant to bend and to support the plants, earthing up was needed, which was not necessary for the other plants or genotypes where the number of offshoots were lower. Moreover, the plants where higher number of offshoots are removed at later stages are more prone to attack of red palm weevil as the base of the trunk (from where the offshoots are removed) are more exposed for the weevil to attack.

This study demonstrates that reducing the number of offshoots to a maximum of four per palm significantly improves date palm growth and yield. Retaining upto four offshoots per plant is sufficient for optimizing fruit production while maintaining sufficient offshoot availability for propagation.

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The author declare that he has no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Table 1: Effect of number of offshoots on various date palm characters (pooled for three years 2021-2023)

Treatment	Plant Height	Plant Spread (E-W)	Plant Spread (N-S)	Number of bunches*	Number of leaves*	Yield (kg/palm)
0 Offshoot	530.26	599.80	630.86	2.44 (6.13)	6.88 (47.53)	37.73
2 Offshoot	511.13	565.93	591.86	2.36 (5.80)	6.81 ^{ab} (46.80)	33.86
4 Offshoot	498.73	529.73	827.33	2.29 (5.46)	6.33 (44.20)	32.80
6 Offshoot	498.20	519.80	516.66	2.21 (5.13)	6.57 (43.33)	29.46
8 Offshoot	482.80	497.33	500.13	1.95 (3.93)	6.40 (41.26)	21.26
Mean	504.22	542.52	553.37	2.25 (5.29)	6.66 (44.62)	31.02
SeM±	7.38	8.12	10.52	0.07	0.11	1.82
C.D. @5%	21.23	23.47	30.09	0.20	0.32	5.21
C.V. %	5.73	5.89	7.41	12.45	6.46	22.90

*Values are sq. root transformed. Value in parenthesis are original value

SHORT COMMUNICATION

Ethnobotanical study of native edible and medicinal plants in Kyrdemkulai, Meghalaya

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ABSTRACT

Neglected underutilized crops can be called the 'future food', especially as the impact of climate change intensifies and drastically affects the global food production system. These crops, which are adapted to local conditions and have lower water and input requirements, offer a sustainable alternative to conventional crops. However, neglecting these crops is leading to a subsequent loss of traditional knowledge regarding their consumption and utilization. Additionally, the preference of local consumers and farmers is shifting towards high-yielding improved varieties, resulting in a loss of biodiversity. Meghalaya, in particular, is endowed with a rich biodiversity of horticultural crops, including indigenous wild crops. Preserving and promoting these underutilized crops is essential for maintaining biodiversity, supporting local economies, and ensuring food security in the face of climate change. A survey-based study focused on the Kyrdemkulai area of the Ri-Bhoi district of Meghalaya identified 63 native edible and medicinal plants, belonging to 33 families, with Euphorbiaceae, Fagaceae, Musaceae, and Zingiberaceae being the most represented. The most commonly used native plants in the area are *Castanopsis purpurella*, *Emblica officinalis*, *Myrica esculenta*, *Baccaurea sapida*, *Rhus chinensis*, *Calamus erectus*, *Elaeagnus pyriformis*, *Morus australis*, *Averrhoa carambola*, *Artocarpus chaplasi*, *Musa spp.*, *Citrus grandis*, *C. esculenta*, *Dendrocalamus hamiltonii*, *Sechium edule*, *Allium spp.*, *Centella asiatica*, *Begonia roxburghii*, *Hibiscus sabdariffa*, *Kaempferia galanga* and *Phlogacanthus thyrsoiflorus*. Most wild edible plants are trees, followed by herbs, shrubs, and climbers. Maximum of these plants are densely wild, then, sparsely wild, and very few are under cultivation. The lack of awareness, processing, and storage facilities hinders these wild species' wider adoption and utilization. Developing value added products of wild edible plants is essential for their acceptance among the consumers. Promoting cultivation of these crops into the existing farming system is crucial for increasing crop diversity and ecosystem balance.

Keywords: Biodiversity conservation, ethnobotany, native edible plants, utilization

India is home to approximately 800 different species of underutilized edible crops. Among these, around 300 species are predominantly used by the tribal and rural populations in the Northeastern region alone (Amrutha *et al.*, 2024). A total of 151 species (49 families and 86 genera) wild edible fruits used by the Khasi tribes of Meghalaya have been recorded (Jeeva, 2009). Most common and potential underutilized edible fruit genetic resources occurring naturally in Meghalaya and NE region have been recorded by scientists and researchers (Rymbai *et al.*, 2015; Kharshandi *et al.*, 2015; Momin *et al.*, 2016; Devi *et al.*, 2018ab; Devi *et al.*, 2022). These species offer notable nutritional, medicinal, and ecological value. For example, *Prunus nepalensis* provides cardiovascular benefits and is traditionally used in foods and beverages (Kuna *et al.*, 2019; Rymbai *et al.*, 2023). *Prunus salicina* fruits offer natural edible color and essential nutrients (Rymbai *et al.*, 2016). *Myrica esculenta* (Sohphie)

supports the treatment of various ailments with its astringent properties (Rymbai *et al.*, 2023). *Docynia indica* (Sohphoh) addresses obesity and digestive issues with its hypoglycemic and antibacterial properties (Rymbai *et al.*, 2023; Deka and Rymbai, 2014). *Flemingia vestita* (Sohphlang), a unique tuber crop, has anthelmintic properties (Talang *et al.*, 2019). Additionally, crops like *Sechium edule* and *Colocasia esculenta*, which are consumed as vegetable, are rich in dietary fiber, vitamins, and minerals, contributing to improved digestion and overall health (Amrutha *et al.*, 2024; Momin *et al.*, 2016). Leafy greens like *Centella asiatica* are recognized for their medicinal properties, promoting cognitive health and wound healing (Amrutha *et al.*, 2024). Medicinal plants such as *Kaempferia galanga* and *Hibiscus sabdariffa* play a pivotal role in traditional healthcare systems (Rymbai *et al.*, 2023; Sarkar *et al.*, 2023). Other valuable species include *Elaeagnus latifolia*, *Haematocarpus validus*, *Baccaurea sapida*, and *Pyrus pashia*, which offer diverse health benefits and support sustainable agriculture and biodiversity conservation in the region (Hazarika *et al.*, 2015; Rymbai *et al.*, 2019ab). Despite their nutritional and ecological advantages, these crops remain largely neglected in mainstream agriculture and markets. The natural diversity of these wild edible plants is under threat in this vulnerable area. Year after year due to forest fires, demand for land for the increasing population, jhum cultivation, forest products and fuel wood collection, the natural forests are being rapidly destroyed. Unprecedented and unscientific collection of wild plants is making the pressure on these species more aggravated. This is narrowing the genetic diversity of wild species. As of now, the genetic resources of such fruits are facing a great threat of extinction due to climate change, large-scale urbanization, changing attitude and taste of peoples and developmental projects. Therefore, there is an

urgent need for conservation and protection of these rich and diverse wild edible fruits before they are completely destroyed and becomes totally extinct from the area (Mozhui *et al.*, 2011). Recognizing their potential and the urgent need for conservation, this study was conducted to identify native edible and medicinal plants in the natural ecosystem of the Kyrdemkulai area of Ri-Bhoi district, Meghalaya.

A village and forest survey was carried out to identify edible wild plants in the region. The study was conducted at the mild tropical mid-hill ecosystem area of Kyrdemkulai, Umsning Block, Ri-Bhoi District, Meghalaya, during 2021-22. The area lies between E 91°77'30" to E 92°27'00" Longitude and N 25°63'00" to N 26°07'00" Latitude, and at a maximum elevation of 1,242 meters above sea level. Information were gathered by filling questionnaire and personal interviews with the village elders and traditional healers residing in various areas (Jain and Goel, 1995), which were identified with the help of village administrators and community leaders. To gather more information, weekly market at Umsning was also visited and interviewed. The fruits and flora were identified according to existing records and literatures to validate all information (Rymbai *et al.*, 2016). Information related to the native edible and medicinal plants, like local name, family, habits, habitat, utilities, etc., is presented in Table 1.

From the survey conducted, a total of 63 native edible and medicinal plants were reported from the natural ecosystem of the Kyrdemkulai area of Ri-Bhoi district, Meghalaya. Among these, the most commonly used native plants as per the frequency of citation or mention (Faruque *et al.*, 2018) are *Castanopsis purpurella* (98%), *Embllica officinalis* (95 %), *Myrica esculenta* (95 %), *Baccaurea sapida* (70 %), *Rhus chinensis* (80%), *Calamus erectus* (68%), *Elaeagnus pyriformis* (80 %), *Morus australis* (75 %), *Averrhoa carambola* (90 %), *Artocarpus*

chaplasi (97%), *Musa* spp. (100%), *Citrus grandis* (96%), *Colocasia esculenta* (98%), *Dendrocalamus hamiltonii* (94 %), *Sechium edule* (95%), *Allium* spp. (80 %), *Centella asiatica* (80%), *Begonia roxburghii* (74 %), *Hibiscus sabdariffa* (85%), *Kaempferia galanga* (78 %) and *Phlogacanthus thyrsoiflorus* (70 %). These edible species are being mentioned in findings by different researcher in similar geographical location (Rymbai et al., 2015; Kharshandi et al., 2015; Momin et al., 2016; Amrutha et al., 2024), which indicates the similarity in preference pattern and overexploitation of some wild species, if not cultivated in the farmsteads. The identified native plants belonged to 33 family, majority of which belonged Euphorbiaceae (5), Fagaceae, Musaceae, Zingiberaceae (4 each), and Rosaceae, Rutaceae, Solanaceae, Araceae (3 each). Devi et al. (2022) have reported four fagaceous species in Kyrdekulai area, while Rutaceae family was reported highest in Garo hills (Momin et al., 2016). With respect to habit-wise distribution of plants, depicts that the number of wild edible tree species was significantly high (30) and it was followed by herbs (17), shrubs (13) and climbers (3). Previous records also showed more numbers of wild edible tree species compared to other forms of growth habits (Kharshandiet al., 2015). Adapting a higher diversity of tree species is relevant to the mild tropical mid-hill condition of the region. The natural frequency of the tree species may suggest a natural multi-tier planting system following the photoperiod requirement of different species. The study reveals 37 % of the species were densely wild and 14 % sparsely wild, which are found growing in the forest area, open areas, river-side and as forest undergrowth. It was observed that out of total wild edible species, 25% are under cultivation. Similar information was also given by Momin et al. (2016), who reported fewer adoption and cultivations of wild species in farmsteads. Local farmers do not

opt to cultivate these native species due to lack of market demand which is because of shift of food preference with the improved and cultivated species, occupying majority of the farmlands, even in these hill areas. Moreover, majority of these edible and medicinal native plants are tree species, which occupies larger area for cultivation. But, majority of the farmers have small landholdings and hence cultivate only the herb, shrub or small trees. On the contrary of cultivation, farmers due to ignorance, are cutting down the native trees to make cultivable lands. This has caused an imbalance in the natural ecosystem, and only a few hardy agro-silvicultural species, like *Schima wallichii*, *Castanopsis* spp., etc., are found to dominate the population in the region, threatening the natural diversity of other species. Hence, proper planning needs to be carried out to bring a natural balance in agroecology through crop diversification using native species into the region's farming system. The consumption pattern was found to be almost similar to previous records (Rymbai et al., 2016; Momin et al., 2016; Kharshandi et al., 2015). Ripe fruits are mostly eaten raw whereas unripe fruits are used variedly and cooked as vegetable, mixed with curry, eaten with salt or cooked with dry fish. Fruits like *Baccaurea sapida*, *Castanopsis* spp., *Dillenia indica*, *Elaeagnus latifolia*, *Artocarpus chaplasi*, *Averrhoa carambola* etc., are consumed raw (Deka & Rymbai, 2014); *Dendrocalamus hamiltonii*, *Momordica dioica*, *Sechium edule*, *Solanum* spp., *Begonia roxburghii*, etc. are cooked as vegetable. The species *Myrica esculenta* is extensively used for preparing sauce (Rymbai, 2023) and help to generate extra income among the indigenous women. Fruits of *Baccaurea sapida*, *Musa* spp., *Artocarpus chaplasi*, *Citrus* spp., *Docynia indica*, *Myrica esculenta*, and leafy vegetables such as *Centella asiatica* and *Houttuynia cordata* are sold in the local market at a large scale. One of the regions of being unable to

popularise these wild species is due to lack of awareness, processing and storage facilities, in the region. Although processing is carried out at a small scale for family or local consumption, intensive and large scale with systematic standardisation of post-harvest processing and development of primary or secondary products need to be developed for wider acceptability and utility in various industries like food processing, nutraceutical, cosmetic and pharmaceutical industries (Amrutha *et al.*, 2024).

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The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Table1:List of native edible and medicinal plants in the mild tropical mid-hill natural ecosystem of Meghalaya

Sl. No.	Scientific name	Family	Local name	Season	Parts used	Habit	Density/Habitat	Local Uses
1	<i>Emblia officinalis</i> Gaertn.	Euphorbiaceae	Soh-Mylleng	Oct-Mar	Fruit	Tree	Dense, grown wild in the forest	Fruit is one of the richest sources of vitamin C. Locally consumed raw and processed.
2	<i>Castanopsis tribuloides</i> (Sm.) A. DC.	Fagaceae	Soh-ot-rit	Sept-Dec	Fruit	Tree	Dense, grown wild in the forest	Locally consumed fresh or roasted
3	<i>Castanopsis indica</i> (Roxb. ex Lindl.) A. DC.	Fagaceae	Soh-ot-langkraw	Dec-Feb	Fruit	Tree	Dense, grown wild in the forest	Locally consumed fresh or roasted
4	<i>Castanopsis purpurella</i> (Mq.) Balak.	Fagaceae	Soh-ot-saw	Dec-Feb	Fruit	Tree	Dense, grown wild in the forest	Locally consumed fresh or roasted
5	<i>Lithocarpus fenestratus</i> (Roxb.) Rehder	Fagaceae	Soh-ot-dieam	Aug-Oct	Fruit	Tree	Dense, grown wild in the forest	Edible but left to be consumed by wild animals
6	<i>Elaeocarpus lanceifolius</i> Roxb.	Elaeocarpaceae	Soh-khyllam	Jan-Mar	Fruit	Tree	Sparse, grown wild in the forest	Edible fruits but not popular for consumption among the local in the area.
7	<i>Myrica esculenta</i> Buch.-Ham. ex D. Don	Myricaceae	Soh-phie	April-July	Fruit	Tree	Dense, grown wild in the forest	Ripe fruits eaten raw, used for preparation of juice
8	<i>Myricanagi</i> . Thunb.	Myricaceae	Soh-phie-rit	April-July	Fruit	Tree	Dense, grown wild in the forest	Same as Soh-phie
9	<i>Passiflora edulis</i> Sim.	Passifloraceae	Soh-brap	Nov-Jan	Fruit	Climber	Sparse, domesticated at homesteads and farm	Ripe fruits consumed after minimal processing and also used for making juice, squash.
10	<i>Baccaurea ramiflora</i> Lour.	Euphorbiaceae	Soh-Myndong/ Soh-Ramdieng	April-July	Fruit	Tree	Very sparse, grown wild in the forest	Ripe fruits consumed fresh
11	<i>B. sapida</i> (Roxb.) Mull.Arg.	Euphorbiaceae	Soh-Langkadaw	May-July	Fruit	Tree	Sparse, grown wild in the forest	Light yellow sour edible fruit. Peel pickled and consumed locally.
12	<i>Rhus chinensis</i> Mill.	Anacardiaceae	Soh-Ma	Oct-Jan	Fruit	Tree	Dense, wild	Sub-acidic pulp much relished. The ripe fruit has a sharp acid taste. It is said to be a remedy for colic.
13	<i>Spondias pinnata</i> (Linn.f.) Kurz.	Anacardiaceae	So-Pa-Ir	Mar-Nov	Fruit	Tree	Sparse, wild	Consumed after minimal processing, decoction for digestion.
14	<i>Prunus jenkinsii</i> Hk.f.& Th.	Rosaceae	Soh-Khwai Dienkyrkhang Sa-tanghi(J)	Sept-Nov	Fruit	Tree	Very sparse, wild	Purple Sweet fruit consumed raw and processed
15	<i>Vangueria spinosa</i> (Roxb. Ex Link) Roxb.	Rubiaceae	Soh Matan Soh Mon	April-Sept	Fruit	Tree	Dense, wild	Leaves consumed as vegetable. Fruit eaten raw after dehydrated.
16	<i>Calamus erectus</i> Roxb.	Arecaceae	Soh-trat-heh	Sept-Dec	Fruit	Shrub	Dense, wild	Fruits antioxidant and antidiabetics
17	<i>Elaeagnus latifolia</i> (Linn.)	Elaeocarpaceae	Soh-shang	Nov-May	Fruit	Shrub	Dense, Semi-domesticated	Fruits consumed fresh, and processed into chutney, pickle.
18	<i>Elaeagnus pyriformis</i> Hk.f.	Elaeocarpaceae	Dieng-soh-blor	March-April	Fruit	Shrub	Dense,	Fruits consumed fresh, and processed into chutney,

							Semi-domesticated	pickle.
19	<i>Syzygium tetragonum</i> (Wt.) Kurz. (Dennst) Balak.	Myrtaceae	Soh-Ra-Moh (Wild jamun) Dieng-soh-sarlei	May-June	Fruit	Tree	Very dense And wild	Anthocyanin rich, small sweet, and oblong edible fruit but mostly neglected
20	<i>Sterculia versicolor</i> Wall.	Sterculiaceae	Soh-Um/ Star-um	April-July	Fruit	Tree	Dense, grown wild near river and stream-side	Anthocyanin rich small sweet and oblong edible fruit but mostly neglected
21	<i>Rubus moluccanus</i> Linn	Rosaceae	SohNypbah	Jan-Dec	Aggregate sweet red berry	Herb	Dense, wild undergrowth of forests.	Fruits are edible. Roots are medicinal and are used for diarrhea and dysentery, constipation, gastric, gripe and diabetes.
22	<i>Musa</i> spp.	Musaceae	Kait-Ja	Perennial	Fruit	Herb	Dense Domesticated	Edible fruit sold at local markets
23	<i>Musa</i> spp.	Musaceae	KaitSyiem	Perennial	Fruit	Herb	Dense Semi-Domesticated	Edible fruit sold at local markets. Popular for feeding infants.
24	<i>Musa</i> spp.	Musaceae	Kait Khar	Perennial	Fruit	Herb	Dense Semi-Domesticated	Edible fruit sold at local markets
25	<i>Musa</i> spp.	Musaceae	Kait-Khlong	Perennial	Fruit	Herb	Dense Semi-Domesticated	Edible fruit sold at local markets
26	<i>Sterculia villosa</i> Roxb .	Sterculiaceae	Soh-star/ Dieng star	Nov-Dec	Fruit	Rambl er shrub	Dense, wild	Leaf edible as vegetable sour
27	<i>Citrus grandis</i> (Brum.) Merr.	Rutaceae	Pumelo	Nov-Dec	Fruit	Tree	Wild/cultivated in open areas	Fruit is consumed fresh or seasoned.
28	<i>Morus australis</i> Poir.	Moraceae	Sohlyngdkhur	April-July	Fruit	Tree	Wild/cultivated forest or jhum areas	Ripe fruit is consumed fresh
29	<i>Averrhoa carambola</i> L.	Oxalidaceae	Sohpyrshong	Mar-Oct	Fruit	Tree	Cultivated and semi-wild	Fruit is consumed fresh, seasoned or pickled.
30	<i>Artocarpus chaplasi</i> Roxb.	Moraceae	Dieng-soh-ram	Mar-August	Fruit	Tree	Wild and cultivated	Ripe fruit is consumed fresh, unripe fruit is cooked or pickled
31	<i>Aegle marmelos</i> (Linn.) Correa ex	Rutaceae	Soh-bel	Oct-Mar	Fruit	Tree	Very sparsely cultivated	Ripe fruit eaten fresh
32	<i>Clausena heptaphylla</i> Wight & Arn.	Rutaceae	Dieng-siang-mat	April-July	Fruit	Shrub or small tree	Wild in deep forest near streams	Plant parts possess medicinal properties and are used for traditional healing purpose

33	<i>Dillenia indica</i> Linn.	Dilleniaceae	Soh-kyrbam	June-April	Fruit	Tree	Very sparsely cultivated	Ripe fruit eaten fresh
34	<i>D. pentagyna</i> Roxb.	Dilleniaceae	Dieng-soh-bar	Mar-July	Fruit, leaf, bark	Tree	Open forest area	Plant parts possess medicinal properties and are used for traditional healing purpose
35	<i>Fragaria nilgerrensis</i> Schlecht.	Rosaceae	-	Feb-May	Fruit	Herb	Herb is found as forest undergrowth	Edible fruits but inferior quality so not consumed
36	<i>Garcinia pedunculata</i> Roxb.	Clusiaceae	Dieng-soh-danei	Sept-Feb	Fruit	Tree	Sparsely found in open areas	Fruits edible fresh or processed
37	<i>Mallotus philippensis</i> Muell-Arg.	Euphorbiaceae	Dieng-chandon	Aug-May	Fruit, roots	Shrub or small tree	Densely found under secondary forest	Fruit possesses medicinal properties. Roots are also used for traditional medicine.
38	<i>Tamarindus indica</i> Linn.	Caesalpiniaceae	Dieng-soh-kyntoi	Jan-April	Fruit	Tree	Sparsely grown under open areas	Edible fruit
39	<i>Viburnum foetidum</i> Wall.	Caprifoliaceae	Soh-lang-ksew	July-Sept	Fruit	Shrub	Densely found under forest	Edible fruit consumed processed
40	<i>Z. mauritiana</i> Lam.	Rhamnaceae	Soh-broi	Feb-June	Fruit	Tree	Sparsely found under forest or open areas	Edible fruit consumed processed or seasoned
41	<i>Melastomam alabathricum</i> Linn.	Melastomataceae	Dieng-soh-khingSohsia	Feb-Dec	Fruit, flower, leave	Shrub	Dense, Common in forest and waste lands	Plant parts used for traditional medicine
42	<i>Manihot esculenta</i> Crantz.	Euphorbiaceae	Phandieng	Nov-Mar	Tubers	Shrub	Dense open areas	Edible tubers
43	<i>C. esculenta</i> Schott.	Araceae	Shriew	Nov-Mar	Corms and stem	Herb	Dense wild in forest and also cultivated	Edible corms
44	<i>Eryngium foetidum</i> Linn.	Apiaceae	Dhania-khlaw	May-Feb	Leaves	Herb	Dense, mostly in open places and along streams, also cultivated	Used for garnishing curry
45	<i>Houttuynia cordata</i> Thunb.	Saururaceae	Ja-myrdoh	April-July	Leaves and roots	Herb	Dense, mostly in open places and along streams	Consumed as salad, chutney.
46	<i>Dendrocalamus hamiltonii</i> Nees&Arn. ex Munro	Poaceae	U sohjew	April-Aug	Shoots	Tree	Dense, Wild in forest and also cultivated	Common local delicacies, consumed cooked, fermented, pickled
47	<i>Parkia roxburghii</i> G. Don	Leguminoceae	-	Oct-April	Flower, Seed and whole pod	Tree	Wild in the open areas	Not popular among Khasi locals. However, pods are consumed raw and cooked.

48	<i>Sechium edule</i> (Jacq.) Sw.	Cucurbitacea	Biskot	Oct-Mar	Fruit	Climber	Dense, cultivated in open and forest	Consumed as cooked as a vegetable
49	<i>Momordica dioica</i> Roxb. ex Wild.	Cucurbitacea	Kroi	June-Sept	Fruit	Climber	Cultivated in Jhum fields	Consumed cooked as a vegetable
50	<i>Cinnamomum tamala</i> Nees&Eberm.	Lauraceae	Dieng-la-tyrpad	Perennial	Leaves	Tree	Cultivated	Used as spice
51	<i>Allium</i> spp.	Alliaceae	Jellang	Perennial	Whole herb	Herb	Cultivated at fields	Condiment, consumed as salad or chutney
52	<i>Centella asiatica</i> (Linn.) Urban	Apiaceae	Badmaina	Perennial	Leaves, runners	Herb	Runs wild in forest and open areas	Consumed raw as salad or chutney
53	<i>Solanum barbisetum</i> Nees.	Solanaceae	Soh-podok	Aug-May	Fruit	Shrub	Densely found in the forest peripheries	Used as for medicinal purpose
54	<i>S. indicum</i> Linn.	Solanaceae	Soh-ngan	June-Feb	Fruit	Shrub	Densely found in the forest peripheries	Used as for medicinal purpose
55	<i>S. xanthocarpum</i> Schrad. & Wendl.	Solanaceae	Dieng-soh-podokbakthang	Dec-Feb	Fruit	Herb	Densely found in the forest peripheries	Used as for medicinal purpose
56	<i>Begonia roxburghii</i> A. DC.	Begoniaceae	DiengJajew	July	Leaves	Herb	Tropical and sub-tropical forests	Shoot used as vegetable
57	<i>Hibiscus sabdariffa</i> L.	Malvaceae	Jajew	May-Dec	Leaf, fruit flower	Shrub	Cultivated	Cooked as a vegetable, made into pickles and fruits used for making jam
58	<i>Kaempferia galanga</i> L.	Zingiberaceae	Syngkhumoh	Sept-Nov	Rhizome	Herb	Very Dense wild undergrowth in the forests	Chutney Consume directly for cough, asthma, respiratory and stomach ailments remedy
59	<i>Cucurma angustifolia</i> Roxb.	Zingiberaceae	La Shiang	April-June	Flower	Herb	Very densely available as forest undergrowth	Consumed as vegetable
60	<i>Cheilocostus speciosus</i> (J. Koenig) C. Specht	Zingiberaceae	Syngkhlaw	Oct-Dec	Rhizome	Herb	Densely found in swampy and shaded undergrowth	Edible rhizome, important source of diosgenin
61	<i>Phlogacanthusthysiflorus</i> (Roxb.) Nees	Acanthaceae	Tdongksew Dieng-soh- kajut	Dec-April	Leaf and flower	Shrub	Densely cultivated at homesteads and wild races found in deep forests	Flowers are consumed as vegetables. Decoction for respiratory steam and skin ailments.
62	<i>Hedychium coronarium</i> Koen.	Zingiberaceae	Syngkhlaw	Perennial	Rhizome and flower	Herb	Densely found in swampy areas and streamside in open areas	Flowers and rhizome are consumed as vegetables
63	<i>Caryo taurens</i> Linn.	Arecaceae	Kwai-cha	Jan-April	Seed	Tree	Sparsely found in forest areas	Traditional sources of dietary starch in the past

SHORT COMMUNICATION

Ecological condition of walnut forests during the development of green economy

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ABSTRACT

Walnut-fruit forests represent a rich and important source of genetic biodiversity and are one of the sources of income for the people living in this region, especially through the collection of fruits, nuts and other non-timber forest products, food products as well as a base for wild animals. It has been shown that under the influence of anthropogenic factors, unsystematic grazing of cattle, felling of trees, collection of nuts and fruits, as well as other types of anthropogenic impact lead to deterioration of the physical and chemical properties of the soil, soil erosion. This article is aimed at the development of forestry in the territory of the republic in accordance with the directions of implementation of the state policy in the field of forest horticulture development, mitigation of the effects of climate change and its development, obtaining environmentally friendly products, realizing the advantages of the "green economy".

Keywords: Anthropogenic factor, biodiversity, climate change, ecological system, walnut forest,

The nut-fruit forests of Kyrgyzstan are the largest and only natural massif of nut-fruit trees in the world, and it is noted that they have a unique gene pool and landscape in terms of size, value and beauty of the territory they occupy. The walnut-fruit forests of Arstanbap, Kara-Alma, Kyzyl-Unkur and Dashman are among the most beautiful places in the South of Kyrgyzstan. These forests are of great importance for the overall ecology. It maintains the climate balance and prevents the land from turning into a desert. In addition, the walnut forests in the South of Kyrgyzstan are also of great importance for the local population. In recent years, some areas of walnut-fruit forests have been allocated to local residents as land plots, but

not all locals are concerned about preserving the future of the forest. Currently, 60% of forests in southern Kyrgyzstan are on the verge of disappearing. If the current neglect by the public continues, then within fifty years there is a risk of losing walnut and fruit forests. Scientists have proven that the origin of walnuts is millions of years old. Walnut is the longest-living tree. With good care and no damage from humans or nature, it can grow for 1000 years. The reason why walnut forests do not recover naturally is because they are neglected. It is alarming that the number of forests in Kyrgyzstan is decreasing.

According to the Forest Code of the Kyrgyz Republic, forests, flora and fauna are

the exclusive property of the Kyrgyz Republic, are used to maintain a unified ecological system as the basis for the life of the people of the Kyrgyz Republic and are under special protection of the state (Forest Code of KP, 1999). The area of forests in the Kyrgyz Republic is 1,123,045.2 hectares, or 5.61% of the total area of the country (forest vegetation). Among the forest areas of Kyrgyzstan, the rare nut and berry forests located in the Jalal-Abad and Osh regions are of particular value. Under the influence of anthropogenic factors that have a long-term impact on mountain forest biogeocenoses, unsystematic and disorderly grazing, tree felling, collection of nuts and fruits, collection of medicinal herbs, as well as other types of anthropogenic impact lead to deterioration of the physical and chemical properties of the soil and its erosion (Gryza *et al.*, 2008).

Studying the soil cover and clarifying the relationships between soils and forests allows us to identify the changes that occur in forest landscapes due to human activity: deforestation, grazing, haymaking, fruit harvesting, etc. Ultimately, this will allow us to make fuller use of the fertility of mountain soils and ensure rational forest management.

The study was conducted in the Kara-Alma walnut-fruit forest (elevation – 1801m., latitude -41°12'54.66"N, longitude -73°23'00.05"E) of the Jalal-Abad region of Kyrgyzstan. The research work was carried out in 2020-2024 at the Jalal-Abad State University named after B. Osmonov. To study the fertility of walnut-fruit forests, soil samples were taken from genetic horizons. At the same time, soil sections were described based on the morphological characteristics of the genetic horizons of the soil profile. Laboratory analyzes were done according to generally accepted methods adopted in the Kyrgyz Republic, in the Republican laboratory of soil-agrochemical station of Kyrgyzstan (Arinushkina, 1963). The humic and fulvic acids were analyzed in the laboratory of the Institute of Soil Science and Plant Nutrition of the

Federal Center for Agricultural Research in Germany (Faithfull, 2002).

In walnut-fruit forests, there are mountain-forest black-brown soils with very high fertility. They are characterized by high fertility and are distinguished by a high content of humus, mineral substances and digestibility (Roychenko, 1960). Table 1 shows the state of humus in mountain-forest black-brown soils.

The composition of humus reflects such an important aspect of the soil structure as the nature of the processes of accumulation and decomposition of organic matter (Kononova, 1963, Sakbaeva *et al.*, 2012). The most important characteristics of the soil are the composition of humus and the thickness of the humus horizon. Soil fertility, the formation of its structure and microbial activity depend on the humus content (Sakbaeva *et al.*, 2013). The humus state of the mountain-forest black-brown soils of the walnut-fruit forests of the Kara-Alma forestry enterprise is characterized by very high fertility, the amount of humus in them is up to 11.3-12.0%, and the humus-accumulative horizon contains 8.3-9.3% humus, and its volume decreases sharply along the soil profile.

The soil under nut-fruit forests has the highest water permeability. However, due to the increase in cattle grazing, deforestation, and the collection of nuts and fruits, the landscape changes dramatically, which leads to a sharp deterioration in the water-regulating and anti-erosion properties of forests. Plants are often trampled by roads that cross hillsides from all sides. Therefore, it is known that slopes covered with vegetation are more susceptible to erosion. On these slopes, 50-60% of the soil is washed away, and in some places even more. First, the upper humus horizon is washed away, then the lower one, as a result of which the entire territory becomes unsuitable for use.

Leaching of mountain forest black-brown soils of walnut-fruit forests is closely and

directly dependent, first of all, on the water resistance of the structural elements of the soil, which, in turn, largely depends on the vegetation cover (Sakbaeva *etal.*,2022).As can be seen from Table 2, as a result of leaching, the chemical and physical properties of soils change significantly. The bulk density of unwashed soils in the upper horizon is 0.78-0.82 g / cm³, and washed - 1.20-1.24 g / cm³. The physical properties of soils are of great ecological importance, since they largely determine the exchange processes between the soil and other components of the biogeocenosis (Sakbaeva *etal.*,2022).In mountainous conditions, the physical properties of soils are of primary importance, since the resistance of the soil to erosion largely depends on them.

Measures to mitigate the effects of climate change should include measures aimed at maximum protection, restoration and reforestation of forest areas. In connection with the above, it is necessary to carry out reforestation and afforestation in the territories of the Kara-Alma forestry, where anthropogenic pressure is observed.

In conclusion, it should be noted that the goal is to improve (reconstruct) growing trees of nut crops, create walnut plantations aimed at obtaining a bountiful harvest, meeting the needs of the local market, processing nuts and exporting competitive, environmentally friendly products, promoting food security, and raising the standard of living of the country's population. Improving the quality and productivity of nut crops can be achieved through the introduction of modern agricultural technologies for growing planting material, widespread introduction of valuable forms and varieties of nut crops into production, creating highly productive plantings using advanced domestic and foreign experience, including modern grafting methods.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal

relationships that could have appeared to influence the work reported in this paper.

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Table 1:Humus state of mountain-forest brown and black-brown soils of the walnut-fruit forest of the Kara-Alma forestry

Land and soil	Depth, cm	Humus, %	Carbon %	Total nitrogen,%	C:N
Walnut-fruit forest of Kara-Alma (mountain-forest brown soil)	0-2	11,33	6,58	0,95	6,9
	2-14	8,30	4,82	0,55	8,76
	14-52	2,70	1,56	0,20	7,84
	52-105	0,88	0,51	0,09	5,68
	105-165	0,68	0,39	0,05	7,9
Walnut-fruit forest of Kara-Alma (mountain-forest black-brown soil)	0-4	12,0	6,9	0,98	7,04
	4-18	9,30	5,4	0,64	8,43
	18-57	3,80	2,20	0,30	7,30
	57-91	2,65	1,54	0,14	11,0
	91-130	1,09	0,63	0,10	6,3
	130-185	0,88	0,51	0,06	8,5

Table-2:Density of unwashed and washed mountain-forest brown and black-brown soils of walnut-fruit forests, g/cm³

Depth, cm	Soil			
	P.61 Mountain-forest black-brown, unwashed	P.62 Mountain-forest black-brown, unwashed	P.63 Mountain- forest black brown, washed	P.64 Mountain-forest black- brown, unwashed
0-5	0.82	0.78	1.24	1.20
5-10	1.01	0.96	1.27	1.25
10-20	1.06	1.00	1.38	1.36
20-30	1.15	1.07	1.39	1.35
30-40	1.24	1.28	1.41	1.38
40-50	1.36	1.35	1.45	1.39

SHORT COMMUNICATION

Unveiling of physico-chemical changes of some underutilized fruits at different stages of maturity

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ABSTRACT

A study on physico-chemical properties of some underutilized fruits grown in Nagaland was conducted in School of Agricultural Sciences, Nagaland University in 2023 to evaluate the morphological and biochemical changes from fruit set to maturity. Three different locations furnished the fruits for study of *Averrhoa carambola*, *Eleagnus latifolia*, and *Phyllanthus acidus*. *Averrhoa carambola* fruit had the diameter of 5.0 ± 0.62 cm, 8.47 ± 0.81 cm in length and 68.69 ± 15.41 g in weight at 75 days after fruit set (DAFS). There was a discernible biochemical synthesis trend in *Averrhoa carambola* fruit in TSS (5.27 ± 0.64 °Brix to 6.77 ± 0.36 °Brix), total sugar (6.10 ± 0.33 to 7.96 ± 0.09 %) and vit-C (12.64 ± 7.20 to 22.68 ± 10.04 mg/100 g pulp) from 15 DAFS to 75 DAFS respectively. The significant variation of *Eleagnus latifolia* were found in fruit weight (3.70 ± 0.62 to 11.27 ± 0.76 g), fruit length (2.80 ± 0.36 to 3.77 ± 0.48 cm) and fruit diameter (1.59 ± 0.20 to 2.40 ± 0.09 cm) from 15 days after fruit set to 75 DAFS respectively. A progression of biochemical synthesis in the fruit of *Eleagnus latifolia* was noticed in TSS (5.70 ± 0.46 °Brix to 9.87 ± 0.52 °Brix), total sugar (4.39 ± 0.04 % to 6.59 ± 0.36 %) and vit-C (14.08 ± 1.32 to 9.19 ± 0.99 mg/100 g pulp) from 15 DAFS to 75 DAFS. *Phyllanthus acidus* showed a fruit weight of 4.67 ± 0.74 g, length of 1.60 ± 0.01 cm and diameter of 2.22 ± 0.03 cm at 60 DAFS. The biochemical composition of *Phyllanthus acidus* was noticed in TSS (6.40 ± 0.57 °Brix), total sugar (5.87 ± 0.26 %), and Vit-C (22.20 ± 1.22 mg/100 g pulp) at 60 DAFS.

Keywords: Morphology, physico-chemical properties, underutilized fruits

Indigenous and minor horticultural crops available in the region are not being exploited properly, but they have the potential to alleviate poverty, food, and nutritional security through processing, value addition, and diverse use. These fruits and vegetables are rich in vitamins and minerals as well. Besides, they are rich in secondary metabolites and medicinal properties which could be exploited in industrial and medicinal sectors (Deka and Rymbai, 2014). A survey in Nagaland was initiated and identified several underutilized fruits, including *Averrhoa carambola*, *Elaeagnus latifolia*, and *Phyllanthus acidus*, which are important in local diets and traditional medicine (Sarkar *et al.*, 2023). These fruits, though abundant locally, are underrecognized and lacked in scientific study concerning their physiological, agronomic, and ecological characteristics. The study aims to address this gap by examining the morphological and biochemical characteristics of

Averrhoa carambola, *Eleagnus latifolia*, and *Phyllanthus acidus*.

The study was conducted in Chumoukedima district of Nagaland. The region falls under the geographical coordinates of 25°6' and 27° 4' North latitude and 93° 20' and 95° 15' East longitude covering a humid subtropical climate with a typical average temperature of 25.06°C and receives about 150-250 cm of annual precipitation. Soil in exploration area is predominantly acidic inceptisols, with fine clay, clay loamy, and fine loamy clay textures, and a pH ranging from 4.5 to 6.0. The season of flowering and harvesting times were determined through local interactions with village elders, farmers, and growers. For fruit morphological characters, weight, diameter, and length were measured by weighing of five random fruits on an electronic balance and using a vernier calliper to determine average measurements. Biochemical analysis was executed preparing a juice extract by removing seeds, crushing the pulp with a mortar and pestle, and filtering it through a muslin cloth. Total soluble solids (TSS) were determined with the help of hand refractometer calibrated in °Brix at 20 °C with necessary correction factor. Total sugar was determined using Fehling's reagents with methylene blue as an indicator, while reducing sugar was calculated through a similar titration process, with the endpoint indicated by a deep brick-red colour (A.O.A.C, 1995). Titratable acidity was measured by titrating the extracted juice against N/10 NaOH using phenolphthalein as an indicator and ascorbic acid using 2, 6-dichlorophenol indophenols dye titration method following the standard procedure of A.O.A.C (1995).

***Averrhoa carambola* L.**

It is locally known as Charkhona in Ao and Karmal in Hindi. The fruit was collected from the location of surrounding Chumoukedima district, Nagaland. The

fruit grows well all over the north east region of India (De, 2017). *Averrhoa carambola* is an evergreen tree which flowers during the months of July-August, October-November, and January-February and is ready to harvest in September-October, December-January, and March-April in Chumoukedima region of Nagaland. *Averrhoa carambola* are generally oblong and 5-angled, weighing about 68.69±15.41 g at 75 DAFS, having a diameter of 5.00±0.62 cm, and a length of 8.47±0.81 cm on average per fruit (Table 1). The colour of the fruit when harvested is usually yellow in colour with yellow-coloured flesh. The fruit of *Averrhoa carambola* at 75 DAFS had the highest TSS of 6.77±0.36 °Brix, titratable acidity of 0.51±0.06%, total sugar of 7.96±0.09%, reducing sugar of 6.39±0.07% (Table 1). Pongener *et al.* (2022) also reported similar findings of titratable acidity (0.576%), total sugar (6.10±0.33% to 7.96±0.09%) and reducing sugar (5.20±0.26% to 6.39±0.07%). The vitamin C content was the highest at 75 DAFS (22.68±10.04 mg/100 g pulp), which was in consonance with Pawar *et al.* (2014) for vit-C (15.6 to 32.8 mg/100 g).

Eleagnus latifolia

The fruit is vernacularly known as Metsüsera (Ao) and Pedüchüdi (Angami). *Eleagnus latifolia* L. is a large evergreen spreading type woody shrub which flowers in the month of December to January and is harvested when the fruits turn deep red or orange during the month of March to April. The fruits of *Elaeagnus latifolia* are oblong in shape with orange to red flesh and skin colour, weighing about 11.27±0.76 g, diameter of 2.40±0.09 cm, and a length of 3.77±0.48 cm at 75 DAFS (Table 2). The fruit of *Eleagnus latifolia* showed a TSS of 9.87 °Brix at 75 DAFS, while 5.70 °Brix was noticed at 15 days after fruit set (DAFS). The notable changes occurred in titratable acidity (1.47±0.10%), total sugar (6.59 ±0.36%), reducing sugar (4.75

$\pm 0.26\%$) and vitamin C ($9.19 \pm 0.99 \text{ mg}/100 \text{ g}$) during harvesting stages of fruits (Table 2). The vitamin C content was also at par with Devachandra *et al.* (2018) where they reported vitamin C content of $11.49 \text{ mg}/100 \text{ g}$. The finding of total sugar was at par with Sarkar *et al.* (2023) who reported total sugar and reducing sugar of 6.89% and 4.44% , respectively in silver berry.

***Phyllanthus acidus*(L.)**

Star gooseberry was collected from home gardens at Medziphema Town, Seithekima C and Pherima village of Nagaland. The star gooseberry is a deciduous tree. It is small to medium tree which grows about 2-9 m. It was also noted that it flowered twice any time of the year and fruits and flowers appeared simultaneously. The fruits of star gooseberry are oblate in shape, light green to yellow in skin colour, with white to light yellow colour in flesh. The weight of the fruit is around $4.67 \pm 0.74 \text{ g}$, with a diameter of $2.22 \pm 0.03 \text{ cm}$ and length of $1.60 \pm 0.01 \text{ cm}$ at harvesting stage (60 DAFS) (Table 3). *Phyllanthus acidus* had shown TSS of 6.40°Brix , titratable acidity of $1.31 \pm 0.08\%$, total sugar of $5.87 \pm 0.26\%$, reducing sugar of $4.08 \pm 0.42\%$, non-reducing sugar of 1.70% and vitamin C content of $22.20 \pm 1.22 \text{ mg}/100 \text{ g}$ pulp at 60 DAFS (Table 3). Sarkar *et al.* (2023) also reported TSS of $6.66 \pm 0.17^\circ \text{Brix}$, titratable acidity of $1.82 \pm 0.10\%$, total sugar of $5.33 \pm 0.23\%$, reducing sugar of $3.77 \pm 0.04\%$ and vitamin-C content of $24.00 \pm 1.20 \text{ mg}/100 \text{ g}$ pulp in *Phyllanthus acidus* fruits.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Table 1. Study on physicochemical properties of carambola (*Averrhoa carambola*) at different stages of maturity

DAFS (Days after fruit set)	Fruit weight (g)	Fruit diameter (cm)	Fruit length (cm)	TSS (°Brix)	Titrateable acidity (%)	Total sugar (%)	Reducing sugar (%)	Vitamin-C (mg/100g pulp)
15 DAFS	1.31±0.22	1.50±0.36	2.70±0.13	5.27±0.64	0.22±0.05	6.10±0.33	5.20±0.26	12.64±7.20
30 DAFS	7.33±0.93	2.44±0.14	4.72±0.16	4.70±0.46	0.32±0.09	6.53±0.63	5.55±0.53	16.67±7.09
45 DAFS	25.57±4.78	4.17±0.95	7.60±0.78	5.50±0.53	0.35±0.07	6.80±0.20	6.07±0.27	17.44±8.83
60 DAFS	61.22±17.15	4.67±0.64	8.40±0.79	6.03±0.49	0.46±0.05	7.39±0.36	6.16±0.39	18.60±6.96
75 DAFS	68.69±15.41	5.00±0.62	8.47±0.81	6.77±0.36	0.51±0.06	7.96±0.09	6.39±0.07	22.68±10.04

Table 2. Study on physicochemical properties of silver berry (*Eleagnus latifolia*) at different stages of maturity

DAFS (Days after fruit set)	Fruit weight (g)	Fruit diameter (cm)	Fruit length (cm)	TSS (°Brix)	Titrateable acidity (%)	Total sugar (%)	Reducing sugar (%)	Vitamin C (mg/100g pulp)
15 DAFS	3.70±0.62	1.59±0.20	2.80±0.36	5.70 ±0.46	0.12 ±0.09	4.39 ±0.04	3.89 ±0.02	14.08 ±1.32
30 DAFS	5.80±1.93	1.94±0.25	3.35±0.70	8.13 ±0.42	0.38 ±0.12	4.77±0.16	4.11 ±0.10	12.64 ±6.96
45 DAFS	9.70±0.56	2.11±0.09	3.50±0.48	8.67 ±0.58	1.08 ±0.13	5.16 ±0.09	4.34 ±0.42	15.51 ±6.21
60 DAFS	11.10±0.72	2.32±0.07	3.67±0.56	6.00±0.56	1.20±0.16	5.53±0.30	4.63±0.37	12.80±3.48
75 DAFS	11.27±0.76	2.40±0.09	3.77±0.48	9.87 ±0.52	1.47 ±0.10	6.59 ±0.36	4.75 ±0.26	9.19 ±0.99

Table 3. Study on physicochemical properties of star gooseberry (*Phyllanthus acidus*) at different stages of maturity

DAFS (Days after fruit set)	Fruit weight (g)	Fruit diameter (cm)	Fruit length (cm)	TSS (°Brix)	Titrateable acidity (%)	Total sugar (%)	Reducing sugar (%)	Vitamin C (mg/100g pulp)
15 DAFS	0.34±0.03	1.05±0.07	0.84±0.04	8.15±0.21	1.02±0.36	3.63±0.36	2.03±0.21	24.93±6.10
30 DAFS	1.09±0.16	1.35±0.07	1.05±0.04	7.90±1.27	1.18±0.37	4.21±0.02	2.52±0.08	25.86±7.32
45 DAFS	3.56±0.35	2.05±0.07	1.48±0.11	5.65±0.49	1.20±0.08	5.20±0.12	3.04±0.06	28.65±1.22

SHORT COMMUNICATION

Impact of biofertilizers on growth, yield and nutritional composition of Coriander (*Coriandrum sativum* L.)

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ABSTRACT

Organic farming benefits greatly from the use of biofertilizers because they are affordable, environmentally friendly and quickly increase soil fertility. Eight treatments were undertaken to evaluate the impact of *Azospirillum brasilense*, *Pseudomonas putida* and *Brevibacillus brevis* on the growth, yield and nutritional characteristics of coriander. The study used a Randomized Block Design for its execution. In comparison to the control (T_0), the treatment involving the combined inoculation of *Azospirillum brasilense* + *Pseudomonas putida* + *Brevibacillus brevis* (T_7) exhibited considerably higher collar diameter, plant height, leaf count, fruit count, fresh weight of leaves & fruits and various nutrition. The enhanced intake of nitrogen, phosphorus and other critical nutrients for coriander growth may be the cause of the increases in growth & yield parameters and nutritional composition in treatments with biofertilizers.

Keywords: Biofertilizers, coriander, growth, nutrients, yield

Another strategy for achieving potential agriculture is the utilization of biofertilizers, which are beneficial microorganisms that play a crucial role in meeting the nutrient requirements of plants (Bhardwaj *et al.*, 2014; Raghad *et al.*, 2021). Moreover, bio-fertilizers are administered to the soil, fruit, or root and promote the establishment of microflora, enhanced nutrient availability and nitrogen fixation (Afnan *et al.*, 2021; Datta *et al.*, 2024).

Azospirillum brasilense is the most researched PGPR, which shows a great deal of promise for commercial uses. It increased the development and productivity of a wide

variety of plant species (Vidotti *et al.*, 2019; Peng *et al.*, 2021). *Pseudomonas putida* is a drought-tolerant variant having traits that encourage plant growth. Additionally, it improved the uptake of water and nutrients, which sustains the rate of photosynthesis (Tanveer *et al.*, 2023). *Brevibacillus brevis* is considered as plant growth-promoting rhizobacteria (PGPRs) and generally occurring in sediment and soil. Several research works have highlighted the impact of these bacteria as a PGPR in different types of crops (Nehra *et al.*, 2016; Dutta and Thakur, 2017).

Coriander (*Coriandrum sativum* L.) is a spice crop with a nice perfume across the entire plant and is used extensively to flavor cuisine (Kumar *et al.*, 2023). About 80% of the coriander seeds produced worldwide are grown in India, mostly in Madhya Pradesh, Gujarat, Rajasthan, Assam, West Bengal, Orissa, Andhra Pradesh and Tamil Nadu (Thakur *et al.*, 2021). Research from a variety of sources is necessary to regulate nutrients at different sites and assess the effect of biofertilizers particularly PGPR on the development and production of different crops, such as coriander. Having the above mentioned facts, the current research was conducted to estimate the effect of different biofertilizers on growth and yield attributes of coriander. According to the literature review, this is the first study on *Pseudomonas putida* and *Brevibacillus brevis* promoting plant growth of coriander.

The field experiment was carried out in Suranam village of Sivagangai district in Tamil Nadu during September 2023. The study area lies between 78.7186° E longitude and 9.6929° N latitude at an altitude of 41 m above mean sea level. The annual rainfall of this area is about 20 – 35 mm. This location has varying temperatures between 22° and 40°C. The study employed the cultivar variety *Coriandrum sativum* cv. CO₃, whose seeds were sourced from organic growers in Suranam location. For this investigation, 2 m × 2 m (4 m²) plots were prepared and each plot received 15 g of hand-sown seeds. Irrigation was applied both at the time of planting and on a regular basis starting on the third day after sowing, when the soil moisture content was reduced. Every patch was manually kept free of weeds. The experiment was laid out in Randomized Block Design with three replications. The biofertilizers employed for present research work include *Azospirillum brasilense*, *Pseudomonas putida* and *Brevibacillus brevis* and all these were procured from Department of Agricultural

Microbiology, Agricultural College and Research Institute, Madurai. The treatments consist, T₀ – control (without fertilizer), T₁ – *Azospirillum brasilense* (15 g), T₂ – *Pseudomonas putida* (15 g), T₃ – *Brevibacillus brevis* (15 g), T₄ – *Azospirillum brasilense* + *Pseudomonas putida* (7.5 g each), T₅ – *Azospirillum brasilense* + *Brevibacillus brevis* (7.5 g each), T₆ – *Pseudomonas putida* + *Brevibacillus brevis* (7.5 g each) and T₇ – *Azospirillum brasilense* + *Pseudomonas putida* + *Brevibacillus brevis* (5 g each).

After the completion of full growth circle (on 90th day after sowing), secondary growth data on collar diameter, shoot length, root length, total length and number of leaves were recorded along with number of fruits. Then the plants were uprooted carefully and clean off soil particles from roots. The fresh and dry weight of foliage, root and fruits were estimated separately by using electronic weighing balance. Fruit yield was measured by harvesting the entire crop from a net plot area, allowed to air-dry and threshed by hand. After being separated from the plant, the fruits were cleaned and weighed. The proximate analysis of coriander leaves and seeds was carried out by standard methods. Moisture content was determined by using a laboratory oven method provided by Association of Official Analytical Collaboration (AOAC) (Latimer, 2016), carbohydrate was estimated using anthrone method given by Kejla *et al.* (2023), unsaturated fat was estimated by the method given by Hauser (2023), fiber was determined by Weende method given by Association of Official Analytical Collaboration (AOAC) (Latimer, 2016) and proteins was estimated by kjeldahls method as described by Mæhre *et al.* (2018). The vitamins such as vitamin A, B₁, B₂, B₃ and C content of leaves and seeds of coriander were estimated by using the method described by Association of Official Analytical Collaboration (AOAC) (Latimer, 2016). The minerals like magnesium, iron, phosphorus, sodium, zinc and potassium in both foliage and

seeds of coriander were estimated by using standard procedures as described by Carolina *et al.* (2022). All the results obtained by present study were statistically analysed by Duncan's Multiple Range Test ($P < 0.05$) (Duncan, 1955).

Data revealed that highest collar diameter (5.35 mm) was recorded in the plants treated with combined inoculation of *Azospirillum brasilense* + *Pseudomonas putida* + *Brevibacillus brevis* (T₇) followed by T₅ (*Azospirillum brasilense* + *Brevibacillus brevis*). The maximum plant height (53.82 cm) and number of leaves (69.25) were noted in the coriander plants inoculated with *Azospirillum brasilense* + *Pseudomonas putida* + *Brevibacillus brevis* (T₇). In dual inoculation, T₅ plants treated with *Azospirillum brasilense* + *Brevibacillus brevis* exposed superior collar diameter (5.20 mm), shoot length (37.30 cm), root length (11.05 cm) and number of leaves (47.0) than other dual inoculations T₄ (*Azospirillum brasilense* + *Brevibacillus brevis*) and T₆ (*Pseudomonas putida* + *Brevibacillus brevis*). Among the coriander plants inoculated with *Azospirillum brasilense*, *Pseudomonas putida* and *Brevibacillus brevis* individually, they were exhibited their own impact on various growth parameters. Overall, the growth parameters were increased by the combined application of *Azospirillum brasilense* + *Pseudomonas putida* + *Brevibacillus brevis* (T₇) contrasted with untreated control plants (Table 1). In case of yield attributes, maximum fresh weight (g/plot) of foliage (1758.56), root (140.68) and fruit (493.03) was obtained from the plants treated with combined inoculation of *Azospirillum brasilense* + *Pseudomonas putida* + *Brevibacillus brevis* (T₇) like growth parameters. Similarly, maximum dry weight of each attributes (foliage-351.71, root-28.13, fruit-493.03 g/plot) was attained in the same plants (Table 2).

Numerous previous investigations supported the results of the current study. Davaran-Hagh *et al.* (2015) found that the

fresh weight of corn plants infected with *Azospirillum brasilense* was 30% higher than that of non-inoculated plants. Salazar-Garcia *et al.* (2022) documented that inoculation with *A. brasilense* resulted a notable raise in both waterlogged and non-waterlogged plants, demonstrating the crucial function of this bacterium in radish growth.

Tanveer *et al.* (2023) found that inoculating with *Pseudomonas putida* significantly increased root and shoot length and leaf area when compared to the uninoculated plants in canola. Bacteria that promote plant growth enhance the flexibility of the root membrane, and the improvement in root and shoot development has been related with the production of phytohormones by *Pseudomonas putida* (Chieb and Gachomo, 2023).

Brevibacillus brevis has also been studied by several researchers as a PGPR on a variety of crops. Chandel *et al.* (2010) found *B. brevis* as a potential biological control agent for reducing the impacts of *Fusarium oxysporum* ssp. *lycopersici* on tomato plants. Iqbal *et al.* (2016) conducted research in a saline area and they have found that IAA-producing rhizobacteria such as *Brevibacillus* and *Pseudomonas* greatly improved the growth, physiological traits and yield of maize plants. Rasouli *et al.* (2022) evaluated the essential function of organic fertilizers in increasing seed production of coriander. The enhancement in growth characteristics resulting from the inoculation with nitrogen-fixing bacteria has also been documented by Sahu *et al.* (2014), Sahu and Sahu (2018), Swain (2020), Ali *et al.* (2023) on coriander.

Proximate analysis of both coriander leaves and seeds showed less significant variations among the plants treated with various biofertilizers when compare to control. In case of leaves, the moisture content was in the range of 86.6–87.9%. Maximum moisture content (87.9%) was observed in the

plants treated with combined inoculation of *Azospirillum brasilense* + *Pseudomonas putida* + *Brevibacillus brevis* (T₇) and also in *Azospirillum brasilense* + *Pseudomonas putida* inoculated plants (T₄). Likewise, the biochemical contents such as carbohydrate, unsaturated fat, fiber and protein were also have been found maximum in leaves of coriander plants treated with combined inoculation of *Azospirillum brasilense* + *Pseudomonas putida* + *Brevibacillus brevis* (T₇) and they were determined as 6.8, 1.8, 5.5 and 3.8 g/100g respectively (Table 3). The proximate analysis of the seeds also exhibited the same results as in case of leaves of coriander thus the plants treated with combined inoculation of *Azospirillum brasilense* + *Pseudomonas putida* + *Brevibacillus brevis* (T₇) showed maximum values in all the biochemical characteristics including moisture content (Table 3).

Regarding the vitamin content of leaves, all the vitamins of coriander inoculated with biofertilizers had significantly increased over untreated plants (Table 4). The highest vitamin A concentration (183 mg/100g fresh) was estimated in plants inoculated with *Azospirillum brasilense* + *Pseudomonas putida* + *Brevibacillus brevis* (T₇) followed by double inoculation of *Pseudomonas putida* + *Brevibacillus brevis* inoculated plants (T₆) (178 mg/100g). The vitamins B₁, B₂ and B₃ were found maximum (1.42, 1.72 and 5.70mg/100g respectively) in the plants treated with combined inoculation of *Azospirillum brasilense* + *Pseudomonas putida* + *Brevibacillus brevis* (T₇). These plants also contained maximum concentration (135.9 mg/100g) of vitamin C also (Table 4). In case of seeds, the concentration of vitamin A, B₁, B₂, B₃ and C was found maximum in the plants treated with *Azospirillum brasilense* + *Pseudomonas putida* + *Brevibacillus brevis* (T₇) as 154, 1.72, 1.96, 7.78 and 13.8 mg/100g respectively (Table 4).

By the present study, it was noted that all plants treated with biofertilizers were exhibited notable variations in the different mineral concentrations of coriander leaves and seeds when compared to plants that were not treated (Table 5). In case of calcium concentration of leaves, all treated plants showed significant variations, with the exception of the uninoculated plants. It was recorded that 255 mg/100g of calcium as highest in T₇ (*Azospirillum brasilense* + *Pseudomonas putida* + *Brevibacillus brevis*) plants. The highest concentration of magnesium (134 mg/100g), iron (6.4 mg/100g), phosphorus (95 mg/100g), sodium (0.43 mg/100g), zinc (0.98 mg/100g) and potassium (550 mg/100g) were also found in the coriander plants treated with combined inoculation of *Azospirillum brasilense* + *Pseudomonas putida* + *Brevibacillus brevis* (T₇) (Table 5). In case of seeds, the highest concentration of calcium (350 mg/100g), magnesium (178 mg/100g), iron (8.8 mg/100g), phosphorus (120 mg/100g), sodium (0.50 mg/100g), zinc (1.82 mg/100g) and potassium (678 mg/100g) were determined in the plants treated with combined inoculation of *Azospirillum brasilense* + *Pseudomonas putida* + *Brevibacillus brevis* (T₇) (Table 5). The overall findings regarding the concentration of minerals of seeds showed higher content when compared to leaves of coriander (Table 5). Research has demonstrated that the process of photosynthesis in plants is influenced by various nutrient elements like calcium, magnesium, iron, phosphorus, sodium, zinc and potassium. These components play a critical role in plant photosynthesis (Meng and Gao, 2011). The measured elemental content in coriander by present study indicated that the subjected microbial strains (*Azospirillum brasilense*, *Pseudomonas putida* and *Brevibacillus brevis*) enhanced the absorption of several components in treated plants. Facilitating the absorption of macroelements

and microelements in plants is a key function of PGPR. Earlier research indicated that plant hormones generated by microorganisms encourage root growth, which increases the root surface area and aids in the absorption of additional nutrients (Kumar *et al.*, 2014), which leads to the maximum growth and higher biomass.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Table 1: Effect of biofertilizers on growth and yield attributes of coriander

Treatments	Growth and yield parameters					
	Collar diameter (mm)	Shoot length (cm)	Root length (cm)	Total length (cm)	Number of leaves	Number of Fruits
T ₀	4.10 ± 0.14	23.0 ± 1.54	7.20 ± 0.49	30.20 ± 1.70	23.50 ± 3.41	49.75 ± 4.87
T ₁	3.92 ± 0.63	26.87 ± 1.70	7.25 ± 0.20	34.12 ± 2.82	32.0 ± 2.94	114.25 ± 44.47
T ₂	4.0 ± 0.78	29.05 ± 4.86	7.87 ± 0.98	36.92 ± 3.31	32.50 ± 5.44	130.25 ± 30.26
T ₃	3.35 ± 0.36	33.97 ± 2.58	8.67 ± 2.68	42.65 ± 3.21	27.0 ± 7.39	133.0 ± 30.39
T ₄	4.17 ± 0.38	35.42 ± 5.61	9.57 ± 2.32	44.99 ± 4.18	32.75 ± 3.77	152.50 ± 48.39
T ₅	5.20 ± 0.43	37.30 ± 3.26	11.05 ± 3.03	48.35 ± 5.79	47.0 ± 4.16	201.0 ± 58.67
T ₆	4.50 ± 0.41	36.22 ± 5.11	8.32 ± 1.02	44.54 ± 6.13	42.25 ± 20.45	155.50 ± 25.54
T ₇	5.35 ± 0.34	39.70 ± 3.60	14.12 ± 2.49	53.82 ± 2.88	69.25 ± 18.31	243.25 ± 67.48

T₀ – control (without fertilizer), T₁ – *Azospirillum brasilense* (15 g), T₂ – *Pseudomonas putida* (15 g), T₃ – *Brevibacillus brevis* (15 g), T₄ – *Azospirillum brasilense* + *Pseudomonas putida* (7.5 g each), T₅ – *Azospirillum brasilense* + *Brevibacillus brevis* (7.5 g each), T₆ – *Pseudomonas putida* + *Brevibacillus brevis* (7.5 g each) and T₇ – *Azospirillum brasilense* + *Pseudomonas putida* + *Brevibacillus brevis* (5 g each). Values are Mean + Standard Error

Table 2: Effect of biofertilizers on fresh and dry weight of coriander

Treatments	Fresh weight (g/plot)				Dry weight (g/plot)			
	Foliage	Root	Fruits	Total	Foliage	Root	Fruits	Total
T ₀	908.09 ^a	72.64 ^a	200.12 ^a	1180.85 ^a	181.61 ^a	14.52 ^a	200.12 ^a	396.26 ^a
T ₁	983.17 ^b	78.65 ^a	240.29 ^b	1302.11 ^b	196.63 ^b	15.73 ^a	240.29 ^b	452.65 ^b
T ₂	998.34 ^b	79.86 ^a	300.09 ^c	1378.29 ^c	199.66 ^b	15.97 ^a	300.09 ^c	515.73 ^c
T ₃	1166.72 ^c	93.33 ^b	313.42 ^c	1573.47 ^d	233.34 ^c	18.66 ^b	313.42 ^c	565.43 ^d
T ₄	1209.84 ^c	96.78 ^b	351.57 ^d	1658.19 ^e	241.96 ^c	19.35 ^b	351.57 ^d	612.89 ^e
T ₅	1342.32 ^d	107.38 ^c	407.41 ^e	1857.11 ^f	268.46 ^d	21.47 ^b	407.41 ^e	697.35 ^f
T ₆	1301.17 ^d	104.09 ^c	362.18 ^d	1767.44 ^g	260.23 ^d	20.81 ^b	362.18 ^f	643.23 ^g
T ₇	1758.56 ^e	140.68 ^d	493.03 ^f	2392.27 ^h	351.71 ^e	28.13 ^c	493.03 ^g	872.87 ^h

T₀ – control (without fertilizer), T₁ – *Azospirillum brasilense* (15 g), T₂ – *Pseudomonas putida* (15 g), T₃ – *Brevibacillus brevis* (15 g), T₄ – *Azospirillum brasilense* + *Pseudomonas putida* (7.5 g each), T₅ – *Azospirillum brasilense* + *Brevibacillus brevis* (7.5 g each), T₆ – *Pseudomonas putida* + *Brevibacillus brevis* (7.5 g each) and T₇ – *Azospirillum brasilense* + *Pseudomonas putida* + *Brevibacillus brevis* (5 g each). Means followed by a common letter in the same column are not significantly different at 5% level by DMRT.

Table 3: Effect of biofertilizers on proximate composition of coriander leaves and seeds (per 100g)

Treatments	Moisture (%)		Carbohydrate (g)		Unsaturated fat (g)		Fiber (g)		Protein (g)	
	Leaves	Seeds	Leaves	Seeds	Leaves	Seeds	Leaves	Seeds	Leaves	Seeds
T ₀	86.8 ^a	65.3 ^a	6.4 ^a	12.2 ^a	1.2 ^a	1.5 ^a	4.4 ^a	5.6 ^a	3.0 ^a	2.1 ^a
T ₁	87.8 ^b	67.2 ^a	6.6 ^a	14.4 ^{ab}	1.6 ^a	1.8 ^a	4.8 ^a	6.8 ^{ab}	3.4 ^a	2.2 ^a
T ₂	87.0 ^a	66.0 ^a	6.7 ^a	13.5 ^a	1.5 ^a	1.7 ^a	5.0 ^{ab}	5.9 ^a	3.2 ^a	2.0 ^a
T ₃	87.2 ^a	65.2 ^a	6.5 ^a	13.8 ^a	1.7 ^{ab}	1.9 ^a	4.6 ^a	5.8 ^a	3.2 ^a	2.5 ^a
T ₄	87.9 ^b	67.2 ^a	6.5 ^a	14.2 ^{ab}	1.7 ^{ab}	1.8 ^a	4.8 ^a	5.6 ^a	3.3 ^a	2.3 ^a
T ₅	86.6 ^a	68.5 ^a	6.8 ^a	13.0 ^a	1.4 ^{ab}	1.6 ^a	5.1 ^{ab}	6.1 ^b	3.5 ^a	2.7 ^{ab}
T ₆	86.9 ^a	68.7 ^a	6.7 ^a	13.4 ^a	1.5 ^a	1.7 ^a	5.3 ^{ab}	6.5 ^{ab}	3.4 ^a	2.8 ^{ab}

T₀ – control (without fertilizer), T₁ – *Azospirillum brasilense* (15 g), T₂ – *Pseudomonas putida* (15 g), T₃ – *Brevibacillus brevis* (15 g), T₄ – *Azospirillum brasilense* + *Pseudomonas putida* (7.5 g each), T₅ – *Azospirillum brasilense* + *Brevibacillus brevis* (7.5 g each), T₆ – *Pseudomonas putida* + *Brevibacillus brevis* (7.5 g each) and T₇ – *Azospirillum brasilense* + *Pseudomonas putida* + *Brevibacillus brevis* (5 g each). Means followed by a common letter in the same column are not significantly different at 5% level by DMRT.

Table 4: Effect of biofertilizers on vitamin composition of coriander leaves and seeds (mg/100g)

Treatments	Vitamin A		Vitamin B ₁		Vitamin B ₂		Vitamin B ₃		Vitamin C	
	Leaves	Seeds	Leaves	Seeds	Leaves	Seeds	Leaves	Seeds	Leaves	Seeds
T ₀	162 ^a	135 ^a	1.02 ^a	0.25 ^a	1.25 ^a	0.25 ^a	1.50 ^a	3.56 ^a	120.5 ^a	11.0 ^a
T ₁	165 ^a	148 ^b	1.25 ^a	0.55 ^b	1.50 ^a	0.50 ^b	2.60 ^b	4.64 ^b	129.0 ^a	12.7 ^b
T ₂	168 ^{ab}	150 ^b	1.28 ^a	0.48 ^b	1.64 ^{ab}	0.84 ^{bc}	3.62 ^{bc}	5.68 ^c	120.6 ^a	12.9 ^b
T ₃	155 ^a	148 ^b	1.31 ^a	0.51 ^b	1.46 ^{ab}	0.48 ^b	2.70 ^b	4.78 ^b	128.8 ^a	11.9 ^b
T ₄	165 ^a	153 ^c	1.25 ^a	0.35 ^{ab}	1.30 ^a	0.80 ^c	4.54 ^d	6.54 ^d	129.8 ^a	12.8 ^a
T ₅	176 ^{ab}	146 ^b	1.32 ^{ab}	0.62 ^c	1.42 ^{ab}	0.42 ^b	3.28 ^{bc}	5.29 ^c	125.3 ^a	13.2 ^c
T ₆	178 ^b	150 ^b	1.26 ^a	0.62 ^c	1.63 ^{ab}	1.63 ^d	4.50 ^d	6.50 ^d	130.0 ^{ab}	12.8 ^b

T₀ – control (without fertilizer), T₁ – *Azospirillum brasilense* (15 g), T₂ – *Pseudomonas putida* (15 g), T₃ – *Brevibacillus brevis* (15 g), T₄ – *Azospirillum brasilense* + *Pseudomonas putida* (7.5 g each), T₅ – *Azospirillum brasilense* + *Brevibacillus brevis* (7.5 g each), T₆ – *Pseudomonas putida* + *Brevibacillus brevis* (7.5 g each) and T₇ – *Azospirillum brasilense* + *Pseudomonas putida* + *Brevibacillus brevis* (5 g each). Means followed by a common letter in the same column are not significantly different at 5% level by DMRT.

Table 5: Effect of biofertilizers on mineral composition of coriander leaves and seeds (mg/100g)

Treatments	Calcium		Magnesium		Iron		Phosphorous		Sodium		Zinc		Potassium	
	Leaves	Seeds	Leaves	Seeds	Leaves	Seeds	Leaves	Seeds	Leaves	Seeds	Leaves	Seeds	Leaves	Seeds
T ₀	235 ^a	290 ^a	130 ^a	165 ^a	5.0 ^a	6.8 ^a	65 ^a	98 ^a	0.34 ^a	0.41 ^a	0.76 ^a	0.96 ^a	430 ^a	560 ^a
T ₁	246 ^a	318 ^b	133 ^a	176 ^b	5.2 ^a	7.9 ^b	70 ^b	112 ^b	0.33 ^a	0.48 ^b	0.95 ^{ab}	1.75 ^b	458 ^a	558 ^a
T ₂	251 ^a	335 ^b	132 ^a	170 ^b	5.8 ^a	8.2 ^b	85 ^b	115 ^b	0.41 ^a	0.42 ^a	0.91 ^{ab}	1.65 ^b	510 ^b	624 ^b
T ₃	230 ^a	303 ^b	131 ^a	168 ^a	5.4 ^a	7.0 ^{ab}	69 ^a	102 ^b	0.32 ^a	0.43 ^a	0.93 ^{ab}	1.40 ^b	450 ^a	590 ^{ab}
T ₄	242 ^a	310 ^b	131 ^a	171 ^b	6.0 ^b	8.0 ^b	80 ^b	118 ^b	0.36 ^a	0.47 ^b	0.72 ^a	1.54 ^b	528 ^b	648 ^b
T ₅	240 ^a	328 ^b	132 ^a	172 ^b	5.6 ^a	7.4 ^{ab}	83 ^b	115 ^b	0.32 ^a	0.45 ^a	0.80 ^b	1.43 ^b	502 ^b	657 ^b
T ₆	248 ^a	324 ^b	132 ^a	173 ^b	6.0 ^b	7.2 ^{ab}	90 ^c	113 ^b	0.35 ^a	0.43 ^a	0.83 ^b	1.35 ^b	532 ^b	645 ^b

T₀ – control (without fertilizer), T₁ – *Azospirillum brasilense* (15 g), T₂ – *Pseudomonas putida* (15 g), T₃ – *Brevibacillus brevis* (15 g), T₄ – *Azospirillum brasilense* + *Pseudomonas putida* (7.5 g each), T₅ – *Azospirillum brasilense* + *Brevibacillus brevis* (7.5 g each), T₆ – *Pseudomonas putida* + *Brevibacillus brevis* (7.5 g each) and T₇ – *Azospirillum brasilense* + *Pseudomonas putida* + *Brevibacillus brevis* (5 g each). Means followed by a common letter in the same column are not significantly different at 5% level by DMRT.

SHORT COMMUNICATION

Genetic resources, distribution area, cultivation history and results of breeding almonds in Azerbaijan

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ABSTRACT

The article discusses the features of genetic resources, areas of distribution of species and historical regions of almond cultivation in Nakhchivan, Azerbaijan, also the results of the study of local and introduced varieties of almond (*Prunus dulcis* (Mill.) D.A. Webb), common in the Shakhbuz and Julfa districts. These varieties - ripening at different times, with high biomorphological and economic indicators, resistant to biotic and abiotic stress factors of the environment have great prospects for growing almonds in Azerbaijan. The agrobiological characteristics of these almond varieties were studied and pomological indicators were given - flowering period, average weight, height, diameter, peel color, kernel color, weight and taste of the fruit of each variety were studied separately and assessed on a 5-point scale. The studied varieties received the following ratings - Dash Badam - 4.9, Sarayi - 4.7, Ketan Koynek - 4.6, Gosha Lepe - 4.8, Kurdashi - 4.6, Sugra - 4.4, Kaghız Badam - 4.8, Nonpareil - 4.7 and Nec Plus Ultra - 4.8 points. The study of almond genetic resources in the region not only contributes to the preservation of biodiversity but also supports the development of agronomy and agriculture in the country.

Keywords: Almond, introduced, local, pomology, variety.

INTRODUCTION

Almonds are one of the promising crops grown in the arid conditions of USA (California), Australia Spain, Turkey, Morocco, Iran, Iraq, Central Asia, Turkmenistan, Tajikistan, Afghanistan, Portugal, etc. These countries contain the main almond growing areas (Rahemi, 2002, Chepinoga 2020, Llompartet *al.*, 2024). Almonds are rich in beneficial nutrients, including proteins, healthy fats, vitamins (especially E and B), minerals

(magnesium, calcium, iron), and fiber (Hasanov and Aliev 2011). This makes them an excellent addition to the diet for maintaining health. Almonds contain monounsaturated fats, which help lower levels of bad cholesterol (LDL) and increase levels of good cholesterol (HDL). This supports heart and vascular health. Vitamin E and other antioxidants in almonds help protect cells from oxidative stress and inflammation, which can reduce the risk of chronic diseases. Despite their high calorie

content, almonds can aid in weight management. Their high fiber and protein content creates a feeling of fullness, which can reduce overall calorie intake. Almonds may also promote improved metabolism due to their magnesium content, which plays a crucial role in carbohydrate and fat metabolism. Almonds can be used in various forms: whole, sliced, as oil or milk. This makes them an ideal ingredient for both sweet and savory dishes, snacks, desserts, and even beverages. Almonds and almond-based products (such as almond flour) are excellent alternatives to wheat for people with gluten intolerance or celiac disease. Regular consumption of almonds is associated with a lower risk of cardiovascular diseases, type 2 diabetes, and certain types of cancer due to their nutritional properties and ability to enhance overall health. Replacing some ingredients with almonds can help reduce the calorie content of dishes without sacrificing flavor and texture. For example, adding almond milk instead of cow's milk in smoothies or coffee. The study of the biological and agricultural characteristics of almond varieties and forms and other fruit crops includes several key aspects that help understand their agronomic traits and conditions for successful cultivation (Musayev 2019, Musayev, and Hajiyeu 2024). Different almond varieties may exhibit varying levels of resilience to climatic conditions such as temperature, humidity, and precipitation. Research helps identify which varieties are best suited for specific regions (Bayramov 2022, Musayev and Hajiyeu 2023).

OBSERVATIONS

Along with other fruit plants, almond is one of the oldest fruit plants in the Republic of Azerbaijan. There are three species of almonds found in the wild flora of Azerbaijan. The Common or sweet almond (*Prunus dulcis* (Mill.) D.A.Webb) grows in Azerbaijan on the northeastern slopes of the Greater Caucasus, as well as in the central and southern parts of the Lesser Caucasus. The Mountain or fenzls almond (*Prunus fenzliana* Fritsch.) is found in the

central and southern regions of the Lesser Caucasus in Azerbaijan (Ladizinsky, 1999) (in Zangilan), in the highlands of the Nakhchivan Autonomous Republic, and in Diabar (Talış region). It occurs on rocky areas and in coastal thickets in the middle and upper mountain belts. The Nairi almond (*Prunus nairica* (Fed. & Takht.) Eisenman) is noted on dry, stony, and rocky slopes in the southern part of the Lesser Caucasus and Nakhchivan. As a cold-resistant species of almond, it can be found in the Nakhchivan autonomous Republic at altitudes ranging from 1,700-1,900 meters above sea level to 2,300-2,400 meters. Of these, only one species - common or sweet almond (*Prunus dulcis* (Mill.) D.A.Webb) is cultivated (Martínez-Gómez *et al.*, 2007).

Prunus dulcis are an important agricultural crop in Azerbaijan, where their cultivation has a long history. The country has favorable climatic conditions for almond cultivation, especially in regions with a warm and dry climate. The remaining almond forests around the famous "Badamly" spring (the name means "Almond" or "With Almond") and in the mountainous part of the Shahbuz district of the Nakhchivan Autonomous Republic testify to the fact that there were once large almond forests here since ancient times.

In Azerbaijan, almonds are mainly cultivated in the following 4- regions:

1. Absheron region: Almond cultivation in the Absheron region of Azerbaijan has a long and interesting history closely linked to the climatic and geographical features of this area. The Absheron Peninsula, located on the coast of the Caspian Sea, has a mild climate with warm summers and moderately cold winters, creating favorable conditions for growing various fruit crops, including almonds. Almonds have been cultivated in this region since ancient times, as evidenced by archaeological finds and historical sources. In traditional agriculture in Absheron, almonds held an important place among nut-bearing crops. Local residents used almonds not only as a food product but

also in folk medicine. Almond cultivation was common in private gardens and small farms. During the Soviet period, attempts were made to systematize and expand almond production in the Absheron region. Specialized nurseries were established, and new agronomic methods aimed at increasing yield and disease resistance of trees were introduced. Today, almond cultivation in Absheron continues to develop. Almond orchards are combined with other crops, contributing to sustainable agricultural development (Khidirova *et al.*, 2016, Khidirova and Asgarbeyli 2017, Khidirova and Mamedova 2019).

2. Nakhchivan AR: This autonomous region also has traditions of almond cultivation that are adapted to local conditions. A large number of local varieties and forms of almonds are concentrated here. Almond cultivation in Nakhchivan has deep cultural roots and is an important part of the local economy and agriculture (Taghiyev *et al.*, 1990, Bayramov 2022).

3. Shirvan region: This region is also known for its agriculture, including almond cultivation. The region is suitable for growing almonds due to its warm climate, sufficient sunny days, and good solar insolation that contribute to the healthy growth and fruiting of trees (Taghiyev *et al.*, 1990, Hasanov and Aliev 2011).

4. Karabakh region: After the restoration of agriculture in the liberated territories of Karabakh, there is also a growing interest in reviving traditional crops, including almonds.

Since ancient times, folk breeders have created valuable varieties and forms of almonds that are distinguished by fruit size, appearance, yield, taste, aroma, oiliness and resistance to diseases and pests. Below are descriptions of 9 local and introduced almond varieties common in Shahbuz and Julfa districts.

1. Dash Badam (meaning - Stone almond): Commonly found in personal subsidiary farms in the villages of Badamly, Selesuz,

Shada, Ayrndzh, and others in the Shahbuz and Julfa districts. The tree reaches a height of 5–6 meters with a pyramidal crown. The bark of the trunk is grayish-brown. Annual shoots are 25–35 cm long. The leaves are broad-lanceolate, pointed, and light green in color. It blooms in early March. The fruit is wide, elongated, large, weighing 9–12 grams, and has a flat shape. The fruit stalk is very short, as if attached directly to the fruit itself. The length of the fruit stalk is 0.3–0.4 mm, and its thickness is 0.5–1 mm. The shell color is gray, while the kernel is light brown and not very hard. Each tree yields an average of 25–28 kg of fruit. It ripens in early August. The dry pericarp easily separates from the stone upon ripening. The fruits are mainly used fresh for obtaining kernels. The tasting score is 4.9 points.

2. Sarayi: This variety is commonly found in private yards, mainly in the villages of Selesg, Daylahli, Kolani, Mahmudoba, and Nurs in the Shahbuz district. It is one of the most widespread ancient local varieties in the Nakhchivan Autonomous Republic. The tree is of medium height, reaching 4–6 meters, with a high inverted pyramidal crown. The trunk is brown in color. Depending on the growing area, it blooms from late March to the second decade of April. The fruits are medium-sized, oval in shape, weighing 8–10 grams, with a height of 9–14 mm, a length of 30–32 mm, and a diameter of 11–13 mm. The pericarp is of medium thickness, cracked on the surface, and dark gray in color. The fruit kernel is soft and oily. This is a very productive variety, yielding an average of 15–17 kg of fruit per tree. The fruit stalk is almost completely fused with the fruit branch. Harvesting occurs at the end of August. It is resistant to diseases and pests. The tasting score is 4.7 points.

3. Ketan Koynek (meaning -Linen shirt):- This variety is mainly found in the regions of Badamly, Garababa, and Shahbuz. Recently, the variety has spread to other villages as well. It is a late-ripening variety. The tree is of medium height with a wide crown, averaging 3–4 meters tall, with a spherical,

drooping crown that has a diameter of 6–7 meters. It is resistant to frost and drought. It begins to bear fruit 3–4 years after planting and blooms in early March. The flowers are lilac in color, with each flower having one pistil and 10–12 stamens. The lifespan of each flower varies among almond varieties, lasting 4–7 days. Typically, flowering lasts 12–16 days on the tree. This is a very productive variety. The fruits are medium-sized, weighing 9–11 grams, with a height of 10–12 mm and a diameter of 13–16 mm. The pericarp is slightly thin, gray in color, with surface cracks. The shell is very soft. The kernel is brittle and oily. Each tree yields an average of 12–15 kg of fruit. The fruit stalk measures 1.5–2 mm in length and 1 mm in thickness. The stems are not very firmly attached to the stem. The harvest is collected at the end of August. It is not susceptible to diseases and pests. The tasting score is 4.6 points.

4. Gosha Lepe (meaning - Double Kernel): - This variety is found in private household plots in the villages of Tyurkesh, Badamly, Nursu, and Kolani in the Shahbuz district. As the name suggests, the kernel is very large and has a double kernel. The tree is of medium height, reaching 3–4 meters, with a pyramidal-spherical crown. The lower branches are drooping and spreading. The crown diameter is up to 4–5 meters. It blooms in the first decade of March, with white and lilac flowers. The fruits are medium-sized, weighing about 8–9 grams. The surface of the pericarp is bumpy. The length of the fruits is 12–15 mm, and the width is 16 mm. The height of the kernel is half its length. Unlike other varieties, the kernel is very oily and full. The fruit stalk is almost connected to the fruit branch. As they ripen, the fruits do not fall from the tree. The leaves are light green and long-lanceolate. This is a productive variety, with each tree yielding an average of 20–24 kg of fruit. It is indispensable for fresh use and desserts. The trees are resistant to diseases and moderately resistant to pests. The tasting score is 4.8 points.

5. Kurdashi: - This variety is found in household plots and newly established

almond orchards in the villages of Kolani, Ashagy Kishlag, Gyunay Kishlag, Kyuku, and Nursu in the Shahbuz district. Although it is an imported variety, it produces high-quality, abundant yields that are fully adapted to the soil and climatic conditions. The tree is of medium height, reaching 3–4 meters, with a drooping crown that has a diameter of 5–6 meters, an ash-colored trunk, gray buds, and glossy dark green leaves. The leaves are long and lanceolate. It blooms in the first and second decades of April, thus avoiding spring frost due to late flowering. The petals are white and pink. Each flower has 5 petals, 1 pistil, and 9–11 stamens. This is a very productive variety, with medium-sized fruits averaging 9–11 grams, a length of 28–30 mm, a thickness of 10–12 mm, and a diameter of 14–16 mm. The pericarp is of medium thickness, gray in color, and cracked. The kernel is brittle, oily, and tasty. Each tree yields 13–15 kg of fruit. The fruit stalk is longer compared to other varieties, measuring 3–4 mm in length, with a hairy surface and a thickness of 2 mm, firmly attached to the fruit. The stems are tightly attached to the branch, and as the fruit ripens, the pericarp easily cracks, causing the fruits to fall to the ground. Therefore, it's important to harvest the fruits immediately after the skin cracks and they fully ripen; otherwise, most of the fruits may drop. Depending on the growing region, the fruits ripen from late August to mid-September. It is resistant to diseases and pests. The tasting score is 4.6 points. Due to the fragility of the fruits, they are recommended for consumption as a snack.

6. Sugra (meaning - Female name): - This variety is mainly found in the villages of Jamaldin, Gulistan, and Bananiyar. It is a late-ripening variety. The tree is of medium height with a wide crown, averaging 3–4 meters tall, with a spherical, drooping crown that has a diameter of 5–6 meters. It is resistant to frost and drought, beginning to bear fruit 3–4 years after planting. It blooms in early March, with lilac-colored flowers. Each flower has 1 pistil and 10–12 stamens. The lifespan of each flower varies among

almond varieties, lasting 6-7 days, and flowering typically lasts 12-16 days on the tree. This is a very productive variety, with medium-sized fruits weighing 9-11 grams, a width of 15-16 mm, and a length of 20-25 mm. The pericarp is slightly thick, gray in color, with surface cracks. The shell is not very hard, and the kernel is brittle and oily. The fruit stalk measures 1.5-2 mm in length and 1 mm in thickness, with the part connecting to the fruit being slightly larger. The fruit stalks are not very firmly attached to the stem. The harvest is collected at the end of August. It is resistant to diseases and pests, although during very rainy weather, this variety can be affected by aphids. The tasting score is 4.4 points.

7. *Kaghız Badam* (meaning - *Paper Almond*): - This variety is planted and grown in private household plots in most villages of the Julfa district. As the name suggests, its pericarp is very soft and easily breaks with fingers. The tree is of medium size, reaching 3-4 meters in height, with a pyramidal crown. The lower branches are drooping and spreading, with a crown diameter of up to 4-5 meters. It blooms in the first decade of March, with white and lilac flowers. The fruits are medium-sized, weighing about 8-9 grams, with a pericarp surface that is bumpy. The fruits measure 15-20 mm in length and 15 mm in width. The height of the kernel is half its length. Unlike other varieties, the kernel is very oily and full. The fruit stalk is almost completely fused with the fruit branch, and as it ripens, it easily falls from the tree. The leaves are light green and long-lanceolate. This is a productive variety, yielding an average of 25-30 kg of fruit per tree. It is indispensable for fresh use and desserts. The trees are resistant to diseases and moderately resistant to pests. The tasting score is 4.8 points.

8. *Nonpareil*: This variety was recently introduced to the Nakhchivan Autonomous Republic. Although it is an imported variety, it is fully adapted to the soil and climatic conditions of Nakhchivan, including the Shahbuz region, and produces high-quality

products. *Nonpareil* is cultivated in the villages of Mahmudoba, Nursu, and Gyzyk Kishlag. Recently, its cultivation area has expanded, and it is widely used for establishing new almond orchards. The trees reach a height of 3.5-4 meters with a flat-pyramidal crown. The trunk is silvery and shiny, the branches are dark gray, and the leaves are thin and long, lanceolate. The variety blooms late and bears fruit every year, starting to bloom in the Shahbuz region at the end of April, making it resistant to spring frosts. The fruits are 35-40 mm long, 15-20 mm wide, and 10-12 mm thick, with an average weight of each fruit ranging from 0.9 to 12 grams. The yield per tree is 15-18 kg. The skin of the fruit is very thin and light gray. The kernel is flat, very oily, and sweet, with a kernel yield of 56% and an oil content of 53%. When ripe, the kernel splits, but the shell remains in place. The peduncle is firmly attached to the branch, with a thickness of 3 mm and a length of 1.5-2 mm. The fruits ripen at the end of September and are resistant to diseases and pests. The tasting score is 4.7 points.

9. *NecPlus Ultra*: This variety was introduced to the Shahbuz region in the last 20 years. It produces high-quality and abundant yields, fully adapted to the soil and climatic conditions. It is widespread in the villages of Daylahli, Selesuz, and Badamly. This variety has become popular in many parts of the world and is considered very valuable. The tree is large, reaching a height of 3-4 meters with dense foliage. The leaves are broad and dark green. The trunk has a dark silvery color and cracked bark. The variety blooms late and annually produces a rich, high-quality harvest. The fruits have an oval shape, with an average weight of each fruit being 0.7-0.9 grams, a length of 25-30 mm, a thickness of 18-20 mm, and a width of 1.5-1.7 mm. These fruits are consumed as snacks. The kernel constitutes 57% of the fruit, and the oil content is 53%. The peduncle is firmly attached to the branch, so even if the shell splits upon ripening, the fruit does not fall from the tree. The variety is

resistant to diseases and pests, and the tasting score is 4.8 points.

CONCLUSION

The study of biomanagement characteristics of almond varieties includes several key aspects that help to understand their agronomic characteristics and conditions for successful cultivation. Here are some of them:

- **Resistance to diseases and pests:** The study of biological and agricultural features involves assessing the resistance of varieties to common diseases (such as fungal infections) and pests. This is crucial for reducing crop losses and minimizing the use of chemical pesticides.
- **Fruiting and yield:** Research focuses on fruiting characteristics, such as flowering time, quantity, and quality of fruits. This allows for the evaluation of which varieties provide the highest yields and best flavor qualities.
- **Nutrient requirements:** Different varieties may have different nutrient requirements. Studying these needs helps optimize fertilization and improve growth conditions to achieve maximum productivity.
- **Environmental aspects:** The examination of biological and agricultural features also includes assessing environmental impacts, including resource use and effects on biodiversity. Sustainable practices can help minimize negative impacts on ecosystems.
- **Fruit quality:** Analyzing the chemical composition of fruits (such as oil, sugar, and vitamin content) allows for the evaluation of nutritional value and market appeal of various varieties. The study of the biological and agricultural characteristics of almond varieties and forms plays a vital role in advancing agronomy and sustainable

agriculture, contributing to increased productivity and product quality.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Thesis: Singh Harsimranpreet. 2009. Evaluation of new peach and nectarine varieties under Punjab conditions. M.Sc. Thesis, Department of Horticulture, PAU Ludhiana.

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