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Diagnostic approaches for differentiating between true fruit allergy and oral allergy syndrome

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ABSTRACT

The purpose of the present study was to identify diagnostic markers that would allow distinguishing true food allergy to fruits from oral allergy syndrome, as well as to evaluate the efficacy of various diagnostic approaches. The study included 182 adult patients from Poland with immediate allergic reactions after consuming fresh fruits. Diagnosis was conducted in stages and included the collection of clinical data, allergy testing with fruit and pollen allergens, laboratory determination of total and specific immunoglobulin E (IgE), molecular methods of sensitisation identification, and assessment of cell activation. A comparative analysis of skin tests and specific immunoglobulin E determination revealed a 69.2% overall concordance, but diagnostic discrepancies were noted in more than 20% of cases, highlighting the need for a comprehensive approach. The use of component-resolved diagnostics helped to identify sensitisation to individual stable protein structures that are not accessible with standard testing and to clarify the form of allergy – true or cross-reactive. The data obtained demonstrated the need to integrate molecular diagnostics and functional methods into clinical practice to improve the accuracy of the differential diagnostics of fruit allergy.

Keywords: Cellular reactivity, cross-sensitisation, molecular diagnostics, thermolabile proteins.

INTRODUCTION

Food allergy to fruits is a pressing issue in clinical allergology, considering its widespread prevalence, diverse clinical manifestations, and the complexity of differential diagnostics. It is particularly challenging to distinguish between true allergy and oral allergy syndrome (OAS), which is a form of allergic reaction primarily triggered by cross-reactions between pollen allergens (such as birch or grass pollen) and proteins found in certain fresh fruits. While OAS typically presents as localized symptoms, such as itching, swelling, and burning in the mouth, true fruit allergies can cause more severe, systemic reactions, including skin rashes, gastrointestinal distress, and respiratory symptoms. This distinction is crucial for effective diagnosis and treatment, as OAS is often triggered by heat-labile proteins, with patients tolerating cooked fruits, while true food allergies are not dependent on the fruit's preparation. The problem of cross-reactivity between pollen and food allergens is particularly pronounced in regions with a high prevalence of pollen sensitisation (Bekov *et al.*, 2023; Pashova *et al.*, 2019). Research found that the structural similarity between the main birch allergen Bet v 1 and its homologues in apples, cherries, peaches, and other fruits is the cause of symptoms that occur in patients after consuming these products. Knyziak-Mędrzycka *et al.* (2024) investigated the molecular structure of homologous proteins and concluded that it is thermolability and sensitivity to gastric enzymes that determine the limited clinical manifestations of cross-reactive oral allergy.

The clinical variability of fruit allergy manifestations requires the development of reliable diagnostic algorithms that include both primary medical history and laboratory and challenge tests. Kuźniar *et al.* (2024) analysed the effectiveness of skin tests in patients with suspected fruit allergy and concluded that low standardisation of extracts and the influence of

concomitant pollen sensitisation reduce the reliability of the method. However, the researchers did not consider the possibility of component diagnosis, which limits the practical applicability of their conclusions.

Modern methods of component-resolved diagnostics enable the identification of specific protein components associated with severe reactions (Alimardanova *et al*., 2015; Uikassova *et al*., 2022). De Rose *et al.* (2021) applied a molecular approach to the differentiation of fruit allergies and demonstrated high sensitivity in the detection of stable components such as lipid transfer proteins and profilins. Functional tests, such as the basophil activation test (BAT), allow assessing the reactivity of blood cells to allergen contact in vitro. This allows confirming the clinical significance of the detected Immunoglobulin E (IgE) antibodies. Yoshida *et al.* (2022) conducted a BAT study in patients with borderline serology results and found that the method can serve as a reliable tool in doubtful cases. Double-blind placebo-controlled food challenge (DBPCFC) tests continue to be the gold standard for confirming food allergies, despite their complexity and risks. Haidar *et al.* (2024) conducted a large-scale study of the effectiveness of DBPCFC in patients with suspected fruit allergy and found that reproducible symptoms were observed in only some patients, with a considerable proportion of reactions being mild.

Thus, despite the availability of various diagnostic methods, the problem of differentiating between OAS and true allergy is still unresolved. The development of a diagnostic approach based on a stepwise evaluation of clinical symptoms, immunological markers, and functional test results appears necessary. The purpose of the study was to identify diagnostic criteria for differentiating between true food allergy to fruit and OAS and to evaluate the sensitivity and specificity of the methods used.

MATERIALS AND METHODS

The study was conducted in Poland between January and December 2023 at the allergology department of the University Medical Centre in Warsaw, with the participation of the private diagnostic centre MedGen, which specialises in molecular allergology. The study included 182 patients aged 18 to 65 years (74 men and 108 women), with an average age of 34.6±12.1 years. All participants complained of itching, burning, and swelling of the mucous membranes of the mouth and lips, as well as skin and gastrointestinal symptoms after consuming fresh fruit. Notably, all subjects had a history of pollinosis symptoms, primarily during the spring and summer. The cohort demonstrated a predominance of women (59.3%) compared to men (40.7%)

The initial clinical stage included a standardised questionnaire based on the European Academy of Allergy and Clinical Immunology (EAACI) questionnaire (Gromek *et al.*, 2024), a physical examination, and skin prick tests with allergens from birch, hazel, artemisia, and the 12 most commonly consumed fruits using diagnostic extracts (Stallergenes Greer, France). To assess sensitisation, total and specific IgE levels were determined using the Immuno CAP method (Thermo Fisher Scientific, Sweden). Particular attention was paid to the components Bet v 1, Mal d 1, Cor a 1, Pru p 1, Act d 1, and Ara h 8. In cases where conventional tests were insufficiently informative, component-resolved analysis was performed using Allergens Evaluation by Multiplex Diagnostics (ALEX2) (MacroArray Diagnostics, Austria), which enables quantitative and qualitative assessment of sensitisation to more than 280 allergens and their protein components. In 17 patients, a BAT was additionally performed by flow cytometry using Flow Cytometry Equipment (BD Biosciences, USA), which supported the clinical significance of sensitisation at the cellular level.

In 38 patients with conflicting data, a series of oral provocation tests (DBPCFC) was performed following the EAACI protocol (Santos *et al.*, 2025) using lyophilised fruits (in doses of 5-100 g) and placebo, administered at 20-minute intervals. Patients were kept under constant monitoring in a day hospital equipped with anti-shock measures. The Sampson severity grading scale was used to assess the severity of the reaction.

Statistical data processing was performed using Statistica 13.3 (TIBCO Software Inc.,

USA). For quantitative variables, the Student's t-test or Mann-Whitney U-test was employed, and for categorical variables, the chi-square test or Fisher's exact test was used. Multivariate logistic regression analysis was used to identify predictors of true allergy. Differences were considered significant at $p<0.05$. The study followed the Declaration of Helsinki (1964) and the principles of good clinical practice; all participants signed informed consent forms.

RESULTS AND DISCUSSION

Clinical manifestations and symptom profiles

Most often, patients reported symptoms occurring within the first 15 minutes after eating fresh fruit. The most typical manifestations were itching and swelling of the lips and palate (89.6%), a burning sensation in the mouth (76.4%), and itching in the throat (71.4%). Skin reactions in the form of urticaria or localised itching were observed in 41.2% of patients, and dyspeptic disorders in 24.2%. All patients had a history of seasonal allergic rhinitis, mainly in the spring and summer, with 68.7% associating the worsening of symptoms with the birch flowering season. It was noted that in more than half (52.7%) of patients, reactions occurred exclusively to raw fruits, while heat-treated fruits did not cause symptoms, which already at this stage suggested a possible association with thermolabile allergens (Table 1).

At the same time, the presence of skin manifestations in the form of urticaria and itching outside the orofacial area in 41.2% of patients, as well as gastrointestinal symptoms in 24.2% (Table 1), indicates a broader spectrum of clinical reactions that do not always fit within the framework of a localised form of OAS. These manifestations can be considered as potential markers of systemic hypersensitivity and require further evaluation with specific tests. Notably, all patients had a history of seasonal allergic symptoms, confirming the pollen-based sensitisation. It is particularly significant that 68.7% of patients associated the exacerbation of symptoms with the spring period, which indicates a possible role of cross-reactivity, although at this stage this remained a hypothesis that was yet to be corroborated by laboratory tests.

Interestingly, 52.7% of the subjects reported the development of symptoms exclusively after eating raw fruits (Table 1), while heat-treated products were tolerated without discomfort. This observation is an essential clinical indicator of differences in the thermal stability of allergens, but without laboratory verification, it does not allow a definitive conclusion to be drawn about the nature of the sensitisation.

Diagnostic approaches and results

A comparative evaluation of the results of skin testing and the determination of specific IgE levels in the blood serum of the patients examined revealed a general trend of high frequency of sensitisation to fruits using both methods. The most frequent sensitisation was to apples, kiwis, and peaches. Despite the significant level of agreement between the two methods, discrepancies were noted in a series of cases, requiring further interpretation. The summary data is presented in Table 2.

Frequency of positive skin tests and specific IgE to fruits

Analysis of the diagnostic data presented in Table 2 demonstrated prominent features of sensitivity and diagnostic concordance of the two basic approaches – skin prick testing and determination of specific IgE levels in blood serum – in the examination of patients with suspected allergy to fresh fruits. Skin tests performed using standard extracts revealed sensitisation in a significant proportion of patients. The greatest number of positive reactions was recorded with the introduction of apple extract (63.7%), followed by kiwi (44.5%) and peach (41.2%). This supported that these fruits were the most medically significant allergens in the study cohort. For cherries and plums, the rates were significantly lower – 27.5% and 21.4%, respectively, which could suggest both a lower prevalence of sensitisation and potentially weaker allergenic activity of the extracts used in the tests.

In parallel with the skin tests, specific IgE levels were determined, which showed slightly lower overall sensitivity. Thus, IgE to apple was detected in 58.2% of patients, to kiwi – in 39.0%, to peach – in 36.3%, to cherry – in 22.5%, and to plum – in 19.2% (Table 2).

Although the quantitative differences between the methods were not radical, they were systematic and suggested that skin testing more often revealed reactivity, possibly due to a local effect and greater skin sensitivity to allergenic extracts.

The concordance between skin test results and serological data varied depending on the particular allergen. The greatest level of concordance was observed for apple (72.5%), which was probably explained by the good representation of apple allergenic components in both skin test extracts and the laboratory analysis panel. For peaches and kiwis, the concordance rates were 66.2% and 61.5%, respectively, while for cherries and plums – 58.9% and 60.1%, respectively (Table 2). This data suggested that even when using standard diagnostic panels, discrepancies between in vivo and in vitro methods are possible, especially with less typical fruits.

Cases of discrepancies between the results of the two methods deserved special attention. In 11.5% of patients, skin tests were positive with negative specific IgE values (Table 2). This situation could be conditioned by skin hypersensitivity, the inclusion of non-specific irritants in the extract, individual characteristics of the immune response, or low antibody titres that did not reach the diagnostic threshold in laboratory analysis. In contrast, 9.3% of the subjects showed the opposite situation: detection of IgE antibodies with negative skin tests (Table 2). In these cases, the possible influence of antihistamine use, reduced skin reactivity, or characteristics of the allergen poorly represented in the prick test used were significant. The overall concordance between the two methods was 69.2% (Table 2), which, on the one hand, suggested an acceptable level of diagnostic convergence, but on the other hand, highlighted the existence of diagnostic "blind spots" covering over 20% of cases.

Results of component diagnostics (ALEX2) and basophil activation test

Refined diagnostics using component-resolved methods and cell tests were performed in patients in whom basic methods (skin tests and specific IgE determination) did not allow for the unambiguous establishment of clinically relevant sensitisation. The ALEX2 test was used in 56 patients with inconclusive results, while the BAT was used in 17 patients, mainly with severe symptoms but no laboratory confirmation. These methods enabled a more accurate determination of allergen profiles and establishment of the clinical significance of previously unidentified sensitisations. The summarised results are presented in Table 3.

The use of component-resolved diagnostics involving the ALEX2 multiplex platform in 56 patients with inconclusive or conflicting results from previous diagnostic stages substantially improved the understanding of their sensitisation profile. In 85.7% of cases (48 patients), positive results were obtained, i.e., sensitisation to concrete protein components of allergens that could not be detected by conventional tests was identified. This associated with the high sensitivity of the method and its ability to detect "hidden" sensitisation that is not accessible using extract-based tests. In 14.3% of cases (8 patients), the component test was negative, which corresponded to clinically mild or questionable symptoms in these individuals (Table 3).

The performance of BAT in 17 patients, predominantly with borderline IgE levels and no clinically verified skin reactions, helped to further assess the functional activity of sensitising allergens. A positive BAT result was obtained in 82.4% of cases (14 patients), and all those patients had previously experienced symptoms caused by the consumption of particular fruits in their daily lives. This confirmed that the detected IgE was clinically significant and caused the activation of effector cells. In the remaining 3 cases (17.6%), BAT was negative in the presence of specific IgE, which allowed classifying the sensitisation as subclinical or cross-reactive, with no prognostic value (Table 3). This refers to the role of IgE in triggering a functional immune response, as evidenced by positive BAT results, which indicate that the immune system actively responded to allergens. In cases where BAT was negative, sensitization was considered functionally irrelevant, indicating that it did not contribute to clinical symptoms and therefore had no prognostic significance.

Advanced diagnostic techniques and clinical implications

DBPCFC tests were performed in 38 patients with ambiguous results of skin testing, specific IgE, and/or component diagnosis. All tests were performed in a day hospital setting, with sequential administration of lyophilised fruits and placebo at 20-minute intervals. Reactions were recorded using the Sampson severity grading scale. A positive response, defined as the reproduction of typical symptoms after administration of the active substance, was recorded in 21 patients (55.3%). In 17 cases (44.7%), the provocation tests did not cause any symptoms, which allowed ruling out the clinically relevant allergy. Most of the positive reactions occurred within the first 15-30 minutes and were limited to mild orofacial manifestations. Systemic reactions developed in 6 cases (15.8%), including skin, respiratory, and gastrointestinal symptoms. No patient experienced anaphylaxis or life-threatening conditions. Placebo did not cause reactions in any participant, confirming the specificity of the responses. Positive reactions were most frequently observed with the administration of freeze-dried apples (42.8%), peaches (28.5%), and kiwis (23.8%). The summary data are presented in Table 4.

DBPCFC tests in 38 patients with conflicting results from previous diagnostic stages helped to reliably determine the clinical significance of sensitisation and finally classify the type of allergic reaction. A positive response was recorded in 21 patients (55.3%), suggesting the presence of a reproducible reaction to fruit components under controlled exposure conditions (Table 4). Orofacial manifestations dominated – itching, swelling of the lips and mucous membranes, and a burning sensation, which corresponded to the typical clinical picture of OAS. However, 6 patients (15.8%) experienced systemic reactions, including skin rashes, dyspeptic symptoms, and respiratory symptoms (Table 4). These cases were considered clinically significant true food allergies requiring exclusion of the corresponding product from the diet and consideration of allergen-specific immunotherapy. Thus, DBPCFC played a key role in verifying the diagnosis, especially in cases where laboratory and skin tests gave ambiguous or inconclusive results.

Analysis of the distribution of positive reactions by fruit type showed the highest frequency of medically significant sensitivity to apples (42.8% of all positive responses), followed by peaches (28.5%) and kiwis (23.8%) (Table 4). These data correlated with the results of skin and serological tests, where these fruits also showed the greatest frequency of sensitisation. Thus, apples supported their significance as the leading allergen in the study population. Notably, no cases of anaphylaxis or severe life-threatening conditions were reported. Negative tests recorded in 44.7% of the subjects allowed ruling out the clinically relevant allergy and suggested that the previously identified sensitisation had no pronounced clinical significance.

The presented study identified key clinical features inherent in patients with suspected oral allergy syndrome or true food allergy to fresh fruits. The established predominance of localised symptoms – itching and swelling of the mucous membranes of the mouth and lips (89.6%), burning sensation in the mouth (76.4%), and itching in the throat (71.4%) – was consistent with the typical manifestation of OAS. This is confirmed by data from Gromek *et al.* (2024) and Guvenir *et al.* (2024), who described an analogous symptom profile in patients sensitised to pollen allergens. Combined with the patients' reports of worsening symptoms in the spring and summer, these factors associated with a high probability of cross-reactivity between pollen and food proteins. However, the detection of skin reactions (41.2%) and dyspeptic symptoms (24.2%) broadens the clinical picture beyond classic OAS. Analogous findings were also reported by Şirin *et al.* (2024), who emphasised the possibility of systemic manifestations in patients with latent true food allergy. Thus, the present study demonstrated the existence of a wide range of symptoms requiring a stratified diagnostic approach. The fact that 52.7% of the subjects examined had reactions exclusively to raw fruits suggests the involvement of heat-labile allergens, which is consistent with the findings of Schmitt (2024).

Diagnostic evaluation based on a comparison of skin prick tests and specific IgE levels demonstrated a satisfactory level of agreement (69.2%), especially for apples (72.5%). This confirms the high clinical significance of this fruit, which was also reflected in studies by Torres-Arroyo *et al.* (2024). However, the discrepancies between the methods (in 20.8% of cases) highlighted the diagnostic limitations of each, especially towards less typical allergens. Cases of positive skin tests with negative IgE results and vice versa require separate analysis. Barni *et al.* (2022) also described analogous diagnostic discrepancies, attributing them to individual characteristics of the immune response and variability in the allergenic composition of extracts.

The use of ALEX2 in patients with inconclusive results of standard tests helped to identify the specific sensitisation in 85.7% of cases, demonstrating the high effectiveness of this method in clarifying allergic status. Apart from the ability of ALEX2 to detect previously undiagnosed sensitisation, especially to individual protein structures, a valuable advantage is the ability to distinguish between primary sensitisation and cross-reactions. Terlouw *et al.* (2024) obtained analogous findings, highlighting the high sensitivity of the method and its ability to differentiate between primary and cross-sensitisation. Furthermore, the multiplex test format enables the simultaneous detection of a wide range of allergens, which expedites diagnostics and reduces the need for multiple tests.

Of particular significance is the detection of sensitisation to thermolabile proteins – specifically, recombinant homologues of Bet v 1 – in patients who react only to raw fruits. Such proteins are homologues of the major birch pollen allergen and rapidly denature under the influence of temperature, which explains the lack of reaction to heat-treated products (Petrenko *et al*., 2022). This allows Bet v 1-like structures to be considered key markers of cross-allergy between pollen and fruits (Pashova and Radev, 2021; Shahini *et al*., 2023). These data are consistent with the findings of Wang *et al.* (2024), who described analogous molecular mechanisms in pollen-food syndrome, pointing to a high prevalence of sensitisation to these proteins in populations with pollen rhinoconjunctivitis.

The BAT performed in 17 patients showed positive results in 82.4% of cases and associated with the clinical significance of previously detected IgE. Corresponding data were presented by Shah *et al.* (2023) emphasising the high specificity of BAT in assessing the functional activity of allergens. In the present study, BAT supported its diagnostic value in situations where serological and skin tests gave conflicting results. However, Skypala *et al.* (2022) questioned the advisability of routine use of BAT in allergological practice, pointing to its excessive cost and limited reproducibility.

DBPCFC tests allowed confirming the diagnosis definitively in complex clinical cases. The data obtained (55.3% positive responses) correlated with the findings of Costa and Mafra (2022), who emphasised the need to use DBPCFC as the “gold standard” for diagnosing food allergies. The predominance of mild reactions in most of the subjects corroborated the dominance of OAS. However, the presence of systemic manifestations in 15.8% of patients requires special attention, as the spread of symptoms beyond the orofacial area may suggest a more pronounced degree of sensitisation and a potential risk of developing severe allergic reactions.

The results of the study have significant practical implications for clinical allergology. Component-specific diagnosis, in particular the ALEX2 method, is useful in cases where standard methods, such as skin tests or specific IgE determination, give ambiguous or contradictory results. It allows for the accurate determination of sensitization to specific protein components of allergens, which is important for differentiating between cross-reactivity and true allergy. The use of the BAT is particularly indicated for patients with borderline IgE levels and no clinically confirmed skin reactions, as it helps to assess the functional activity of sensitizing allergens. At the same time, traditional methods such as skin tests and IgE determination remain important tools in diagnosis, especially for the general assessment

of sensitization to the most common allergens, such as fruits.

CONCLUSIONS

The presented study of the clinical and diagnostic characteristics of 182 patients with suspected allergic reactions to fresh fruit found that the most widespread symptoms were itching and swelling of the mucous membranes of the mouth and lips (89.6%), burning in the mouth (76.4%), and itching in the throat (71.4%), which corresponded to the typical clinical picture of oral allergy syndrome. The average age of the patients was 34.6 years, with a predominance of females (59.3%). All patients had a history of seasonal allergic rhinitis, and 68.7% associated the exacerbation of symptoms with birch flowering, indicating a high probability of pollen-food cross-sensitisation. At the same time, the presence of systemic symptoms (in 15.8% with Double-blind placebo-controlled food challenge), skin manifestations (41.2%), and dyspepsia (24.2%) suggested a more heterogeneous nature of reactions that extended beyond the scope of classic oral allergy syndrome.

The combined use of skin tests and serological analyses (specific IgE) helped to identify the allergen-specific sensitisation in most patients, with apples, kiwis, and peaches being the most significant allergens. The concordance between the two methods reached 69.2%, but discrepancies (over 20%) revealed the limitations of each approach. Component-resolved diagnostics refined the sensitisation profile in 85.7% of patients with ambiguous results, identifying clinically significant IgE to both heat-labile and heat-stable proteins. Functional assessment using the basophil activation test showed positive results in 82.4% of subjects, confirming the biological activity of the detected antibodies. Double-blind placebo-controlled food challenge tests finally supported the diagnosis in patients with borderline results from previous diagnostics. The study found that more than half of the reactions were limited to mild symptoms characteristic of oral allergy syndrome, while some patients had systemic manifestations indicating true food allergy.

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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Table 1: Clinical profile of study participants

Indicator	Value
Total number of patients	182
Male/Female	74/108 (40.7%/59.3%)
Average age (years±SD)	34.6±12.1
Itching and swelling of the mucous membrane of the mouth/lips	89.6%
Burning sensation in the mouth	76.4%
Itching in the throat	71.4%
Skin reactions (hives, itching)	41.2%
Gastrointestinal symptoms	24.2%
Seasonal allergic rhinitis in medical history	100%
Worsening of symptoms during birch blossoming	68.7%
Reaction only to raw fruit	52.7%

Table 2: Frequency of positive skin tests and specific IgE to fruits

Allergen (fruit)	Positive skin tests (%)	Positive IgE (%)	Coincidence of results (%)
Apples	63.7	58.2	72.5
Kiwis	44.5	39.0	61.5
Peaches	41.2	36.3	66.2
Cherries	27.5	22.5	58.9
Plums	21.4	19.2	60.1
Overall consistency	-	-	69.2
Divergence (skin+/IgE–)	-	-	11.5
Divergence (IgE+/skin–)	-	-	9.3

Table 3: Results of component diagnostics (ALEX2) and basophil activation test

Diagnostic procedure	Number of patients	Positive results	Negative results	Clinical significance corroborated (%)
ALEX2	56	48 (85.7%)	8 (14.3%)	85.7%
BAT	17	14 (82.4%)	3 (17.6%)	82.4%

Table 4: Results of double-blind food challenge tests with fruits

Indicator	Value
Number of patients	38
Positive response	21 (55.3%)
Negative response	17 (44.7%)
Systemic responses	6 (15.8%)
Anaphylaxis	0 (0%)
Placebo responses	0 (0%)
Most reactive fruits	Apples (42.8%), peaches (28.5%), kiwis (23.8%)

Morphological and chemotypic diversity of a cultivated cinnamon (*Cinnamomum verum* J. Presl) collection at the mid-country research station in Sri Lanka

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ABSTRACT

Though Sri Lankan cinnamon (Cinnamomum verum J. Presl) poses a significant morphological and chemical diversity, challenging standardization, it presents opportunities for breeding tailored to niche market products. This study evaluated 71 cultivated C. verum accessions at the Mid-Country Research Station (MRS), Dalpitiya, Sri Lanka, including elite varieties Sri Gemunu and Sri Wijaya. Nine leaf traits were assessed using TURIS 2013 descriptors. Principal Component Analysis (PCA) and cluster analysis grouped accessions into six morphological clusters. Gas Chromatography-Mass Spectrometry (GC-MS) analysis of leaf essential oils revealed 34 compounds. Eugenol ranging from 92.88-98.09% (accessions 52,64,65 and 70) and accession 41, which was unique for 94.94% benzyl benzoate and no eugenol, suggested the existence of distinct chemotypes. The study supports the idea of divergent biosynthetic pathways. These findings highlight the potential of Sri Lankan C. verum for breeding high-value varieties with tailored phytochemical profiles for use in food, pharmaceuticals, and cosmetics.

Keywords: Benzyl benzoate, *Cinnamomum verum*, cinnamon chemotypes, eugenol, germplasm characterization, leaf morphology.

INTRODUCTION

Cinnamon exports from Sri Lanka increased from 17,860 metric tons in 2018 to 19,195 metric tons in 2021, with earnings rising from 124.95 million USD to 167.07 million USD (Central Bank of Sri Lanka, 2023). Beyond the traditional use as a spice, cinnamon has gained increasing recognition in the cosmetic and pharmaceutical industries. Despite this strong global demand and recognition for quality, Sri Lankan cinnamon is still primarily exported in raw form to countries such as the USA, Mexico, Colombia, Ecuador, Peru, Spain, Guatemala, Chile, and Bolivia, often at relatively low prices. To access premium markets and enhance export value, there is a clear need for strategic value addition and cultivar development.

The genus *Cinnamomum* (family Lauraceae) comprises approximately 250 species and subspecies distributed across Asia and other parts of the world (Mabberly, 2008). There are two cinnamon varieties named, *Sri Gemunu* and *Sri Wijaya* in Sri Lanka, which were introduced by the Department of Export Agriculture. These were superior accessions selected from a germplasm collection of approximately 700 accessions and propagated vegetatively to maintain uniformity and desirable traits.

Essential oils from bark, leaves, roots, fruits, and inflorescences contain key volatile compounds such as cinnamaldehyde, eugenol, camphor, cadinene, and cinnamyl acetate (Paranagama *et al*., 2001; Kaul *et al*., 2003). These compounds have been shown to possess diverse biological activities (Ranasinghe and Galappaththy, 2016; Abeysekera *et al*., 2017; Gulcin *et al*., 2019). As such, chemical composition is not only important for quality assessment but also for functional use in various industries.

Studies have consistently shown that *C. verum* germplasm in Sri Lanka exhibits significant morphological and chemical diversity. Leaf morphology in particular has emerged as a useful trait for distinguishing among genotypes (Azad *et al*., 2016a; 2018; 2019; Geekiyanage *et al*., 2025). A significant correlation between leaf shape and oil yield was reported by Wijesinghe and Gunarathna (2001). Azad *et al* (2024) found morpho-chemo correlations of the constituents in cinnamon bark oil while Prathibhani *et al* (2024) identified two distinct cinnamon chemotypes based on leaf essential oil composition, one eugenol-dominant and the other rich in benzyl benzoate. These chemotypes were also linked to visible morphological traits such as flush colour.

Currently, cinnamon genetic resources are maintained in several major repositories across Sri Lanka. National Cinnamon Research and Training Center hosts approximately 700 cultivated accessions, while a wild cinnamon collection is conserved at the Royal Botanical Garden, Peradeniya. Mid-Country Research Station, Dalpitiya, Sri Lanka (MRS) houses a collection of 71 morphologically diverse cultivated accessions. Given this background, the identification and evaluation of morphologically and chemically diverse accessions are essential for cinnamon breeding programmes aimed at developing elite cultivars with targeted chemical profiles. Morphological characterization provides a cost-effective, early-stage screening tool to guide the selection of chemically valuable accessions, especially in resource-limited breeding programs.

Therefore, this study focuses on evaluating the leaf morphological diversity in a cultivated collection of 71 *C. verum* accessions at the MRS, including the *Sri Gemunu* and *Sri Wijaya* varieties. In parallel, we analyzed the essential oil composition of selected accessions to identify distinct chemotypes with potential applications in breeding, product differentiation and commercialization.

MATERIALS AND METHODS

This study was conducted using a cultivated cinnamon (*Cinnamomum verum*) collection of 71 accessions maintained at the MRS (GPS coordinates: 7.1333031 N, 80.590026 E).

Leaf morphological data collection and chemical profiling were carried out to assess phenotypic and biochemical diversity among these accessions. Nine leaf morphological traits {leaf arrangement (LA), leaf length (LL), leaf width (LW), petiole length (PL) leaf shape (LS), leaf margin (LM) leaf apex (LAP), leaf base (LB) and leaf venation (LV)} were assessed using standardized descriptors (Azad *et al*., 2016b). For each accession, measurements were taken from five fully expanded, mature leaves. Sampling was standardized by selecting leaves located between the 5^{th} and 6^{th} nodes below the apical

shoot tip of a healthy branch, to minimize developmental variability.

Essential oil extraction and Gas Chromatography-Mass Spectrometry (GC-MS) analysis were performed following the same protocols described by Prathibhani *et al* (2024). Briefly, leaf essential oils were extracted using hydro-distillation with a Clevenger-type apparatus. The extracted oils were analyzed using Gas Chromatography-Mass Spectrometry (GC-MS) to determine their chemical composition.

Instrument specifications, operational parameters, and compound identification methods were consistent with those described by Prathibhani *et al* (2024), including the use of the National Institute of Standards and Technology (NIST) library for spectral matching and retention index confirmation where applicable.

Principal Component Analysis (PCA) was carried out to identify the major sources of variation among the accessions based on the morphological parameters. Subsequently Hierarchical Cluster Analysis was performed to group morphologically similar accessions. Both analyses were conducted using the FactoMine R package in R (Le *et al*., 2008).

A dendrogram was generated using Ward's method based on Euclidean distances. Five morphologically diverse accessions representing major clusters were selected for further chemical analysis.

RESULTS AND DISCUSSION

Diversity of Accessions based on leaf morphological variation

Leaf is reported to be the most variable, age and environment-independent morphological character in the genus *Cinnamomum* (Ravindran *et al*., 2004). In the present study, leaf morphological traits of 71 cultivated cinnamon (*C. verum*) accessions maintained at the MRS were characterized. Leaf length (LL) ranged from 10.54 cm (accession 26) to 17.64 cm (accession 60), leaf width (LW) from 4.3 cm (accession 26) to 7.4 cm (accession 22), and petiole length (0.9-1.94 cm). The overall means were 14.3±0.2 cm (LL), 5.8±0.1 (LW) and 1.5±0.03 cm (PL). Commercial varieties *Sri Gemunu* recorded 14.0±1.0 cm (LL), 6.7±0.5 cm (LW) and 1.6±0.1 cm (PL) while *Sri Wijaya* recorded 11.9±0.5 cm (LL), 5.9±0.3 cm (LW) and1.2 ±0.1 cm (PL).

The descriptors developed by Azad *et al* (2016b) classified four types of leaf arrangements (LA): opposite, sub-opposite, opposite to sub-opposite in different branches within the same plant and opposite to sub-opposite in the same branch. Representative leaves from the collection are presented in Figure 1. LA of all accessions in the collection at MRS was either opposite or sub-opposite in different branches, but in the same plant or opposite to sub-opposite in the same branch in the same plant, except for accession 40, which showed a purely opposite arrangement. Frequencies of qualitative variables are summarized in Table 1. Both *Sri Gemunu* and *Sri Wijaya* exhibited LA, described as opposite to sub-opposite in different branches of the same plant. According to Prathibhani *et al* (2024), among 40 accessions at the Faculty of Agriculture, University of Ruhuna, the four LA categories were recorded in a 2:1:1:9 ratio. A national survey by Azad *et al* (2019) showed that the "opposite to sub-opposite in same branch" category was the most common (125 out of 269 accessions).

Azad *et al* (2016a) also described nine types of leaf shape (LS), including elliptic, narrowly elliptic, ovate, oval, lanceolate, ovate-lanceolate, oblong-lanceolate, broadly elliptic and broadly ovate. In the Dalpitiya collection, observed LS types included elliptic, narrowly elliptic, ovate, oval and lanceolate (Table 1). Accession 53 exhibited ovate-lanceolate leaves, and accession 66 showed oblong-lanceolate leaves. The broadly ovate leaf shape was not observed in this collection, but was presented only in *Sri Gemunu*. *Sri Wijaya* displayed ovate leaf-shaped leaves. LS

distribution was reported as elliptic (15/40), broadly elliptic (1/40), narrowly elliptic (12/40), ovate (2/40), broadly ovate (2/40), oval (3/40), lanceolate (4/40), ovate-lanceolate (1/40), and oblong-lanceolate (0/40) (Prathibhani *et al*., 2024). In accessions from their original locations, these proportions were: elliptic (112/269), broadly elliptic (31/269), narrowly elliptic (45/269), ovate (42/269), broadly ovate (4/269), oval (2/269), lanceolate (29/269), ovate-lanceolate (2/269), and oblong-lanceolate (2/269) (Azad *et al*., 2019). The most common LS types in Sri Lankan cinnamon are elliptic and narrowly elliptic.

The leaf apex (LAP) types observed in this study included acute, obtuse, acuminate, long acuminate, narrowly acuminate, and acuminate with broad acumen. Leaf base (LB) types included acute, subacute, cuneate, rounded, subcordate, obtuse, and contracted into the petiole, then shortly cuneate (Table 1). At the Faculty of Agriculture, University of Ruhuna, LAP types were observed in the following proportions: acute (20/40), obtuse (9/40), acuminate (7/40), long acuminate (2/40), narrowly acuminate (1/40), and acuminate with broad acumen (1/40) (Prathibhani *et al*., 2024). Azad *et al* (2019) reported acuminate as the most common LAP in the national collection. LB types in the Ruhuna collection were recorded as acute (6/40), subacute (8/40), rounded (3/40), obtuse (10/40), and obtuse, contracted into the petiole then shortly cuneate (13/40). Azad *et al* (2019) found acute to be the most common LB type. *Sri Gemunu* and *Sri Wijaya* both exhibited acute LAPs, and rounded LBs.

Leaf venation (LV) types included three-veined and three- or five-veined patterns. All accessions at Dalpitiya displayed one of these two types (Figure 1). *Sri Gemunu* showed both three-veined and five-veined venation, while *Sri Wijaya* had exclusively three-veined venation. Leaf margins (LM) were mostly undulate; only Accession 26 and *Sri Gemunu* had entire margins. These findings provide a valuable baseline for understanding leaf morphological variation in the WM2 agro-ecological zone of Sri Lanka.

Principal Component Analysis and Cluster Analysis

Principal Component Analysis (PCA) identified three components (PC1, PC2, and PC3) with eigenvalues above 1, explaining 69.6%, 18.2%, and 12.3% of the total variation, respectively. PC1, primarily influenced by petiole length (PL) and leaf width (LW), accounted for the largest variation, while PC2 contributed to differentiation mainly associated with leaf length (LL) (Figure 2).

Cluster analysis at a distance of 0.5 revealed three clusters: Cluster 1 with low PC1 values, Cluster 3 with high PC1 values, and Cluster 2 in between. The continuous nature of trait variation resulted in overlapping clusters. The PCA-biplot showed the contribution of PL and LW to PC1. There is a clear separation along PC1, reflecting PL and LW differences. Accessions on the right exhibited higher trait values, while those on the left had lower values. PC2 added minor separation, likely due to LL, with closer clusters indicating phenotypic similarity and wider separation suggesting greater diversity (Figure 2).

Variation of leaf oil composition

GC-MS analysis of leaf essential oil samples from five accessions revealed 34 chemical compounds. Accession 70 reported had the highest eugenol content (98%) followed by Accessions 64 (96%), 52 (95%), 65 (93%) and 41 (0%). Conversely, Accession 41 had the highest benzyl benzoate (BB) content (95%), while Accessions 64, 52, 65 and 70 had only 1%, 1%, 0.3%, and 0.2% respectively. Additional constituents included linalool (0.3% in Accession 41; 0.6% in Accession 65), caryophyllene oxide (0.4% in Accession 70), and α-Pinene (ranging from 0.08% to 0.25%) (Table 2).

Azad *et al* (2024) previously documented essential oil composition from bark samples of 30 cinnamon accessions. Identifying major compounds such as cinnamaldehyde (27.14-82.65%), eugenol (1.62-8.84%), cinnamyl acetate (0.70-47.57%), and BB (0.55-13.14%). They reported that cinnamaldehyde and cinnamyl acetate are negatively correlated (-0.81, $p < 0.01$), while eugenol and BB are positively correlated (0.49, $p < 0.01$). Leaf width correlated moderately with eugenol ($r = 0.37$, $p < 0.05$), and bark thickness correlated with caryophyllene ($r = 0.54$, $p < 0.01$).

Prathibhani *et al* (2024) documented eugenol levels from 0-79.47%, caryophyllene from 0.46-3.08%, linalool from 0-5.59%, and BB from 0-91.92% across different accessions. Seventeen accessions were eugenol-dominant, while two green flush accessions had BB levels of 86.8% and 91.9%, and no eugenol. They reported strong negative correlations between eugenol and linalool (-0.630) and between eugenol and BB (-0.886, $p < 0.001$). For the first time, they reported BB-rich accessions in Sri Lanka: KA11, GB17 (both >85% benzyl benzoate, 0% eugenol), and HB12 (16.5% eugenol, 22.3% benzyl benzoate). In the present study, Accession 41 from Dalpitiya is confirmed as a BB chemotype (95% benzyl benzoate, 0% of eugenol), expanding the known pool of such accessions.

Additional BB chemotypes have been reported by several researchers. Nath *et al.* (1996) identified similar accessions in India. Farias *et al* (2020) reported two chemotypes in Brazil. One accession was with 93.6% of eugenol in leaves and 89.3% of cinnamaldehyde in bark; the other was with 95.3% BB in leaves and 23.3% cinnamaldehyde in bark. Xavier *et al* (2022) also confirmed BB-rich genotypes in Brazil. These findings collectively support the mutual exclusivity of eugenol and BB biosynthesis.

Our results indicate that the chemical composition of *Cinnamomum verum* accessions at the MRS reveals substantial biochemical diversity, with clear potential for applications in food, pharmaceutical, and cosmetic industries.

This diversity holds significant value for cinnamon breeding programmes and industrial applications. Eugenol-rich accessions have strong potential in culinary and therapeutic markets, while BB-dominant chemotypes are promising for use in cosmetics, perfumery, and pharmaceuticals.

CONCLUSION

The cultivated cinnamon (*Cinnamomum verum*) germplasm collection at the MRS, Sri Lanka, demonstrated a morphological and chemical diversity. Six distinct clusters were produced based on leaf morphological trait variation of 71 accessions. GC-MS analysis of selected accessions confirmed the presence of two major chemotypes: a eugenol-rich type and a benzyl benzoate-rich type, with clear mutual exclusivity between these constituents.

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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: Variation in qualitative leaf morphological traits of 71 cultivated cinnamon accessions at Mid-Country Research Station, Dalpitiya, Sri Lanka.

Qualitative trait	Pattern	Frequency
Leaf arrangement (LA)	Opposite	2
	Sub-opposite	0
	Opposite or sub-opposite in different branch but in same plant	11
	Opposite to sub-opposite in the same branch in same plant	58
Leaf shape (LS)	Elliptic	20
	Broadly elliptic	0
	Narrowly elliptic	23
	Ovate	7
	Broadly ovate	2
	Oval	13
	Lanceolate	5
	Ovate-lanceolate	1
	Oblong-lanceolate	0
Leaf apex (LAP)	Acute	37
	Obtuse	12
	Acuminate	6
	Long-acuminate	4
	Narrowly acuminate	2
	Acuminate with broad acumen	10
Leaf base (LB)	Acute	13
	Subacute	5
	Cuneate	3
	Rounded	15
	Subcordate	2
	Obtuse	2
	Obtuse, contracted into petiole, then shortly cuneate	31
Leaf venation (LV)	3-veined	41
	5-veined	0
	3-veined or 5-veined	30
Leaf margin (LM)	Entire	1
	Undulate	70

Table 2: Variation in major chemical constituents of five selected *Cinnamomum verum* accessions from the germplasm collection at Mid-Country Research Station compared to the *Sri Gemunu* and *Sri Wijaya* varieties

Chemical constituent	Acc. 41	Acc. 52	Acc. 64	Acc. 65	Acc. 70	*Sri Gemunu**	*Sri Wijaya**
Benzyl benzoate	94.94	0.98	1.12	0.26	0.19	-	-
Eugenol	-	95.21	96.09	92.88	98.09	82.11	90.80
Linalool	0.25	0.30	-	0.55	-	2.36	4.94
Caryophyllene	1.61	0.85	0.53	2.45	0.40	4.68	2.32

*Prathibhani *et al*., 2021

Figure 1. Representative leaves from *Cinnamomum verum* accessions 2, 4, 8, 18, 46, 49 and 55 in the germplasm collection at the Mid-Country Research Station, Dalpitiya, Sri Lanka. Bar indicates 1 cm.

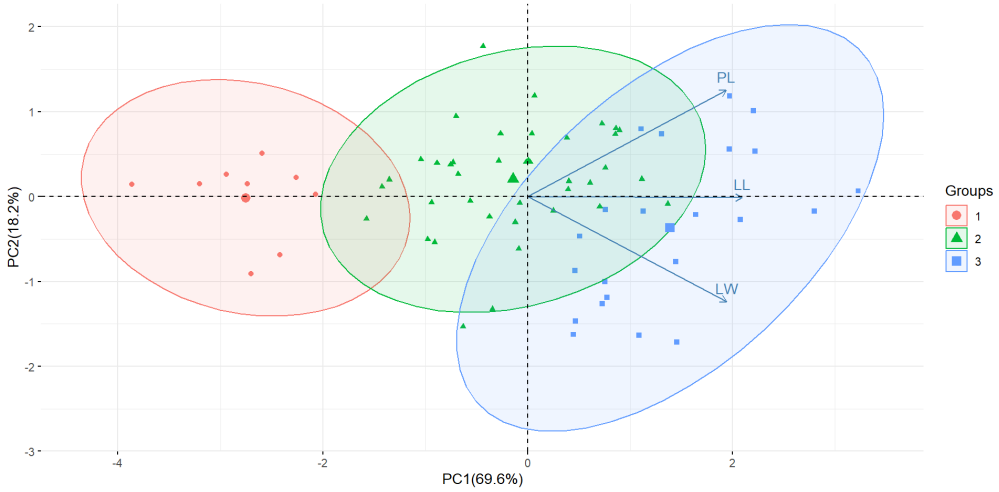

Figure 2. Principal Component Analysis (PCA)- biplot of morphological traits of cinnamon accessions.

Dots (Group 1), triangles (Group 2), and squares (Group 3) represent accessions from clusters 1, 2, and 3, respectively. The vectors PL, LL, and LW indicate the contribution of petiole length, leaf length, and leaf width to the principal components.

Post-harvest dynamics of canker-affected acid lime: Implications for shelf life and market value

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ABSTRACT

Citrus canker, caused by Xanthomonas axonopodis pv. citri (Hasse) Vauterin et al., represents a major threat to acid lime (Citrus aurantifolia (Christm.) Swingle) production, significantly impairing fruit quality and reducing marketability. Throughout the year, monthly average market prices for acid lime were recorded across five quality grades (0 to IV). The percentage decrease in market price for grades I to IV, relative to grade 0, was calculated. Standard procedures were used to estimate cultivation costs, and the Benefit-Cost Ratio (BCR) was calculated to assess profitability. Post-harvest keeping quality is crucial for storage, transportation, and obtaining higher market prices. The presence of visible citrus canker symptoms on the fruit surface led to reduced quality and lower prices, with heavily infected fruits fetching the lowest market values. Fluctuations in market prices over the months appeared to correlate with fruit availability, size, and the extent of canker-related lesions. Fruits not infected by the disease exhibited no signs of rotting after 8 days of storage at room temperature. In contrast, infected fruits demonstrated varying levels of deterioration based on their grade. The potential market price loss could be lowered by 14.26% through effective disease management practices, considering only grade 0 fruits in the BCR calculations. This study underscores the post-harvest challenges posed by citrus canker and highlights the need for effective disease management strategies to maintain fruit market value.

Keywords: Acid lime, citrus canker, keeping quality, post-harvest loss.

INTRODUCTION

Citrus crops are susceptible to various diseases caused by fungi, bacteria, viruses, and phytoplasmas. Among bacterial and phytoplasma-related diseases, the most significant include citrus canker (*Xanthomonas axonopodis* pv. *citri*), citrus greening (*Candidatus liberibacter* spp.), blast or black pit (*Pseudomonas syringae*), citrus variegated chlorosis (*Xylella fastidiosa*), Australian citrus dieback (an unidentified prokaryote), stubborn disease (*Spiroplasma citri*), witches' broom of lime (phytoplasma) and abiotic stresses (Anonymous, 2012a; Anonymous, 2012b; Das *et al.,* 2025). Citrus canker is particularly notorious due to its global significance and destructive nature. The disease affects citrus production severely, with intensity influenced by cultivar susceptibility and climatic conditions. It is prevalent in various parts of Africa, Asia, Australia, South America, and recently in some regions of Florida, USA. In India, citrus canker was initially reported in Punjab (Kalita *et al.*, 1996), with subsequent observations in Tamil Nadu, Andhra Pradesh, Karnataka, Rajasthan, Madhya Pradesh, Assam, Uttar Pradesh (Kalita *et al.*, 1996), and later in West Bengal. Among commercial citrus cultivars, acid lime (*Citrus aurantifolia*) is particularly susceptible (Das *et al.,* 2014), and orchards free from

infection are exceedingly rare. Yield losses of up to 50–60% have been reported globally due to the disease (Das, 2003). The citrus leaf miner (*Phyllocnistis citrella* Stainton) plays a significant role in spreading and intensifying the severity of citrus canker (Das *et al.*, 2012). In West Bengal, citrus canker has emerged as a major issue, inflicting serious damage particularly on lime and lemon crops. However, limited data exist on its impact in the Red and Lateritic Agro-climatic Zone of West Bengal. This study investigates how varying levels of citrus canker infection impact acid lime's shelf life and market price.

MATERIALS AND METHODS

Assessment of loss caused by the disease: The economic loss due to the disease was assessed by conducting market surveys across various locations, including Bolpur, Sriniketan, Illambazar, Nalhati, Bankura Bazaar, and Manbazar-I. The survey focused on the price received by farmers for marketable acid lime fruits across different seasons. In fruits, citrus canker is recognized by small, raised, corky lesions with a distinct yellow halo and a characteristic crater-like appearance. In severe cases, lesions may coalesce, leading to rough, cracked surfaces and blemishes that lower market quality. Infected fruits may also drop prematurely. Fruits were categorized into five grades based on disease incidence: Grade 0 (no incidence), Grade I (1–5%), Grade II (6–30%), Grade III (31–60%), and Grade IV (61–100%) (Das *et al.*, 2012). During 2013–14 and 2014–15, monthly average market prices for each grade were obtained through unstructured interviews with sellers across various markets, and grade-wise market prices of individual fruits were then calculated. The percentage decrease in market price for Grades I to IV was calculated in comparison to Grade 0. Additionally, the cost of cultivation was estimated using standard procedures, and the Benefit-Cost Ratio (BCR) was calculated to determine the actual profitability.

Studies on keeping quality of canker-infected acid lime fruit: An experiment was conducted in August 2014 and August 2015 at the Laboratory of the Department of Plant Pathology, Palli-Siksha Bhavana (Institute of Agriculture), Visva-Bharati, to evaluate the keeping quality of canker-infected acid lime fruits. Fruits from different disease grades (0 to IV) were collected and stored separately at room temperature (25 ± 1°C). Observations were recorded every two days over an eight-day period, focusing on changes in lesion colour, enlargement of lesions with corky texture, onset of rotting, and fruit decay.

RESULTS AND DISCUSSION

Seasonal and grade-wise fluctuation in acid lime market prices

Table 1 presents a two-year average of monthly market prices (₹ per fruit) for acid lime, categorized by fruit grade (0 to IV). The data revealed significant variation in market prices across both grades and seasons, emphasizing how disease severity and seasonal supply-demand dynamics affect the economic value of the produce.

Grade-wise price variation: As expected, the highest average market price was consistently obtained for Grade 0 fruits (₹3.71), which were free of visual canker symptoms. In contrast, heavily infected fruits classified as Grade IV fetched the lowest average price (₹2.33). The per cent decrease in market price over Grade 0 increases sharply with disease severity: from 6.74% in Grade I to 37.20% in Grade IV (Table 1). These findings align with earlier studies showing that citrus canker significantly reduces fruit quality and marketability due to visible blemishes and consumer rejection (Gottwald *et al.*, 2002; Das, 2003). The presence of external symptoms such as lesions and oozing on the fruit surface appears to influence consumer preference and pricing more than internal quality, particularly in fresh produce markets where visual appeal is critical (Behlau *et al.*, 2010). This highlights the economic importance of

managing citrus canker effectively at both pre- and post-harvest stages.

Seasonal price fluctuation*:* Market prices also showed clear seasonal trends, with the highest prices recorded in June (₹4.25) and lowest in November–February (₹2.60) (Table 1). The early summer months (May to July) reflect peak pricing, likely due to increased demand and relatively lower supply of disease-free fruits. In contrast, prices drop during the winter months, possibly due to increased market availability and higher incidence of infected fruits, as disease severity may accumulate post-monsoon. The main fruiting season extends from April to July, with peak harvesting season for mature fruits spans from mid-August to February. Storms during summer promote the spread of citrus canker, which causes visible surface symptoms on fruits. Although the infection does not affect internal fruit quality, visible lesions reduce both market value and consumer appeal, as reflected in the price drop with increased disease severity. These seasonal trends reflect the interplay between supply, disease dynamics, and consumer demand, as also reported by Timmer *et al.* (2000). The market is likely more competitive in off-peak seasons, and fruits showing even minor symptoms are heavily penalized in price. The data show that both fruit grade and season significantly influence acid lime pricing, with citrus canker being a major economic determinant. Effective disease control strategies, along with improved post-harvest grading and storage, are essential to sustain profitability and market competitiveness.

Estimation of cost of cultivation and profitability in citrus Canker-affected acid lime

Table 2 & 3 outlines the economic implications of citrus canker by presenting the estimated cost of cultivation, fruit yield, potential market returns based solely on Grade 0 pricing, and associated profit and loss. This focused analysis isolates the economic impact of disease-induced fruit downgrading.

Cost analysis and yield: The total production cost per 10 acid lime plants per year was ₹9,700.00, including ₹4,300.00 as input costs (e.g., manure, fertilizers, micronutrients, irrigation, herbicides, and plant growth regulators) and ₹5,400.00 as management costs, mostly labour-related activities such as pruning, spraying, and disease monitoring. This investment yielded 7,230 fruits, with the potential for high economic return if all fruits were free from disease symptoms (i.e., Grade 0) (Table 2).

Profit potential based on grade 0 pricing*:* Using the Grade 0 market price of ₹3.71 per fruit (from Table 1), the total potential revenue was ₹26,823.30. Subtracting the production cost of ₹9,700.00, the maximum possible profit would be ₹17,123.30, assuming the entire harvest was of Grade 0 quality (Table 3). However, this is a theoretical maximum. When compared with actual revenue from Table 2 (₹22,997.08), the data reveal a 14.26% loss in potential market value due to the presence of lower-grade (infected) fruits. This loss is directly attributable to citrus canker infection, which affects fruit quality and downgrades market prices significantly (Gottwald *et al.*, 2002; Das, 2003).

Implications for profitability and management*:* If only Grade 0 fruits are considered in BCR analysis, the price loss from lower-grade fruits could potentially be reduced by 14.26%. Benefit-Cost ratio (1.63) was calculated on the basis of profit obtained over the total management cost which indicated that the BCR is quite high (Table 2 & 3). This reinforces the need for effective disease management and post-harvest handling practices, such as sorting, grading, and cold storage, to maintain fruit quality and reduce economic losses (Gottwald, 2000; Schubert *et al.*, 2001). Furthermore, ensuring higher proportions of Grade 0 produce can improve profitability for growers by maximizing returns and reducing the proportion of downgraded, low-value fruit in the market channel. The data clearly demonstrate the dual impact of fruit grade and season on acid lime pricing, with citrus

canker being a major economic determinant. Effective disease control strategies, along with improved post-harvest grading and storage, are essential to sustain profitability and market competitiveness. The Benefit-Cost Ratio of 1.63 highlights that acid lime cultivation remains profitable under managed citrus canker conditions (Table 2). However, the economic returns can be further improved by enhancing fruit quality through better disease control and grading practices. The data emphasize the need for growers to invest in integrated disease management strategies, which not only reduce disease incidence but also enhance economic returns by increasing the proportion of higher-grade fruits. The findings from Table 3 clearly indicate that citrus canker causes a measurable economic loss of 14.26% due to fruit quality degradation. By aligning production strategies to favour high-grade fruit output, farmers can recover a significant portion of this loss, thereby improving the overall profitability of acid lime cultivation.

This analysis confirms that the presence of citrus canker directly translates into economic loss, not just through reduced yield but more importantly through reduced fruit grade and market value. If effective management strategies can improve the proportion of Grade 0 fruits, the overall profit margin could increase substantially, justifying the investment in inputs and labour. These include the use of copper sprays, resistant varieties, orchard sanitation, and integrated nutrient management (Timmer *et al.*, 2000; Schubert *et al.*, 2001).

Post-harvest keeping quality of citrus canker-infected acid lime fruits

Table 4 presents a comparative observation of the post-harvest keeping quality of acid lime fruits infected with citrus canker, categorized by grade and monitored over 8 days of room temperature storage during August 2014 and 2015. The findings provide clear evidence of progressive deterioration in fruit quality correlated with the severity of canker infection.

Grade 0 (Healthy fruits): These fruits showed minimal degradation during the 8-day storage period. Colour changes were gradual—from green to light brown by the 6th day, and a dirty brown surface by the 8th day. Notably, internal tissues remained unaffected, highlighting good post-harvest shelf life.

Grade I (1–5% Infection): Slight yellowing appeared by day 2, progressing to lesion enlargement and water-soaked patches by day 6. By the 8th day, rotting began, affecting both surface and internal tissues, indicating a notable decline in storability even at low infection levels.

Grades II to IV (6–100% Infection): These categories exhibited rapid and severe deterioration. By day 4, visible discoloration and softening set in. By days 6 and 8, severe oozing, cracking, and tissue decay were recorded. Grade IV fruits (with 61–100% infection) showed complete decay of internal and external tissues by day 8, confirming their unsuitability for storage or market.

The keeping quality of acid lime is drastically compromised in the presence of citrus canker. Fruits with higher grades of infection deteriorate faster due to the breakdown of rind tissues and internal colonization by the bacteria, *Xanthomonas axonopodis* pv. *citri*. This correlates with existing research that suggests pathogen-induced physiological degradation accelerates post-harvest spoilage (Das, 2003; Graham *et al.*, 2004). The progression from colour changes to full tissue rotting aligns with the characteristic symptoms of canker—lesion expansion, water-soaking appearance, cracking, and bacterial oozing (Gottwald *et al.*, 2002; Schubert *et al.*, 2001). Once lesions compromise the fruit's epidermis, moisture loss and microbial invasion accelerate the decay process, particularly under ambient (room temperature) conditions.

The study has significant implications for storage, transportation, and marketing – (a). Grade 0 fruits retained acceptable post-

harvest quality and are suitable for longer storage and distant markets. (b). Lower-grade fruits (I–IV) exhibited poor keeping quality, rendering them economically unviable for storage or transport. These should be either processed immediately (e.g., for fresh juice, concentrated juice, pickle, dried lime products, lime oil etc.) or discarded, as their deterioration increases the risk of cross-contamination and post-harvest losses. This aligns with earlier conclusions (Behlau *et al.*, 2010) that emphasize the importance of sorting and grading at harvest, especially under disease stress, to maintain quality and protect market value.

CONCLUSION

Citrus canker causes significant direct and indirect economic losses in acid lime cultivation. This study clearly demonstrates that citrus canker significantly reduces the post-harvest shelf life of acid lime fruits, especially in moderate to heavily infected grades. Maintaining high-grade fruit quality through pre-harvest disease management is critical not only for market pricing but also for preserving post-harvest value. By projecting profitability under ideal Grade 0 conditions, findings of the paper can serve as a benchmark for farmers and policy-makers.

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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Table 1: Fluctuation of market price of acid lime in relation to grade and season

Month	Grade					Month wise avg. market price (₹) per fruit
	0	I	II	III	IV	
	Two years average market price (in ₹ per fruit)					
May	4.50	4.25	4.00	3.50	3.00	3.85
June	5.00	4.50	4.25	4.00	3.50	4.25
July	4.50	4.00	3.50	3.00	2.50	3.50
August	4.00	3.50	3.00	2.50	2.00	3.00
September	3.50	3.00	2.75	2.50	2.00	2.75
October	3.50	3.00	2.50	2.50	2.00	2.70
November	3.00	3.00	2.50	2.50	2.00	2.60
December	3.00	3.00	2.50	2.50	2.00	2.60
January	3.00	3.00	2.50	2.50	2.00	2.60
February	3.00	3.00	2.50	2.50	2.00	2.60
March	3.50	3.50	3.25	3.00	2.50	3.15
April	4.00	3.75	3.50	3.00	2.50	3.35
Grade wise average	3.71	3.46	3.06	2.83	2.33	-
% decrease of market price over grade '0'	-	6.74	17.52	23.72	37.20	-

Table 2: Assessment of Benefit-Cost Ratio (BCR)

Production cost/10 plants			Fruit production (Number) /10 plants						Total market value (₹)/10 plants	Profit (₹)/10 plants	BCR/10 plants
Input cost (₹)*	Other cost (₹)**	Total cost (₹)	Grade wise#					Total			
			Grade 0	Grade I	Grade II	Grade III	Grade IV				
4300.00	5400.00	9700.00	1121	2248	1802	1497	562	7230	22997.08	15767.08	1.63

*Input cost means, Manure ₹ 300.00 + Fertilizers and micronutrients ₹ 2000.00 + PGR ₹ 1000.00 + Irrigation ₹ 600.00 and Herbicides ₹ 400.00 (for 10plants/year), **Other cost i.e. labour cost for different activities at various stages of crop growth, total 30 labours/year/10plants @ ₹ 180/-, #Grade wise average market price for Grade 0-₹ 3.71; Grade I-₹ 3.46; Grade II-₹ 3.06; Grade III-₹ 2.83; Grade IV-₹ 2.33 (Table 1)

Table 3: Estimation of cost of cultivation

Production cost/10 plants		**Total cost (₹)/10 plants**	**Total fruit yield (Number) /10 plants**	**Total Market value (₹) for Grade 0/10 plants**	**Profit (₹)/10 plants**	**% loss over grade 0/10 plants**
Input cost (₹)	**Management cost (₹)**					
4300.00	5400.00	9700.00	7230	26823.30	17123.30	14.26
Average market price for grade 0 – ₹ 3.71/- (Table 1) Total Market value of all grades (0, I, II, III, IV) - ₹22997.08 (Table 2)						

Table 4: Studies the keeping quality of canker infected acid lime at room temperature

Grade	**Scale**	**Days after storing (DAS)**	**Variation of keeping quality**
0	No infestation, fresh lime	2 DAS	No much colour change, become light green
		4 DAS	No much colour change, become light green
		6 DAS	Light brown in colour
		8 DAS	Fruit surface becomes dirty brown, internal tissues were not affected
I	1-5 % infection	2 DAS	Colour become yellowish
		4 DAS	Developed yellow colour
		6 DAS	Size of the lesions increased and become brownish and watery
		8 DAS	Fruit surface becomes deep brown and rotting start including internal tissues
II	6-30% infection	2 DAS	Colour become yellow
		4 DAS	Developed yellowish brown colour
		6 DAS	The fruits become brownish with water soaked
		8 DAS	Huge oozing from deep brown cracky fruit surface and start to rot including internal tissues
III	31-60% infection	2 DAS	Colour become yellowish-orange
		4 DAS	Cankerous lesions become watery and yellowish brown in colour
		6 DAS	Softening of fruit surface and starts to rot
		8 DAS	Decaying of both internal and external tissues of fruits
IV	61-100% infection	2 DAS	Colour become deep yellow
		4 DAS	Lesions become yellow watery and soft
		6 DAS	Huge oozing from cracky surface and rotting start
		8 DAS	Decaying of both internal and external tissues of fruits

Potential of *Rosmarinus officinalis* aqueous extract in managing *Tuta absoluta* Meyrick infestations

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ABSTRACT

Tuta absoluta is the primary pest of tomatoes in its native Latin America. It was first detected in Spain in 2006 and quickly spread to Mediterranean countries, including Algeria by 2008. Medicinal plants, known for their bioactive compounds, offer a natural alternative to chemical pesticides. To promote the use of medicinal plants from the Batna region and combat tomato leaf miner, a laboratory study evaluated the efficacy of aqueous rosemary (Rosmarinus officinalis) leaf extract. Four doses (0.5%, 0.75%, 1%, and 1.5%) were tested against an untreated control and laboratory sucrose (100 ppm). The foliar treatment was applied every 15 days from 6 May to 1 July 2023 in the morning (8:00–10:00 am) using a hand sprayer for uniform coverage. Efficacy was assessed based on infestation levels, mine counts, and overall effectiveness. Mean values with standard errors were calculated for each parameter. Normality was verified using Shapiro-Wilk tests ($\alpha = 0.05$); for non-normal distributions, non-parametric Kruskal-Wallis and Dunn's tests were applied. Results showed the control group had the highest infestation (50.10 ± 5.76%) and mine count (1.72 ± 0.22). The 0.5% and 1.5% rosemary extracts significantly reduced infestation by 35.15% and 37.20%, respectively ($p < 0.05$). The study confirms that rosemary leaf extract has toxic effects on T. absoluta, making it a viable alternative to chemical pesticides, capable of replacing conventional treatments without yield loss.

Keywords: Foliar spraying, infestation rate, Rosemary, SAADA variety, tomato leaf miner.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is a globally significant vegetable crop, with annual production surpassing 189 million tonnes. In Algeria, production grew notably after the 2000 National Plan for Agricultural Development (PNDA), reaching 1.64 million tonnes in 2021. However, regional disparities exist, with Batna province declining to 58,057 quintals in 2020-2021 (DSA, 2024), underscoring cultivation challenges.

The tomato leafminer (*Tuta absoluta* Meyrick) is a highly destructive pest, first identified in South America in 1935 and now globally widespread, causing 80-100% crop

losses. Its invasive success stems from high reproductive rates, multiple annual generations, and effective dispersal (Biondi *et al.,* 2018). Larvae damage all plant growth stages and attack other solanaceous crops like potatoes and eggplants (Mansour *et al.*, 2018; Ponti *et al.,* 2021). Recognized as a quarantine pest by EPPO (2008), its threat to agriculture remains severe.

While effective short-term, traditional chemical methods present significant drawbacks, synthetic pesticides cause environmental pollution, harm non-target organisms, disrupt beneficial microbes, and leave food chain residues (Campos *et al.*, 2021; Kumar *et al.,* 2023). Of particular concern is *T. absoluta* resistance development from overuse (Guedes and Siqueira, 2012), creating a cycle of increasing pesticide dependence with reduced efficacy. These issues have spurred interest in sustainable pest management alternatives.

Botanical pesticides derived from medicinal plants present an effective and eco-friendly pest management solution (Chama *et al.,* 2022). Studies confirm their efficacy against *T. absoluta*, including *Zygophyllum album* extract (Abderrahmene *et al.,* 2019), as well as rosemary essential oils exhibiting significant insecticidal activity (Pavela and Benelli, 2016). These alternatives combat pest resistance while meeting demands for sustainable agriculture.

Rosemary (*Rosmarinus officinalis* L.), a Lamiaceae family member, shows significant biopesticidal potential due to its bioactive compounds (phenolic acids, flavonoids, essential oils) with antioxidant, antimicrobial, and insecticidal properties (Lesnik *et al.,* 2021; Chama *et al.,* 2022). While most research focused on essential oils (Pavela and Benelli, 2016; Campos *et al.*, 2021), aqueous extracts offer practical advantages: lower cost, simpler preparation, and reduced non-target toxicity (Isman, 2020).

Despite their potential, key questions remain about rosemary aqueous extracts' practical use against *T. absoluta*. Optimal concentrations, application frequency, and mode of action require further study (Pavela *et al.,* 2019). Additionally, rosemary's phytochemical variability due to environmental factors, a phenomenon observed in other medicinal plants used for green pesticides (Benelli *et al.,* 2019). This variability demands region-specific research for consistent efficacy. Addressing these gaps could provide farmers with effective, accessible alternatives to synthetic pesticides.

The objective of this study is to evaluate the insecticidal efficacy of aqueous rosemary extracts at four concentrations (0.5%, 0.75%, 1%, and 1.5%) against *T. absoluta* larvae under controlled conditions. The research will assess the impact of foliar application on both pest mortality and tomato plant health, while comparing the performance of the extract to that of a laboratory sucrose-based biopesticide (100 ppm) and untreated controls.

MATERIALS AND METHODS

The rosemary aerial parts (leaves and stems) were collected during the pre-flowering phase (14 October 2022), in the morning between 10.00 and 11.00, in Batna Province, a semi-arid high-altitude region (>1000 m) with calcareous soils that enhance secondary metabolite production (Batna Forestry Department, 2023). These environmental conditions yield rosemary with significant phytochemical potential, ensuring authentic plant material aligned with regional ethnopharmacological practices.

The widespread adoption of pre-flowering harvesting (González-Minero *et al.*,2020) in medicinal and aromatic plant production ensures dual benefits: (i) optimization of bioactive compound (rosmarinic acid, carnosic acid, and essential oils) profiles to meet pharmacopoeial quality thresholds, and (ii) stabilization of secondary metabolite accumulation (e.g., tanshinones in *Salvia miltiorrhiza*; rosmarinic acid in *Rosmarinus officinalis*). Autumn harvesting in Batna's Mediterranean climate maintained moderate temperatures and solar exposure,

preserving volatile compounds and antioxidant capacity (Chetia *et al.,* 2025).

The study employed tomato seedlings (Saada F1 hybrid) grown from seeds in potting soil. After germination, seedlings with two true leaves were transplanted into pots and maintained under laboratory conditions. Natural infestation by tomato leaf miner occurred spontaneously, confirmed on 19 March 2023 through observation of characteristic translucent leaf spots.

Fresh rosemary leaves were shade-dried to preserve heat-sensitive compounds and ground into a fine powder to maximize extraction efficiency. Different concentrations were prepared by suspending specific weights of powder in 100 ml of distilled water, followed by 2.5 hours of agitation (with covered beakers to prevent evaporation). After 24 hours of settling, supernatants underwent triple filtration to remove residual material. Final extracts were stored in airtight, foil-wrapped amber bottles at 4°C for up to 72 hours to prevent microbial growth and degradation of active compounds (Sharma *et al.,* 2020; Lesnik *et al*., 2021). The laboratory sucrose solution (1.6 g/L) was freshly prepared before each application to avoid contamination risks (Brahim and Lombarkia., 2020). Sucrose is approved in France for use against *Cydia pomonella* (codling moth) and *Ostrinia nubilalis* (European corn borer) under biocontrol regulations (ANSES/EC 1107/2009). Studies by Brahim and Lombarkia (2020) demonstrate that sucrose enhances plant resistance by disrupting oviposition behavior, likely through alterations to leaf surface metabolites that deter egg-laying. Notably, higher doses do not improve efficacy; optimal results are achieved at low concentrations (e.g., 0.01% for *C. pomonella*), as excessive sucrose may lose its deterrent effect or even attract pests

Foliar sprays were applied from 6 May to 3 July 2023 at 15-day intervals (five total applications). Treatments were conducted between 8:00 and 10:00 am to coincide with peak stomatal opening and minimal evaporation, optimizing absorption (Sharma *et al.,* 2020). Applications were made using calibrated sprayers maintaining a 15-20 cm distance from plants, with volumes adjusted according to growth stage (50 ml-100 ml). Treated pots were physically isolated to prevent cross-contamination. This protocol specifically targeted vulnerable *Tuta absoluta* developmental stages while minimizing potential phytotoxic effects.

A completely randomised one-factor design was employed to evaluate the insecticidal effects of aqueous rosemary extracts at four concentrations (0.5%, 0.75%, 1%, and 1.5%) and a sucrose solution (100 ppm) against *Tuta absoluta*, with an untreated control. The experiment comprised six treatments, each of which was replicated three times across 18 experimental units that were arranged in a standardised six-column × three-row grid. The sucrose treatment was used as a biopesticide reference rather than as a control, while the untreated group provided baseline mortality data.

The study evaluated treatment effects on *Tuta absoluta* infestation in tomato plants through systematic weekly sampling. Three quantitative parameters were measured: (1) infestation levels, determined by monitoring larval presence throughout the experimental period; (2) average mines per leaf, calculated as the ratio of total mines to total leaves examined; and (3) treatment efficacy, assessed using Abbott's formula. All measurements were conducted through careful visual inspection of experimental plants to ensure accurate data collection.

$$\text{Efficiency} = 100 \times ((T0 - Tt)/T0)$$

T0: % total of leaflets affected in the control treatment;
Tt: % total of leaflets affected in the treated treatment.

All data analyses were performed using Statistica 8. For each measured parameter, including overall infestation rates, leaf-level infestation patterns, and larval population dynamics, mean values were calculated with corresponding standard errors. Prior to

conducting comparative analyses, the assumptions of parametric testing were verified through the implementation of Shapiro-Wilk normality tests ($\alpha = 0.05$) on all datasets. In the case of significant deviations from normality. Non-parametric tests Kruskal-Wallis and Dunn's test was used to compare between groups. This conservative approach ensured robust detection of statistically meaningful differences while maintaining an overall type I error rate of 5%.

RESULTS AND DISCUSSION

Effect of modalities concentration on *Tuta absoluta* infestation rate in tomato leaves

The findings indicated that the most effective method of reducing the infestation rate was attributed to rosemary extract at a concentration of 1.5%, with a result of 37.25 ± 4.89, in comparison to the rate obtained by the control group (50.10 ± 5.76). The infestation rates resulting from the treatment with 0.75% sucrose and sucrose are 44.10 ± 5.92 and 45.32 ± 6.63, respectively. A statistical analysis of *Tuta absoluta* infestation data was conducted, which yielded several significant findings. The Kruskal-Wallis test revealed significant differences among the groups ($H = 15.72$, $p = 0.01$). This finding indicates that rosemary extract exerts a globally significant effect on potato infestation. The Dunn's Post-Hoc Test demonstrated that the doses (0.5%, 1%, and 1.5%) exhibited significantly lower values in comparison to the Control group. Conversely, the doses 0.75% and sucrose 100 ppm versus the Control group did not demonstrate a significant outcome (Fig. 1).

These results align with the growing resistance of *Tuta absoluta* to conventional pesticides, necessitating sustainable alternatives. The dose-dependent efficacy of rosemary extract (RE) supports findings by Benelli *et al.* (2019) on neurotoxic and antifeedant mechanisms of plant-derived compounds. The lack of efficacy from sucrose reinforces that RE's pesticidal properties are mediated by specific bioactive compounds (Pavela and Benelli, 2016).

Efficacy of treatments of rosemary extracts and sucrose

The findings indicated that the most efficacious agent in significantly reducing the infestation rate was rosemary extract at a concentration of 1.5% (37.20 ± 4.14), in comparison to the control group (0.00 ± 0.00). This outcome was in contrast to the lower concentrations of 0.75% and sucrose 100 ppm, which exhibited (16.95 ± 6.43) and (14.44 ± 3.97), respectively, in relation to the control group. A statistical analysis of *Tuta absoluta* infestation data was conducted, which yielded several significant findings. The Kruskal-Wallis test revealed significant differences among the groups ($X2 = 27.43$, $p \leq 0.001$). This finding indicates that rosemary extract has a globally significant efficacy on potato infestation. The Dunn's post-hoc test demonstrated that the doses (0.5%, 1%, and 1.5%) exhibited significantly elevated efficacy values in comparison to the control group, with p-values of ≤ 0.001, ≤ 0.05, and ≤ 0.001, respectively. Conversely, the efficacy values of the dose (0.75%) and sucrose 100 ppm, in comparison to the control group did not reach statistical significance (Fig. 2).

The 25.7% reduction in infestation with 1.5% RE (vs. control) supports the threshold effect hypothesis for botanical insecticides (Isman, 2020). Divergent results at 0.75% may reflect temporal larval mortality or spray coverage variability (Campos *et al.,* 2021). These findings highlight RE's potential for integrated pest management, particularly in organic systems (Biondi *et al.,* 2018).

Dose effects of rosemary extracts and sucrose on tomato leaf mines

The data presented in Figure 3 shows the variability in the average number of leaf mines per leaf according to the treatment modalities. This study revealed a significant decrease in the average number of leaf mines in the group sprayed with 1.5% rosemary

extract, with the lowest value recorded as (1.0 ± 0.1), compared to the control group, which had an average of 1.72 ± 0.22. Conversely, the 100 ppm dose showed a non-significant increase in value to (1.39 ± 0.14) compared to the control. In fact, Kruskal-Wallis Test (H-statistic = 28.6, $p < 0.001$) revealed significant differences in average number of leaf mines among potatoes groups. Dunn's Post-Hoc Test indicated that the doses (0.5%, 0.75%, 1%, and 1.5%) had significantly reduced the average number of mines per leaf, contrary to sucrose that had no significant effect on the number of mines per leaf.

The reduction in leaf mines aligns with documented antifeedant mechanisms of botanical extracts (Benelli *et al.*, 2019). Sucrose's lack of effect suggests RE's activity is mediated by specific bioactive compounds (Pavela and Benelli, 2016). These results could guide concentration optimization under field conditions.

Abbott treatment efficacy

Analysis of the results showed the occurrence of wide differences in efficacy between the compared treatments against *Tuta absoluta*. The aqueous extracts of rosemary registered great performances as far as efficacy was concerned. Indeed, Abbott's formula efficacy application resulted in highlighting following evidences: the most efficacy concentration was 1.5% with 26.4% (Table 1).

These findings reinforce botanical extracts as viable alternatives to synthetic pesticides (Isman, 2020). However, study limitations (controlled conditions, unassessed long-term effects) warrant further field validation and mechanistic research (Kumar *et al*., 2023).

CONCLUSION

The present study demonstrates the efficacy of rosemary extract as a natural pesticide against *Tuta absoluta* in tomato crops. It was demonstrated that higher concentrations were more efficacious in terms of pest control, thus confirming a clear dose-response relationship. The findings provide a robust rationale for the incorporation of rosemary extract into sustainable farming practices, serving as a viable alternative to synthetic pesticides. It is recommended that future research endeavours concentrate on the execution of field testing and the identification of active compounds. This work contributes to the development of eco-friendly solutions for the management of this destructive pest.

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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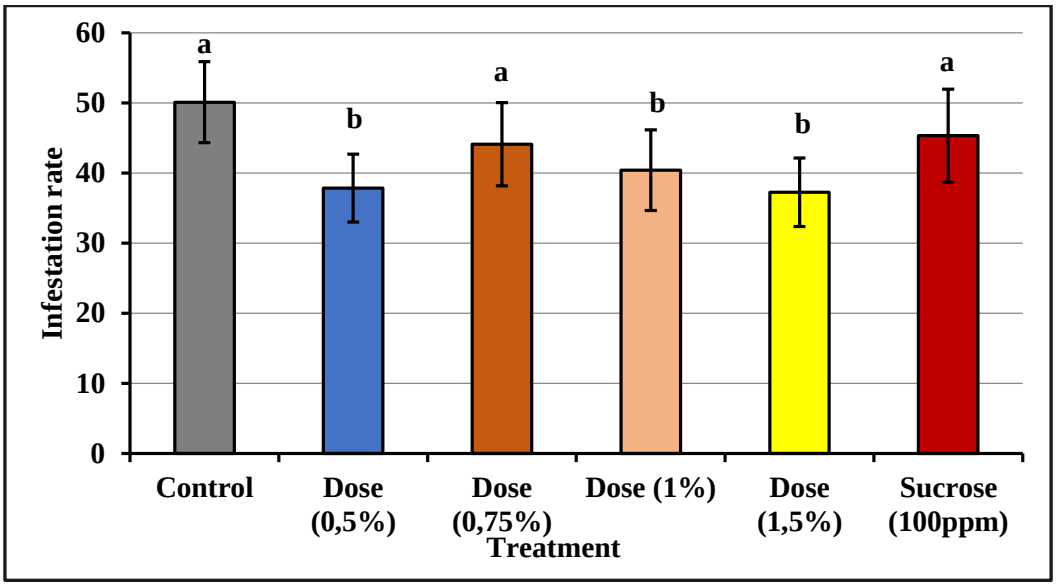

Fig.1. Variation of *Tuta absoluta* infestation rate on tomato leaves, depending different concentrations of rosemary extracts and sucrose. *Values are means ± SD, (n= 10); Dunn's Post-Hoc comparisons test was used to compare different modalities of treatments with control; letters (a, b) : design homogeneous groups.*

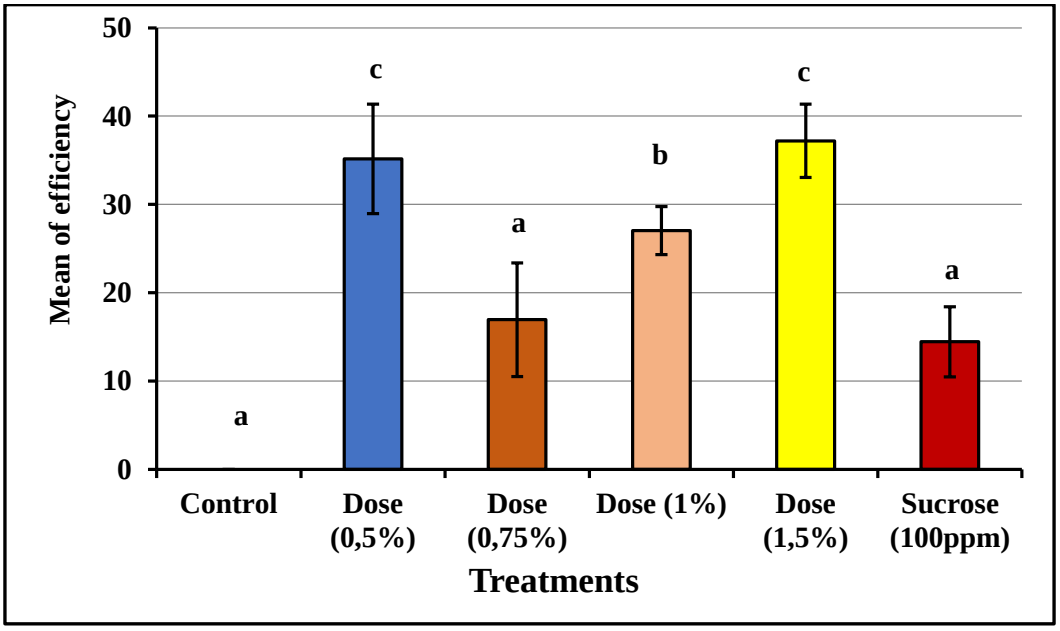

Fig.2. Variation of different treatments efficacy of rosemary extracts and sucrose against *Tuta absoluta* infestation rate in tomato leaves. *Values are means ± SD, (n= 10); Dunn's Post-Hoc used to compare different modalities of treatments efficacy with control; letters (a,b,c) design homogeneous groups.*

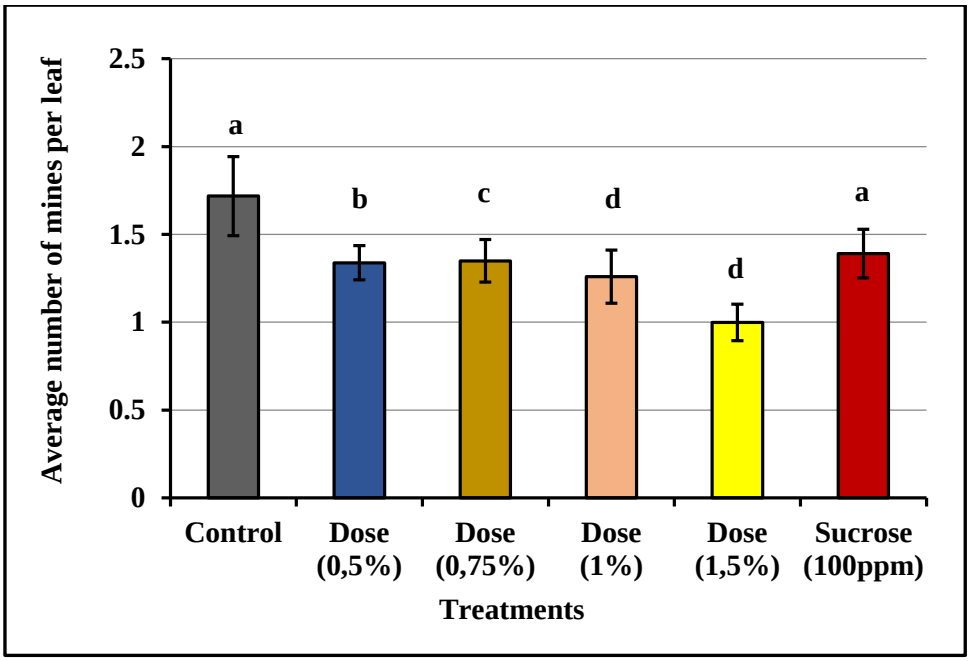

Fig.3. Variation of different treatments of rosemary extracts and sucrose on the average number of *Tuta absoluta* leafs. Values are means ± SD, (n= 10); *Dunn's Post-Hoc* used to compare different modalities of treatments on average number of leaf mines with control; letters (a, b, c, d) design homogeneous groups.

Table 1. Calculated treatment efficacy rates using Abbott's formula:

Treatment		Average Efficacy
Rosemary aqueous extract	0.5% Dose	24.5%
	0.75% Dose	12.5%
	1% Dose	20.9%
	1.5% Dose	26.4%
Sucrose (100ppm)		11.3%

Efficacy of kaolin and salicylic acid in reducing heat stress damage and enhancing yield of dragon fruit

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ABSTRACT

Dragon fruit holds high economic value due to its rich nutrition and growing export demand throughout the world. However, sunburn caused by high temperatures and intense solar radiations is a major constraint, leading to yellowing, bleaching, rotting and drying of cladodes, poor growth, higher disease susceptibility, and yield loss. To mitigate this, foliar sprays of kaolin (4%, 6%, 8%), salicylic acid (SA, 300 ppm), and their combinations were evaluated, with water spray as control. Among treatments, 8% kaolin was most effective, reducing sunburn incidence and severity in April (65.63% SI, 22.08% SS) and May (57.29% SI, 23.75% SS), and lowering disease incidence (32.42%) and severity (13.96%). It also improved physiological traits including new shoot emergence (14.00), chlorophyll (3.25 mg/g FW), and NDVI, as well as reproductive traits such as buds (92.00), flowers (76.00), and fruits (48.33). The 4% kaolin + SA treatment enhanced fruit set (91.23%). Highest yield (15.77 kg/plant; 15.01 t/ha) was recorded with 8% kaolin, with SA also contributing positively. Kaolin sprays enhanced phenols and flavonoids, while 6% kaolin improved vitamin C (45.68 mg/100 g). Overall, 8% kaolin effectively mitigated heat stress by reducing sunburn and disease, while SA, though less effective against disease, significantly boosted yield alone or with kaolin.

Keywords: Kaolin, salicylic acid, sunburn management, yield.

INTRODUCTION

Dragon fruit (*Selenicereus undatus*) is a perennial climbing cactus of the Cactaceae family. Its earlier genus name *Hylocereus* is derived from the Greek word *hulos* meaning "forest," reflecting its natural habitat and drought tolerance (Le Bellec *et al*., 2006). Dragon fruit is a nutrient-rich super-fruit, consumed fresh and processed into juice, jam, and wine. Its pulp and pectin-rich peel are also useful for food products and natural colorants. The fruit offers health benefits due to antioxidants, vitamins (C and B3), phenolics, flavonoids, fibres, and minerals such as phosphorus and calcium, while being relatively low in sugar (Doke *et al.,* 2024; Patil *et al*., 2024). Its cultivation is profitable in regions with 500–1500 mm rainfall and temperatures of 20–30 °C (Kokani *et al*., 2024; Kakade *et al*., 2025). However, with expansion into hot-arid and humid zones, heat stress has emerged as a major challenge

(Jinger *et al*., 2024; Patil *et al.,* 2024). Heat stress occurs when temperatures exceed the tolerance threshold for extended periods, causing irreversible damage (Sharma *et al*., 2018). It disrupts chlorophyll synthesis, photosynthesis, and photosystem II activity, resulting in poor vigour, reduced flowering, lower yield, and weaker fruit quality. Plants also become more prone to sunburn, fruit drop, pests, and diseases (Patil *et al*., 2024). Being a CAM plant, dragon fruit closes stomata during the day to conserve water, limiting cooling through transpiration and increasing tissue heat load. Succulent stems are particularly vulnerable, showing chlorosis, bleaching, or even rot and then dry under stress (Patil *et al.,* 2024). High light intensity combined with day–night temperature fluctuations aggravates heat stress, often resulting in sunburn (Chang *et al.,* 2016). Yield losses due to sunburn can range from 6% to 30% (Lal and Sahu, 2017). In India, 80–95% of growers reported sunburn, especially in Rajasthan, Gujarat, and Maharashtra, when temperatures exceed 38 °C in March–April (Kakade *et al*., 2025). Farmers are using shade nets, anti-transpirants, and intercropping to reduce damage.

Management strategies include kaolin sprays, protective fruit bagging, and shade nets. Kaolin forms a reflective white film that reduces sunlight exposure, lowering fruit surface temperature by 5–10 °C and protecting against heat, UV, and insects (Lal and Sahu, 2017). It is widely applied in fruit crops, it improves yield and quality. Salicylic acid (SA) is a natural phenolic growth regulator involved in signalling and defence. It regulates growth, photosynthesis, and water relations and enhances resistance by activating defence genes and systemic acquired resistance (Kumar *et al.,* 2023). This study was conducted to evaluate kaolin and salicylic acid sprays at different concentrations for reducing heat stress and sunburn in dragon fruit. Their effects on physiological and biochemical responses were assessed to develop cost-effective and practical management strategies supporting plant growth, yield, and resilience under stress.

MATERIALS AND METHODS

The study was conducted in 2024 at Plot I4, North Block orchard, ICAR-NIASM, Baramati, India (18.167°N, 74.498°E; 570 m). The site lies in the Deccan Plateau (AZ-95, AER-6) with a hot semi-arid climate and ~56 cm annual rainfall, mostly from the southwest monsoon. Mean monthly temperatures range 21.8–22.7 °C, peaking in May (38.9/22.3 °C). During the experiment (Mar–May), maximum temperatures were 36.1–38.9 °C with 7.8–8.7 sunshine hours/day, 20–72% RH, negligible rainfall, and winds of 5.7–8.5 km/h. The shallow soil (0.1–0.3 m) was rocky, alkaline (pH 7.86–7.98), low in organic matter (0.82–0.83%), and contained 199–202, 12–17, and 428–495 kg/ha available N, P, and K, respectively. The trial was conducted on 11-year-old white-fleshed dragon fruit spaced at 3.5 × 3.0 m (952 poles/ha, four plants/pole). Eight foliar treatments were imposed: kaolin (4, 6, 8%), salicylic acid (SA; 300 ppm), kaolin + SA combinations, and a water control. Kaolin (400–800 g) was mixed in 10 L water, and SA was dissolved in ethanol before dilution. Sprays were applied at 25-day intervals, starting in second week of March (19th March) and last spray taken in first week of May (2nd May). So, 3 sprays were taken during March-May.

Plant growth and physiological parameters

New shoots were counted manually after treatment. The moisture content of cladodes (%) was determined by drying them in an oven at 65°C for 3 to 4 days until a stable weight was reached. Chlorophyll content was estimated using N, N-dimethyl formamide as the solvent, based on the method described by Inskeep and Bloom (1985). Absorbance readings were taken at wavelengths of 647 nm and 664 nm using a UV-VIS spectrophotometer (Lab India, Hyderabad, India). The Normalized

Difference Vegetation Index (NDVI) was recorded during the summer season using a Green Seeker handheld crop sensor (Trimble, Ukraine). Light intensity was measured under the canopy centre at a depth of 30 cm using a digital lux meter (HTC LX-102A) between 11:00 a.m. and 1:00 p.m. Membrane stability index (MSI) of cladodes was estimated according to Sairam, (1994) and canopy temperature (CT) was assessed using an IR gun during summer. Sunburn incidence (SI) in the field was assessed by counting chlorotic (yellowing) cladodes, following the method of Chang *et al.* (2016). Sunburn and disease symptoms were predominantly observed during periods of elevated temperatures, which caused visible damage to the cladodes. For consistent evaluation, eight cladodes were selected from each direction of the plant north, south, east and west. Healthy green cladodes turned yellow when affected by sunburn or heat stress. Disease incidence was calculated by counting number cladodes showing disease symptoms (mainly stem canker was observed) and expressed in percentage. Disease severity (DS) and sunburn severity (SS) were visually rated using a grading scale with slight modifications from the method described by Hieu et *al.* (2018). The formula was used to calculate SI, DI and SS, DS are as follows;

$$\text{SI (Sunburn incidence)} = \frac{\text{Total number of cladodes infected1}}{\text{Total number of cladodes}} \times 100$$

$$\text{DI (Disease incidence)} = \frac{\text{Total number of cladodes infected}}{\text{Total number of cladodes}} \times 100$$

$$\text{SS (Sunburn severity)} = \frac{\text{Sum of total score}}{\text{No. of observations} \times \text{maximum score}} \times 100$$

$$\text{DS (Disease severity)} = \frac{\text{Sum of total score}}{\text{No. of observations} \times \text{maximum score}} \times 100$$

Flowering and Fruit yield parameters

The number of flower buds and fruits was manually recorded on each plant during every flush from May to December and reported as cumulative counts per pole. Flower bud drop (%) was determined by comparing the number of dropped buds to the total number of flowers produced on the plant. Fruit set was calculated by counting the number of fruits to the total number of flowers. Fruit yield was estimated by summing fruit weight from all flushes and cumulative yield expressed in kg per plant and tons per hectare.

Physical and biochemical fruit quality parameters

Six fruits were randomly selected from each treatment and weighed using an electronic balance to determine the average weight of the whole fruit, pulp and peel with results expressed in grams. Fruit length and width and also peel thickness measured using vernier calliper and expressed in mm. Fruit firmness was evaluated using a texture analyzer (XT Plus, Stable Micro Systems, UK) by measuring both penetration and cutting force at a speed of 0.5 mm/sec, with the results expressed in grams. Total soluble solids (TSS) were determined using a handheld refractometer following AOAC (2005) guidelines. Titratable acidity was estimated by titrating 1 g of fruit sample with 0.1 N NaOH using phenolphthalein as an indicator and the results were expressed as a percentage of citric acid. The pH of the fruit was measured using a pH meter. A 10 mL sample was taken and distilled water was added. The pH meter was then used to record the reading. The ratio was determined by dividing the TSS by the fruits titratable acidity content. Total phenolic content was measured using the Folin-Ciocalteu method as outlined by Singleton *et al.* (1999). For this, 20 g of pulp was homogenized in 80% methanol and centrifuged at 7500 rpm for 15 minutes at 4°C. From the supernatant, 0.5 ml extract was mixed with 0.2 ml Folin-Ciocalteu reagent, 3.3 ml distilled water and 1 ml of 20% sodium carbonate. After 30 minutes of incubation in darkness, absorbance was read at 765 nm using a UV-VIS spectrophotometer and results were expressed in gallic acid equivalents. Total flavonoid content was analysed following the

method of Zhishen *et al.* (1999). The sample was crushed in 80% methanol centrifuged at 7500 rpm for 15 minutes at 4°C and 0.5 ml of the extract was mixed with 0.4 ml distilled water and 0.3 ml of 5% sodium nitrite. After a 5-minute reaction 0.3 ml of 10% aluminum chloride was added and allowed to react for another 5 minutes. Then 3.4 ml of 4N sodium hydroxide was added and the mixture was incubated at room temperature for 30 minutes. Absorbance was recorded at 415 nm and expressed in mg catechin equivalent. Total sugars were estimated using the method described by Nielsen (2010). Ascorbic acid content was measured by titrating a known amount of sample with 2,6- dichlorophenol indophenol dye using metaphosphoric acid as a stabilizer (Ranganna, 1986). Antioxidant activity through DPPH radical scavenging was evaluated as per Brand-Williams *et al.* (1995). Samples were extracted in 80% methanol centrifuged at 12000 rpm for 8 minutes and 0.5 ml of extract was combined with 3 ml of 0.001 M DPPH solution. The mixture was kept in the dark for 45 minutes at room temperature and absorbance was read at 517 nm. Results were expressed as mg Trolox equivalent per 100 g sample. Ferric Reducing Antioxidant Power (FRAP) was estimated following Benzie and Strain (1999). The FRAP reagent was prepared using 1 mM 2,4,6-TPTZ and 20 mM ferric chloride in 0.25 M sodium acetate buffer. After extraction in 80% methanol and centrifugation, 0.2 ml of supernatant was mixed with 1.8 ml of FRAP reagent and incubated for 40 minutes at room temperature. Absorbance was taken at 593 nm, and results were expressed as mg Trolox equivalent per 100 g of sample.

The experiment was conducted using a randomized block design (RBD) with 8 treatments and 3 replications. Statistical analysis was performed through ANOVA to obtain F-values followed by a least significant difference (LSD) test using R Studio (Versions 4.1.1 and 1.4.1417) for Windows. A significance level of $p < 0.05$ was used.

RESULTS AND DISCUSSION

Plant growth and physiological parameters

Significant variation in new shoot formation was observed among treatments, with the maximum (14.00) in T3 (8% kaolin) and the minimum (4.67) in T8 (control) (Table 2). Cladode moisture content ranged from 81.47% (T2, 6% kaolin) to 86.23% (T1, 4% kaolin), though differences were non-significant, reflecting the CAM physiology and succulent nature of dragon fruit that favour water conservation. Treatments significantly influenced chlorophyll content, with T3 recording the highest chlorophyll a (1.72 mg/100 g), chlorophyll b (1.53 mg/100 g), and total chlorophyll (3.25 mg/100 g), while the lowest values were in T8. The chlorophyll a/b ratio ranged from 1.07 (T6) to 1.48 (T5). Enhanced chlorophyll under kaolin and salicylic acid treatments was attributed to reduced heat stress and prevention of pigment degradation, consistent with earlier reports (Doke *et al*., 2024; Patil *et al*., 2024). NDVI values also varied significantly: in April, the highest (0.54) was in T1 and T3, while the lowest (0.42) occurred in T8; in May, T1 and T6 had the highest (0.58) and T7 the lowest (0.47). Higher NDVI under kaolin treatments indicated improved canopy health and stress tolerance.

Sunburn incidence did not differ significantly in April (Table 3), although the control (80.21%) recorded the highest values and 8% kaolin the lowest (65.63%). By May, differences were significant, with 8% kaolin (57.29%) showing the least incidence, comparable to 4% (61.46%) and 6% (63.54%) kaolin, while the control remained highest (88.54%). Kaolin was more effective than salicylic acid combinations, likely due to its reflective properties, consistent with reports in mandarin, pomegranate, and mango (Ennab *et al*., 2017; Hamdy *et al.,* 2022). Disease incidence was also

significantly reduced by treatments, from 60.42% in the control to 35.42% with 8% kaolin, statistically similar to 6% kaolin (43.75%), 4% kaolin (46.88%), and salicylic acid (47.92%). Similar effects of kaolin against pathogens were reported in apple (Sharma *et al*., 2020). As shown in Table 4, MSI varied significantly among treatments, with the highest in 8% kaolin (59.23%), at par with 6% kaolin + SA (56.58%), and the lowest in the control (22.41%). Canopy temperature also differed, with maximum values being non-significant (51.03 °C in T1 vs. 48.67 °C in T3), while minimum temperatures were significantly lower in 8% kaolin (17.37 °C) compared to the control (25.73 °C).

Flowering and yield parameters

Significant differences were observed in flowering and yield parameters (Table 5). The highest flower bud initiation (92.00%) and flower number (76.00) occurred in 8% kaolin, compared to the lowest in the control (47.33% and 36.00, respectively). Kaolin reduced canopy temperature and sunburn stress, thereby enhancing photosynthesis and bud initiation, consistent with Patil *et al.* (2024) and Khayyat and Samadzadeh (2023). Flower bud drop was lowest with 8% kaolin (17.23%) and highest with 6% kaolin + SA (28.61%), reflecting the stress-mitigating role of kaolin (Al-Saif *et al*., 2022). Fruit number was maximum in 8% kaolin (48.33) and lowest in SA (24.66) and control (32.66), while fruit set was highest in 4% kaolin (91.23%) and control (90.46%) but lowest in 8% kaolin + SA (60.87%). Differences were attributed to kaolin's role in alleviating heat stress and reduced competition in the control (Al-Saif *et al.,* 2022). Fruit yield also varied significantly, with 8% kaolin producing the highest values (15.77 kg/plant; 15.01 t/ha) and the control the lowest (7.12 kg/plant; 6.78 t/ha). The improvement was linked to enhanced photosynthesis and stress mitigation, in agreement with Khayyat and Samadzadeh (2023) and Nazari *et al.* (2021).

Fruit physical and biochemical parameters

Fruit weight differed significantly, with the maximum in 6% kaolin (433.00 g) and the minimum in control (214.87 g) (Table 6). Kaolin reduced heat stress and improved fruit growth, while salicylic acid (SA) further enhanced tolerance, corroborating earlier findings (Ennab *et al.,* 2017; Ogiela, 2020). Fruit length and width were highest in 6% kaolin (108.17 mm, 88.68 mm) and lowest in control (77.37 mm, 66.09 mm). Similarly, fruit volume peaked in 6% kaolin (419.00 cm^3) and was lowest in control (144.67 cm^3), reflecting improved microclimate, photosynthesis, and assimilate supply.

Pulp weight was highest in 4% kaolin (228.07 g) and lowest in control (110.80 g), while peel weight peaked in 6% kaolin (220.60 g). Pulp and peel percentages were non-significant, though peel thickness varied, with the maximum in 6% kaolin + SA (7.50 mm). Fruit firmness also showed no significant variation (Table 6).

Antioxidant activity (DPPH) was highest in 6% kaolin (83.25), with similar effects under 8% kaolin and SA treatments (Table 7). FRAP values were non-significant. Phenols (50.66 mg/ml) and flavonoids (23.18 mg/g) were highest in 6% and 8% kaolin, respectively, while ascorbic acid peaked in 6% kaolin (45.68). These improvements reflect kaolin's role in stress alleviation and antioxidant induction (Hamdy *et al.,* 2022). Sugar content varied significantly, with the maximum in 8% kaolin + SA (10.87%), while TSS was highest in 8% kaolin (11.23 ºBrix) (Table 7). Titratable acidity ranged from 0.32% (8% kaolin) to 0.57% (6% kaolin + SA). The TSS: acidity ratio was highest in 8% kaolin (36.07), enhancing sweetness, whereas the lowest was in 6% kaolin + SA (13.90). Fruit pulp pH ranged from 2.81 (6% kaolin) to 3.27 (8% kaolin + SA), indicating improved fruit quality and shelf life (Table 7).

CONCLUSION

Sunburn damage in dragon fruit cladodes was observed when average monthly temperatures reached ~38 °C with high solar radiation, low relative humidity (20–25%), extended sunshine (8.7 h/day), and moderate winds (10–15 km/h) during March–May. Application of 8% kaolin effectively reduced sunburn and disease incidence while significantly improving reproductive and yield traits such as bud, flower, and fruit numbers, fruit set, and yield per plant and per hectare. Kaolin (6%) enhanced fruit weight, length, and width, while 8% and 4% kaolin treatments improved phenol and flavonoid contents. Antioxidant activity (DPPH and FRAP) was highest under kaolin compared to control. Kaolin is easily washed off by rain, so farmers need to reapply it after rainfall. Therefore, spraying should be timed with sunny weather and avoided on cloudy days. Further, frequent stirring is required during its application as it settle down. Overall, kaolin mitigated heat stress, with 8% kaolin proving most effective; however, as this study was conducted for one-year, further research is required before final recommendations can be made.

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: Monthly Meteorological data collected during experimental period (Jan 2024 to Dec 2024)

Month	Temperature (°C)			Relative Humidity (%)			Sunshine (hr/day)	Rainfall (mm)	Wind speed (km/hr)
	Max	Min	mean	Max	Min	Mean			
Jan	29.9	13.8	21.8	85	38	62	7.4	0.2	4.5
Feb	33.4	15.0	24.2	76	25	50	8.8	0.4	4.0
Mar	36.1	17.5	26.8	63	20	41	7.8	0.2	5.7
Apr	38.6	21.9	30.2	59	23	41	8.6	2.1	5.8
May	38.9	22.3	30.2	72	31	52	8.7	2.6	8.5
Jun	32.7	22.0	27.4	88	58	73	4.9	25.0	8.4
Jul	29.6	21.7	25.6	92	71	82	1.6	9.5	9.5
Aug	30.4	21.1	25.8	92	65	78	3.7	14.8	7.5
Sep	30.7	20.4	25.6	91	60	76	5.4	8.7	8.5
Oct	32.0	20.1	26.1	93	52	72	7.1	8.3	5.4
Nov	30.1	14.9	22.5	84	37	60	6.9	0.0	3.5
Dec	29.8	15.6	22.7	87	42	65	5.3	0.0	3.9

Table 2: Effect of kaolin and salicylic acid (SA) on plant growth and physiological parameters in dragon fruit

Treatment	Treatment details	New shoots (nos.)	Cladode moisture (%)	Chlorophyll a (mg/g of FW)	Chlorophyll b (mg/g of FW)	Total Chlorophyll (mg/g of FW)	Chlorophyll a/b (mg/g of FW)	Normalized Difference Vegetation Index (NDVI) APR 24	Normalized Difference Vegetation Index (NDVI) MAY 24
T1	4 % Kaolin	10.33^{b}	86.23^{a}	1.47^{abc}	0.95^{bc}	2.41^{ab}	1.38^{ab}	0.54^{a}	0.58^{a}
T2	6 % Kaolin	6.67^{c}	81.47^{b}	1.41^{abc}	1.23^{ab}	2.64^{ab}	1.17^{bc}	0.47^{ab}	0.56^{ab}
T3	8 % Kaolin	14.00^{a}	82.55^{ab}	1.72^{a}	1.53^{a}	3.25^{a}	1.12^{c}	0.54^{a}	0.54^{ab}
T4	4 % Kaolin + SA 300 ppm	5.33^{c}	84.43^{ab}	1.25^{bc}	1.12^{bc}	2.36^{b}	1.12^{c}	0.48^{ab}	0.49^{ab}
T5	6 % Kaolin + SA 300 ppm	4.67^{c}	85.91^{ab}	1.60^{ab}	1.08^{bc}	2.68^{ab}	1.48^{a}	0.47^{ab}	0.53^{ab}
T6	8 % Kaolin +SA 300 ppm	6.33^{c}	82.77^{ab}	1.20^{bc}	1.13^{bc}	2.34^{b}	1.07^{c}	0.49^{ab}	0.58^{ab}
T7	SA 300 ppm	6.33^{c}	83.75^{ab}	1.51^{abc}	1.19^{b}	2.70^{ab}	1.27^{abc}	0.44^{b}	0.47^{b}
T8	Control	4.67^{c}	84.09^{ab}	1.12^{c}	0.80^{c}	1.92^{b}	1.40^{a}	0.42^{b}	0.48^{ab}
LSD $_{(p<0.05)}$		3.10	4.71	0.41	0.34	0.87	0.22	0.08	0.11
SEm (±)		1.02	1.55	0.13	0.11	0.28	0.07	0.03	0.04

Values with the same letter were not significantly different within column in LSD test ($p< 0.05$).

Table 3: Effect of Kaolin and Salicylic acid (SA) on sunburn, disease incidence and severity index in dragon fruit

Treatment	Treatment details	Sunburn incidence (%) APR	Sunburn incidence (%) MAY	Sunburn severity (%) APR	Sunburn severity (%) MAY	Disease incidence (%)	Disease severity (%)
T1	4 % Kaolin	72.92^{a}	61.46^{cd}	27.71^{abc}	27.92^{bc}	46.88^{abc}	18.75^{ab}
T2	6 % Kaolin	69.79^{a}	63.54^{bcd}	20.83^{c}	22.71^{c}	43.75^{bc}	14.17^{b}
T3	8 % Kaolin	65.63^{a}	57.29^{d}	22.08^{bc}	23.75^{c}	35.42^{c}	13.96^{b}
T4	4 % Kaolin + SA 300 ppm	79.17^{a}	77.08^{ab}	24.79^{bc}	32.08^{abc}	51.04^{ab}	24.38^{a}
T5	6 % Kaolin + SA 300 ppm	76.04^{a}	78.13^{ab}	29.17^{abc}	31.25^{abc}	55.21^{ab}	23.33^{a}
T6	8 % Kaolin +SA 300 ppm	75.00^{a}	73.96^{abc}	28.96^{abc}	31.04^{abc}	51.04^{ab}	24.17^{a}
T7	SA 300 ppm	78.13^{a}	72.92^{bc}	30.21^{ab}	34.79^{ab}	47.92^{abc}	24.58^{a}
T8	Control	80.21^{a}	88.54^{a}	34.59^{a}	40.00^{a}	60.42^{a}	24.38^{a}
LSD $_{(p<0.05)}$		16.97	15.02	8.67	9.65	13.84	6.23
SEm (±)		5.60	4.95	2.86	3.18	4.56	2.06

Values with the same letter were not significantly different within column in LSD test ($p< 0.05$)

Table 4: Effect of Kaolin and Salicylic acid (SA) on Membrane stability index and Canopy temperature

Treatment	Treatment details	Membrane stability index (%)	CT maximum (°C)	CT minimum (°C)	CT average (°C)
T1	4 % Kaolin	38.66^{bc}	51.03^{a}	23.53^{abc}	41.77^{a}
T2	6 % Kaolin	39.07^{bc}	48.90^{a}	17.47^{bc}	40.57^{a}
T3	8 % Kaolin	59.23^{a}	48.67^{a}	17.37^{c}	40.83^{a}
T4	4 % Kaolin + SA 300 ppm	36.81^{c}	49.53^{a}	19.77^{abc}	41.30^{a}
T5	6 % Kaolin + SA 300 ppm	56.58^{a}	49.93^{a}	23.83^{abc}	42.57^{a}
T6	8 % Kaolin +SA 300 ppm	43.98^{b}	50.67^{a}	26.77^{a}	43.53^{a}
T7	SA 300 ppm	30.24^{d}	49.40^{a}	24.63^{ab}	42.80^{a}
T8	Control	22.41^{e}	50.37^{a}	25.73^{a}	41.50^{a}
LSD $_{(p<0.05)}$		5.68	3.44	7.26	3.31
SEm (±)		1.87	1.14	2.39	1.10

Values with the same letter were not significantly different within column in LSD test ($p< 0.05$).

Table 5: Effect of Kaolin and Salicylic acid (SA) on Flowering and Fruit yield parameters in dragon fruit.

Treatment	Treatment details	Buds (nos.)	Flowers (nos.)	Fruit (nos.)	Bud drop (%)	Fruit set (%)	Plant yield (kg/plant)	Yield (tons/ha)
T1	4 % Kaolin	50.66^{de}	38.66^{cd}	35.33^{b}	23.69^{bcd}	91.23^{a}	13.54^{ab}	12.89^{ab}
T2	6 % Kaolin	60.00^{c}	47.33^{c}	33.33^{b}	20.79^{d}	70.34^{cd}	14.45^{ab}	13.75^{ab}
T3	8 % Kaolin	92.00^{a}	76.00^{a}	48.33^{a}	17.23^{e}	63.72^{d}	15.77^{a}	15.01^{a}
T4	4 % Kaolin + SA 300 ppm	59.33^{c}	46.00^{c}	36.00^{b}	22.65^{cd}	78.93^{bc}	9.92^{bcd}	9.44^{bcd}
T5	6 % Kaolin + SA 300 ppm	54.00^{cde}	38.66^{cd}	34.66^{b}	28.61^{a}	89.98^{ab}	11.18^{bcd}	10.64^{bcd}
T6	8 % Kaolin +SA 300 ppm	74.66^{b}	57.33^{b}	34.66^{b}	23.09^{bcd}	60.63^{d}	13.42^{abc}	12.77^{abc}
T7	SA 300 ppm	55.33^{cd}	40.66^{cd}	24.66^{c}	26.06^{ab}	60.87^{d}	8.89^{cd}	8.47^{cd}
T8	Control	47.33^{e}	36.00^{d}	32.66^{bc}	24.16^{bc}	90.46^{a}	7.12^{d}	6.78^{d}
LSD $_{(p<0.05)}$		7.87	9.23	8.54	3.34	11.14	4.58	4.36
SEm (±)		2.59	3.04	2.81	1.10	3.67	1.51	1.43

Values with the same letter were not significantly different within column in LSD test ($p< 0.05$).

Table 6: Effect of Kaolin and Salicylic acid (SA) on physical Fruit quality parameter in dragon fruit

Treatment	Treatment details	Fruit weight (g)	Fruit length (mm)	Fruit width (mm)	Fruit volume (m^3)	Pulp weight (g)	Peel weight (g)	Pulp (%)	Peel (%)	Peel thickness (mm)	Fruit firmness (g)	
											Penetration force	Cutting force
T1	4 % Kaolin	379.60^{abc}	102.22^{ab}	81.65^{abc}	325.33^{b}	228.07^{a}	151.53^{bc}	59.83^{ab}	40.17^{bc}	4.03^{c}	477.60^{a}	6944.03^{a}
T2	6 % Kaolin	433.00^{a}	108.17^{a}	88.68^{a}	419.00^{a}	212.40^{ab}	220.60^{a}	49.04^{c}	50.96^{a}	7.10^{ab}	495.97^{a}	6557.23^{a}
T3	8 % Kaolin	324.67^{bc}	89.00^{bcd}	77.21^{abc}	278.00^{bc}	178.80^{b}	145.87^{bcd}	53.33^{abc}	46.67^{abc}	5.93^{ab}	320.47^{b}	5440.20^{a}
T4	4 % Kaolin + SA 300 ppm	358.80^{abc}	92.65^{abcd}	77.16^{abc}	313.33^{bc}	226.00^{a}	132.80^{cd}	62.49^{a}	37.51^{c}	3.98^{c}	452.77^{ab}	6154.97^{a}
T5	6 % Kaolin + SA 300 ppm	281.47^{cd}	80.42^{cd}	69.02^{bc}	243.33^{c}	133.47^{c}	148.00^{bcd}	47.30^{c}	52.70^{a}	7.50^{a}	376.53^{ab}	6157.87^{a}
T6	8 % Kaolin +SA 300 ppm	391.13^{ab}	95.65^{abc}	83.65^{ab}	325.00^{b}	210.73^{ab}	180.40^{ab}	53.94^{abc}	46.06^{abc}	5.67^{bc}	454.80^{ab}	4976.73^{a}
T7	SA 300 ppm	318.38^{bc}	85.66^{cd}	76.93^{abc}	250.89^{bc}	184.96^{b}	133.41^{cd}	57.26^{abc}	42.74^{abc}	5.61^{bc}	426.21^{ab}	5910.98^{a}
T8	Control	214.87^{d}	77.37^{d}	66.09^{c}	144.67^{d}	110.80^{c}	104.07^{d}	51.48^{bc}	48.52^{ab}	5.40^{bc}	499.33^{a}	5859.97^{a}
LSD $_{(p<0.05)}$		98.78	15.71	15.82	76.70	36.30	45.63	10.30	10.30	1.74	150.54	2086.55
SEm (±)		32.56	5.17	5.22	25.29	11.96	15.05	3.40	3.40	0.57	49.63	687.90

Values with the same letter were not significantly different within column in LSD test ($p< 0.05$).

Table 7: Effect of Kaolin and Salicylic acid (SA) on Biochemical Fruit quality parameter in dragon fruit

Treatment	Treatment details	DPPH (mg TE/100 g)	FRAP (mg TE/100 g)	Phenol (mg/100 g GAE)	Flavonoids (mg/100 g CE)	Ascorbic acid (mg/100g)	TSS (°B)	Acidity (%)	Total Sugars (%)	TSS: Acid ratio	pH
T1	4 % Kaolin	74.72^{b}	17.38^{ab}	44.77^{ab}	21.39^{abc}	13.82^{e}	9.93^{abc}	0.37^{cd}	5.93^{c}	29.20^{abc}	3.19^{a}
T2	6 % Kaolin	83.25^{a}	20.13^{a}	50.66^{a}	21.94^{ab}	45.68^{a}	6.83^{d}	0.48^{abc}	8.38^{b}	14.33^{d}	2.81^{b}
T3	8 % Kaolin	81.10^{a}	16.59^{ab}	48.47^{a}	23.18^{a}	19.96^{cd}	11.23^{a}	0.32^{d}	7.57^{bc}	36.07^{a}	2.85^{b}
T4	4 % Kaolin + SA 300 ppm	81.67^{a}	16.80^{ab}	42.66^{abc}	19.69^{abc}	38.03^{b}	10.70^{ab}	0.40^{bcd}	5.79^{c}	31.83^{ab}	3.21^{a}
T5	6 % Kaolin + SA 300 ppm	73.79^{b}	14.40^{b}	34.42^{cd}	19.17^{abc}	36.80^{b}	7.47^{cd}	0.57^{a}	7.49^{bc}	13.90^{d}	3.04^{ab}
T6	8 % Kaolin +SA 300 ppm	79.25^{ab}	17.66^{ab}	33.16^{d}	19.84^{abc}	25.75^{c}	9.27^{abcd}	0.50^{ab}	10.87^{a}	22.18^{bcd}	3.27^{a}
T7	SA 300 ppm	81.67^{a}	18.38^{ab}	38.75^{bcd}	18.57^{bc}	24.36^{cd}	8.46^{abcd}	0.45^{bc}	7.49^{bc}	19.53^{cd}	3.11^{ab}
T8	Control	74.76^{b}	16.05^{ab}	32.02^{d}	17.60^{c}	19.10^{de}	8.00^{bcd}	0.45^{abc}	5.48^{c}	18.29^{cd}	3.17^{a}
LSD $_{(p<0.05)}$		6.07	4.89	8.69	4.14	5.87	2.85	0.11	2.35	11.69	0.30
SEm (±)		2.00	1.61	2.87	1.36	1.93	0.94	0.04	0.78	3.86	0.10

Values with the same letter were not significantly different within column in LSD test ($p< 0.0$)

Selection of elite male date palm (*Phoenix dactylifera* L.) genotypes based on floral phenology, stability and pollen production potential at Kachchh, India

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ABSTRACT

Date palm (Phoenix dactylifera L.) is a vital crop in arid and semi-arid regions, where genetic diversity of the females is majorly studied for its commercial point of view. While, worldwide there are a few selected male varieties, overall, their presence is limited compared to females. In dates males are equally important as they contribute to the fruit yield and quality. Thus, a study has been conducted at Date Palm Research Station, Mundra for nine male date palm genotypes for the reproductive traits during 2020 to 2023. The data were analyzed using principal component analysis and hierarchical cluster analysis. The results revealed that MDP-M1, MDP-M3 and MDP-M5 are early flowering genotypes with stable flowering period, while MDP-M3 and MDP-13 are superior in pollen production. Male dates showed diversity in spathe opening time ranged for 45 days (27 January to 12 March). The flowering duration for each genotype was 23.25 to 30.50 days with a narrow range of duration. The genotypes showed early, medium and late flowering period. PCA with first three principal components with eigen value greater than unity explained 87.6 % of total variance majorly based on pollen yield and spathe traits. The genotypes, MDP-M3 and MDP-M13 depicted association with pollen production quantity per spathe and per palm making them efficient pollinizers and HCA further clustered these genotypes into same cluster. This study helps to accumulate the data for high pollen yielding ability and early flowering genotypes for identification of superior male as pollinizers.

Keywords: Date palm, hierarchical clustering, male genotypes, PCA, variability

INTRODUCTION

Date palm (*Phoenix dactylifera* L.) is a dioecious perennial fruit crop of importance in the arid and semi-arid regions of the world. Around the world there are more than 5000 varieties have been identified, among them, few are of prime importance around the world (Jaradat and Zaid, 2004). Most identified female genotypes had significant variation, however, variability among the male is also play a crucial role in artificial pollination, which is essential for optimizing fruit yield and quality (Jaradat and Zaid, 2004). The flowering period and fruiting has genotypic specificity and it also depends on geographic location and environmental conditions of the area (Sharma *et al.*, 2025).

Farmers often use stored pollen grains from the known male for pollination, however in some cases inability of the stored pollens led farmers to use pollens grains from unknown male which might cause reduced fruit set and/or may produce negative metaxenic effects on fruit yield and quality (Kadri *et al*., 2019, Sharma *et al*., 2023). In such cases, there is need to study pollinisers to be used for pollinating the female dates (Abdel-Sattar *et al.*, 2024). The selection of good quality male pollinisers for flowering duration, spathe related traits and pollen production help us to identify the desirable male dates for further use. In this regards, Sharma *et al*. (2023) reported important traits to identify the superior male genotypes as a pollinator such as pollen production, pollen viability, spathe size and related characters. Despite their importance, male date palm genotypes remain relatively unexplored compared to female genotypes or cultivars, leading to a lack of systematic characterization and conservation efforts. Kadri *et al.* (2022) reported pollens collected from spathes developed at the middle of flowering stage depicted high viability up to 90% with germinability of 85%.

In India, particularly in the Kachchh region of Gujarat, date palm cultivation is present for 450 years, however, a major exploration and expansion has taken place in the last two decades (Baidiyavadra *et al*., 2019; Sharma *et al*., 2019). However, the information on the diversity and characteristics of locally available male genotypes is limited. Previously, in few of the date palm growing region, the male genotypes are characterized for their variability and the identification of their distinctness which can contribute to improve fruit production (El-Kadri *et al*., 2019; Raza *et al*., 2020). Such assessment helps in the identification of genetically distinct and superior male palms. The comprehensive study of the available genetic resource of the location help us to generate data which contribute to preserve the diversity of the region also support the development of conservational aspects of agricultural practices in the country (Musayev and Bayramov, 2025). This study aims to evaluate the morphological diversity among the male date palm genotypes in Kachchh, India using standard descriptors and identify the relationship between the characters and genotypes using principal component analysis and hierarchal study.

MATERIALS AND METHODS

The experiment was conducted at Date Palm Research Station, Sardarkrushinagar Dantiwada Agricultural University, Mundra-Kachchh, Gujarat, India (22°49’19.7”N,69°43’16.7”E) during 2020-2023. The nine male genotypes under study (MDP-M1, MDP-M2, MDP-M3, MDP-M4, MDP-M5, MDP-M6, MDP-M13, MDP-M19 and MDP-M29) are the selection from the in-situ germplasm at Date Palm Research Station, Mundra which were primarily evaluated for further screening. The plants selected for the study were uniform with age of 15 years old. The soil conditions of the research station were sandy soil with pH of 8.22, EC of 0.170 dSm^{-1}. The selected palms for the present study were maintained under uniform agronomic practices including pollination, pruning, dethorning, etc. Irrigation was provided through drip with an average of 200 liters of water per palm per day. The water quality for irrigation during the experimental period was pH of 7.57, EC of 7.5 dSm^{-1}, Sodium Adsorption Ratio (SAR) of 25.40, Residual Sodium Carbonate (RSC) of 3.30 meq/lit. The climatic condition of the regions was semi-arid with an annual temperature ranges from 6°C to 42 °C and average of annual rainfall 472 mm (average of 30 years), falling under Gujarat North Agro-Climatic Zone -V.

Key phenological, reproductive, and morphological parameters were recorded to assess variability among male date palm genotypes, i.e., phenological traits like the day of first spathe opening, day of last spathe opening, number of spathes per plant, and flowering duration (measured as the number of days between the first and last spathe opening); reproductive characters like the

average amount of pollen per spathe (g) and estimating the approximate total pollen production per palm (g) based on the number of spathes; and morphological characters of spathe like its length (cm), width (cm), average number of strands per spathe, and average number of florets per strand. The characters and flowering duration were recorded for four years for each plant.

Statistical analyses were analyzed using R programming to examine the variability in recorded traits. Descriptive statistics, including mean, standard deviation were computed to quantify the extent of variation among the genotypes. Each genotype was having single plant which were analyzed for four years treated as local replications for analysis. Principal component analysis, biplot analysis and hierarchical cluster analysis were generated using R programming (R Core Team, 2024) using FactoMineR and ggplot2 packages.

RESULTS AND DISCUSSION

Morphological characterization

The flowering period with first and last date of spathe opening for different date palm genotypes over four years are presented in the Table 1. In all the four years, genotypes like MDP-M1, MDP-M2, and MDP-M3 shows consistent early flowering, while MDP-M6 and MDP-M29 tended to flower late. Early flowering is desirable as it facilitates synchronisation of pollination for early maturing females. The last spathe opening also followed a pattern of stability, with MDP-M1, MDP-M3, and MDP-M5 completing flowering by end of February or in March first week in most years proposing predictable timing for pollination making it more economical trait, whereas MDP-M6 and MDP-M29 often ends flowering in mid or late of March. This makes the genotype MDP-M1, MDP-M3, and MDP-M5 advantageous for predictable pollination timing. In contrast, MDP-M6 and MDP-M29 exhibited the most variation, extending flowering periods in certain years, which might require additional pollen availability for effective pollination. There is around 45 days of span for male spathe openings in all genotypes. Some late flowering genotypes could be used for female flowered lately in the field. This staggered flowering among the genotypes facilitates pollen availability over a time. For efficient pollination, early maturing male genotypes are preferred as fresh pollen is available for current season pollination (Sharma *et al*., 2023).

The phenotypic traits of male date palm genotypes are presented in the Table 2. The analysis of phenotypic traits across nine date palm genotypes reveals significant variation in reproductive characteristics, pollen production, and spathe morphology. The number of spathes per plant ranged from 15.25 in MDP-M4 to 31.25 in MDP-M3, with an average of 21.00 and a standard deviation of 5.86, indicating moderate variability among genotypes. The flowering duration varied from 23.25 days (MDP-M3) to 30.50 days (MDP-M2), with a mean of 26.08 days, suggesting a relatively narrow range of flowering time among the genotypes. Pollen yield among the various genotype showed that MDP-M3 produced the highest average pollen per spathe (21.20 g) followed by MDP-M13 (20.60 g) and the total pollen per palm (662.18 g and 418.19 g, respectively), while MDP-M4 produced lower amount of pollen (11.07 g per spathe and 168.44 g per palm). This shows that MDP-M3 and MDP-M13 are important genotype for selection for pollen production. Since manual pollination is practiced, male dates with high pollen yield could be advantageous for large scale dates cultivation. Maturation of early flowering allows availability of pollen for pollinating female plants. It has been observed that pollen receptivity of plants varies from one day to one week based on the genotype but after that period, the pistillate fails to receive the pollen and may cause parthenocarpic fruits which are of inferior quality and does not possess any market value (Sharma *et al*., 2023; Muralidharan *et al*., 2020). Moreover, higher pollen production may allow pollinating a greater number of spathes or

plants (Sharma *et al*., 2021). Additionally, pollen has commercial value and are sold at premium prices during peak flowering season. Hence, late availability of pollen may be useful for late flowering females.

The length of the spathe ranged from 50.82 cm in MDP-M5 to 83.57 cm in MDP-M1, with an average of 60.61 cm, while spathe width varies from 9.16 cm in MDP-M6 to 18.87 cm in MDP-M3. The average number of strands per spathe was highest in MDP-M2 (203.01) followed by MDP-M13 (195.25) and lowest number of strands were observed in MDP-M5 (127.83), while, the average number of florets per strand varied between 38.79 (MDP-M29) and 71.30 (MDP-M4), with a mean of 57.31. Earlier, El-Kadri *et al.* (2019) reported the presence of largest spathe with 40.00 cm long, 10.00 cm wide and 413.33 average number of spikelets, suggesting the potential of the number of strands in males. The spathe weight ranges from 0.87 kg (MDP-M5) to 1.84 kg (MDP-M1), and spathe weight without cover followed a similar trend, varying from 0.33 kg in MDP-M5 to 0.81 kg in MDP-M1 and MDP-M13. It can be noted that MDP-M1 have largest spathes, both in length (83.57 cm) and weight (1.84 kg), however, the overall pollen production is low, while MDP-M5 and MDP-M4 exhibited smaller spathes and lower pollen production suggesting making them unfit for commercial usage. For prioritizing high pollen yielding genotypes MDP-M3 and MDP-M13 are better, while selecting genotypes with early flowering and moderate spathe structures MDP-M13 and MDP-M2 may be beneficial. Other traits of MDP-M3 and MDP-M 13 showed being moderate in length, number of strands per spathe, florets per strand and spathe weight its pollen load was superior making them better pollinizers with medium flowral structure. Early flowering of male allows higher success of pollination thus allows better revenue, while higher pollen production enhances commercial value. Also, medium length of the spathe might be efficient pollen producers and dispersal, while larger spathe could be less manageable.

Principal Component Analysis

The variance and eigen value of the principal component analysis are presented in Table 3. The first two principal components (PCs) accounts for high variability with 49.6% and 24.3 %, respectively, representing cumulative variability of 73.9 % explaining major variability of the data set. The eigen value of first two PCs were 5.578 and 2.739, respectively. PC3 and PC4 explains 13.7 % and 6.7% and from PC5 to PC9 combined explains 5.6% of the variance. This indicates that the first two or three components are sufficient for visualizing genotype differences and reducing dimensionality while, retaining most of the data's information. The high variance explained by PC1 and PC2 suggests strong correlations among the original traits, making them useful for genotype differentiation and classification in breeding programs.

The PC scores for are presented in Table 4 and the character loading is presented in Table 5, which provides insight of how different date palm genotypes are distributed across the first three principal components (PCs). The genotype, MDP-M1 (3.12) and MDP-M3 (2.83) have high positive values for PC1, which indicates that they exhibit strong characteristics related to the spathe characters like spathe weight (with or without cover), spathe width, number of strands, and number of spathes per plant. These characters can be considered as key characters to differentiate among the genotypes. On the other hand, MDP-M6 (-2.97) and MDP-M5 (-2.85) have low PC1 values, suggesting they show opposite trends in these traits. In PC2, high scores were observed in MDP-M3 (2.92) and MDP-M5 (1.58) which shows strongly association of traits like amount of pollen per spathe, pollen per palm and negatively linked with flowering duration, and number of florets per strand. This also indicates that MDP-M3 and MDP-M5 are important genotypes for pollen

production but have shorter flowering period and lower number of florets. In PC3, MDP-M4 (2.44) stands out with the highest value, suggesting it has distinct features with negatively linked traits like flowering duration, length of spathe, and number of spathes per plant (-0.387), whereas MDP-M29 (-1.27) and MDP-M6 (-0.99) have negative values, indicating their negative relationship.

The principal component analysis biplot is presented in Figure 1. The collective presentation of PC1 and PC2 explains 73.9 % of the total variation, where, MDP-M3 and MDP-M13 are positioned in the upper right quadrant, showing a strong association with amount of pollen per spathe pollen per palm, suggesting these genotypes are superior in pollen production. While, genotypes MDP-M1 and MDP-M2 are located towards the right which are strongly influenced by characters like number of spathes per plant and weight of spathe without cover, indicating their relationship with spathe characters. In contrast, MDP-M19 and MDP-M4 are positioned on the negative PC1 axis, meaning they exhibit opposing traits compared to those in the right quadrant. The direction and length of the arrow for the trait represents the contribution of the trait towards variation and its correlation with other trait. The traits such as average amount of pollen per spathe, approximate pollen per palm, number of spathes per plant and width of the spathe are grouped together and pointing upper right quadrant suggesting these traits are positively associated. The weight and length of the spathe could be indicator of overall male flower quality and pollen production ability of the male dates.

Hierarchical Cluster Analysis

The hierarchical cluster analysis (HCA) dendrogram is presented in the Figure 2 providing a structured representation of the genetic relationships among different genotypes using Ward's method. The different genotypes are presented in x-axis while the dissimilarity (distance) among the genotypes is presented in y-axis, with larger values indicating greater differences and they are grouped based on their similarity. The analysis reveals three distinct clusters, each highlighted in different colors, *i.e.*, pink, red and orange. The first cluster (pink) is the largest, comprising MDP-M19, MDP-M4, MDP-M1, and MDP-M2, implying that these genotypes have closely related characteristics like high number of florets. The second cluster (red) consists of MDP-M13 and MDP-M3, suggesting moderate similarity between them but distinct differences from the first group. These two genotypes are high pollen producing genotypes. The third cluster (orange) includes MDP-M5, MDP-M29, and MDP-M6, and as per PCA they are distinct group with limited similarity.

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: Flowering period of different date palm male genotypes

Genotype	2020		2021		2022		2023	
	Day of First spathe opening	Day of Last spathe opening	Day of First spathe opening	Day of Last spathe opening	Day of First spathe opening	Day of Last spathe opening	Day of First spathe opening	Day of Last spathe opening
MDP-M1	27-Jan	01-Mar	30-Jan	28-Feb	28-Jan	25-Feb	10-Feb	28-Feb
MDP-M2	30-Jan	06-Mar	02-Feb	08-Mar	01-Feb	02-Mar	05-Feb	27-Feb
MDP-M3	02-Feb	28-Feb	05-Feb	25-Feb	10-Feb	10-Mar	08-Feb	02-Mar
MDP-M4	10-Feb	01-Mar	05-Feb	03-Mar	09-Feb	03-Mar	03-Feb	28-Feb
MDP-M5	01-Feb	28-Feb	06-Feb	03-Mar	05-Feb	03-Mar	10-Feb	26-Feb
MDP-M6	15-Feb	20-Mar	08-Feb	10-Mar	09-Feb	01-Mar	05-Feb	05-Mar
MDP-M13	11-Feb	03-Mar	05-Feb	01-Mar	04-Feb	05-Mar	10-Feb	01-Mar
MDP-M19	11-Feb	01-Mar	03-Feb	06-Mar	01-Feb	10-Mar	12-Feb	02-Mar
MDP-M29	06-Feb	05-Mar	10-Feb	03-Mar	05-Feb	11-Mar	10-Feb	12-Mar

Table 2: Phenotypic variation in different male date palm genotypes (averaged for the year 2020-2023)

Genotype	Number of spathes per plant	Flowering Duration (days)	Avg. Amount of pollen per spathe (g)	Approx. pollen per palm (g)	Length of spathe (cm)	Width of spathe (cm)	Avg. No. of strands per spathe	Avg. No. of Florets per strand	Weight of spathe (kg)	Weight of spathe without cover (kg)
MDP-M1	26.25	26.75	13.72	360.70	83.57	14.32	187.95	70.68	1.84	0.81
MDP-M2	27.50	30.50	11.19	309.21	60.94	14.58	203.01	63.08	1.53	0.52
MDP-M3	31.25	23.25	21.20	662.18	61.77	18.87	177.63	49.92	1.46	0.73
MDP-M4	15.25	23.50	11.07	168.44	52.07	16.00	188.60	71.30	1.32	0.55
MDP-M5	16.00	23.50	17.93	285.83	50.82	11.74	127.83	45.51	0.87	0.33
MDP-M6	15.75	28.00	16.93	266.30	56.11	9.16	129.67	46.47	0.99	0.35
MDP-M13	20.25	23.75	20.60	418.19	65.22	15.71	195.25	60.49	1.26	0.81
MDP-M19	18.00	27.25	11.54	208.89	55.89	15.36	153.96	69.54	1.31	0.58
MDP-M29	18.75	28.25	12.22	229.96	59.11	10.55	141.58	38.79	0.91	0.51
Mean	21.00	26.08	15.15	323.30	60.61	14.03	167.27	57.31	1.28	0.58
S.D.	5.86	2.66	4.08	148.27	9.77	3.03	29.28	12.36	0.32	0.18
Max.	31.25	30.50	21.20	662.18	83.57	18.87	203.01	71.30	1.84	0.81
Min.	15.25	23.25	11.07	168.44	50.82	9.16	127.83	38.79	0.87	0.33

Table 3: Eigen value and variance of principal component for variables

Principal Component	Eigen value	Variance Explained	Per cent cumulative variation
PC1	5.578	0.496	49.6
PC2	2.739	0.243	73.9
PC3	1.539	0.137	87.6
PC4	0.759	0.067	94.4
PC5	0.269	0.024	96.7
PC6	0.216	0.019	98.7
PC7	0.140	0.012	99.9
PC8	0.010	0.001	100.0
PC9	0.000	0.000	100.0

Table 4: PC scores for different genotypes across PCs

Genotype	PC1	PC2	PC3
MDP-M1	3.12	-1.32	-1.04
MDP-M2	1.09	-1.88	-1.05
MDP-M3	2.83	2.92	-0.22
MDP-M4	-0.13	-1.31	2.44
MDP-M5	-2.85	1.58	0.54
MDP-M6	-2.97	0.35	-0.99
MDP-M13	1.69	1.30	0.73
MDP-M19	-0.39	-1.44	0.85
MDP-M29	-2.40	-0.20	-1.27

Table 5: Character loading of different principal components for various traits

Principal Components	Number of spathes per plant	Flowering Duration	Avg. Amount of pollen per spathe (g)	Approx. pollen per palm (g)	Length of spathe (cm)	Width of spathe (cm)	Avg. No. of strands per spathe	Avg. No. of Florets per strand	Weight of spathe (kg)	Weight of spathe without cover (kg)
PC1	0.358	-0.078	0.078	0.286	0.318	0.366	0.380	0.262	0.405	0.407
PC2	0.103	-0.412	0.596	0.456	-0.100	0.093	-0.176	-0.394	-0.227	0.034
PC3	-0.387	-0.576	-0.026	-0.203	-0.396	0.375	0.142	0.392	-0.065	0.031
PC4	0.416	0.270	-0.207	0.187	-0.609	0.377	0.183	-0.126	-0.021	-0.337
PC5	-0.182	0.202	0.106	-0.115	-0.087	-0.105	0.705	-0.276	-0.423	0.361
PC6	0.095	-0.168	-0.573	-0.073	0.068	0.295	-0.325	-0.395	-0.202	0.485
PC7	-0.196	0.557	0.291	0.095	-0.202	0.255	-0.396	0.331	-0.158	0.400
PC8	0.501	-0.047	0.193	-0.442	0.292	0.200	-0.038	0.249	-0.546	-0.165
PC9	0.359	-0.190	-0.187	0.240	-0.319	-0.597	-0.038	0.392	-0.211	0.293

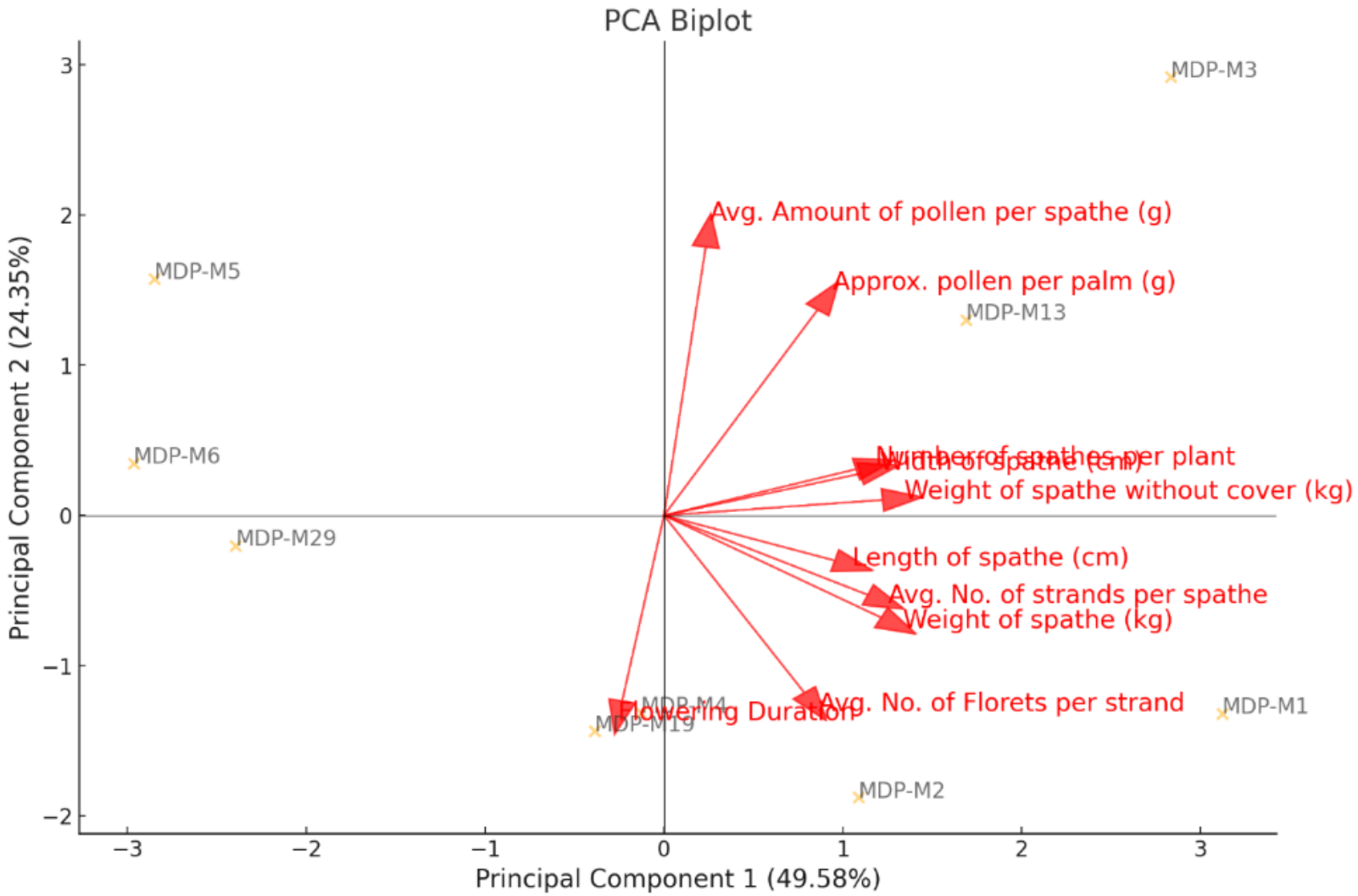

Figure 1: PCA Biplot of male date palm genotype for different genotypes and traits

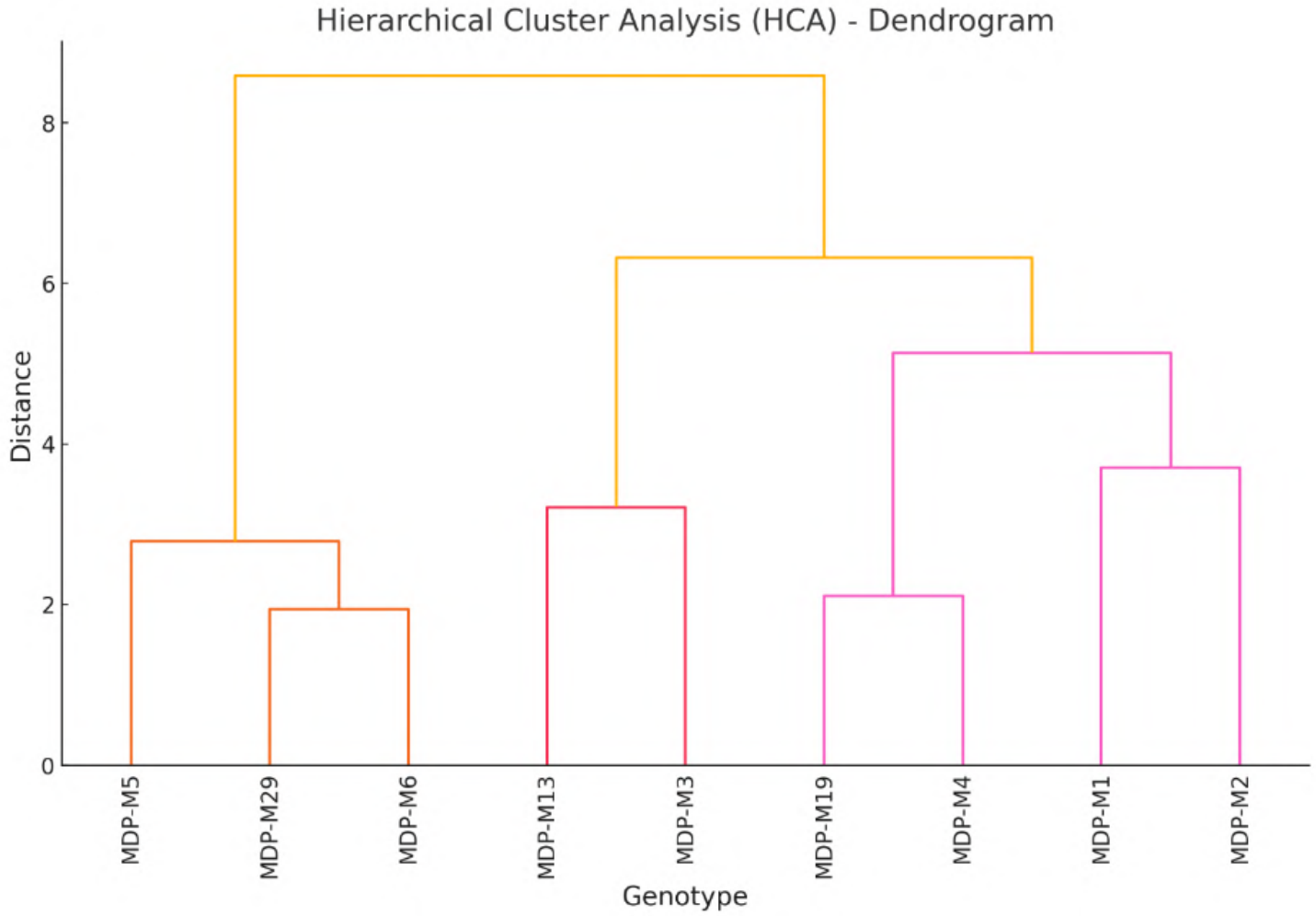

Figure 2: Hierarchical cluster analysis for different male date palm genotypes

Screening of chrysanthemum genotypes for quality traits under Ayodhya regions of Uttar Pradesh

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ABSTRACT

Fifteen chrysanthemum genotypes were screened under pot conditions in a Completely Randomized Design (CRD) with three replications during 2023–2024 at the Main Experiment Station, College of Horticulture and Forestry, Acharya Narendra Deva University, Ayodhya (U.P.). Significant variations were observed among genotypes for morphological, physiological, qualitative, and yield traits. 'Autumn White' recorded maximum plant height (59.33 cm) and inter-nodal length (4.33 cm), while 'Kusum' and 'Phyllis' showed early bud (71 days) and flower initiation (88 days), respectively. 'Sport' produced the highest number of ray florets (322), largest flower diameter (12.52 cm), and highest flower fresh (8.72 g) and dry weight (0.74 g). 'Liliput' had the highest flower count per plant (164), and 'Sunny' showed maximum leaf area (23.82 cm²). 'Phyllis' also had the highest chlorophyll content (0.31 mg/g). 'Zembla' exhibited maximum stem diameter (3.97 mm), flowering duration (30.33 days), and shelf life (6.67 days). These superior genotypes hold potential for use in cut flowers, loose flowers, and pot plant production, and in future breeding programs.

Keywords: Chrysanthemum, genotypes, morphological traits, quality, yield

INTRODUCTION

Chrysanthemum (*Dendranthema grandiflorum* Tzvelev), a key member of the Asteraceae family, holds significant global importance as both a cut flower and a potted ornamental plant. Renowned for its diverse flower forms, vibrant colors, and extended vase life, chrysanthemum ranks second after rose in spray types and seventh among standard cut flowers globally (Anonymous, 2017). Commonly known as "mums" or "chrysanths," it is regionally referred to as *Guldaudi*, *Chandramalika*, *Samanti*, and *Shevanti* across various parts of India. Characterized by a capitulum inflorescence composed of numerous small florets, chrysanthemum is a short-day plant, requiring less than 13.5–14.5 hours of daylight for flower bud initiation. This photoperiodic response restricts natural flowering to a limited seasonal window, typically spanning three months.

In India, chrysanthemum is cultivated over approximately 30.13 thousand hectares, yielding 463.73 thousand metric tons (MT) of loose flowers and 18.60 thousand MT of cut flowers. Chrysanthemum exhibits extensive phenotypic variability influenced by genotype, region, season, and environmental conditions (Singh *et al*. 2017). In Northern India, traditional varieties are favored for ornamental and display purposes, whereas in the south, spray types dominate, primarily for garlands and religious offerings during the festive season.

The commercial value of chrysanthemum has expanded beyond floriculture into

industries such as essential oil, perfumery, aromatherapy, cosmetics, natural dyes, and pharmaceuticals. Major constituents in chrysanthemum (*C. indicum*) oil are Camphor, Isoborneol, α- Tepinene and Caryophyllene oxide and *Chrysanthemum morifolium* oil are Comphor, Curcumene, Pentacosane, Borneol. Chrysanthemum oil is extracted through various methods including distillation, soxhlet extraction (Swati *et al*. 2024). Chrysanthemum species contain high levels of phenolic acids and flavonoids including chlorogenic acid, luteolin, rutin, quercetin, and apigenin which confer strong antioxidant and free-radical scavenging activities. *Chrysanthemum indicum* extracts showed IC_{50} values of ~77.2 µg/mL (flowers) and ~101.9 µg/mL (leaves) in DPPH assay, with similarly potent results in ABTS assays (Kim *et al*. 2024). Technological advancements in photoperiodism and genetics now enable year-round production. However, varietal limitations persist, particularly in meeting the growing demand for novel flower traits and enhanced adaptability. To address these gaps, a systematic screening of 15 chrysanthemum genotypes was undertaken at the Department of Floriculture and Landscaping, College of Horticulture & Forestry, Acharya Narendra Deva University of Agriculture & Technology, Kumarganj, Ayodhya. The study aimed to assess morphological and physiological traits of selected chrysanthemum varieties and evaluate their quality and yield-related characteristics. This investigation seeks to identify superior genotypes suited for commercial cultivation in Eastern Uttar Pradesh, thereby enhancing productivity, profitability, and cultivar diversity.

MATERIALS AND METHODS

The present study was conducted during the 2023–2024 growing season at the main experimental station (Horticulture), Floriculture Unit, Department of Floriculture and Landscaping, Acharya Narendra Deva University of Agriculture & Technology, Kumarganj, Ayodhya, Uttar Pradesh, India. The experimental site is geographically situated at 26.47° N latitude, 82.12° E longitude, and an altitude of 113 meters above mean sea level. The region experiences a subtropical climate, characterized by hot summers, moderate monsoons, and cool winters. The soil type of Kumarganj, Ayodhya was silty loam with average fertility level and pH in the range of 7.0 to 8.0.

Fifteen genotypes of chrysanthemum (*Dendranthema grandiflorum* Tzvelev) were selected for screening, *viz.,* Zembla, Sunny, Autumn Pink, Autumn White, Banglore Button, Sport, White Prolific, Liliput, Kusum, Neelima, Diana Orange, Phyllis, Mayur, Lilia Spray and Pusa Chitraksha. The experiment was laid out in a Completely Randomized Design (CRD) with three replications. Uniform, healthy rooted cuttings were transplanted into 10-inch earthen pots containing a standardized growing medium composed of loamy soil, sand, and well-decomposed farmyard manure (FYM) in a 2:1:1 ratio. The water-soluble fertilizers were applied one week after planting NPK (19:19:19) @ 2g per liter of water and same dose weekly was repeated to promote vegetative growth. At the bud initiation stage, NPK @ 10:20:15 was applied 2g per litre of water to enhance the production and quality of flowers, along with micronutrients such as boron and ferrous were applied @ 2 g/l during the flowering stage and irrigation was scheduled based on crop requirements. Pinching was performed 30 days after transplanting to promote lateral shoot. Plants were regularly monitored for signs of pest infestations and disease symptoms, and appropriate phytosanitary measures were taken as needed.

Observations were recorded on a range of morphological, physiological, quality, and yield-related traits. Morphological and physiological parameters included plant height, number of sprays per plant, plant spread, stem diameter, internodal length, days to first bud initiation, days to first

flower opening, leaf area, total chlorophyll content (Chlorophyll a and b, were measured using a SPAD meter), fresh flower weight, and dry flower weight. Quality and yield traits comprised number of flowers per plant, flowering duration, number of ray florets per flower, flower diameter, shelf life under ambient conditions, flower yield per plant, flower color (categorized using the RHS color chart), and flower type (classified based on standard horticultural descriptors such as decorative, pompon, or spider forms). All collected data were subjected to statistical analysis using analysis of variance (ANOVA) to determine the significance of differences among the genotypes. The critical difference (CD) at 5% probability level was calculated to compare treatment means where applicable.

RESULTS AND DISCUSSION

Morphological and physiological traits

The present study revealed highly significant variability among the fifteen evaluated chrysanthemum genotypes for all the morphological and physiological traits (Table 1), underscoring the influence of genotypic constitution and environmental interactions on plant performance under the agro-climatic conditions of Eastern Uttar Pradesh. Among the genotypes, 'Autumn White' exhibited the highest plant height (59.33 cm), whereas 'Phyllis' recorded the shortest stature (32.00 cm). These variations are indicative of differential growth vigour and are consistent with previous reports by Suvija *et al*. (2016), who emphasized the role of both genetics and micro-environmental factors in determining plant architecture. Similarly, significant variation was observed in the number of leaves per plant, with 'Mayur' producing the highest number (72.67), while 'Bangalore Button' produced the fewest (22.33). Such differences highlight the potential for selecting genotypes with superior vegetative growth for enhanced photosynthetic capacity and overall productivity, as also noted by Thakur *et al*. (2018). Similarly, Lisianthus showed variations in number of leaves among cultivars leaves are the functional unit of photosynthesis, which greatly influenced the growth and flower yield of the crop recorded by Bindhu *et al*. (2024).

Branching behaviour, as measured by the number of sprays per plant, also varied significantly. 'Liliput' showed the highest number of sprays (9.00), while both 'Sunny' and 'Phyllis' showed limited branching with only 3.00 sprays per plant. These observations support the findings of Jamaluddin *et al*. (2015), who reported genotypic differences in lateral shoot formation, which is a desirable trait for increasing the number of flowering stems in ornamental crops. Plant spread ranged from a maximum of 47.00 cm in 'Nilima' to a minimum of 15.67 cm in 'Bangalore Button'. A wider plant spread is advantageous in landscaping and bedding uses, and this variability corroborates earlier findings by Arora *et al.* (1999), suggesting the importance of genotype selection for plant architecture traits.

Variation in stem diameter was evident, with the thickest stems recorded in 'Zembla' (3.97 mm) and the thinnest in 'Liliput' (2.97 mm). Stem robustness is a key trait influencing the plant's ability to support flowers and withstand mechanical stresses, and its genotypic control was similarly highlighted by Rajiv *et al*. (2007). The internodal length, another growth parameter linked to plant compactness, showed considerable variation from 4.33 cm in 'Autumn White' to 1.40 cm in 'Lilia Spray', indicating that genotypic background significantly affects node elongation, a result consistent with Pasha *et al*. (2015).

Phenological traits such as days to bud initiation and flowering also varied significantly among genotypes. 'Kusum' initiated budding the earliest (71 days), whereas 'Phyllis' was the earliest to flower (88 days). Early flowering genotypes are especially valuable for floriculture markets targeting specific festival or sale windows, and these results align with the observations of Behera *et al*. (2002).

In terms of photosynthetic traits, 'Sunny' exhibited the highest leaf area (23.82 cm²), while 'Diana Orange' showed the lowest (7.19 cm²). Leaf area is closely

tied to light interception and photosynthetic potential, and its variability indicates potential selection for genotypes with superior photosynthetic efficiency under regional conditions. The content of total chlorophyll content also varied, with 'Liliput' having the highest concentration (0.22 mg g^{-1}) and 'Pusa Chitraksha' the lowest (0.09 mg g^{-1}), suggesting that physiological efficiency in terms of light harvesting and photosynthetic activity differs significantly across genotypes. Anitha *et al*. (2000) similarly highlighted the role of chlorophyll concentration in plant productivity.

Flower biomass, both fresh and dry, followed a similar genotypic pattern. 'Sport' produced the heaviest flowers (8.72 g fresh and 0.74 g dry), whereas 'Liliput' recorded the lightest flowers in both fresh and dry form. These results indicate strong genetic control over biomass partitioning and flower development, supporting previous studies by Baskaran *et al*. (2010). Overall, the significant genotypic variability observed in morphological and physiological parameters provides a valuable base for identifying elite genotypes for commercial cultivation and breeding programs targeted to the specific agro-ecological conditions of Eastern U.P.

Qualitative and yield traits

Analysis of quality and yield traits (Table 2) revealed wide genotypic variation, confirming that significant potential exists within the screened germplasm for improvement of floral traits important for commercial floriculture. Among the genotypes, 'Liliput' stood out with the highest number of flowers per plant (164), making it a promising candidate for mass flowering applications, while 'Diana Orange' recorded the fewest flowers (12.33). These findings are in line with earlier studies by Kavitha *et al*. (2019) and Kumar *et al*. (2015), which also reported genotype-dependent flower productivity in chrysanthemum.

Floral morphology showed substantial variation as well. The number of ray florets per flower was highest in 'Sport' (322), followed by 'Zembla' (282), suggesting their suitability for use as attractive cut flowers due to their dense floret arrangement. In contrast, 'Liliput', with its compact and minimal floret structure, may be better suited for decorative or potted purposes. Flower diameter also varied greatly among genotypes, ranging from 12.52 cm in 'Sport' to just 2.41 cm in 'Liliput', illustrating the diversity in floral size preferences among market segments. These differences reflect underlying genetic factors and agree with Baskaran *et al*. (2016), who reported genotype-driven variations in flower morphology.

Flowering duration, an essential trait for commercial floriculture, also differed significantly, with 'Zembla' flowering the longest (30.33 days) and 'Phyllis' the shortest (17.00 days). Long flowering duration is advantageous for extended market availability and landscape use, and this variation is likely due to both genetic makeup and environmental adaptation, similar to findings by Srilatha *et al*. (2015).

Post-harvest longevity or flower shelf life, ranged from 1.67 days in 'Kusum' to 6.67 days in 'Zembla', underscoring genotypic differences in post-harvest physiology. Longer shelf life is a critical trait for cut flower markets, and these results are consistent with the work of Roopa *et al*. (2018), who emphasized the need to select genotypes with improved vase life for better marketability.

In terms of flower yield per plant, 'Sport' again emerged as the highest-yielding genotype (206.24 g), followed by 'Nilima' (194.40 g), while 'Bangalore Button' produced the lowest (18.18 g). This trait is a direct indicator of commercial value, and such marked variability provides opportunities for genetic enhancement of yield, as also reported by Singh and Dadlani (1989).

The diversity in flower color and type (Plate 1) was notable across the evaluated genotypes, ranging from white, yellow, orange, pink, red, and purple hues to an array of forms including decorative, pompom, incurve, single Korean, double Korean, and

anemone types. Based on the RHS colour chart, white-flowered genotypes included 'Zembla', 'Sunny', 'Autumn White', and 'White Prolific'; red shades were represented by 'Lilia Spray'; dark red by 'Pusa Chitraksha'; yellow by 'Bangalore Button', 'Liliput', 'Kusum', 'Phyllis', and 'Mayur'; orange by 'Diana Orange'; light orange by 'Sport'; light pink by 'Autumn Pink'; and dark purple by 'Nilima'. Such diversity in ornamental features is critical for aesthetic appeal and consumer preferences and aligns with previous observations by Mishra *et al*. (1999).

The observed range of flower types included Anemone ('Mayur'), Pompom ('Zembla', 'Autumn White', 'Diana Orange'), Incurve ('Autumn Pink'), Decorative ('Bangalore Button', 'Sport', 'White Prolific', 'Nilima'), Single Korean ('Kusum', 'Pusa Chitraksha'), and Double Korean ('Sunny', 'Lilia Spray'). These traits greatly influence consumer preferences and are often used as selection criteria in breeding programs. The significant variation observed across all these parameters highlights the potential for targeted breeding and selection to develop improved varieties suited to both market and climatic requirements of the Eastern U.P. region.

CONCLUSION

The study revealed significant variability among chrysanthemum genotypes, identifying 'Autumn White', 'Zembla', and 'Sport' as promising for cut flower production, while varieties like 'Phyllis', 'Sunny', and 'White Prolific' showed potential for loose flowers and pot culture. These genotypes offer valuable traits for breeding and commercial cultivation under Eastern Uttar Pradesh conditions.

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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: Effect of genotypes screening for morphological and physiological traits

Treatments	Plant height (cm)	No. of leaves/ plant	No. of spray/ plant	Spread of plant (cm)	Stem diameter (mm)	Inter-nodal length (cm)	Days to first bud initiation	Days to first flower initiation	Leaf area ($cm^{2)}$	Total chlorophyll (mg g^{-1})	Fresh weight of flower (g)	Dry weight of flower (g)
Zembla	46.33	36.33	4.00	32.00	3.97	2.00	79.67	96.33	15.69	0.21	8.57	0.73
Sunny	33.33	47.33	3.00	30.67	3.25	2.33	74.00	95.00	23.82	0.22	2.14	0.32
Autumn Pink	47.67	33.67	3.00	30.00	3.31	3.17	81.00	96.33	13.32	0.16	6.84	0.53
Autumn White	59.33	62.33	6.67	39.00	2.88	4.33	81.33	104	10.95	0.26	3.97	0.37
Bangalore Button	38.67	22.33	3.33	15.67	3.56	2.17	78.33	98.00	14.39	0.21	1.43	0.14
Sport	44.33	45.33	4.33	25.00	3.64	2.83	88.00	102.00	11.85	0.26	8.72	0.74
White Prolific	50.67	30.33	4.67	27.33	3.22	3.40	78.67	91.33	10.19	0.26	2.74	0.25
Liliput	37.67	43.33	9.00	28.67	2.97	3.00	92.00	104.00	10.85	0.28	0.52	0.09
Kusum	50.67	33.67	5.00	31.67	3.19	2.93	71.00	98.33	23.09	0.21	1.44	0.19
Nilima	53.67	37.33	5.00	47.00	3.86	3.23	73.00	100.00	22.57	0.15	3.30	0.37
Diana Orange	43.33	27.67	3.33	20.33	3.28	3.83	81.33	97.00	7.19	0.21	4.58	0.41
Phyllis	32.00	53.00	3.00	34.33	3.17	2.17	72.33	88.00	10.58	0.31	1.04	0.14
Mayur	32.33	72.67	3.33	24.00	2.87	1.83	72.00	97.67	10.03	0.25	1.12	0.15
Lilia Spray	33.33	65.67	4.00	25.33	3.32	1.40	72.67	96.33	11.16	0.19	0.91	0.11
Pusa Chitraksha	44.33	48.00	4.67	32.33	3.67	2.73	105.67	121.67	14.23	0.13	1.00	0.12
S Em ±	2.67	3.69	0.83	1.17	0.07	0.36	1.41	2.18	1.33	0.018	0.31	0.007
CD@5%	7.76	10.72	2.42	3.41	0.45	1.00	4.11	6.33	3.85	0.053	0.91	0.13

Table 2: Effect of genotypes screening for quality and yield traits

Treatment	No. of flowers/ plant	No. of ray florets/ flower	Diameter of flower (cm)	Flowering duration (days)	Shelf life (days)	Flower yield/ plant(g)	Flower colour	Flower type
Zembla	15.33	282.00	8.44	30.33	6.67	126.79	White	Pompom
Sunny	41.00	118.00	5.98	25.00	3.33	84.76	White	Double Korean
Autumn Pink	22.00	143.67	7.64	28.33	4.67	149.86	Light pink	Incurve
Autumn White	40.33	178.33	6.21	23.00	3.33	155.74	White	Pompom
Bangalore Button	13.00	34.67	5.22	20.00	3.33	18.17	Yellow	Decorative
Sport	23.67	322.00	12.32	29.33	6.00	206.24	Light orange	Decorative
White Prolific	42.33	274.67	5.83	28.00	3.33	113.49	White	Decorative
Liliput	164.00	126.67	2.41	27.67	2.33	86.93	Yellow	Pompom
Kusum	52.33	68.00	7.54	19.67	1.67	76.93	Yellow	Single Korean
Nilima	58.67	153.33	7.47	22.33	2.67	194.40	Dark purple	Decorative
Diana Orange	12.33	150.00	5.54	29.00	3.33	53.26	Orange	Pompom
Phyllis	63.67	121.33	5.29	17.00	2.00	66.76	Yellow	Double Korean
Mayur	87.00	32.33	4.09	20.67	3.67	95.12	Yellow	Anemone
Lilia Spray	83.00	68.33	5.32	21.00	3.00	76.93	Red	Double Korean
Pusa Chitraksha	85.00	35.00	5.70	27.00	2.67	86.77	Dark red	Single Korean
S Em $\pm$	3.47	5.52	0.19	1.28	0.36	11.06		
CD@5%	10.08	16.02	0.55	3.73	1.06	32.11		

 **Zembla**	 **Sunny**	 **Autumn Pink**
 **White Prolific**	 **Bangalore Button**	 **Sport**
 **Kusum**	 **Nilima**	 **Diana Orange**
 **Mayur**	 **Lilia Spray**	 **Pusa Chitraksha**
 **Autumn White**	 **Liliput**	 **Physalis**

Plate: 1 Flowers photograph of screened fifteen chrysanthemum genotypes

Evaluation of *Clematis hedysarifolia* DC. for potential anticonvulsant activity in Pentylenetetrazol and Strychnine induced convulsion in albino mice

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ABSTRACT

Juice of Clematis hedysarifolia DC, has been employed in the treatment of seizures and various neurological disorders. Research objective was to examine anticonvulsant effects of different solvent extracts of leaf and stem parts of Clematis hedysarifolia DC by employing several experimental models. Anticonvulsant activity of plant extracts at dosages of 200 and 400mg/kg (administered orally) was evaluated using pentylenetetrazole (PTZ) as well as strychnine (STN)-induced seizure models in mice. Acute toxicity study indicated that LD50 exceeded 2000mg/kg in mice. Pre-treatment with the aqueous extract of Clematis hedysarifolia DC resulted in a dose-related protective effect against PTZ-induced seizures along with mortality, offering complete protection at highest dose. Extract significantly delayed onset of myoclonic jerks and shortened duration of tonic seizures in a dose-dependent fashion. In STN-induced seizure model, although extract did not prevent seizure occurrence, similar to standard diazepam, it significantly ($p<0.05$) delayed onset of seizures and extended survival period prior to death in dose-dependent manner.

Keywords: Anticonvulsant, *Clematis*, pentylenetetrazol, strychnine

INTRODUCTION Epilepsy is prevalent neurological condition that impacts individuals globally, with over 80% of cases occurring in countries with limited resources or underdeveloped healthcare systems (Beghi, 2020). Global incidence of epilepsy is estimated at 61.4 per 100,000 individuals (Fiest *et al.*, 2017). It manifests through recurring, sudden episodes characterized by consistent behavioral changes, indicating disruptions in neural function (Fisher *et al.*, 2017). Although precise cause of epilepsy is still unclear, it is believed to result from an imbalance between excitatory as well as inhibitory neurotransmission (Fokoua *et al.*, 2021). This imbalance often involves complex interactions among GABAergic, glutamatergic, as well as cholinergic systems (da Guedes, 2022). While various antiepileptic medications are available, many are associated with adverse effects, some of which can be severe or fatal. Additionally, more than 25% of patients do not respond effectively to existing antiepileptic drugs.

Therefore, there is a pressing need for identifying new, safer therapeutic agents, especially those derived from natural sources. Md. Sah *et al.* (2024) noted that tribal communities in hilly areas of Bangladesh are mainly depending on herbal treatment for their illness and primary health care. In most nations, medical plants also known as herbs, herbal remedies, pharmacologically active plants, or phyto-medicinals remain the principal source of medication. In Kashmir Valley, medicinal plants are also a major source of revenue for thousands of families (Khawaja *et al*., 2023). *Clematis hedysarifolia* DC, a member of the Ranunculaceae family, is a climbing plant commonly found in the Sahyadri hills of Maharashtra. Traditionally, its juice has been used to manage seizures and epilepsy. Among tribal communities in Maharashtra, such as Warli, Thakar, and Kokna, this plant continues to be used for treating convulsions and epilepsy without scientific substantiation. Kamble *et al.* (2009 and 2014) also reported the use of ethano-medicinal plants by the tribes of Maharashtra. Our research objective is to investigate anticonvulsant properties of *Clematis hedysarifolia* DC extracts by employing established experimental anticonvulsant models in mice (Ugwah-Oguejiofor *et al*., 2013).

MATERIALS AND METHODS

Fresh leaves along with stems of *Clematis hedysarifolia* DC are collected from area of Trimbakeshwar, District Nashik in October 2019. Plant was identified by the botanist Priyanka A. Ingle and the authentication No.isBSI/WRC/IDEN.CER/2018/112/88 (B). The plant materials were dried by natural drying method. The leaves and stems were dried under the shade of sunlight. The plant materials were pulverized in grinder as fine powder that was sieved through numbers (# 60-80). The powders of plant materials were then used for further study. Powdered plant material (500 g) underwent Soxhlet extraction with solvents including petroleum ether, ethyl acetate, butanol, and ethanol. Resulting extracts were concentrated by evaporation over a water bath, and percentage yield for each was calculated.

Healthy adult Wistar albino mice (20-25g) and Wistar albino rats (175-200g) of either sex were obtained from National Center for Cell Sciences, Pune. Animals were housed in polypropylene cages under controlled environmental conditions, maintaining temperature of 22±2°C, 12-hour light/dark cycle, along with relative humidity of 55±5%. They were provided unrestricted access to a standard pellet diet (food) (Nutrivet Life Sciences, Pune) and water *ad libitum*".

All experimental procedures were reviewed and approved by Institutional Animal Ethical Committee (IAEC) of MGV's Pharmacy College, Panchavati, Nashik, Maharashtra, India (Registration No. 121/1999/CPCSEA), operating under guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA)", Ministry of Environment and Forests, Government of India (GoI) (Certificate No. MGV/PC/CPCSEA/XXXV1/01/2019/19).

Diazepam (Valium®, Roche), "Pentylenetetrazol, Strychnine and Phenytoin (Sigma Chemical Co.), were standard chemicals" employed for the study. All extracts of *Clematis hedysarifolia* DC were analyzed for presence of saponins, alkaloids, glycosides, flavonoids, steroids, phenols, anthraquinones, as well as tannins using procedures outlined by (Trease *et al*., 2002). Quantitative phytochemical estimation was carried out following methodology reported by Narendra *et al*. (2013).

Oral acute toxicity examination was conducted for determining median lethal dose (LD50) among mice following Up and Down procedure using OECD guidelines No.425. In brief, in this method, six female albino mice were used (n=6). In the present study, Wistar albino mice were employed

and administered with different doses of two types of plant parts (leaves and stem) extracts (PE, EA, BE and EE) of *Clematis hedysarifolia* DC to test their lethality. None of the tested male Wistar albino mice died, no change in relative organ weight and there were no discernible behavioural alterations for administered extracts like PE, EA, BE and EE leaf and stem extracts in all three doses i.e. 175, 550, 2000mg/kg up to 2000mg/kg for 14 days. Data proved that the administration of PE, EA, BE and EE leaf and stem extracts from *Clematis hedysarifolia* up to the 2g/kg dose were secure to be used for our different anti-inflammatory and antiepileptic investigations throughout in vivo model, in a single administration (OECD guidelines 425). LD50>2000mg/kg was established.

Evaluation of anticonvulsant activity for all extracts of *Clematis hedysarifolia* was conducted using procedures outlined by Amabeoku (1999), Bum *et al.* (2001), Wannang *et al.* (2008), and Bum *et al.* (2011). Anticonvulsant assessment employed pentylenetetrazol (PTZ) as well as strychnine (STN) induced seizure models. For each test (PTZ and STN), a total of 50 mice were divided into 10 groups (Groups I–X) with 5 mice in each group (n=5). The sample size (n=5) was determined based on ethical guidelines to minimize animal use. Power analysis, considering the expected effect size and variability from prior studies, indicated that n=5 per group provides adequate statistical power to address the study objectives. Group I act as negative control and was given distilled water, whereas Group II functioned as positive control, receiving 5mg/kg diazepam for PTZ model or 25mg/kg for STN model. Groups III-X administered plant extracts at doses of 200mg/kg and 400mg/kg orally. Seizures were triggered by intra-peritoneal injection of 80mg/kg PTZ or 2.5mg/kg STN. Onset and duration of seizures were monitored for 30min. A mouse was considered protected if no seizures occurred during observation period. Mortality was recorded within 24hrs of administration.

Findings were indicated as "mean standard error (S.E.) of mean. Statistical analysis was conducted by employing one-way analysis of variance (ANOVA), and Dennett's post hoc test. The Kolmogorov–Smirnov test was employed to assess the normality of the data, as it is recommended for sample sizes of $n \geq 50$ and is generally preferred for medium to large datasets. Homogeneity of variances was evaluated using Bartlett's test. Data analysis was performed by employing Graph Pad Prism version-9.5.1 (Graph Pad®, San Diego, CA, USA)".

RESULTS AND DISCUSSION

Acute toxicity studies

The acute toxicity of the *Clematis hedysarifolia* extracts was evaluated in female mice at doses up to *2000 mg/kg body weight*, administered orally according to OECD guideline 425. No mortality or significant clinical signs of toxicity (such as tremors, convulsions, salivation, diarrhea, lethargy, or abnormal locomotor activity) were observed during the 14-day observation period. Body weight measurements taken on days 0, 7, and 14 showed normal weight gain compared to the control group, indicating no adverse effect on growth. Food and water intake remained within normal limits. Based on these findings, the oral LD_{50} of the extract was estimated to be greater than 2000 mg/kg, suggesting that the plant extract is relatively safe and non-toxic at the tested dose levels.

Effect of *Clematis hedysarifolia* DC on Pentylenetetrazol-induced seizure

Leaf and stem extracts of *Clematis hedysarifolia* demonstrated a dose-dependent protective effect against PTZ-induced seizures, as presented in Table 1 and 2. Comparable to diazepam, petroleum ether extracts from both plant parts completely prevented onset of PTZ-induced seizures at dosage of 400mg/kg. The extracts

significantly increased seizure latency in animals that were not fully protected and significantly shortened seizure duration compared to control group. Specifically, the extracts led to 5.7-fold rise in latency and a 4-fold reduction in seizure duration at 200mg/kg and 400mg/kg doses. Moreover, complete survival was found in groups III, IV, VI, IX, and X of leaf extract-treated animals, along with groups III, IV, and VI of stem extract group within 24-hour period.

Treatments Vs Latency to Myoclonic spasm (sec) leaves extract

Group II-VI and IX-X demonstrated significant ($p<0.0001$) rise in latency in myoclonic spasm, compared with Group I, except Group VIII ($p<0.001$) showed significant increase in myoclonic spasm and group VII showed non-significant effect as shown in Figure 1. All values were presented as mean±S.E.M. Groups II- X were compared with groupI via "One-Way ANOVA followed by Dunnett's test *p < 0.05, **p < 0.01, ***P < 0.001, **** P< 0.0001. ns non-significant in comparison with disease control.

Treatments Vs Latency to Clonic spasm (sec) leaves extract

Groups II to X exhibited a significant increase ($p<0.0001$) in latency of clonic convulsions, compared with Group I, as illustrated in Figure 2. Data is represented as mean ± S.E.M. Statistical comparisons among Groups II-X and Group I were carried out by employing One-Way ANOVA followed by Dunnett's post hoc test. *p < 0.05, **p < 0.01, ***P < 0.001, **** P< 0.0001. ns non-significant in comparison with disease control.

Treatments Vs Latency to Clonic spasm (sec) stem extracts

Groups II-X exhibited a significant increase in latency of clonic convulsions ($p<0.0001$) when compared to Group I, as illustrated in Figure 4. All data are represented as mean±S.E.M. Statistical comparisons among Groups II-X and Group I were conducted by employing One-Way ANOVA followed by Dunnett's test. *p < 0.05, **p < 0.01, ***P < 0.001, **** P< 0.0001. ns non-significant in comparison with disease control.

Treatments Vs Latency to myoclonic spasm stem extract

Groups II-VI and IX-X indicated significant rise in latency of myoclonic spasms ($p<0.0001$), while Group VIII demonstrated statistically significant rise ($p<0.001$), compared with Group I. Group VII exhibited a non-significant increase in latency. These findings are depicted in Figure 3. All values are represented as mean±S.E.M. Statistical analysis was carried out by employing One-Way ANOVA followed by Dunnett's test, with comparisons made between Groups II-X and Group I. *p < 0.05, **p < 0.01, ***P < 0.001, **** P< 0.0001. ns non-significant in comparison with disease control.

Anticonvulsant activity by using – Strychnine (STR) induced seizure model:

Leaf and stem extracts of *Clematis hedysarifolia* demonstrated a dose-dependent protective effect against STR -induced seizures, as presented in Table 3 and 4. Extract demonstrated dose-dependent protection against STR-induced seizures. At both 200mg/kg and 400mg/kg p.o., animals treated with extract, similar to those given diazepam, exhibited no convulsions. Moreover, in animals that were not completely protected, extract significantly prolonged seizure latency ($p<0.0001$) and markedly decreased seizure duration ($p<0.0001$) when compared to control group. At lower dose of 200mg/kg, extract effectively enhanced latency and" reduced duration of seizures relative to control.

Treatments Vs Latency to myoclonic and clonic spasm Leaves extract

Group II-VI and IX-X demonstrated significant ($p<0.0001$) rise in latency in myoclonic and clonic spasm when compared to Group I, except Group VIII ($p<0.001$) showed significant increase in myoclonic spasm and group VII showed non-significant

effect as indicated in figure 5 and 6. All values "were presented as mean ± S.E.M. All Groups Viz II-X are compared with groupI by employing One-Way ANOVA and Dunnett's test. *p < 0.05, **p < 0.01, ***P < 0.001, **** P< 0.0001. ns non-significant in comparison with disease control.

Treatments Vs Latency to Myoclonic spasm stem extract

Groups II-VI and IX-X exhibited a significant increase in latency to myoclonic spasms (p<0.0001) compared to Group I. Group VIII also demonstrated statistically significant rise in latency (p<0.001). These findings are illustrated in Figure 7. All values are presented as mean± S.E.M and comparisons among Groups II-X and Group I were assessed by employing One-Way ANOVA and Dunnett's test.

Treatments Vs Latency to Clonic spasm stem extract

Groups II to VI and VIII to X indicated significant rise in latency to clonic spasms (p<0.001) when compared with Group I. Group VII also demonstrated significant rise in latency (p<0.05). These observations are presented in Figure 8. All "data are presented as mean±S.E.M. and comparisons of Groups II to X with Group I were carried out by employing One-Way ANOVA and Dunnett's test. One-Way ANOVA followed by Dunnett's test. *$p < 0.05$, **$p < 0.01$, ***$P < 0.001$, **** $P< 0.0001$. ns non-significant in comparison with disease control. Current investigation assessed the anticonvulsant potential of Petroleum ether, Ethyl acetate, Butanol, along with Ethanol extracts derived from leaves as well as stem of *Clematis hedysarifolia* DC in mice subjected to chemically induced seizures. Extracts displayed dose-dependent protective impacts in PTZ as well as STZ-induced seizure models. Among tested extracts, LPE exhibited most pronounced anticonvulsant activity compared to others. PTZ and STZ are recognized as convulsant agents commonly utilized for screening antiepileptic drugs (Brodie *et al.,* 2016). PTZ provokes seizures primarily through inhibition of Gamma-aminobutyric acid (GABA) pathway at GABAA-receptors (Salaudeen *et al.*, 2022) which play a key inhibitory role in central nervous system and are critical in epilepsy. Additionally, action of PTZ involves glutamatergic mechanisms (Chindo *et al*.,2014) particularly via activation of N-methyl-D-aspartate (NMDA) receptor, which contributes to initiation and propagation of PTZ-induced convulsions (Parmar *et al*., 2022). Therefore, agents that counteract PTZ-induced seizures are believed to enhance GAB Aergic transmission or inhibit NMDA receptor-mediated glutamatergic pathways (Ofokansi *et al.,* 2022). Substances that suppress PTZ-evoked seizures in mice are believed to block T-type calcium channels (Son *et al*., 2014) and such agents are effective against myoclonic and absence seizures in clinical practice (Ofokansi *et al*., 2022; Rahimi *et al.,* 2019). In present study, extracts provided full "protection against PTZ-induced convulsions, showing results similar to positive control, diazepam. Diazepam exerts its anticonvulsant effects by facilitating GABA-mediated neural inhibition" (Sarfo *et al*. 2022; Rang *et al*., 2000). Based on observed findings, it is plausible that the anticonvulsant effect of *Clematis hedysarifolia* extracts could be attributed to their role in enhancing GABAergic activity and/or suppressing NMDA receptor-mediated glutamatergic transmission. Consequently, these extracts may prove beneficial in managing clonic and myoclonic seizure types. However, further investigation is essential to clearly elucidate underlying mechanisms involved (Malami *et al*., 2016). CONCLUSIONS Findings of current study indicate that all extracts of *Clematis hedysarifolia* DC exhibit anticonvulsant activity in experimental seizure models. The observed anti-seizure effects may be attributed to the presence of secondary metabolites within the extracts. These outcomes offer scientific support for traditional use of *Clematis hedysarifolia* DC in managing epilepsy. The present study provides *in vivo* pharmacological evidences

for antiepileptic potential of secondary metabolites like saponine, steroids, volatile oils etc. present in Indian traditional plant *Clematis hedysarifolia DC* act as cheapest sources for multi-target agent with immense antiepileptic potentials.

List of Abbreviations: LPE: Leaf Pet ether extract; **LBU:** Leaf Butanol extract; **LEA**: Leaf Ethyl acetate **LET**: Leaf Ethanol Extract; **SPE:** Stem Pet ether extract; **SBU**: Stem Butanol extract; **SEA**: Stem Ethyl acetate extract; **SET**: Stem Ethanol extract; **PTZ**: Pentylenetetrazol; **STZ**: Strychnine; **NMDA**: N-methyl-D-aspartate receptors; **GABA**: Gamma- amino butyric acid; **Ca_2+:** Calcium; **CPCSEA:** Committee for the Purpose of Control and Supervision of Experiments on Animals; **IAEC:** Institutional Animal Ethical Committee; **ANOVA**: Analysis of Varian.

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: Anticonvulsant activity of *Clematis hedysarifolia* DC leaves extracts on PTZ induced seizure Drug effect on Latency to Myoclonic Spasm and Clonic spasm.

Group	Treatment	Dose mg/kg Orally	Mean Duration of Myoclonic Spasm +/- SEM(s)	Mean Duration of Clonic Spasm +/- SEM(s)	No of Animals Recovered	Protection against Mortality (%)
I	Control	PTZ 80 mg/kg +Water(10ml /kg)	30.06 ± 3.2	43.38 ± 4.03	0/5	0%
II	Diazepam + PTZ	Diazepam 1 and PTZ 80	290.83 ± 3.93	480.90 ± 2.08	5/5	100 %
III	SPE+PTZ	200	178.03 ± 1.08	220.70 ± 3.15	5/5	100 %
IV	SPE+PTZ	400	220.80 ± 3.19	333.30 ± 6.08	5/5	100 %
V	SEA+PTZ	200	75.17 ± 2.90	101.72 ± 1.02	2/5	60 %
VI	SEA+PTZ	400	117.30 ± 3.19	143.10 ± 2.87	5/5	100 %
VII	SBU+PTZ	200	35.17 ± 2.90	78.72 ± 1.02	3/5	40 %
VIII	SBU+PTZ	400	50.73 ± 6.18	97.43 ± 2.41	3/5	40 %
IX	SET+PTZ	200	65.18 ± 5.32	126.87 ± 2.71	1/5	80 %
X	SET+PTZ	400	105.03 ± 1.08	135.70 ± 3.15	5/5	100 %

Table 2: Anticonvulsant activity of *Clematis hedysarifolia* DC Stem extracts on PTZ induced seizure Drug effect on Latency to Myoclonic Spasm and Clonic spasm

Group	Treatment	Dose mg/kg Orally	Mean Duration of Myoclonic Spasm +/- SEM(s)	Mean Duration of Clonic Spasm +/- SEM(s)	No of Animals Recovered	Protection against Mortality (%)
I	Control	PTZ 80 mg/kg +Water(10ml/kg)	30.06 ± 3.2	43.38 ± 4.03	0/5	0 %
II	Diazepam + PTZ	Diazepam 1 and PTZ 80	290.83 ± 3.93	480.90 ± 2.08	5/5	100 %
III	LPE+PTZ	200	209.03 ± 1.08	270.70 ± 3.15	5/5	100 %
IV	LPE+PTZ	400	253.80 ± 3.19	396.30 ± 6.08	5/5	100 %
V	LEA+PTZ	200	75.17 ± 2.90	101.72 ± 1.02	4/5	80 %
VI	LEA+PTZ	400	117.30 ± 3.19	143.10 ± 2.87	5/5	100 %
VII	LBU+PTZ	200	35.17 ± 2.90	97.72 ± 1.02	2/5	40 %
VIII	LBU+PTZ	400	50.73 ± 6.18	78.43 ± 2.41	3/5	60 %
IX	LET+PTZ	200	123.18 ± 5.32	143.87 ± 2.71	5/5	100 %
X	LET+PTZ	400	150.03 ± 1.08	196.70 ± 3.15	5/5	100 %

Table 3: Anticonvulsant activity of *Clematis hedysarifolia* DC leaves extracts on STR induced seizure Drug effect on Latency to Myoclonic Spasm and Clonic spasm

Group	Treatment	Dose mg/kg Orally	Mean Duration of Myoclonic Spasm +/- SEM(s)	Mean Duration of Clonic Spasm +/- SEM(s)	No of Animals Recovered	Protection against Mortality (24hr) (%)
I	Control	STR 1mg/kg +Water(10ml/kg)	35.06 ± 3.1	48.28 ± 5.03	0/5	0%
II	Diazepam + STR	Diazepam 1 and STR 1mg/kg	460.83 ± 3.43	680.90 ± 4.08	5/5	100 %
II	LPE+STR	200	230.03 ± 2.08	310.70± 5.15	5/5	100 %
IV	LPE+STR	400	397.80 ± 3.50	520.30± 6.08	5/5	100 %
V	LEA+STR	200	85.17 ± 2.80	125.72± 5.02	5/5	100%
VI	LEA+STR	400	147.30 ± 3.19	183.10± 6.75	5/5	100 %
VII	LBU+STR	200	65.17 ± 2.85	112.72± 4.02	5/5	100 %
VIII	LBU+STR	400	102.73 ± 5.28	123.42 ±5.31	5/5	100 %
IX	LET+STR	200	165.18 ± 5.42	176.87± 4.61	5/5	100 %
X	LET+STR	400	264.03 ± 2.08	225.70± 5.20	5/5	100 %

Table 4: Anticonvulsant activity of *Clematis hedysarifolia* DC Stem extracts on STR induced seizure Drug effect on Latency to Myoclonic Spasm and Clonic spasm

Group	**Treatment**	**Dose mg/kg Orally)**	**Mean Duration of Myoclonic Spasm +/- SEM(s)**	**Mean Duration of Clonic Spasm +/- SEM(s)**	**No of Animals Recovered**	**Protection against Mortality (%)**
I	Control	STR 1mg/kg +Water(10ml/kg)	45.06 ± 4.5	83.38 ± 4.03	0/5	0%
II	Diazepam + STR	Diazepam 1 and STR 1mg/kg	463.83 ± 5.93	681.90 ± 5.08	5/5	100 %
III	SPE+STR	200	215.03 ± 5.08	298.70 ± 4.35	5/5	100 %
IV	SPE+STR	400	365.73 ± 5.29	502.25 ± 6.08	5/5	100 %
V	SEA+STR	200	81.17 ± 5.90	132.72 ± 5.22	5/5	100%
VI	SEA+STR	400	132.30 ± 4.19	162.10 ± 5.27	5/5	100 %
VII	SBU+STR	200	54.57 ± 5.20	104.62 ± 6.12	5/5	100 %
VIII	SBU+STR	400	98.73 ± 6.28	118.43 ± 4.41	5/5	100 %
IX	SET+STR	200	142.18 ± 5.32	156.87 ± 5.51	5/5	100 %
X	SET+STR	400	204.03 ± 4.08	258.70 ± 4.35	5/5	100 %

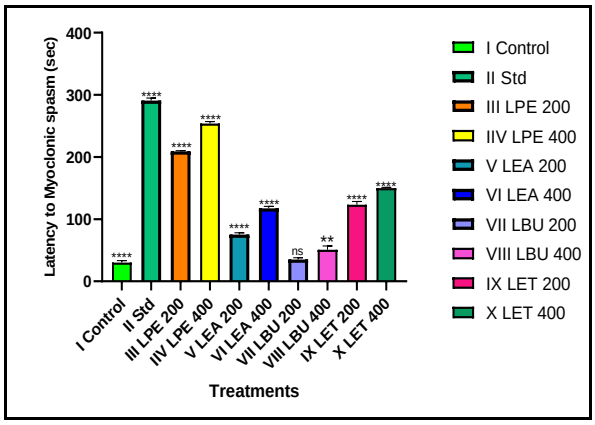

Figure 1: Effect of *Clematis hedysarifolia* leaf extracts on on PTZ induced Myoclonic spasm seizure model

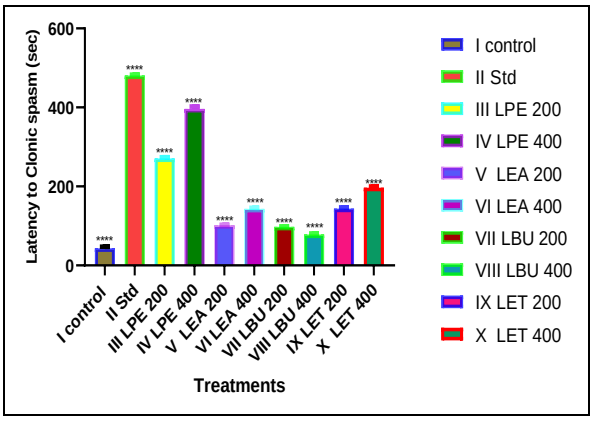

Figure 2: Effect of *Clematis hedysarifolia* leaf extracts on PTZ induced clonic spasm seizure model

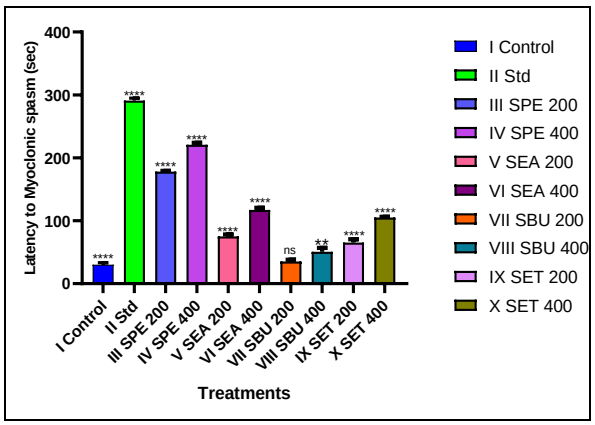

Figure 3: Effect of *Clematis hedysarifolia* stem extracts on on PTZ induced Myoclonic spasm seizure model

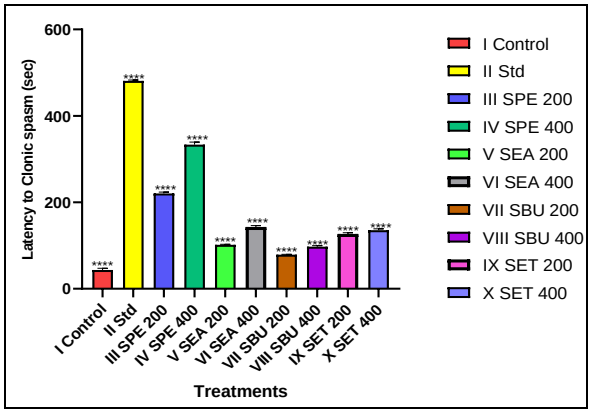

Figure 4: Effect of *Clematis hedysarifolia* stem extracts on PTZ induced clonic spasm seizure model

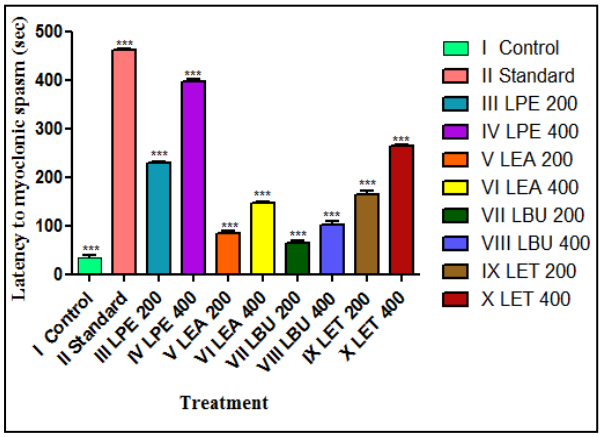

Figure 5: Effect of *Clematis hedysarifolia* leaf extracts on on STR induced Myoclonic spasm seizure model

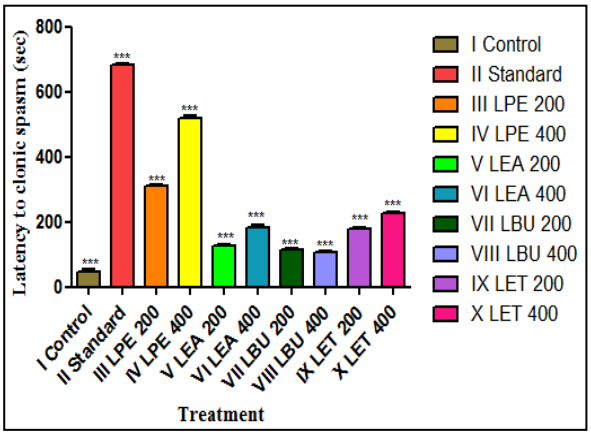

Figure 6: Effect of *Clematis hedysarifolia* leaf extracts STR induced clonic spasm seizure model

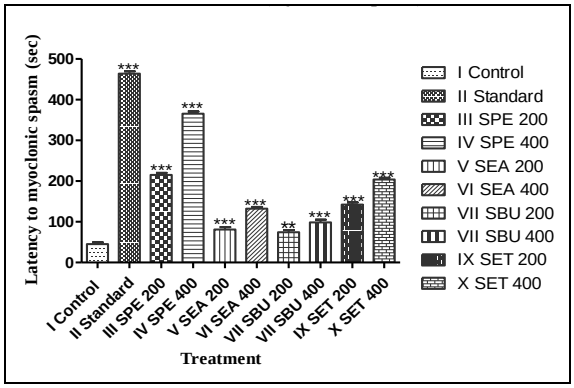

Figure 7: Effect of *Clematis hedysarifolia* stem extracts on on STR induced Myoclonic spasm seizure model

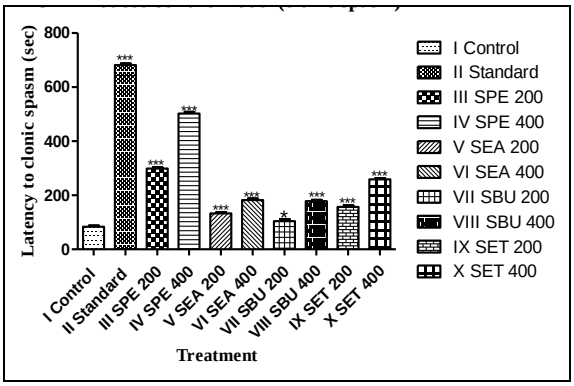

Figure 8: Effect of *Clematis hedysarifolia* stem extracts STR induced clonic spasm seizure model

Consumer acceptability and sensory profiling of Rhododendron cider vinegar

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ABSTRACT

Rhododendron arboreum (Burans) was used to develop a value-added cider vinegar infused with mint and basil to enhance flavour and health benefits. Sensory evaluation and consumer acceptability were assessed through hedonic, JAR, and CATA tests with 400 respondents. Results revealed significant differences among samples ($F = 91.15$, $p < 0.001$). The 12% basil-infused vinegar (B3) achieved the highest overall liking (5.56 ± 1.25), while the 12% mint–basil blend (C3) was least accepted (3.58 ± 1.01). Consumers associated positive attributes such as good aroma, taste, and appearance, while negative feedback included offensive odour and dull colour. Overall, basil-infused rhododendron vinegar demonstrated high acceptance, with aroma and colour being key drivers of preference. According to these findings, rhododendron cider vinegar has a great chance of becoming a commercial product and being well-liked by consumers in a variety of market niches, especially when it is infused with basil at the right amounts.

***Keywords**: CATA, JAR, consumers, sensory evaluation vinegar,*

INTRODUCTION

Vinegar, derived from the French word *vinaigre* (sour wine), is a non-alcoholic beverage valued for its therapeutic properties. It is produced through double fermentation of starch- or sugar-rich agricultural resources, yielding acetic acid, which is linked to health benefits such as lowering blood pressure, aiding weight management, reducing inflammation, and providing antibacterial effects (Shah and Banerjee, 2013). Fermentation also enhances the nutritional value and bioavailability of phenolic antioxidants. Globally, various vinegars, *viz*., apple cider, fruit, wine, balsamic, and malt are produced, with processing methods significantly affecting quality. In India, where 80% of the population relies on plant-based remedies, fermenting fruits, flowers, and herbs into functional vinegars is gaining popularity. *Rhododendron arboreum*, the state flower of Uttarakhand and national flower of Nepal, grows at elevations of 1500–3300 m and is known for its medicinal properties (Bisht *et al*., 2023). Medicinal plants are defined as having active ingredients used in herbalism or used in drug development and synthesis (Ariyawansha *et al*., 2024). Medicinal plants, widely used in traditional and modern healthcare, contain bioactive compounds that can treat ailments ranging from digestive disorders to metabolic and osteoarticular problems (Maryama *et al.,* 2025).

Consumer acceptance is the crucial for the success of any value added products. Sensory analysis provides insights into consumer preferences, guiding product development and positioning (Ruiz-Capillas *et al*., 2021). Rapid sensory profiling methods, such as Check-All-That-Apply (CATA), Just-About-Right (JAR) scales, and hedonic testing, are increasingly used due to their efficiency and reliability. CATA, in particular, has been applied successfully across diverse food products, offering a simple yet powerful tool to capture consumer perceptions (Lee, 2021). JAR scales

complement hedonic ratings by identifying product attributes that are "too much" or "too little," enabling targeted improvements (Gera, 2017). Penalty analysis further quantifies how these attributes influence overall liking.

MATERIALS AND METHODS

An interviewer-administered questionnaire was used to collect information on food quality characteristics of Vinegar sample. A well-structured questionnaire comprising of 9 points hedonic test, Just About Right test (JAR) and Check-All That-Apply test (CATA) was administered to 400 consumers**.**

Product development

Indigenous variety of Rhododendron *arboreum* flowers was procured from the Rudrprayag district, Uttarakhand (Garhwal) while mint and basil were collected from the local market of Jaipur, Rajasthan. All the collected materials were thoroughly cleaned and washed with the help of distilled water, to remove dirt and pests. Extraction of *Rhododendron arboreum* flower juice was done by cold-pressing technique that is used by the processor to overcome the problem of thermo-sensitive phytochemical properties that get affected and to provide a wholesome product to the consumers.

After that, the juice's sugar content was brought down to 10 °Brix before adding of *Saccharomyces cerevisiae* yeast. The most common and practical method used is controlled dilution with water or low-sugar juice. First, the initial sugar level of the juice is accurately measured using a calibrated refractometer. Based on this value, the amount of water required for dilution is calculated using the dilution formula $C_1V_1 = C_2V_2$, where $C_1$and $C_2$are the initial and desired °Brix values, and $V_1$and $V_2$are the initial and final volumes, respectively. Theen, filtered water is then added gradually to the juice under hygienic conditions while stirring continuously to ensure uniform mixing. The °Brix is rechecked after mixing, and adjustments are made if necessary to achieve the target 10 °Brix. Alcoholic fermentation was then conducted for five to ten days at 26 ± 2 °C. Following this phase, acetic acid fermentation was carried out by inoculating the fermenting medium with *Acetobacter aceti*, a bacterium that produces acetic acid, and keeping it at 26–29 °C for three to four weeks. The vinegar was fermented, clarified, bottled, and pasteurized for 30 to 40 minutes at 75 to 80 °C. The finished product had a six-month shelf life and was kept in glass bottles at ambient temperature (15–25 °C) or in a refrigerator (4–7 °C) for preservation.

During the study, three different variants of value-added vinegar were prepared using Rhododendron as the base ingredient, with the incorporation of mint and basil in varying proportions. **Variant 1** was developed by adding mint at three different concentrations (8%, 10%, and 12%) to Rhododendron. **Variant 2** involved the addition of basil at similar concentrations (8%, 10%, and 12%) to Rhododendron. **Variant 3** was formulated by combining both mint and basil, each added at 8%, 10%, and 12% levels, to the Rhododendron base.

Consumers Acceptability Tests (CAT)

Rhododendron cider vinegar made from rhododendron flower pulp. Extraction of Rhododendron juice was done by cold-pressing technique that is used by the processor to overcome the problem of thermo-sensitive phytochemical properties and was tested for consumer acceptance with 400 informed participants. Using a convenience selection technique with purposive criteria, respondents were chosen to ensure that only Dehradun-based adults (18-50 years) who were acquainted with *Rhododendron arboreum* flowers, food and beverage items, and potential users of functional drinks were included. Nine coded samples were evaluated using three successive tests, firstly a 9-point hedonic scale (1 = extremely dislike, 9 = extremely like) was used for overall liking. Before the 9-point hedonic sensory exam, each participant received comprehensive information on the study's goals, methods, and possible risks. Participants were made aware that participation was completely optional and that there would be no repercussions if they decided to stop at any

moment. Before the sensory test began, each participant provided written informed consent. To preserve participant anonymity, data were evaluated collectively and individual responses were guaranteed confidentiality. Panel members were screened for absence of allergies or aversions to vinegar and for their willingness to participate in sensory evaluations. Nine coded samples were served in identical cups at room temperature with water available to wash the palate in between samples, and they were evaluated under carefully monitored serving settings. To prevent positional bias, samples were served in a randomized order. After giving informed consent, received a brief explanation of methods and then completed a two-step questionnaire i.e. a 5-point JAR scale (1 = much too little, 3 = just right, 5 = much too much) to assess attribute intensity, and a CATA test with 11 sensory descriptors (Meyners *et al.,* 2016). First, they rated overall liking; second, they identified characteristics using CATA and judged their appropriateness with JAR. A descriptor list was provided for clarity, and finally, panelists compiled essential traits of an "ideal" Rhododendron Vinegar. Further, The Just-About-Right (JAR) evaluation was followed by a penalty analysis to ascertain how variations in sensory qualities affected consumers' overall satisfaction with the product. The JAR scale determines whether an attribute is viewed as too low, just right, or too high, but it doesn't show how much these differences affect customer approval. When an attribute is assessed as "too low" or "too high" in comparison to the "just right" category, the mean decrease in overall liking is calculated. This is how penalty analysis tackles this issue.

Data analysis

In order to generate frequencies and to analysed the data using descriptive statistics including percentages, frequencies, charts and Correlation analysis to better understand the interrelationships among sensory attributes, a correlation analysis was conducted between overall liking (9-point hedonic scale) and the key attributes evaluated in the JAR test (aroma, colour, taste, sourness, and after-mouth taste) from the Statistical Package for Social Sciences (SPSS version 16.0) and Excel statistical package (XLSTAT).

RESULTS AND DISCUSSION

Consumer's Acceptability Test Using 9-point Hedonic Test,

Overall liking was significantly impacted by sample type, according to the analysis of variance ($F = 91.15$, $p<0.001$). The results of the post hoc Tukey's HSD test showed that sample B3 (5.56 ± 1.25^{e}) outperformed all other treatments and had the highest customer acceptability. Although sample AX (5.05 ± 1.11^{d}) had a good score as well, it was still much lower than B3. Samples C1 (3.70 ± 1.14^{a}) and C3 (3.58 ± 1.01^{b}), however, obtained the lowest scores, suggesting a decrease in customer preference. The majority of A-series and B-series samples (A1, A2, A3, B1, B2, C2, and C3) clustered within an intermediate acceptance group, showing no significant differences among them. These findings suggest that formulation B3 was the most preferred by the panel, while C-series samples tended to be less favoured as shown in (Table 1).

Check-All-That-Apply (CATA) test

The Check-All-That-Apply (CATA) test identified both positive and negative attributes of the preferred sample (B3). Favourable qualities included good aroma (100%), neatness (84%), good taste (83.5%), good appearance (81%), slightly sour (62.5%), and fermented odour (60%). Negative descriptors were offensive odour (74.75%), dark/dull colour (71.75%), no taste (69%), sour (56.25%), and turbid appearance (53.75%) represented in (Table 2, 3) Overall, B3 vinegar showed desirable sensory traits such as a pleasant aroma, neat appearance, balanced sourness, and mild fermented smell, highlighting its potential as a refreshing and functional beverage (Osunbade *et al*., 2021).

Just About Right (JAR) test

Just-About-Right (JAR) test further confirmed B3 variant as the most preferred

sample by (77.00 %) consumers in terms of aroma and colour (77.00%), (75.0 %) in terms of taste, (70.5%) in terms of after mouth taste and (60.25%) in terms of sourness (Figure 1, 2, 3, 4). JAR test showed that B3 sample was most preferred variant in terms of aroma, colour, taste. It was in accordance with the 9-point hedonic results that scored B3 variant highest.

Penalty analysis

Penalty analysis was performed to identify how deviations from the "Just-About-Right" (JAR) level of sensory attributes affected overall liking of the Rhododendron cider vinegar. The analysis revealed that for aroma, 77% of panelists perceived it as "too little," resulting in a mean drop in liking from 5.597 to 5.467, with a significant penalty effect ($p<0.001$), indicating that insufficient aroma negatively impacted overall acceptance (Table 4). Similarly, for colour, 77% of respondents rated it as "too little," leading to a comparable decrease in mean liking from 5.467 to 5.597 ($p<0.001$), suggesting colored samples were less preferred. Regarding taste, 16.75% of panelists found it "too much" and 6.25% "too little," producing minor mean drops (5.578–5.448) and non-significant penalties ($p< 0.05$), indicating taste deviations had less impact on overall liking. For sourness, 57.5% of participants rated it as "too little," leading to a reduction in mean liking from 5.617 to 5.500 ($p <0.001$), highlighting that insufficient sourness reduced consumer preference. Finally, after-mouth taste showed mixed responses, with 70.5% perceiving it as "too little" and 12.25% as "too much," generating a mean drop from 5.610 to 5.306 ($p = 0.030$), indicating that an inadequate lingering taste negatively affected acceptance as mentioned in Table 4. Overall, penalty analysis identified aroma, colour, sourness, and after-mouth taste as key attributes where deviation from the ideal JAR level significantly decreased consumer liking. Properly stored vinegar can maintain its physicochemical properties and antioxidant activity for 12–24 months, although minor changes in color, aroma, and flavor may occur during prolonged storage. To maximize shelf life, vinegar should be stored in airtight, dark containers at ambient or slightly cool temperatures, avoiding direct sunlight and contamination. Regular monitoring of pH, acidity and sensory attributes can help ensure product quality and consumer acceptability throughout its shelf life.

Correlation analysis

The relationships between the sensory elements were further examined by comparing overall liking with the sensory qualities evaluated by the JAR test (aroma, color, taste, sourness, and after-mouth taste). High positive correlations were found between all sensory attributes and overall liking as mentioned in Table 5, with coefficients ranging from $r = 0.950$ to $r = 0.959$ ($p \leq 0.01$). Among these, sourness ($r = 0.959$) and aroma ($r = 0.955$) were most highly linked to overall-liking, suggesting that a balance between sourness and pleasant aroma significantly influenced consumer acceptance. Additionally, the sensory qualities themselves had a good association ($r > 0.99$ in most cases), indicating that consumers evaluated aroma, color, taste, sourness, and after-mouth taste holistically rather than separately. According to this study, enhancing one attribute, such as scent, is likely to simultaneously improve aftertaste and flavor perceptions, which will raise total consumer satisfaction. There are strong correlations between sensory attributes and overall preference, which is consistent with earlier studies on functional beverages that found aroma and flavor balance to be significant acceptability determinants. Studies on vinegars made from fruits and flowers, where acidity is a determining factor in customer preference, are consistent with the prevalence of sourness as a primary determinant. The strong connections between aroma, color, taste, and after-mouth taste further demonstrate that consumers evaluate product quality holistically rather than attribute-by-attribute. This highlights how important it is to simultaneously improve multiple sensory components while creating new products to boost customer satisfaction.

Consistent with findings from earlier research, the current study showed that rhododendron-based functional meals had a strong taste appeal and possible health advantages because of its bioactive ingredients. Similar studies on *Rhododendron arboreum* flower extracts added to yogurt and drinks, for example, demonstrated increased antioxidant activity and improved sensory qualities, which are consistent with the increases in overall liking as seen in the investigation made by Postolache *et al*. (2023). Previous studies showing that vinegars derived from flowers or fruits maintain a considerable phenolic content and the ability to scavenge free radicals throughout storage are consistent with the reported antioxidant activity in the generated vinegar products (Antoniewicz *et al.,* 2021).

CONCLUSION

Collectively, the findings suggest that optimizing basil infusion levels can support the development of Rhododendron vinegar as a novel, functional, and commercially viable product with strong consumer appeal. Further research on nutritional profiling, long-term stability, and market feasibility is recommended to facilitate product scaling and commercialization.

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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Table 1: Mean Overall liking based on 9-point hedonic scale

Sample code	AX	A1	A2	A3	B1	B2	B3	C1	C2	C3	F-value
Overall Liking	5.05 ±1.11^{d}	4.27± 1.60^{bc}	4.53±1 .26^{c}	4.08± 1.00^{b}	4.24± 1.09^{b}	4.24±1. 27^{b}	5.56±1 .25^{e}	3.70± 1.14^{a}	4.19±1 .45^{b}	3.58±1. 01^{b}	91.15

Results are expressed as mean ± S.D. F- Values are significant at P ≤ 0.001. Values with the same letters are not significantly different from each other, while different letters indicate a significantly different. AX- Standard *Rhododendron* vinegar, A1- 8% mint infused+ *Rhododendron* vinegar, A2- 10% mint infused+ *Rhododendron* vinegar, A3- 12% mint infused+ *Rhododendron* vinegar, B1- 8% basil infused+ *Rhododendron* vinegar, B2- 10% basil infused+ *Rhododendron* vinegar, B3- 12% basil infused+ *Rhododendron* vinegar, C1- 8% basil + 8% mint each+ *Rhododendron* vinegar, C2- 10% mint + 10% basil+ *Rhododendron* vinegar and C3- 12% mint+ 12% basil+ *Rhododendron* vinegar

Table 2: Good descriptors of vinegar sample using CATA test for the most preferred variety *i.e.,* B3= 12% basil infused R. arboreum vinegar

Attributes	Respondents	Rank
Good aroma	100%	1
Neat	84%	2
Good taste	83.5%	3
Good appearance	81%	4
Slightly sour	62.5%	5
Fermented odour	60%	6

Table 3: Bad descriptors of vinegar sample using CATA test for the most preferred variety i.e. B3= 12% basil infused R. arboreum vinegar

Attribute	Respondent	Rank
Offensive odour	74.75%	1
Dark/dull colour	71.75%	2
No taste	69%	3
Sour	56.25%	4
Turbid appearance	53.75%	5

Table 4: Penalty analysis (product B3)

Variables	Level	%	Sum (Overall-liking)	Mean liking	Mean drop	Penalties	p-value
Aroma	Too much aroma JAR Too little aroma	0.00% 77.0% 23.00%	 1724.00 503	 5.597 5.467	 0.130	-	0.000
Colour	Too much colour JAR Too little colour	23.00% 77.00% 0.00%	 503.00 1724.00	 5.467 5.597	 0.130	-	0.000
Taste	Too much taste JAR Too little taste	16.75% 77.0% 6.25%	365.00 1718.00 144.00	5.448 5.578 5.760	0.130	-	0.762
Sourness	Too much sour JAR Too little sour	0.00% 42.50% 57.50%	 935.00 1292.00	 5.500 5.617	 0.117	-	0.000
After-mouth taste	Too much after-mouth taste JAR Too little after-mouth taste	12.25% 70.50% 17.25%	260 1582 385	5.306 5.610 5.580	0.304 0.030	0.144	0.296

Table 5: Correlation coefficients between overall liking and sensory attributes of basil infused Rhododendron vinegar (B3)

Attribute	Overall Liking	Aroma	Colour	Taste	Sourness	After taste
Overall Liking	**1.000**	0.955	0.951	0.950	0.959	0.947
Aroma	0.955	**1.000**	0.998	0.992	0.992	0.994
Colour	0.951	0.998	**1.000**	0.995	0.997	0.997
Taste	0.950	0.992	0.995	**1.000**	0.994	0.999
Sourness	0.959	0.992	0.997	0.994	**1.000**	0.996
Aftertaste	0.947	0.994	0.997	0.999	0.996	**1.000**

Results are expressed as Pearson's correlation coefficients (r). All correlations are significant at $p \leq 0.01$. Values close to 1.0 indicate strong positive correlations between attributes

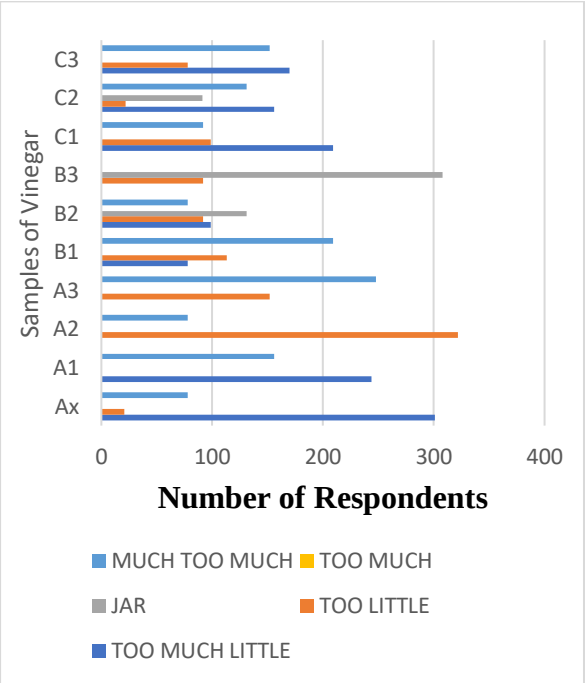

Figure 1: JAR scale for Aroma

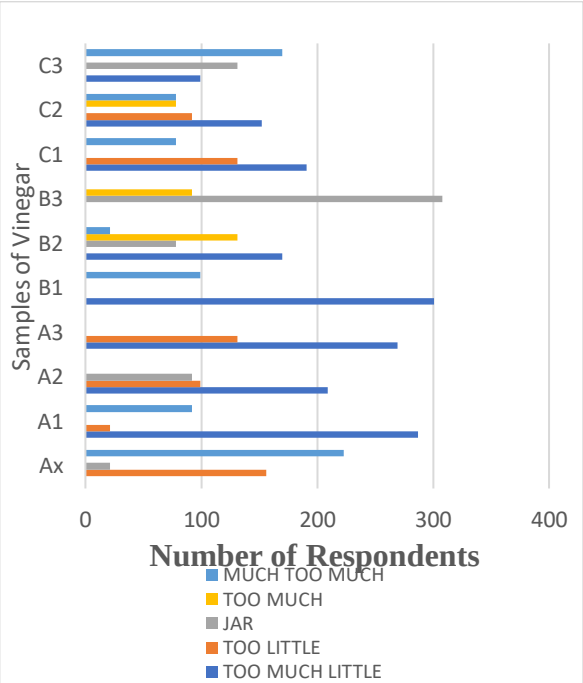

Figure 2: JAR scale for Colour

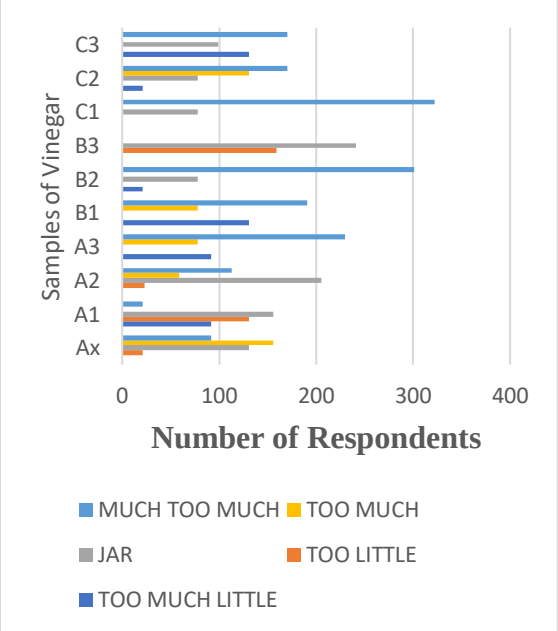

Figure 3: JAR scale for taste

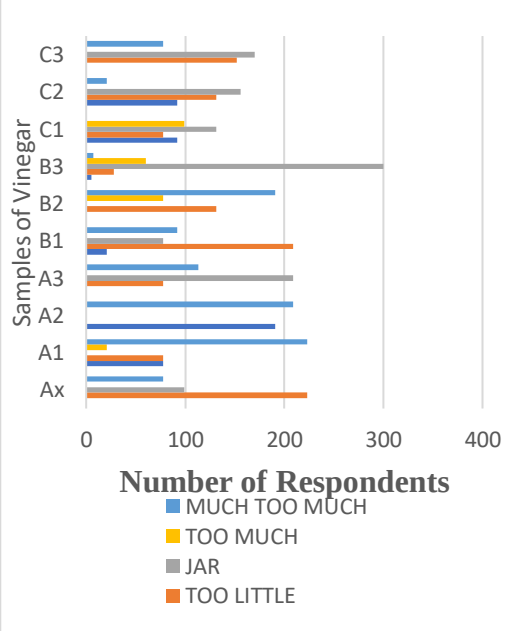

Figure 4: JAR scale for Sourness

Pharmacogenetic properties of *Mentha spicata* L. leaves and isolation of L-Carvone from its leaves

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ABSTRACT

The present study aimed to explore the phytochemical constituents, antioxidant properties, and characterization of M. spicata L. leaves, with a focus on isolating and identifying L-Carvone. Phytochemical screening was performed using the condensation method, while extracts were prepared with solvents of increasing polarity. Qualitative analysis of phytochemicals was carried out on different solvent extracts, whereas quantitative estimation and antioxidant assays were performed on the hydroethanolic (50:50) extract. GC-MS and FT-IR analyses were used for characterization, and Soxhlet extraction followed by column chromatography enabled isolation of compounds. TLC and HPLC confirmed the presence of L-Carvone. Results indicated that the hydroethanolic extract was rich in steroids and exhibited strong antioxidant activity, with GC-MS, FT-IR, and chromatographic methods confirming L-Carvone in the isolated fraction.

Keywords: Analytical standardization, isolation, L-Carvone, phytochemistry, spearmint

INTRODUCTION

Spearmint (*Mentha spicata* L.), a member of the Lamiaceae family rich in essential oils (Amel *et al*., 2022), is widely cultivated and valued for its antifungal, antimicrobial, insecticidal, antioxidant, and therapeutic properties (Kumar *et al*., 2023), including treatment of fever, bronchitis, gastritis, headaches, and nausea (Choudhury *et al*., 2006). With restrictions on synthetic antioxidants due to health concerns (Gomez, 2003; Mitra *et al*., 2009), natural sources like mint are important antioxidants. Often the fresh leaves of *M. spicata* L., are used as condiments (Hasan Jone *et al*., 2022). Peppermint (*Mentha* × *piperita*), a hybrid of *M. spicata* and *M. aquatica*, also shows potential in cardiovascular disorders (Nagarajan and Doss, 2023). The main constituent of spearmint oil is L-Carvone, along with limonene (Snoussi *et al*., 2015), responsible for its aroma and strong antimicrobial activity (Scherer *et al*., 2013). Since spearmint oil contains at least 28 organic compounds (Diaz-Maroto *et al*., 2003), rationalizing its traditional use requires focused studies. This work was aimed to isolate L-Carvone using Soxhlet extraction followed by column chromatography, with TLC, FTIR, and HPLC to confirm its presence. Our microscale method reduced solvent and silica gel use and employed FTIR and HPLC as confirmatory techniques.

MATERIALS AND METHODS

All analytical-grade chemicals and reagents were procured from HiMedia Laboratories Pvt. Ltd., India. Leaves of spearmint were collected from farms in Coimbatore, authenticated by the Botanical Survey of India, Southern Regional Centre

(BSI/SRC/5/23/2021/Tech/137). Fresh leaves were washed, shade-dried (5–10 days), powdered, and extracted with solvents of increasing polarity (petroleum ether, acetone, chloroform, ethanol, hydroethanol 50:50, and water) using cold maceration (10 g/100 mL, 72 h). Filtrates were concentrated, lyophilized, and stored for analysis.

Qualitative and quantitative analysis

Hydroethanolic (50:50) extracts of spearmint leaves were concentrated at 45 °C and stored at –20 °C. Screening confirmed carbohydrates, proteins, alkaloids, flavonoids, glycosides, terpenoids, phenols, tannins, saponins, steroids, and fats. Quantitative assays estimated carbohydrates, proteins, phenols, flavonoids, steroids, and tannins (Sugumar *et al*., 2019).

Antioxidant analysis

Antioxidant potential was evaluated by DPPH dot-blot, DPPH, nitric oxide, and hydroxyl radical scavenging assays. Enzymic assays included catalase, SOD, and glutathione peroxidase, while non-enzymic antioxidants measured were ascorbic acid (Omaye *et al*., 1979), tocopherol, and reduced glutathione. Total antioxidant capacity was assessed by reducing power, phosphomolybdenum, and FRAP (Jethinlalkhosh *et al*., 2016) determinations.

Characterization

Phytocomponents were analyzed by GC-MS (Shimadzu QP2010, Elite-1 fused silica column, 100% dimethyl polysiloxane) and FTIR (Shimadzu spectroscope, 400–4000 cm^{-1}, 16 cm^{-1} resolution) (Nagarajan and Victor Arokia, 2024).

Soxhlet extraction

Mint leaf powder (10 g) was extracted in a 250 mL Soxhlet using ethanol (99.9%), which gave higher yield than hydroethanol. Extraction with 100 mL ethanol at 78 °C for 1.5 h produced a crude extract, which was filtered (Whatman No.1), concentrated on a hot water bath, weighed, and stored in amber vials at –8 °C (Alelign *et al*., 2020).

Column, TLC, and HPLC

L-Carvone isolation was carried out by Elmastas (2006) method with modifications. Crude extract (2 g) was fractionated on silica gel (50 g) using solvent mixture (benzene:toluene:ethyl acetate:ethanol, 3.5:2.5:2.5:1.5), which yielded L-Carvone fractions. TLC (silica gel 60F254, Merck) separation using toluene:ethyl acetate:ethanol (4.5:3.5:2) fractionated terpenoid which was vizualized using vanillin–sulfuric acid (Mar *et al*., 2020). HPLC was performed on a Shimadzu LC-2030 Plus with UV detection (272 nm). Injected 20 µL sample in acetonitrile:water (45:55, v/v) at a speed of 1.0 mL/min, and at 40 °C, for 20 minutes, which confirmed L-Carvone peaks.

RESULTS AND DISCUSSION

Qualitative and Quantitative phytochemical analysis

Phytochemical screening revealed the highest metabolite content in hydroethanolic extract, followed by chloroform, benzene, acetone, petroleum ether, ethanol, and water, (Table 1) consistent with Paikara & Pandey (2018). Quantitative analysis of the hydroethanolic extract (Figure 1) showed carbohydrates (34.60 ± 0.79 mg/g) and proteins (20.67 ± 0.53 mg/g), comparable to Scherer *et al*. (2013). Phenols (40.13 ± 2.5 mg/g) exceeded aqueous extract values reported by Kanatt *et al*. (2007), supporting their usefulness in cardiovascular, cancer, metabolic, and aging-related disorders (Yuan *et al*., 2011). Flavonoids (54.50 ± 0.7 mg/g) were significantly of higher values than those reported with aqueous solution (Kanatt *et al*., 2007). The other antimicrobial, anticancer, and anti-inflammatory properties were as reported earlier (Panche *et al*., 2016). Steroids (80.56 ± 0.40 mg/g) and tannins (54.03 ± 0.35 mg/g) were also abundant of which, the latter is known for antioxidant, cardioprotective, and anticancer effects (Labieniec *et al*., 2003).

Antioxidant activity

Antioxidant evaluation confirmed strong free radical scavenging ability. As shown in the Table 2, in the DPPH assay, the extract showed IC_{50} = 221.34 µg/ml, close to ascorbic acid (255.75 µg/ml) and ethanolic

extract reported by Mata *et al*. (2007), IC_{50} = 140.97 µg/ml). NO scavenging activity (IC50 = 288.18 µg/ml) slightly outperformed the standard (314.47 µg/ml), while hydroxyl radical scavenging (IC_{50} = 316.46 µg/ml) activities was stronger than aqueous extract values reported by Kanatt *et al*. (2007), IC_{50} = 498.3 µg/ml), likely due to phenolic hydrogen-donating ability. Dot-blot assay (200–1000 µg/ml) further confirmed free radical scavenging through visible yellow spot formation against a purple background (Figure 2).

Enzymic and non-enzymatic antioxidants

Enzymatic antioxidants were also prominent, with catalase (1.948 ± 0.02 µmol H_2O_2 /min/mg protein), SOD (1.308 ± 0.05 U/mg), and glutathione peroxidase (2.36 ± 0.24 U/mg) activities (Figure 3), in line with the SOD activity (aqueous extracted) reported by Kanatt *et al*. (2007). These enzymes provide anti-inflammatory and anticancer-preventive benefits. Non-enzymic antioxidants (Figure 4) included ascorbic acid (23.06 ± 0.02 mg/g), α-tocopherol (31.63 ± 0.12 mg/g), and glutathione (18 ± 0.6 mg/g), reinforcing the strong antioxidant capacity of the hydroethanolic extract (Klimczak *et al*., 2007).

Total antioxidant assay

Phenolics and flavonoids largely contributed to the antioxidant capacity of spearmint. Reducing power assay showed 59.75% inhibition at 500 µg/ml, comparable to ascorbic acid (62.25%) with RC_{50} values of 351.62 and 336.47 µg/ml (Table 3). Phosphomolybdenum assay gave IC_{50} of 254.20 µg/ml (extract) vs. 246.18 µg/ml (standard), while FRAP assay showed IC_{50} values of 261.64 µg/ml (sample) and 257.47 µg/ml (standard), as reported by Mandana *et al*. (2011), who had observed high antioxidant activity (71.00 ± 2.65%) *via* Supercritical Carbon Dioxide ($SC\text{-}CO_2$) extraction.

GC-MS and FTIR analysis of spearmint leaves

GC-MS identified 37 compounds (Figure 5), with major constituents β-sitosterol (46.05%), L-carvone [2-Cyclohexen-1-one, 2-methyl-5-(1-methylethenyl)-(R)] (17.28%), phytol (7.07%), and ergost-5-en-3-ol (4.88%) at retention times 22.285, 9.053, 17.385, and 20.974 min, respectively. L-Carvone, the bioactive signature compound, was selected for isolation. FTIR spectra (Figure 6) confirmed functional groups including phenols, aldehydes/ketones, carboxylic acids, ethers, alkanes, aliphatic amines, alkenes, and aromatics as showed in Table 4.

L-Carvone extraction

Soxhlet extraction followed by column chromatography (benzene to ethanol; ethyl acetate: toluene:ethanol, 5.5:3:1.5 v/v) (Figure 7) yielded 50 mg of L-carvone as a pale-yellow oil. Fractions 3–12 showed a pale-green TLC spot (Rf = 0.42), consistent with the reported Rf value of carvone (0.44) (Stecher, 1968), confirming its identity (Figure 8). The compound was further verified by FTIR.

FTIR analysis of L-Carvone

FTIR spectra of the hydroethanolic extract (Figure 9) confirmed the compound as L-carvone, matching the standard (NIST, 2018). In Table 5 and Figure 9, peak values for phenols (3618.46 cm^{-1}), aromatics (1435.04 cm^{-1}), aldehydes (2731.20 cm^{-1}), benzene derivatives (894.97 cm^{-1}), and halogens (570.93 cm^{-1}) were consistent with Truzzi *et al*. (2022).

High Performance Liquid Chromatography

Standard L-carvone of 20–100 µg/mL showed linearity (R^2 = 0.9927) with retention times of 14.819–15.079 minutes as shown in Table 6. The standard and isolated compound results of HPLC Figure 10 & 11 showed a similar retention time of 14.994 and 14.041 minute respectively. Clear, sharp peaks confirmed purity and successful extraction using benzene:ethyl acetate:toluene:ethanol (3.5:2.5:2.5:1.5), validating identity and suitability for bioassays.

CONCLUSION

Spearmint leaves are rich in L-carvone, isolated and characterized using simple, effective techniques. Such refined methods meet the growing demand for bioactive compounds as food supplements. Despite modern medicine, traditional remedies remain vital, especially for underprivileged populations. Hence, documenting and standardizing phytoconstituents like L-carvone is crucial, alongside innovations in extraction, analysis, and clinical evaluations.

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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: Preliminary phytochemical analysis of *M. spicata* L. leaves.

Phytochemical analysis test name	Aqueous	Ethanol	Hydro Ethanol	Petroleum Ether	Chloroform	Acetone	Benzene
Carbohydrate							
Benedict's	+	+	-	+	-	+	-
Fehling's	+	++	+	+++	++	+	+++
Molisch's	-	+	+	+	+	-	+
Protein							
Biuret's	+	-	-	-	+	+	-
Ninhydrin	++	+	+	-	-	++	-
Alkaloids							
Dragendorff's	-	+	+	++	+	-	++
Wagner's	++	-	+	++	+	-	++
Mayer's	-	-	+	-	-	-	+
Flavonoids							
Alkaline reagent	+	-	+	+	+	-	+
Zinc HCl	+	+	++	+	++	+	++
Glycosides							
Borntrager's	+	+	+++	-	+	-	++
Keller- Killani	-	-	+	+	+	++	++
Legal's	++	-	+++	++	+++	+++	++
Terpenoids							
Salkowski	+	+++	++	-	+	++	++
Libermann-Burchard's	+	++	+++	+	+	++	+
Phenols							
Ferric chloride	-	++	++	-	+	+	-
Lead acetate	+	++	+++	+	+	+	+
Tannins							
Ferric chloride	+	+	+	-	+	+	+
Lead acetate	-	++	+	+	-	+	+
Saponin							
Foam	+	++	++	++	+++	+	+
Steroids							
Salkowsi's	+	+++	++	-	+	++	++
Libermann-Burchard	+	++	+++	+	+	+	+
Fats							
Cupric hydroxide	+	+++	++	+	++	+	++

+++ indicates **excess,** ++ indicates **strong,** + indicates **weak** concentrations, and
- indicates **absence** of phytochemicals in different extracts of leaves of spearmint.

Table 2: Free radical scavenging activity of spearmint sample.

Concentration (µg/mL)	DPPH (%)		Nitric Oxide Free Radical Scavenging (%)		Hydroxyl Radical Scavenging (%)	
	Ascorbic acid	*M. spicata* L.	Ascorbic acid	*M. spicata* L.	Ascorbic acid	*M. spicata* L.
100	61.63 ± 1.12	58.75 ± 2.04	42.86 ± 0.98	38.57 ± 1.62	42.00 ± 1.05	40.00 ± 1.75
200	75.25 ± 1.35	62.50 ± 2.30	48.57 ± 1.12	44.29 ± 1.85	50.00 ± 1.20	45.00 ± 1.90
300	81.13 ± 1.42	67.50 ± 2.55	57.14 ± 1.25	50.00 ± 2.05	55.00 ± 1.28	51.00 ± 2.10
400	85.13 ± 1.50	73.75 ± 2.80	64.29 ± 1.35	60.00 ± 2.20	62.00 ± 1.35	59.00 ± 2.15
500	89.25 ± 1.62	78.75 ± 2.95	77.14 ± 1.48	71.43 ± 2.40	74.00 ± 1.45	70.00 ± 2.25

Table 3: Total antioxidant activity of spearmint sample.

Concentration (µg/mL)	Reducing Power Assay (%)		Phosphomolybdenum Assay (%)		FRAP Assay (%)	
	Ascorbic acid	*M. spicata* L.	Ascorbic acid	*M. spicata* L.	Ascorbic acid	*M. spicata* L.
100	38.63 ± 0.75	33.75 ± 1.25	58.41 ± 1.12	53.74 ± 2.05	43.00 ± 0.86	41.37 ± 1.60
200	44.25 ± 0.88	42.50 ± 1.35	64.02 ± 1.22	63.41 ± 2.15	57.28 ± 1.05	55.89 ± 1.85
300	50.13 ± 0.95	48.50 ± 1.45	70.41 ± 1.35	68.74 ± 2.30	62.08 ± 1.10	61.18 ± 1.95
400	57.13 ± 1.05	54.75 ± 1.55	77.05 ± 1.45	74.85 ± 2.45	69.44 ± 1.20	69.00 ± 2.05
500	62.25 ± 1.15	59.75 ± 1.65	82.27 ± 1.55	79.08 ± 2.55	89.26 ± 1.35	87.71 ± 2.20

Table 4: FTIR frequency values and functional groups of ethanolic extract of spearmint leaves

S.No	Frequency (cm^{-1})	Reference frequency (cm^{-1})	Functional group	Compounds	Intensity
1.	3726.47	3749.62	O-H	Phenol	Strong
2.	2978.09	2975	C-H	Alkenes	Medium
3.	2360.87	2400-3200	N-H	Ammonium ions	Multiple broad peaks
4.	2337.72	2400-3200	N-H	Ammonium ions	Multiple broad peaks
5.	1728.22	1725	C=O	Aldehyde/Ketone	Influenced by conjugation
6.	1604.77	1550-1610	C=O	COOH derivates	Medium
7.	1381.03	1380	N-O	Nitro compounds	Weaker
8.	1249.87	1220-1260	C-O	Ether	Strong
9.	1149.57	1100-1200	C-X	Fluoroalkanes	Two strong broad bands
10.	1072.42	1020-1220	C-N	Aliphatic amines	Often overlapped
11.	956.69	956.69	C-H	Alkenes	Strong
12.	810.10	800-860	C-H	Aromatic	Strong
13.	671.23	670-700	C-H	Alkenes	Strong
14.	547.78	540-760	C-X	Chloroalkanes	Weak to medium
15.	509.21	500-600	C-X	Bromoalkanes	Medium to strong

cm^{-1} can be abbreviated as centimetre power minus 1.

Table 5: FTIR frequency values and functional groups of isolated terpene compound L-Carvone.

S.No.	Frequency (cm^{-1})	Reference Frequency (cm^{-1})	Functional Group	Compound	Intensity
1.	3618.46	3610	O-H	Phenol	Medium
2.	2924.09	2925	C-H	Alkyl	Medium to strong
3.	2731.20	2720	C-H	Aldehyde derivates	Medium
4.	1674.21	1675	C=C	Alkenes	Medium
5.	1435.04	1450	C=C	Aromatic	Weak to strong
6.	1242.16	1220- 1260	C-O	Aromatic ethers	Medium
7.	964.41	965	C-H	Alkene	Strong
8.	894.97	860- 900	C-H	Aromatic benzene	Strong

Table 6: Data for the calibration curve of L-Carvone quantification using HPLC.

S No	Injection Volume (ul)	Concentration (ug/ml)	Area Under the Curve (AUC)
Stock Standard (1000ug/ml)			
1	20	20	2041372
2	20	40	4332665
3	20	60	5939478
4	20	80	7571862
5	20	100	9059949
***M. spicata* L. Extract Sample**			
1	L-Carvone Extract	60	5027556.81

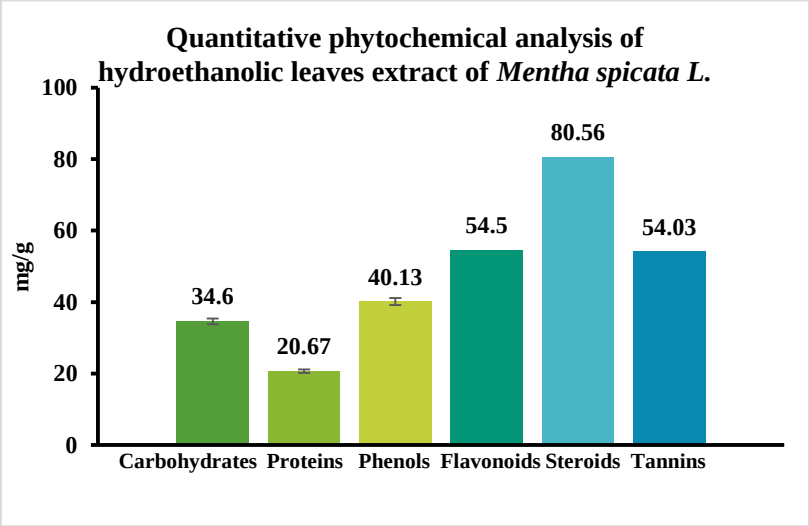


Figure 1. Quantitative phytochemical analysis of hydroethanolic leaves extract of spearmint.

200µg 400µg 600µg 800µg 1000µg

Figure 2. DPPH-dot-blot of spearmint sample.

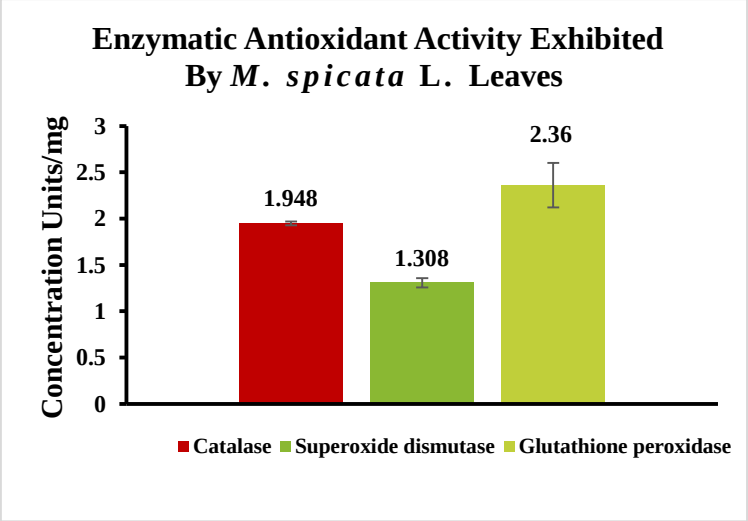


Figure 3. Enzymatic antioxidant activity of spearmint sample.

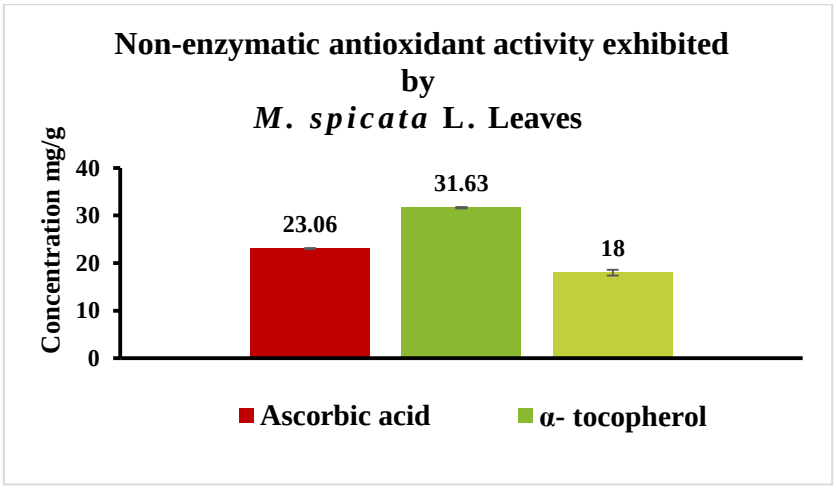


Figure 4. Non-enzymatic antioxidant activity of spearmint.

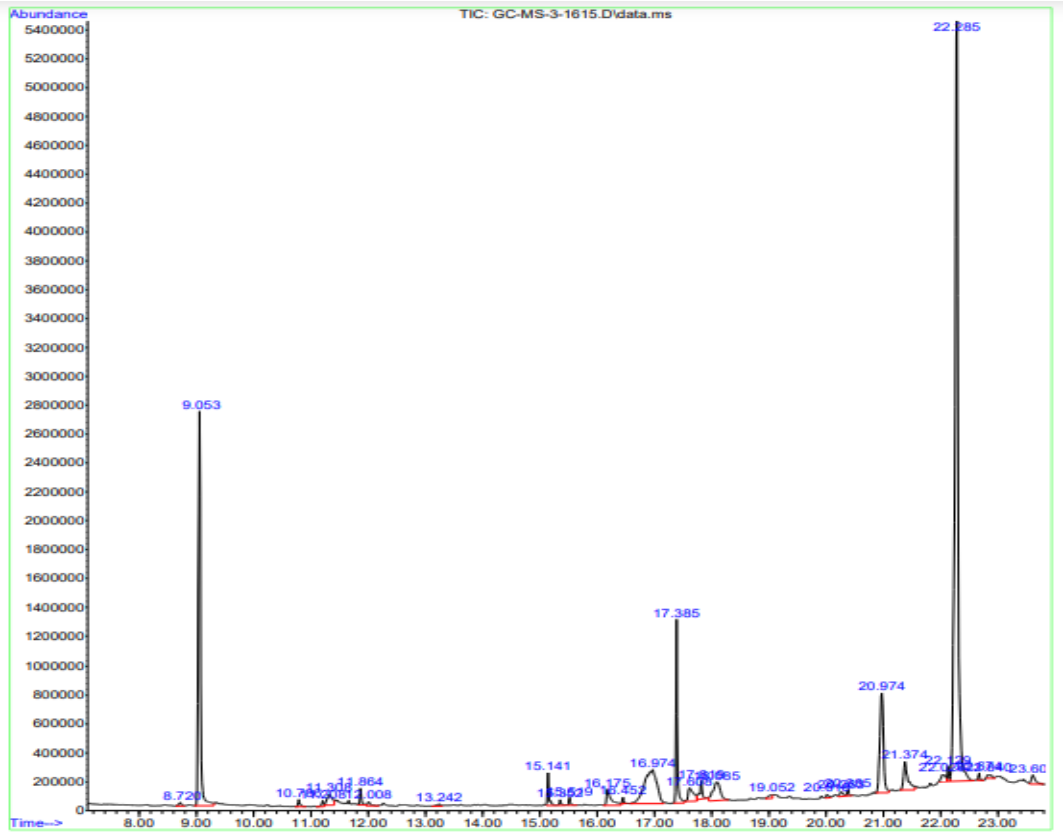


Figure 5. The result of spearmint GCMS chromatogram.

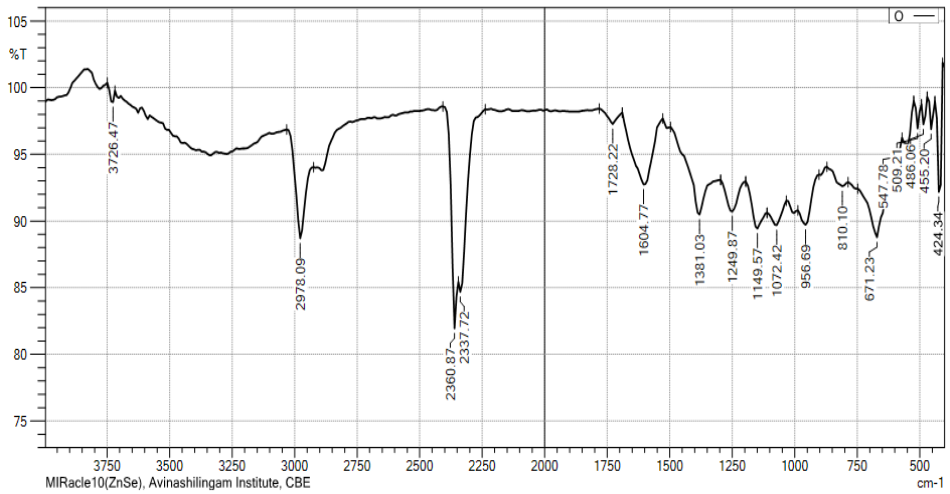


Figure 6. FTIR- spectrum wave numbers of ethanolic extract of spearmint leaves.

Figure 7. Column chromatography setup for isolating terpene compound L-Carvone by using increasing polarity of the eluent by beginning with pure benzene and ending with ethanol.

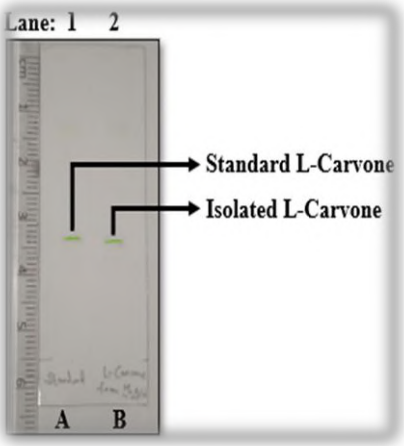


Figure 8. TLC separation of L-Carvone: - A and B represent Standard L-Carvone and isolated L-Carvone respectively. Green spot (Lane - 2) is that of isolated L-Carvone and the Rf value (0.42) of it matches with Rf value (0.44) reported by Stecher 1968. The solvent system used is benzene:toluene:ethyl acetate:ethanol (3.5:2.5:2.5:1.5, v/v).

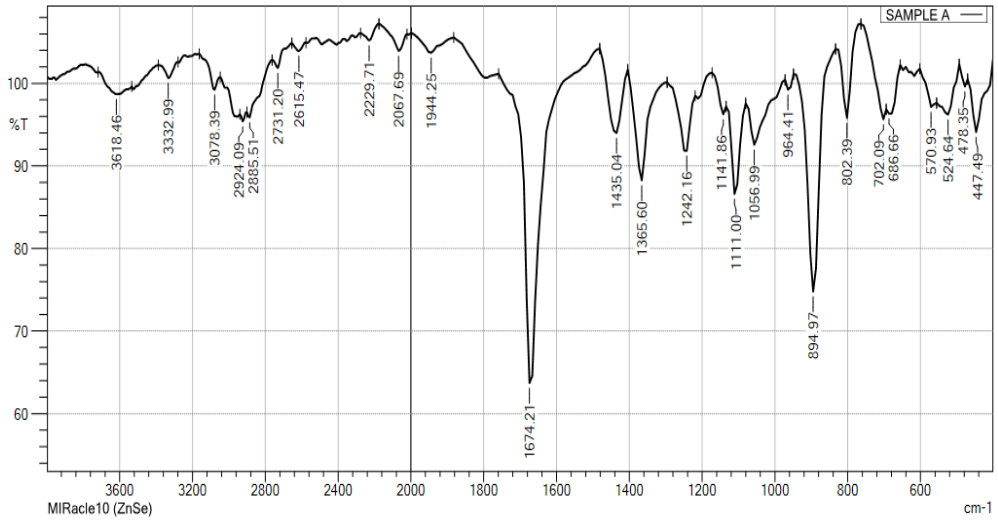
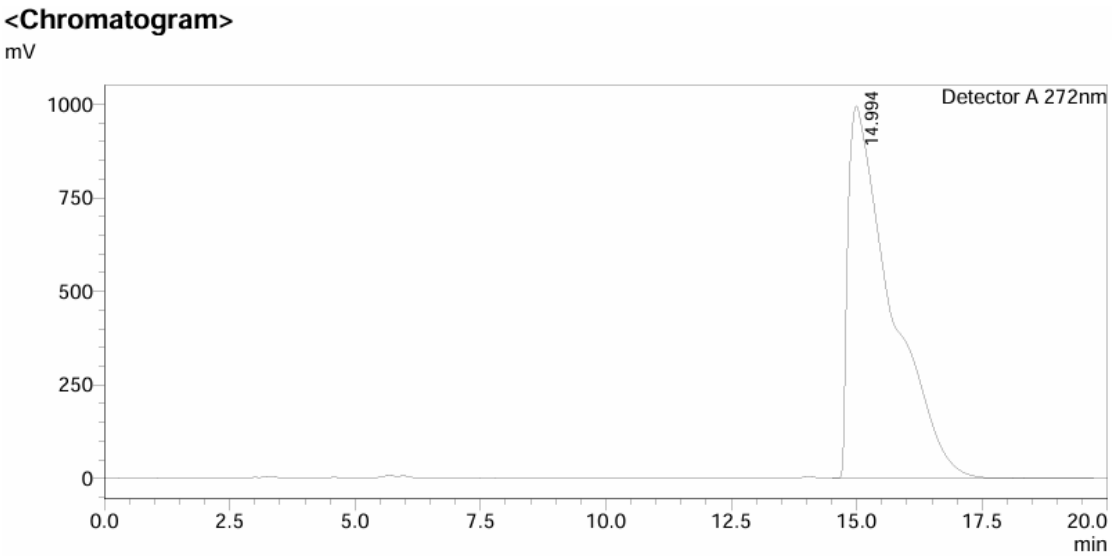


Figure 9. FTIR- spectrum of isolated terpenoid compound L-Carvone.

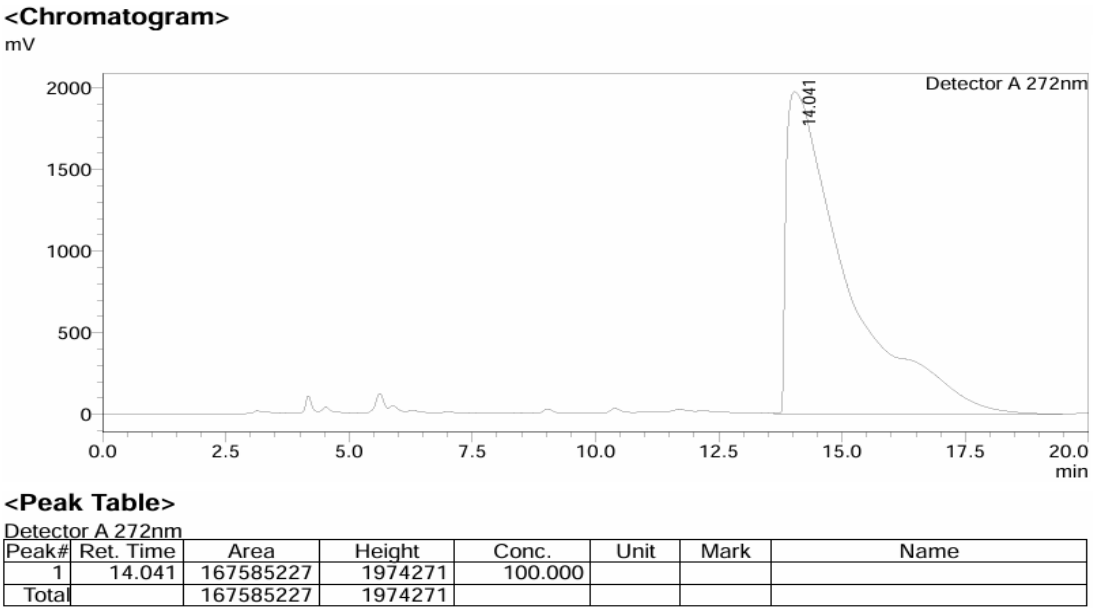


<Peak Table>

Detector A 272nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	14.994	59394787	994068	100.000			
Total		59394787	994068				

Figure 10. HPLC chromatogram of L-Carvone standard (100 µg/mL): A distinct peak appeared at 14.994 minutes at 272 nm, confirming the pure L-Carvone standard. The peak area and height were 59,394,787 mVs and 994,068 mV, respectively, validating the standard's retention and peak characteristics.



<Peak Table>

Detector A 272nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	14.041	167585227	1974271	100.000			
Total		167585227	1974271				

Figure 11. HPLC chromatogram of isolated L-Carvone sample (200 µg/mL). A distinct peak was observed at a retention time of 14.041 minutes at 272 nm, confirming the elution of L-Carvone. The peak area and height were 167,585,227 mVs and 1,974,271 mV, respectively, validating the similar retention and peak characteristics of the standard compound.

Ethnomedical inventory of the leaves of *Brassica Rapa var rapa* in a northwestern Algeria: qualitative and quantitative approaches

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ABSTRACT

This research aims to conduct an ethnobotanical inventory of the therapeutic use of Brassica rapa var rapa (Brr) leaves in the Tlemcen region, covering three distinct localities: Ghazaouet, Nedroma and Maghnia. To this end, a group of 419 participants was surveyed using an organised questionnaire, of whom 191 reported using the plant. There was a predominance of women (64.39%) in the 60-70 age group (29.84%). The majority of users had varying levels of education, ranging from illiteracy to university level. Although most of them belonged to disadvantaged socio-economic categories, their knowledge of the plant's use was mainly derived from family traditions. The methods of preparation included infusing the leaves in herbal tea and steaming them, with internal use being the most common. Most participants have incorporated the leaves into their daily diet, generally favouring oral consumption. Observations have shown significant consumption of Brr leaves, particularly for treating hypercholesterolemia (17.5%), digestive problems (14.8%) and fever (10.5%). Although the benefits are noticeable but several participants reported adverse effects, including gastrointestinal problems such as vomiting, nausea and diarrhoea, especially associated with long-term use. This research suggests that Brr leaves are a major source of phytoparticles, which makes them a promising natural alternative for use in the pharmaceutical and cosmetics industries.

Keywords: *Brassica rapa. L, Ethnobotany index, Ethnobotany, leaves,*

INTRODUCTION

According to the WHO, 80% of developing countries continue to use traditional medicines derived from medicinal plants (Vaou *et al*., 2021). Ethnobotanical knowledge, transmitted orally from generation to generation, represents a treasure trove of knowledge about medicinal plants and their therapeutic properties.

The Cruciferae family (also known as Brassicaceae) is a group of plants that includes approximately 338 genera and more than 3,700 species. Among these species, *Brassica rapa (B. rapa)* includes several subspecies cultivated as vegetables or oilseeds (*B. rapa ssp. Pekinensis; B. rapa ssp. chinensis; B. rapa ssp. chinensis var. narinosa; B. rapa ssp. chinensis var. nipposinica; B. rapa ssp. rapa)* (Li *et al*., 2024). Similarly, turnips (*Brassica rapa var. rapa L.*) are an excellent source of nutrients, bioactive compounds, minerals, vitamins, fiber, and glucosinolates (glucoraphanin, glucobrassicin, and gluconasturtiin) (Yang *et al*., 2023)**.** These phytochemicals possess the ability to produce biological activities beneficial for therapeutic applications (Mirihagalla and Fernando, 2021).

Several researchers have highlighted the anticancer, antioxidant, antibacterial, anti-inflammatory, and antihypertensive

properties of *Brassica rapa (B. rapa)* (Elhouda-Mekhadmi *et al*., 2024). Because of its high content of bioactive compounds, it is widely used to treat diabetes, hypercholesterolemia, heart disease, and photosensitivity disorders (El kadi *et al*., 2024). However, the aerial parts of *Brassica rapa* remain unexploited despite their biological properties. In this context, the aim was to document local knowledge and practices concerning the therapeutic use of *Brassica rapa* leaves in the Tlemcen region.

MATERIALS AND METHODS

The study was conducted in the Tlemcen region in the communes of Ghazeouette, Nedroma, and Maghnia (Table 1). The information was collected in accordance with ethnobotanical research guidelines (N'Do *et al*., 2024). The study was conducted in three areas of the Tlemcen region and involved a total of 419 participants. The questionnaire included socio-demographic data on the surveyed population and ethnopharmacological data on the plant. Before the interview, informants were given comprehensive information about the questionnaire and its objectives. They were given formal assurances regarding the confidentiality and purely educational use of their data. The questionnaire was designed to be anonymous, optional, and respectful of participants' confidentiality. Furthermore, to facilitate communication with local populations who have low literacy levels, the interview was conducted in Algerian dialect. It is important to note that participants were free to end the interview at any time.

Relative frequency of citation, RFC: This index was calculated by dividing the number of informants citing a plant species as useful (frequency of citation, FC) by the total number of informants participating in the study (N). It was evaluated on a scale of [0-1], with a value close to 0 indicating a lower frequency of citation and, consequently, less cultural importance. Conversely, values close to 1 indicate a higher frequency of citation and, consequently, greater cultural importance among the population. The following formula was used to determine the RFC index: FC/N = RFC (Balafraj *et al*., 2024).

Fidelity level, FL (%): Measures the reliability of the main therapeutic uses of plants for a specific condition by healers in the region studied. It is calculated using the following formula: FL (%) = Np /N×100. The NP index corresponds to the number of informants who report using a specific plant species to treat a specific disease, while the N index corresponds to the total number of informants who use plants as medicine to treat a specific disease. The highest level of fidelity corresponds to maximum agreement on the use of a species.

Cultural Importance Index, CI: This is used to assess the importance of a plant in a specific category of use and can be expressed using the following formula: UC: UR/N. Where N is the total number of informants and UR is the number of informants citing a species for a specific use.

RESULTS AND DISCUSSION

Demographic characteristics of informants

The study was conducted among 419 people. Among those surveyed, 191 individuals reported that they were familiar with the plant and its therapeutic uses, with a predominance of women (64.39%) and an age group of 61-70 years with a threshold of (29.84%) (Table 2). Similarly, illiterate individuals had the highest level of knowledge, with a percentage of 30.89%. In addition, individuals with a university education had a similar level of knowledge to illiterate individuals (28.27%).

According to the results, individuals with a low socioeconomic status are the most frequently exposed to herbal medicine, with a percentage of 54.97%. On the other hand, married people represent the highest percentage of knowledge**,** (85.34%) (Table 2).

Our survey confirmed that people over the age of 41 have the highest level of knowledge about the plant studied, compared to other age groups. This is consistent with their experience with modern therapy, which causes side effects and is ineffective, prompting them to search for safer and more natural alternatives. This trend has been demonstrated in other studies (Li *et al*., 2024). In addition, women show a dominance (64.39%) compared to men, which is in agreement with the findings of (Lazli *et al*., 2019), who reported a higher proportion of women (71.8%). It is known that women in North Africa are involved in ethnobotanical practices and have some experience with traditional alternative medicine, which is passed down from generation to generation. Statistical analysis showed that the level of education had no influence on participants' responses regarding knowledge of the target plant, which is similar to some previous studies (Hedidi *et al*., 2024).

The population with low socioeconomic status has a good knowledge of plants (54.97%). Due to their limited income, many people turn to herbal medicine, which remains less expensive than modern medicine. Several previous studies have confirmed this result (James *et al*., 2018). Similarly, 85.34% of the population report being married, it is possible that this is due to the fact that married people can avoid or reduce the material expenses necessary to pay doctors and pharmacists by turning to herbal medicine (Benlarbi *et al*., 2023). Several studies confirm our results (Yabrir *et al*., 2019).

Characteristics related to the use of the plant

According to Table 3, 45,9% of the population interviewed indicated that they had learned about the plant through a family source. Similarly, more than half of the population uses the leaves of the plant in fresh form (51.4%) with internal application (52%). On the other hand, the population under study uses eight methods for preparing the leaves of the plant, the most common of which is in the form of herbal tea (22.8%). A large percentage of our population (68%) consumes the aerial parts of *Brassica rapa var rapa* as food. Users confirmed their use of the plant with other liquid additives. It was noted that *Brassica rapa var rapa* leaves were used with water by 49,8% of respondents, followed by olive oil at a usage rate of 17%, honey 31% and eggshell 0.3%.

According to our survey, we found that the aerial parts of *Brassica rapa* var rapa are commonly used to treat several conditions, the most common of which are hypercholesterolemia (17.5%), digestive disorders (14.8%), and fever (10.5%). Furthermore, the majority of the population that uses Br leaves as a treatment are adults (42.6%), followed by the elderly (38.8%) (Table 3).

It appears that the majority of our informants (45.9%) acquired their knowledge of the uses of this plant as an alternative medicine from their families and family traditions. The trust we place in our ancestors about this subject illustrates why this plant has been valued for many generations and continues to hold significance over time (Jadid *et al*., 2020). A significant proportion of our population (17.5%) confirmed the use of Br leaves mainly to treat cholesterol. This therapeutic property may be explained by its high flavonoid and polyphenolic compound content, which regulate and control the biosynthesis of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG CoA reductase), leading to a decrease in cholesterol levels (Fard *et al*., 2015). Our informants (14.8%) use the leaves to treat digestive disorders. It is known that Brassica rapa L leaves contain high levels of carbohydrates, mainly in the form of polysaccharides, which protect the gastrointestinal mucosa and facilitate intestinal transit (Abid *et al*., 2022). They are also rich in glucosinolates, which are biologically inactive compounds that are converted into active isothiocyanates by myrosinase, known for their anti-

inflammatory, antioxidant, chemopreventive, cytotoxic and anticancer properties (Dejanović *et al*., 2021). The analgesic effect (against migraines and rheumatic pain) reported by our population is attributed to the abundance of secondary metabolites (Nazar *et al*., 2023).

According to our study, researchers have confirmed the use of leaves to treat obesity, which may be explained by improvements in blood lipid profiles and a reduction in body fat accumulation. Similarly, *Brassica rapa* leaves have hypoglycemic activity by promoting peripheral glucose absorption in tissues, reducing hepatic gluconeogenesis, regulating carbohydrate metabolism and decreasing intestinal absorption of dietary carbohydrates by promoting the production of insulin or other insulin-like substances (Fard *et al*., 2015).

The population surveyed consumed *Brassica rapa* leaves for their anti-flu properties. This therapeutic benefit is due to their high vitamin C content, which can promote iron absorption (Dejanović *et al*., 2021). According to (Swastika *et al.* 2019), boiled Br leaves are an excellent source of vitamins K, A, and C, as well as folate and lutein. They also contain dietary nitrates, which are beneficial for cardiovascular health. These nitrates help lower blood pressure and improve blood vessel health. The vitamins and antioxidants present play an essential role in immune, ocular, and osseous health. Another study showed that extracts from *Brassica rapa* leaves have angiotensin-converting enzyme (ACE) inhibitory activity, which helps lowering blood pressure (Abid *et al*., 2022).

On the other hand, the aerial part of Br is osteoprotective due to its high vitamin C content, which contributes to the creation of collagen, an essential element for bone health. In addition, the administration of vitamin C with calcium in postmenopausal women has shown an increase in bone mineral density (BMD) in the femur. Vitamin K is also responsible for the production of osteocalcin, a protein essential for the regulation of bone muscles.

Our survey found that studied population uses *Brassica rapa* leaves as a natural therapy for nephrolithiasis. *In vivo* studies have shown that *Brassica rapa* leaf extract protects the kidneys in rats with nephrolithiasis induced by a calcium-rich diet, and on diabetic nephropathy (Salem *et al*., 2018). These therapeutic effects are mainly due to the bioactive compounds present in the leaves (Meliani *et al.,* 2023), such as polyphenols, flavonoids, tannins and dietary fiber, which help to reduce oxidative stress and tissue inflammation in general

Duration of treatment

The data presented in Figure 1, indicated that the majority of participants use Brassica rapa leaves for a period of 1 to 7 days using four methods: orally (70.3%), topically (22.3%), through massage (2.1%), and by mastication (13.0%).

Risks and secondary effects

During our survey, most participants confirmed that use of the plant is asymptomatic: no vomiting (74.4%), no nausea (76.4%), no diarrhea (72.3%), no intoxication (63.1%). (Figure 2) However, like any medicinal plant, its many benefits do not prevent it from being harmful if misused. Indeed, participants reported that the plant could cause adverse effects, and these side effects can occur at varying frequencies depending on the duration and frequency of use of the plant, but this situation remains limited.

Influence of demographic factors on knowledge of *Brassica rapa* var rapa

The results of the chi-square test analysis showed that age, level of education, socio-economic status and family situation had a significant association with knowledge and the use of *Brassica rapa* L leaves, as can be

seen in the P values in Table 4 ($P < 0.001$). Cramer's V method allows for a comparison of the strength of the link between two analysed variables. The variables studied are more dependent when V is close to zero. Conversely, it will be 1 when the two variables are completely linked. The standard interpretation rules are as follows: (Amrouche *et al*., 2019).

$0 \leq V < 0.1$: very weak or no dependence; $0.1 \leq V \leq 0.3$: weak dependence; $0.3 \leq V < 0.5$: moderate dependence; $0.5 \leq V$: strong dependence.

As can be seen in Table 4, the results of our study reveal that the link between (age, level of education, socio-economic status and family situation) and knowledge of the plant is respectively (weak: 0.3, 0.3), (moderate: 0.4) and (very weak: 0.2).

The Relative frequency of citation (RFC) of this study is 0.45, showing that less than 50% of the population uses the plant, Although the proportion obtained may seem relatively low, a result probably influenced by the small sample size and which could change as the study area expands, this result can also be explained by the popular perception that traditionally favors the root, considered the most useful and valuable part of the plant, to the detriment of the leaves, which have fewer uses. However, the data collected clearly show that this often-neglected part of Br is not limited to traditional therapeutic uses. But also, it plays a significant role in other fields, as illustrated by the Cultural Importance Index (CI). The leaves are mainly consumed as food (70%), used for Cosmetics (18%), for animal feed (11%). for their aromatic qualities (3%) and as a condiment (1%). (Table 5).

As part of our research, we identified that the aerial parts of *Brassica rapa* var rapa are used to treat 15 categories of diseases and symptoms as detailed in (Table 6). Cholesterol treatment is particularly notable, with a fidelity rate of 20.94%, indicating its frequent use. Digestive disorders follow with a rate of 17.80%, while fever ranks third with a rate of 12.52%, The other ratios were arranged as follows: Rheumatism 9,42%, Obesity 8,37% Rheum 7,85, Osteoporosis 7,85, Migraine 7,32, Immune system booster 5,75, Diabetes 4,71, Skin disease 4,71, Anemia 4,18, Cancer 2,61, kidney disease 2,61%, cardiovascular disease 2,61%, Injurie and fracture 0,52%. (Table 6). In terms of fidelity, our community is interested in this plant and is loyal to its use in treating various diseases. studies such as (Rehman *et al*., 2017) the one conducted in Punjab, Pakistan, have shown that respondents reported using *Brassica rapa* L leaves and roots to treat stomach problems and ulcers with a Fidelity rate of 97.22%.another study (Rao *et al.*, 2015) conducted in India mention that the whole plant of *Brassica rapa* L (root and leaves and seed) used to treat andrological problems, gynecological and birth problems with a fidelity rate of 100%. These benefits were not shown in our study, A study also (Nazli *et al.*, 2022) showed the use of *Brassica rapa L* to treat skin diseases and drug poisoning, but it did not mention the level of fidelity. Nazar *et al*. (2023) showed that *Brassica rapa* L used to treat diabetes with an FL % of 12,5%, For other results, the study by (Swastika *et al.,* 2019) highlighted that *Brassica rapa L.* exhibits cardiovascular and lipid-lowering effects, antioxidant, anti-diabetic, anti-inflammatory, hepatoprotective, nephroprotective, anticancer, anti-obesity, and analgesic properties, confirming its strong therapeutic potential against various metabolic and inflammatory disorders. These findings therefore support the relevance of the traditional uses reported by local populations, particularly for managing cholesterol levels, digestive problems, and fever-related conditions.

CONCLUSION

Our study revealed the notable therapeutic potential of *Brassica rapa* var. rapa leaves in the Tlemcen region, where they are traditionally used to treat various diseases. This local knowledge provides a valuable basis for future research in phytochemistry and pharmacology, highlighting the

importance of traditional knowledge in the development of new therapeutic solutions.

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: Climatic parameters of study areas

Parameters	Regions		
	Ghazaouet	**Maghnia**	**Nedroma**
Latitude (UTM)	35° 05′ 38″	34° 49' 59.99"	35°00′48″
Longitude (UTM)	1° 51′ 37″	-1° 42' 59.99"	1°44′52″
Altitude (m)	20	487	355
Temperature (C°)	17.0	17.1	16.9
Precipitation (mm)	374	365	400
Climate	Mediterranean	arid	Mediterranean

Table 2: Demographic characteristics of informants

Parameters		**Frequency**	**Percentage (%)**
Age	[20-30]	03	1.57
	[31-40]	12	6.28
	[41-50]	29	18.18
	[51-60]	38	19.89
	[61-70]	57	29.84
	[71-80]	52	27.22
Gender	Female	123	64.39
	Male	68	35.26
Level of education	Illiterate	59	30.89
	Primary	36	18.84
	Average	14	7.32
	Secondary	28	14.65
	University	54	28.27
Socio-economic status	Low	105	54.97
	Average	65	34.03
	High	21	10.99
Family situation	Married	163	85.34
	Single	16	8.37
	Divorced	1	0.52
	Widowed	111	5.75

Table 3: Characteristics related to the use of the plant

Characteristics	Frequency	**Percentage (%)**
Source of knowledge about the plant		
Through family	135	45.9
Through studies	14	4.8
Through social environment	90	30.6
Through scientific documentation	55	18.7
Technique for using the plant		
Fresh	145	51.4
Dried	30	10.6
After heat treatment	107	37.9
Mode of utilization		
Internal	158	52.0
External	92	30.3
Both	54	17.8
Preparation technique		
Cataplasm	41	10.7
Infusion	87	22.8
Fumigation	13	3.4
Decoction	56	14.7
Steam	80	20.9
Salad	32	8.4
Raw	52	13.6
Powder	10	2.6
Aqueous extract	11	2.9
Other Uses		
Cosmetics	34	17.3
Animal feed	21	10.7
Food	134	68.0
Condiments	3	1.5
Aromatic properties	5	2.5
Additives associated with the plant		
Other medicinal plants	4	1
Water	191	49,8
Olive oil	65	17
Honey	122	31
Eggshell	1	0.3
Medicinal properties		
Kidney disease	5	2.2
Skin disease	9	3.9
Digestive disorder	34	14.8
Hypercholesterolemia	40	17.5
Immune system booster	11	4.8
Anemia	8	3.5
Cancer	5	2.2
Cardiovascular disease	5	2.2
Osteoporosis	15	6.6
Diabetes	9	3.9
Rheumatism	18	7.9

Obesity	16	7.0
Migraine	14	6.1
Fever	24	10.5
Injurie and fractures	1	0.4
Rheum	15	6.6
Target population for treatment		
Babies	26	5.8
Children	26	5.8
Adults	191	42.6
Pregnant women	31	6.9
Seniors	174	38.8

Table 4: Influence of demographic factors on knowledge of *Brassica rapa* var rapa

	Distribution	Informants – Experts	Informants Non-experts	Chi - squared X^2	P value	Cramer's V
Age	[20-30]	03	27			
	[31-40]	12	32			
	[41-50]	29	46	38.781	0.000	0.304
	[51-60]	38	50			
	[61-70]	57	33			
	[71-80]	52	40			
Gender	Female	123	144	0.69	0.436	0.013
	Male	68	84			
Level of education	Illiterate	59	16			
	Primary	36	49			
	Medium	14	45	47.355	0.000	0.336
	Secondary	28	33			
	University	54	85			
Socio-economic level	Low	105	37			
	Medium	65	173	79.152	0.000	0.435
	High	21	18			
Family situation	Married	163	151			
	Single	16	40	23.578	0.000	0.237
	Divorced	01	15			
	Windowed	11	22			

Table 5: Index of cultural importance of *Brassica rapa var rapa* leaves in the different localities visited.

Parameters	Use Reports (UR)	Cultural Importance Index (CI)
Cosmetics	34	0.18
Animal feed	21	0.11
Food	134	0.70
Condiment	3	0.01
Aromatic properties	5	0.03

Table 6: Percentage of fidelity level of diseases treated by *Brassica rapa L* leaves

Diseases	Citation number	Fidelity Level FL (%)
Kidney disease	5	2.61
Skin disease	9	4.71
Digestive disorder	34	17.80
Hypercholesterolemia	40	20.94
Immune system booster	11	5.75
Anemia	8	4.18
Cancer	5	2.61
Cardiovascular disease	5	2.61
Osteoporosis	15	7.85
Diabetes	9	4..71
Rheumatism	18	9.42
Obesity	16	8.37
Migraine	14	7.32
Fever	24	12.52
Injurie and fractures	1	0.52
Rheum	15	7.85

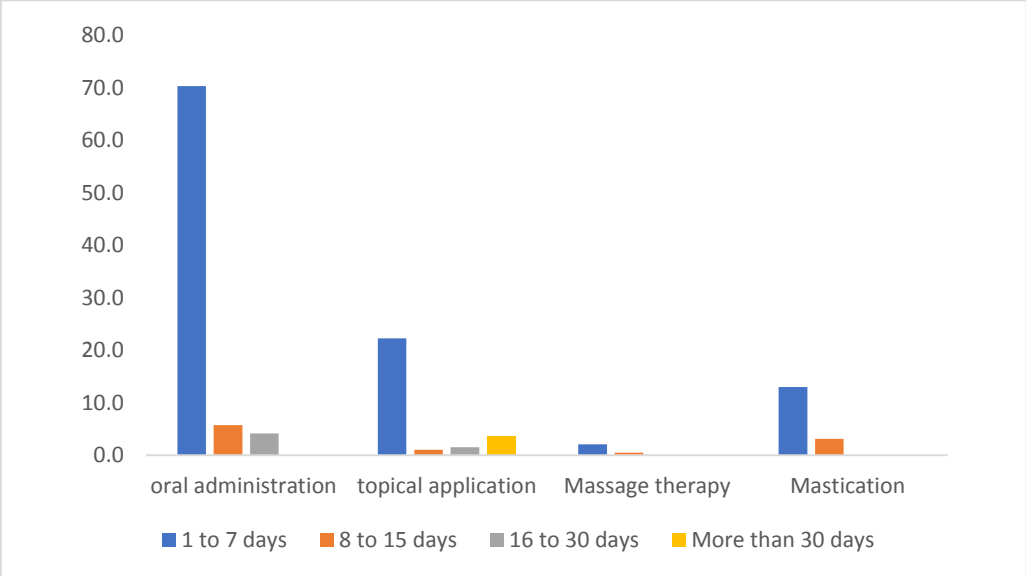

Figure 1: Treatment duration

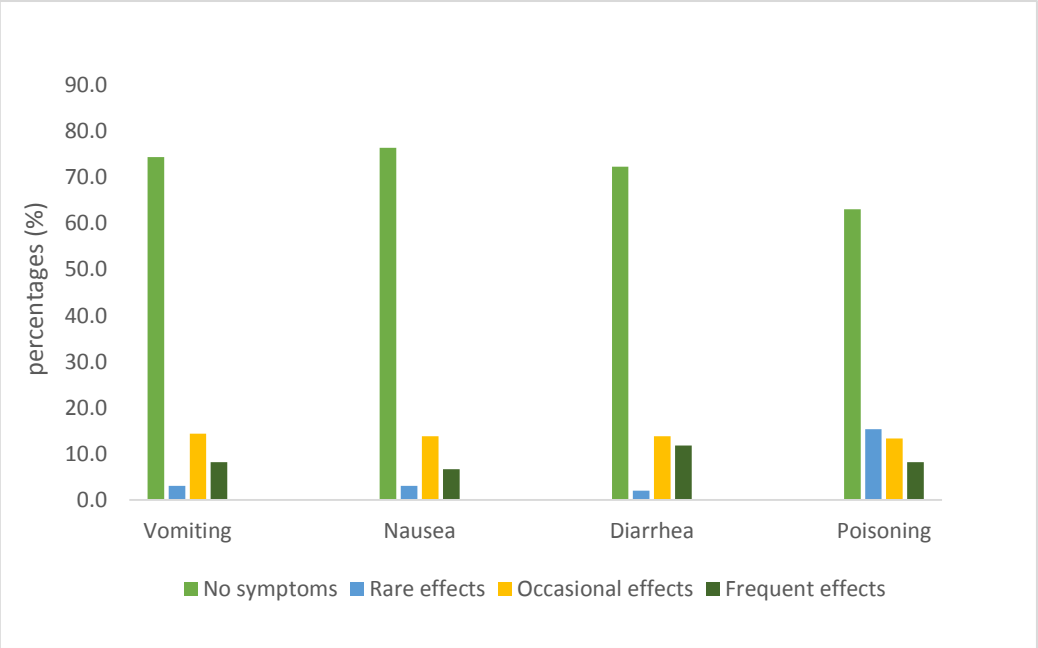

Figure 2: **Risks and secondary effects**

Diversity analysis and correlation study of fruit physical, biochemical characters and antioxidant properties of some palmyrah palm (*Borassus flabellifer* L.) genotypes under Western dry tract of West Bengal

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ABSTRACT

The present study investigates the diversity in fruit morphology, biochemical composition, and antioxidant attributes of eighteen naturally occurring palmyrah palm genotypes growing under the western dry tract of West Bengal, India. The selected region, characterized by lateritic soils and semi-arid climate, supports a wide range of seedling progenies exhibiting considerable phenotypic variation. Fruit morphological evaluation of eighteen seedling progenies (each considered as treatments for mean, variance analysis) of palmyrah palm from different locations of western dry tract of West Bengal in the year 2023-24 and 2024-25 revealed pronounced differences among genotypes in fruit size, shape, and pulp content. Fruit weight varied from 787.2 g to 2381.7 g, and pulp content ranged between 369.8 g and 1340.5 g, indicating substantial potential for yield improvement. Biochemical analyses also displayed significant variability, with total soluble solids (TSS) ranging from 15.7°Brix to 22.0°Brix, total sugars from 13.7% to 20.1%, and ascorbic acid from 19.4 to 46.7 mg 100 ⁻¹ g fresh pulp. Antioxidant activity (DPPH radical scavenging) spanned from 45.2% to 77.3%, strongly correlating with phenolic and ascorbic acid contents. Correlation analysis demonstrated that fruit weight was highly associated with pulp content (r = 0.963), while TSS showed strong positive relations with reducing sugars (r = 0.904) and TSS: acidity ratio (r = 0.842). The results highlight the existence of broad genetic variability within the regional germplasm and identify genotypes such as PPG-17, PPG-12, and PPG-13 as promising candidates for both table and processing purposes. Overall, the findings provide a scientific basis for selection, conservation, and genetic improvement of palmyrah palm for enhanced fruit quality and antioxidant potential under dry land conditions.

Keywords: Bioactive compounds, diversity, fruit morphology and quality, palmyrah palm,

INTRODUCTION

The palmyrah palm (*Borassus flabellifer* L.) is a hardy, multipurpose tree belonging to the family Arecaceae (Palmae). It is a dioecious, long-lived species native to the Indian subcontinent and widely distributed across South and Southeast Asia (Gnanavelrajah *et al*., 2025). Within the genus *Borassus*, which comprises about five species, *B. flabellifer* is the most economically important. It thrives in hot, dry, and semi-arid regions owing to its deep root system, drought tolerance, and ability to grow in poor soils and commonly known as “toddy palm” or “ice-apple palm” (Jayaraj *et al*., 2025). The palm not only contributes to the ecological stability of arid landscapes but also serves as a source of food, fibre, timber,

and income for rural communities, earning it the title of "tree of life" (Rao *et al*., 2021).

Owing to its wide utility and adaptability, palmyrah forms an integral part of the socio-economic fabric of dry-land ecosystems like as many other minor fruits (Deb *et al*., 2024; Mukherjee *et al*., 2023). Nutritionally, the fruit pulp and sap are rich in carbohydrates, sugars, vitamins, minerals, and phenolic compounds, exhibit antioxidant and antimicrobial properties. The fibrous mesocarp is a good source of dietary fibre, while the hardened endosperm (palmyrah nut) provides starch for industrial applications (Penkey *et al*., 2025). Despite these valuable attributes, organized cultivation and systematic research on this species remain minimal. Most palms grow naturally through seed propagation, leading to a highly heterogeneous population structure.

The genetic diversity of palmyrah palm represents an important resource for future crop improvement and conservation. Being a cross-pollinated and dioecious species, each seedling is genetically unique, contributing to wide variability in fruit size, shape, pulp yield, and biochemical composition. Characterizing this variation is essential for identifying superior genotypes for breeding, propagation, and commercial use (Sridevi *et al*., 2020). A broad genetic base also enhances the adaptability of the species to climatic fluctuations and environmental stresses. In India, palmyrah is mainly found in Tamil Nadu, Andhra Pradesh, Odisha, West Bengal, and parts of Gujarat and Maharashtra (Vanitha *et al*., 2025). Within West Bengal, its natural population is concentrated in the western dry tract covering Birbhum, Bankura, and West Burdwan districts. This region, characterized by lateritic soils, high summer temperatures, and low rainfall, provides a favourable environment for the species. Here, palmyrah exists mostly as scattered seedling progenies along field boundaries and wastelands. Over generations, natural regeneration and open pollination have led to the evolution of a diverse genetic pool, yet this remains largely undocumented. The region's palms may therefore represent an unexplored reservoir of genetic variability in fruit morphology and pulp quality, possessing potential for selection and improvement.

The present investigation was undertaken to assess the genetic diversity among seedling progenies of palmyrah palm from the western dry tract of West Bengal using fruit morphological and quality parameters. The specific objectives were to document the variation in fruit traits among different seedling populations in Birbhum, West Burdwan, and Bankura districts; analyze the relationships among fruit morphology and pulp quality attributes; and identify promising genotypes with desirable characteristics for propagation and conservation. By elucidating the extent of variability within natural populations, this study aims to highlight the hidden potential of palmyrah genetic resources in West Bengal and provide a scientific basis for their systematic improvement, value addition, and sustainable utilization.

MATERIALS AND METHODS

The present investigation was conducted during the fruiting season of 2023–24 and 2024-25 in the western dry tract of West Bengal, India, covering three major districts like Birbhum, Bankura, and West Bardhaman. These districts represent a typical semi-arid ecosystem characterized by lateritic to sandy loam soils, erratic rainfall, and high summer temperatures exceeding 40°C. The region supports a rich natural population of palmyrah palm, which thrives under minimal management. Eighteen mature, fruit-bearing female palms of seedling origin were randomly selected from naturally growing populations in these districts, ensuring representation of diverse phenotypes. Each selected palm of different locations was treated as a distinct genotype and designated as Palmyrah Palm Genotype (PPG-1 to PPG-18) (Table 1). From each genotype, fully mature fruits were randomly

harvested at the ripening stage when the exocarp turned deep yellowish-brown and the pulp attained full sweetness. Care was taken to collect fruits of uniform maturity and to avoid mechanical damage during harvesting and transport. The collected fruits were washed, cleaned, and analyzed immediately after harvest to minimize compositional changes. Each genotype was considered as one biological replicate, and all determinations were performed in triplicate.

Fruit morphology was assessed following standard descriptors used for palm species considering five randomly selected fruits from each genotype to get average value for each parameter. The parameters recorded included polar diameter (cm), equatorial diameter (cm), fruit weight (g), fruit volume (ml), cap diameter (cm), single seed weight (g), pulp content (g), and pomace content (g). The shape index was calculated as the ratio of polar to equatorial diameter to express the overall fruit form. All dimensional traits were measured using a digital Verniercaliper, while fruit weight and seed weight were determined using an electronic balance with ±0.01 g precision. Fruit volume was recorded by water displacement in a graduated cylinder. Pulp and pomace weights were determined after separating the edible mesocarp manually from the fibrous residue. The data were expressed as mean values from three independent measurements per genotype.

The biochemical composition of fruit pulp (from composite samples of five fruits from each palmyrah palm) was determined using fresh, homogenized samples. The following parameters were analyzed: **Total Soluble Solids (TSS):** Measured with a digital refractometer (Atago, Japan) and expressed in degrees Brix (°B). **Total sugar and reducing sugar:** Estimated using the anthrone and DNS methods, respectively, and expressed as percentage of pulp fresh weight (Lane and Eynon ,1923).**Titratable acidity:** Determined by titration with 0.1 N NaOH using phenolphthalein as indicator and expressed as percent citric acid equivalent (AOAC, 1990). **TSS: Acidity Ratio**: Calculated as the ratio of total soluble solids to acidity, serving as an indicator of palatability. **Ascorbic acid content:** Estimated by the 2,6-dichlorophenol indophenol visual titration method and expressed as mg ascorbic acid per 100 g pulp (Rangana, 1986). **Total phenol content**: Determined using the Folin–Ciocalteu reagent and expressed as mg gallic acid equivalents (GAE) per 100 g pulp (Dewanto *et al.*, 2002). **Antioxidant activity:** Measured as percent DPPH radical scavenging activity following the method of (Brand-Williams *et al.,* 1995) and expressed as percentage inhibition. All analyses were performed under controlled laboratory conditions, and each parameter was measured in triplicate to ensure reliability. The instruments were calibrated prior to each set of measurements.

Data collected for all morphological and biochemical parameters were subjected to statistical analysis to assess the extent of variability among genotypes. Descriptive statistics (mean, range, standard deviation, and coefficient of variation) were calculated for each trait. One-way analysis of variance (ANOVA) was performed to test the significance of differences among genotypes. To explore interrelationships among fruit traits, Pearson's correlation coefficients were computed between selected important morphological and biochemical variables.

RESULTS AND DISCUSSION

Morphological characters

All morphological or fruit physical parameters are presented in Table 2 in the current study. **The polar diameter** of fruits ranged from 40.1 cm in PPG-2 to 56.9 cm in PPG-8, while the **equatorial diameter** varied between 35.3 cm (PPG-2) and 52.3 cm (PPG-16). The significantly wide range in these parameters suggests variable fruit shapes and degrees of fruit expansion among genotypes. The **shape index**, calculated as

the ratio of polar to equatorial diameter, fluctuated between 0.92 (PPG-15) and 1.14 (PPG-4 and PPG-8). Values close to 1.00 indicated near-spherical fruits, while higher indices implied slightly elongated forms. The significantly maximum shape index in PPG-4 and PPG-8 reveals their tendency towards an oblong structure, whereas PPG-15 and PPG-3 exhibited more flattened shapes. **Fruit volume**, a major determinant of marketable size, displayed pronounced variation, ranging from 770.6 ml (PPG-2) to 2354.3 ml (PPG-17). Genotypes PPG-4, PPG-7, PPG-12, PPG-13, and PPG-17 produced markedly larger fruits (> 2000 ml), while PPG-2 and PPG-6 bore smaller ones. A similar trend was observed for **fruit weight**, which ranged from 787.2 g in PPG-2 to 2381.7 g in PPG-17. The strong correspondence between fruit volume and weight indicates the uniform density of fruit tissues among genotypes. **Cap diameter**, another important indicator of fruit base expansion, varied between 8.6 cm in PPG-2 and 14.2 cm in PPG-8, suggesting genetic differences in fruit morphology and pedicel attachment. Heavier fruits generally exhibited larger cap diameters, as observed in PPG-7, PPG-8, PPG-13, and PPG-17. The **single seed weight** ranged from 169.2 g (PPG-2) to 334.5 g (PPG-4), reflecting significant variability in seed development and fruit–seed ratio. Genotypes such as PPG-4, PPG-8, and PPG-13 had heavier seeds, while PPG-2 and PPG-6 recorded lower values, implying a greater proportion of pulp relative to seed mass in these accessions. **Pulp content**, which directly influences the fruit's edible yield, varied widely from 369.8 g in PPG-6 to 1340. 5 g in PPG-17. The significantly highest pulp yield in PPG-17, followed by PPG-9, PPG-12, and PPG-13, indicates their potential utility in pulp-based product development. Conversely, the lowest pulp content in PPG-2 and PPG-6 denotes comparatively poor mesocarp development. **Pomace content** (residual fibrous material) ranged from 43.3 g (PPG-2) to 156.8 g (PPG-17), showing a consistent increase with fruit size and pulp mass. Genotypes producing higher pomace content, such as PPG-17 and PPG-12, also possessed thicker fruit walls and denser fibrous structures, traits that may be advantageous for breeding programs targeting bio-fiber extraction.

Marked differences in fruit dimensions, weight, and pulp content among the genotypes demonstrate strong genotypic control over fruit development. The pronounced variation in fruit weight and volume observed across the studied genotypes aligns with previous findings in other perennial palms, where differential assimilate partitioning and mesocarp development are key determinants of fruit size (Garcia-Inza *et al*., 2014). The strong positive relationship between fruit weight and pulp content observed in this study indicates that larger fruits generally allocate more biomass to the mesocarp, the edible portion of the fruit. This relationship highlights the potential of using fruit weight as an indirect selection criterion for identifying high-yielding genotypes (Mori and Cipriani, 2023). Similarly, the strong association between fruit and seed weight reflects coordinated development, suggesting that the genotypes differ in resource allocation efficiency between seed and pulp tissues (Wetzstein *et al*., 2011).

Biochemical composition and quality attributes

The biochemical composition of fruits exhibited wide and statistically meaningful variations among the eighteen palmyrah palm genotypes, confirming the existence of substantial genotypic diversity for fruit quality traits (Table 3). Parameters such as total soluble solids (TSS), sugars, acidity, and antioxidant components revealed clear differentiation, reflecting the nutritional and processing potential of the germplasm.

The total soluble solids (TSS), which reflect soluble carbohydrate accumulation and overall sweetness, ranged from 15.7°Brix in PPG-16 to 22.0°Brix in PPG-17. Genotypes PPG-12 (21.5°Brix), PPG-8

(20.8°Brix), and PPG-13 (20.1°Brix) also recorded higher values, suggesting superior sweetness and flavour intensity. Conversely, PPG-11 and PPG-16 displayed significantly lower TSS, implying comparatively bland taste. These variations may arise from differences in carbohydrate metabolism and fruit maturity at harvest, emphasizing their importance in genotype selection for table or processing purposes. A similar trend was observed in **total sugar content**, which varied between 13.7% (PPG-11) and 20.1% (PPG-17). Genotypes exhibiting significant higher total sugars, such as PPG-12, PPG-8, and PPG-13, correspondingly possessed elevated TSS values, indicating a strong positive association between the two parameters. The lowest sugar concentration in PPG-11 and PPG-16 suggests lower photosynthetic assimilate partitioning into fruit pulp. **Reducing sugars**, which largely determine sweetness perception and fermentation efficiency, fluctuated between 10.6% (PPG-6) and 17.2% (PPG-17). The high reducing sugar levels in PPG-17, PPG-12, and PPG-13 denote enhanced enzymatic conversion of non-reducing sugars, contributing to their higher palatability. On the contrary, PPG-6 and PPG-11 recorded lower reducing sugars, which could influence the sensory appeal and suitability of these genotypes for processing into sweet products. The **titratable acidity** ranged from 0.22% in PPG-7 to 0.37% in PPG-16, indicating significant variable acid–sugar balance across genotypes. Fruits with lower acidity, such as PPG-7, PPG-8, and PPG-12, were correspondingly higher in TSS, yielding a favourable **TSS: acidity ratio**, a key indicator of flavour quality. The highest TSS: acidity ratio (88.0) was recorded in PPG-17, followed by PPG-7 (87.7) and PPG-12 (86.0), highlighting their superior sweetness and consumer acceptability. By contrast, PPG-16 exhibited the lowest ratio (42.4) due to its relatively high acidity, producing a less balanced flavour profile. **Ascorbic acid content** varied extensively, from 19.4 mg/100 g in PPG-11 to 46.7 mg/100 g in PPG-10. The relatively higher ascorbic acid concentration in PPG-10 indicates its greater antioxidant potential and nutritional superiority, while PPG-11 and PPG-16 showed significant lower values, signifying reduced vitamin C accumulation. Interestingly, genotypes with moderate TSS and sugar levels, such as PPG-10 and PPG-7, maintained higher ascorbic acid content. Considerable diversity was also recorded for **phenolic content**, which ranged from 23.4 mg GAE/100 g in PPG-11 to 45.8 mg GAE/100 g in PPG-10. Elevated phenol levels in PPG-10, PPG-17, and PPG-5 indicate their potential as rich sources of natural antioxidants and their suitability for value-added nutraceutical applications. On the other hand, PPG-6, PPG-11, and PPG-16 exhibited comparatively lower phenolic concentrations. The **antioxidant activity**, measured through DPPH radical scavenging, ranged from 45.2% in PPG-11 to 77.3% in PPG-17. The strong antioxidant capacity recorded in PPG-17, PPG-7, and PPG-5 corresponds with their elevated phenolic and ascorbic acid contents, demonstrating a synergistic effect of both classes of compounds. The relatively lower antioxidant activity in PPG-11 and PPG-16 suggests a deficiency in these bioactive metabolites.

Biochemical characterization further revealed substantial variation in total soluble solids (TSS), sugar fractions, acidity, and antioxidant constituents. High TSS and sugar levels in genotypes such as PPG-17 and PPG-12 indicate superior sweetness and better consumer preference. The close association between TSS and reducing sugar content suggests a shared metabolic pathway in carbohydrate accumulation during ripening (Durán-Soria *et al*., 2020). Similar patterns have been reported in date palm and coconut, where sugar enrichment during fruit maturation results from increased enzymatic hydrolysis of polysaccharides and conversion of organic acids into soluble sugars (Sharif, 2019). Acidity levels varied considerably among genotypes, influencing the overall taste and sugar–acid ratio (TSS: acidity), which serves as a reliable indicator

of flavour balance. Genotypes with high TSS: acidity ratios were generally more palatable and suitable for fresh consumption. The inverse relationship between TSS and acidity observed in some genotypes may be attributed to progressive utilization of organic acids as respiratory substrates during ripening (Durán-Soria *et al*., 2020). Antioxidant parameters, including ascorbic acid and phenolic content, also displayed pronounced differences. Fruits rich in these compounds exhibited enhanced antioxidant activity, suggesting a synergistic effect of vitamin C and phenolics in quenching free radicals. This relationship is consistent with earlier observations in tropical fruits such as mango and date palm, where phenolic metabolism contributes significantly to antioxidant defence mechanisms. The high phenol and ascorbic acid contents in certain genotypes imply their potential utility in functional food development and nutraceutical industries (Nowak *et al*., 2018).

Correlation analysis among important morphological and biochemical parameters

Correlation analysis among the studied traits of palmyrah palm genotypes revealed several significant positive relationships, indicating close interdependence between morphological and biochemical parameters influencing fruit quality and yield potential (Table 4 and figure 1). Fruit weight exhibited a very strong and positive association with pulp content (r = 0.963) and seed weight (r = 0.838), suggesting that heavier fruits tend to possess higher edible pulp mass as well as larger seeds. This relationship confirms that fruit size is a reliable indicator of potential pulp yield. A moderately high correlation of fruit weight with the TSS: acidity ratio (r = 0.644) and ascorbic acid (r = 0.551) further implies that larger fruits generally maintain better sweetness–acidity balance and vitamin C content. Among biochemical parameters, total soluble solids showed an exceptionally high positive correlation with reducing sugar (r = 0.904) and TSS: acidity ratio (r = 0.842), reflecting that sweetness and flavour balance are largely governed by sugar accumulation. The significant correlation of TSS with antioxidant activity (r = 0.618) suggests that higher sugar concentration may accompany enhanced antioxidant potential. Phenol content showed a notable positive correlation with antioxidant activity (r = 0.666) and ascorbic acid (r = 0.631), confirming the combined contribution of phenolics and vitamin C to the total antioxidant capacity of palmyrah fruits. Similarly, shape index was moderately correlated with TSS (r = 0.562) and TSS: acidity ratio (r = 0.610), indicating that more elongated fruits may possess comparatively sweeter pulp. Overall, the correlation pattern highlights that selection for higher fruit weight and TSS will simultaneously enhance pulp yield, sweetness, and antioxidant potential. These inter-trait relationships provide a valuable basis for identifying superior genotypes for both nutritional quality improvement and commercial exploitation.

Correlation analysis provided valuable insight into the interdependence of morphological and biochemical traits. The strong positive correlation between fruit weight and pulp content reinforces the importance of size-related parameters as indicators of yield potential. Similarly, the high correlation between TSS and reducing sugars confirms that sweetness is largely governed by the accumulation of simple carbohydrates (Lenucci *et al*., 2022). The association of TSS with antioxidant activity suggests that sugar metabolism may also influence the synthesis of secondary metabolites, possibly through shared metabolic intermediates (Elhadi *et al*., 2023). Furthermore, the positive correlation between phenol content and antioxidant activity confirms that phenolic compounds are major contributors to the antioxidant potential of palmyrah fruits (Ouamnina *et al*., 2024).

Collectively, these findings emphasize the complex interplay between fruit morphology, biochemical composition, and antioxidant metabolism. The observed trait

associations provide a foundation for selection strategies targeting both high yield and superior fruit quality. Genotypes such as PPG-17, PPG-12, and PPG-13, which exhibited large fruits, high pulp recovery, elevated sugar concentration, and strong antioxidant capacity, represent promising candidates for breeding programs and commercial exploitation.

CONCLUSION

The study revealed significant genotypic variability among palmyrah palm accessions in terms of fruit morphology and biochemical composition. Strong associations between fruit weight, pulp content, and sugar traits highlight the potential for simultaneous improvement of yield and quality through targeted selection. Genotypes such as PPG-17, PPG-12, and PPG-13 demonstrated superior performance in sweetness and antioxidant potential and size as well as pulp content, indicating their suitability for large-scale cultivation and processing. The positive correlations among sugars, acids, and antioxidant compounds underscore an integrated metabolic regulation influencing fruit quality. Overall, the findings provide a scientific basis for identifying elite genotypes and developing breeding strategies aimed at enhancing productivity, nutritional value, and industrial utilization of palmyrah palm in diverse growing environments.

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: Geographical position of different palmyrah palm genotypes in three districts of West Bengal.

Sl. No.	Address	Latitute (ºN)	Longitude (ºE)
1.	Atgram, Nalhati, Birbhum	24.25	87.85
2.	Bhagabatipur, Nalhati, Birbhum	24.27	87.77
3.	Hazarpur, Sadinpur, Birbhum	24.23	87.83
4.	MaraBasahar, Hansan, Birbhum	24.19	84.84
5.	Dangram, Rampurhat, Birbhun	24.16	87.80
6.	Sahapur, Tarapith, Birbhum	24.10	87.82
7.	Kharasinpur, Mallarpur, Birbhum	24.05	87.73
8.	Beluti, Kotasur, Birbhum	23.94	87.75
9.	Muradihi, Sainthia, Birbhum	23.93	87.69
10.	Kaferpur, Kirnahar, Birbhum	23.78	87.88
11.	Nohana, Ahmadpur, Birbhum	23.80	87.70
12.	Bahadurpur, Bolpur, Birbhum	23.65	87.62
13.	Barabani, Asansol, West Burdwan	23.70	87.02
14.	Banshra, Ranigunj, West Burdwan	23.63	87.14
15.	Pandabeshwar, West Burdwan	23.69	87.25
16.	Unanshila, Ragunathpur, Bankura	23.51	86.71
17.	Gangajalghati, Bankura	23.45	87.06
18.	Kochdihi, Sonamukhi, Bankura	23.29	87.37

Table 2: Fruit physical characters of different palmyrah palm genotypes under western dry tract of West Bengal:

Palmyrah palm genotype (PPG)	Polar diameter(cm)	Equatorial diameter (cm)	Shape index	Fruit volume (ml)	Fruit weight (g)	Cap diameter (cm)	Single seed weight (g)	Pulp content (g)	Pomace content (g)
PPG-1	46.4	47.5	0.97	1480.5	1476.5	12.7	212.7	724.3	76.6
PPG-2	40.1	35.3	1.13	770.6	787.2	8.6	169.2	404.4	43.3
PPG-3	42.4	44.8	0.94	1210.2	1198.7	11.8	208.7	513.0	61.1
PPG-4	52.8	46.4	1.14	2340.5	1894.6	12.4	334.5	791.7	101.7
PPG-5	47.7	45.2	1.05	1450.0	1444.8	12.2	233.8	628.1	117.4
PPG-6	42.3	40.7	1.03	820.4	1019.6	11.7	197.6	369.8	59.9
PPG-7	54.7	50.4	1.08	2180.7	2049.3	13.5	260.2	1150.4	119.5
PPG-8	56.9	50.1	1.14	2000.2	2147.1	14.2	325.5	1047.9	125.3
PPG-9	55.3	50.3	1.09	2064.3	2178.5	9.7	289.8	1311.3	143.6
PPG-10	49.4	47.0	1.05	1813.6	1942.9	11.8	241.4	1069.5	121.7
PPG-11	47.2	48.2	0.97	1985.5	1740.4	13.3	220.7	962.1	118.5
PPG-12	54.1	51.4	1.05	2156.8	2217.7	12.4	262.1	1261.6	150.2
PPG-13	51.7	46.9	1.10	2202.4	2256.1	13.1	301.6	1239.9	144.8
PPG-14	46.6	47.8	0.97	1679.5	1519.4	9.7	239.2	730.4	72.1
PPG-15	42.0	45.5	0.92	1853.2	1748.2	11.8	251.5	893.6	102.4
PPG-16	49.5	52.3	0.94	1748.1	1899.6	12.0	246.7	1044.3	117.5
PPG-17	53.8	50.6	1.06	2354.3	2381.7	13.1	295.4	1340.5	156.8
PPG-18	54.1	49.2	1.10	2076.9	2154.3	12.3	302.3	1113.1	137.2
Mean	49.28	47.20	1.04	1788.20	1780.9	12.0	255.1	921.9	109.4
CV	15.61	18.69	16.96	20.23	24.8	11.51	17.3	22.3	32.4

Table 3: Fruit biochemical characters and antioxidant activity of different palmyrah palm genotypes under western dry tract of West Bengal:

Palmyrah palm genotype (PPG)	Total soluble solids (^{o}Brix)	Total sugar (%)	Reducing sugar (%)	Acidity (%)	TSS: Acidity ratio	Ascorbic acid content (mg/100g)	Phenol content (mgGAE/100g)	Antioxidant activity (% DPPH)
PPG-1	16.3	14.1	11.5	0.30	54.3	22.4	36.5	61.6
PPG-2	19.6	16.5	14.4	0.32	61.2	26.6	39.6	67.4
PPG-3	18.2	16.6	13.7	0.35	52.0	21.7	26.2	50.8
PPG-4	17.8	14.2	11.9	0.29	61.3	27.3	37.7	58.9
PPG-5	19.5	16.3	13.3	0.33	59.0	25.7	40.1	65.4
PPG-6	17.6	13.7	10.6	0.30	58.6	24.4	25.8	49.0
PPG-7	19.3	17.4	13.1	0.22	87.7	34.9	37.4	71.3
PPG-8	20.8	18.9	14.8	0.25	83.2	32.5	32.0	65.6
PPG-9	19.4	16.4	13.4	0.24	80.8	30.2	26.7	57.0
PPG-10	16.2	15.5	12.7	0.27	60.0	46.7	45.8	51.5
PPG-11	15.9	13.7	11.2	0.33	48.1	19.4	23.4	45.2
PPG-12	21.5	19.3	16.5	0.25	86.0	35.9	31.6	50.1
PPG-13	20.1	18.6	14.5	0.24	83.7	32.4	34.3	57.6
PPG-14	16.5	14.8	11.7	0.28	58.9	27.1	27.7	48.9
PPG-15	18.3	16.3	13.4	0.29	63.1	25.3	32.8	55.3
PPG-16	15.7	14.5	10.9	0.37	42.4	21.7	25.3	48.5
PPG-17	22.0	20.1	17.2	0.25	88.0	34.6	42.1	77.3
PPG-18	18.4	16.0	13.5	0.26	70.7	31.9	35.7	64.6
Mean	18.5	16.2	13.2	0.28	66.6	28.9	33.3	58.1
CV	10.1	11.7	13.3	14.1	14.2	22.4	18.9	15.1

Table 4: Pearson Correlation coefficients of important fruit morphological and biochemical characters of palmyrah palm genotypes:

Parameters	Shape index	Fruit weight	Seed weight	Pulp content	TSS	Reducing sugar	TSS: Acidity ratio	Ascorbic acid	Phenol	Antioxidant activity
Shape index	1.000									
Fruit weight	0.277	1.000								
Seed weight	0.504*	0.838	1.000							
Pulp content	0.223	0.963*	0.692	1.000						
TSS	0.562	0.370	0.398	0.366	1.000					
Reducing sugar	0.400	0.438	0.345	0.469	0.904*	1.000				
TSS:Acidity	0.610*	0.644	0.588	0.648*	0.842*	0.754*	1.000			
Ascorbic acid	0.507	0.551	0.406	0.562	0.387	0.501	0.636*	1.000		
Phenol	0.470	0.153	0.162	0.124	0.303	0.403	0.313	0.631*	1.000	
Antioxidant activity	0.559	0.220	0.307	0.198	0.618	0.542	0.569	0.293	0.666*	1.000

*5% level of significance

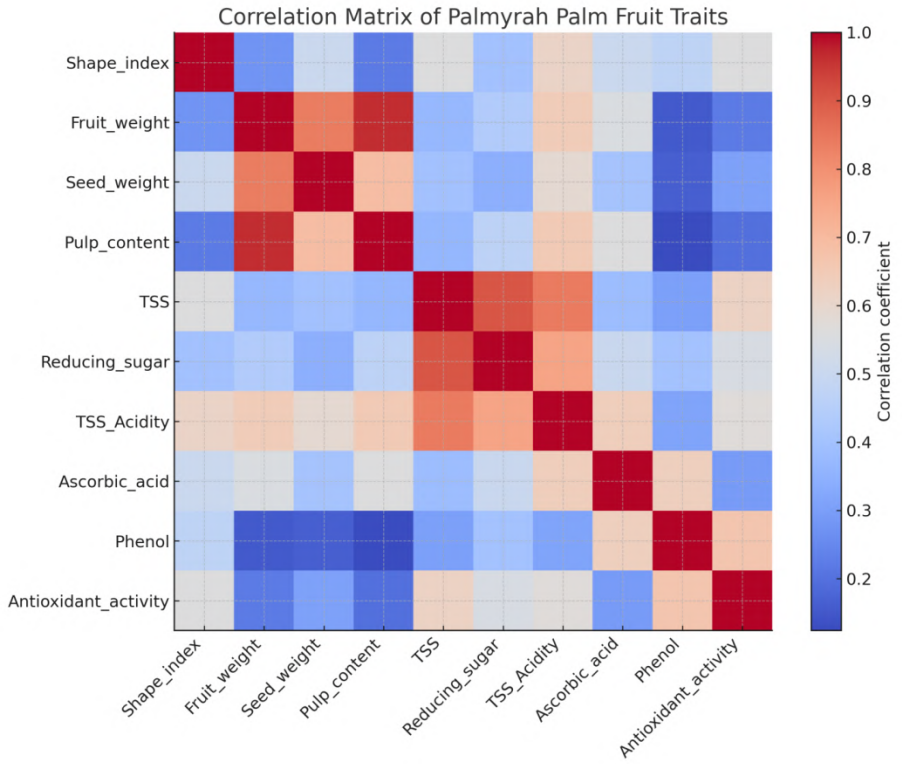

Figure 1: Correlation heat map of important fruit morphological and biochemical characters of palmyrah palm genotypes

Marine metabolites for HIV control: A multi-target *in-silico* approach

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ABSTRACT

This study evaluates the potential of marine sponge-derived metabolites as multi-target inhibitors of Human Immunodeficiency Virus (HIV), aiming to overcome the limitations of current antiretroviral therapies. An in silico molecular docking study was conducted on 15 compounds, including FDA-approved drugs (Efavirenz, Darunavir, Lenacapavir) and marine-derived phytoconstituents from Dysidea, Axinella, and Hippospongia species. The compounds were docked against key HIV targets: reverse transcriptase (RT), capsid protein (CP), and protease (PR). ADMET (absorption, distribution, metabolism, excretion, and toxicity) profiling was performed to assess pharmacokinetic and safety parameters, including blood-brain barrier penetration (logBB) and toxicity indices (LD_{50}, mutagenicity, CYP enzyme inhibition). Hippospongide A showed strong dual-target inhibition against RT (-7.1 kcal/mol) and PR (-9.4 kcal/mol), comparable to Darunavir. Avarone exhibited broad-spectrum efficacy (RT: -7.0, CP: -7.4, PR: -8.7 kcal/mol) and notable CNS penetrability (logBB: 0.26). Hippospongide A was non-mutagenic with low toxicity (LD_{50}: -0.5), although it showed moderate inhibition of CYP2C19/2C9 enzymes. Avarone, despite its mutagenicity, had strong multi-target and CNS potential. Marine sponge metabolites, particularly Hippospongide A and Avarone, show promise as next-generation HIV therapeutics. From this in-silico study, we confirmed that Hippospongide A is ideal for systemic viral suppression, while Avarone offers potential for targeting CNS reservoirs.

Keywords: ADMET profiling, HIV inhibitors, marine sponges, molecular docking

INTRODUCTION

Human Immunodeficiency Virus (HIV) continues to pose a severe worldwide health burden, with approximately 38 million people relying on lifelong antiretroviral regimens to control viral progression and prevent the onset of AIDS (Forsythe *et al*., 2019). While existing FDA-approved medications, including reverse transcriptase blockers such as Efavirenz and protease-targeting drugs like Darunavir, have markedly extended patient survival, persistent therapeutic limitations remain (Rathbun *et al.*, 2006). Long-term treatment frequently encounters obstacles, including adverse reactions, evolving drug-resistant viral variants, and restricted availability in underserved communities (Inzaule *et al*., 2017). These challenges collectively underscore the pressing necessity for innovative antiviral candidates featuring novel mechanisms, enhanced safety profiles, and cost-effective production.

Our investigation employs a strategic multi-target approach against HIV by simultaneously disrupting three essential viral proteins. Reverse transcriptase performs the critical function of converting viral RNA

into DNA capable of integrating into host chromosomes (Hu and Hughes, 2012). HIV protease enables viral maturation by cleaving precursor polyproteins into functional components. The capsid protein visualized through structural modelling (PDB 1E6J) constructs the conical core that safeguards viral genetic material while coordinating early infection processes, including uncoating and nuclear import (Handa *et al.*, 2025). Attacking these distinct targets creates complementary therapeutic effects: RT inhibition blocks the establishment of permanent viral genetic material in host cells, protease suppression halts production of infectious particles, and capsid interference impedes post-entry replication events. This coordinated strategy aims to achieve superior antiviral synergy while minimizing the emergence of resistance, a common weakness of single-target therapies. Figure 1 illustrates this multi-stage intervention approach across the viral lifecycle.

To identify potential candidates, we conducted molecular docking analyses on a curated library of marine sponge metabolites. These included brominated alkaloids, terpenoid derivatives, and lactone-based compounds are selected from *Dysidea, Axinella, and Hippospongia* species (Ariyasoma *et al.,* 2023 and Abdelaleem *et al*., 2020). Marine sponge metabolites were chosen for this study because marine organisms, particularly sponges, are increasingly recognized as prolific and unique sources of bioactive natural products with distinct chemical scaffolds not commonly found in terrestrial plants. (Varijakzhan *et al*., 2021). Their ecological exposure to microbial stressors has driven the evolution of potent chemical defence mechanisms, many of which interact with viral enzymes and structural proteins (Rathore *et al.,* 2023). This unique biochemical reservoir provides a strong rationale for exploring sponge-derived compounds as novel anti-HIV candidates, particularly those capable of multi-target inhibition. Binding affinities and interaction patterns against HIV reverse transcriptase, protease, and capsid targets were systematically compared to clinically approved reference inhibitors. This screening methodology aims to uncover structurally novel scaffolds suitable for developing next-generation therapeutics with improved resistance profiles and reduced toxicity.

MATERIALS AND METHODS

Computational tools

The study employed an integrated computational workflow utilizing specialized software for structure-based drug discovery. PubChem and the RCSB Protein Data Bank served as primary repositories for retrieving ligand structures and target protein coordinates, respectively (Kim, 2021). Molecular format conversions and energy minimization were performed using Open Babel to ensure structural compatibility (O'Boyle *et al.*, 2011). Docking simulations leveraged AutoDockTools for parameterization and grid generation, while binding interactions were visualized and analyzed in Biovia Discovery Studio (Baroroh S.Si. M.Biotek. *et al.,* 2023). Pharmacokinetic and toxicity profiles were predicted using SwissADME and pkCSM to evaluate drug-likeness and safety parameters (Pires *et al.,* 2015).

Selection and preparation of ligands

A diverse panel of reference inhibitors and sponge-derived compounds was curated for screening. Clinically approved drugs included Efavirenz (reverse transcriptase inhibitor), lenacapavir (capsid inhibitor), and Darunavir (protease inhibitor). 6 compounds were selected from the *Axinella* species including 3-Bromohymenialdisine, 12-Chloro-11-hydroxydibromoisophakellin, Debromohymenialdisine, N-Methylmanzacidin C, Stevensine, and Debromohymenialdisine (Yalçın *et al.,* 2007). One compound, namely Hippospongides A selected from the

Hippospongia species (Chang *et al.*, 2012). Remaining 6 compounds are selected from the *Dysidea* species including Avarol, Avarone, Dysideanin A, Dysideanin B, Furodysinin lactone, Spongia-13(16),14-dien-19-oic acid (Fathallah *et al*., 2023). These compounds are selected based on the literature search. All ligands were downloaded from PubChem in SDF format, protonated at physiological pH, converted to PDBQT using Open Babel, and energy-minimized with the MMFF94 force field to optimize 3D conformations (Bakale *et al.*, 2025).

Target protein selection and preparation

Three critical HIV-1 proteins were selected: reverse transcriptase (PDB: 3LP3) , protease (PDB: 1HVR), and capsid (PDB: 1E6J). Crystal structures were obtained from the RCSB PDB, prioritizing resolutions ≤2.5 Å and ligand-bound states. Proteins were prepared by removing water molecules, heteroatoms, and non-essential ions. Polar hydrogens and Kollman charges were added in AutoDockTools, followed by energy refinement using the CHARMM force field in Discovery Studio to correct steric clashes and optimize side-chain orientations.

Active site prediction

Binding sites were defined using crystallographic evidence of native co-crystallized ligands (e.g., Efavirenz for RT, Darunavir for protease) and literature-guided residues. For capsid (1E6J), which lacks a canonical small-molecule binding site, the (NTD) inter-protomer interface implicated in capsid dimerization was targeted (Wilbourne and Zhang, 2021). Discovery Studio's "Binding Site" module supplemented these predictions by identifying pockets with high propensity for ligand engagement based on surface geometry and hydrophobicity.

Molecular Docking Protocol

Docking simulations employed Auto Dock Vina integrated within Auto Dock Tools. A grid box encompassing the active site residues was parameterized with dimensions 25×25×25 Å (x/y/z) and 0.375 Å spacing. Exhaustiveness was set to 20 to ensure robust conformational sampling. Ligands were subjected to flexible docking with rotational bonds enabled, while protein residues remained rigid (Cavasotto and Abagyan, 2004). Each compound underwent 10 independent runs, with the lowest-energy pose retained for further analysis.

The grid box was centered at coordinates (x = 22.5, y = 45.7, z = 37.9) for the protease active site, with dimensions 25 × 25 × 25 Å and a grid spacing of 0.375 Å. For reverse transcriptase and capsid, grid centers were set at (x = 30.4, y = 58.2, z = 62.5) and (x = 41.7, y = 33.8, z = 52.1), respectively. Exhaustiveness was fixed at 20 to ensure thorough conformational sampling.

To validate the docking protocol, co-crystallized ligands (Efavirenz for RT, Darunavir for PR, and Lenacapavir for CP) were re-docked into their native binding sites. The resulting RMSD values were ≤ 2.0 Å, confirming the accuracy and reproducibility of the docking setup.

Docking analysis

Binding poses were evaluated using empirical scoring metrics (ΔG in kcal/mol) and interaction patterns. Discovery Studio analysed hydrogen bonds, hydrophobic contacts, π-stacking, and ionic interactions between ligands and key residues (RT: Asp443, Glu478, Asp498, and His539, PR: Asp25 and Asp29, and CP: Pro1, Met5, Leu7, Gln8, Gly9, Val10 & Leu13). Compounds were benchmarked against reference inhibitors (Efavirenz/Darunavir/Lenacapavir) to identify scaffolds with superior or comparable binding affinities. Stability assessments included root-mean-square deviation (RMSD) calculations for docked poses relative to crystallographic ligands.

ADMET prediction

Drug-likeness and toxicity risks were profiled using SwissADME and pkCSM. Key parameters included Lipinski's Rule of Five compliance, bioavailability scores, cytochrome P450 inhibition, and permeability (Caco-2/BBB). Toxicity endpoints covered AMES mutagenicity, hepatotoxicity, hERG inhibition, and LD_{50} (oral rat acute toxicity). Compounds exhibiting favourable binding energy but poor ADMET profiles were deprioritized to focus on viable therapeutic candidates.

RESULTS AND DISCUSSION

Docking

In this docking study, a total of 15 compounds were evaluated for their potential to inhibit three crucial proteins of the Human Immunodeficiency Virus (HIV): reverse transcriptase (RT), capsid protein (CP), and protease (PR). These proteins play essential roles in the HIV lifecycle, making them important targets for drug design. The test group included three FDA-approved antiretroviral drugs, Efavirenz, Darunavir, and Lenacapavir, as well as twelve bioactive compounds derived from marine sources, often referred to as marine phytoconstituents. Each compound's effectiveness was measured by its binding energy (in kcal/mol), with more negative values indicating stronger binding and, potentially, better inhibitory effects, which is shown in Table 1 (Compounds showing higher negative values demonstrate better binding potential). The binding energy values showed consistency across replicate docking runs, with variations within ±0.3 kcal/mol, indicating stable and reproducible interaction trends. Nevertheless, due to the semi-empirical nature of docking scoring functions, these energies should be interpreted qualitatively, emphasizing relative ranking rather than absolute values.

Among the standard drugs, Lenacapavir showed the strongest interaction with the reverse transcriptase enzyme (-7.7 kcal/mol), highlighting its specialized activity against this target. Darunavir displayed the highest affinity for the HIV protease (-9.8 kcal/mol), confirming its role as a highly effective protease inhibitor. Efavirenz, although widely used, showed moderate binding across all targets (RT: -7.2, CP: -5.4, PR: -7.5), suggesting a broader but less potent interaction profile.

Interestingly, several marine compounds demonstrated competitive or even superior binding compared to the standard drugs. Avarone emerged as a promising multi-target inhibitor, binding strongly to all three proteins (RT: -7.0, CP: -7.4, PR: -8.7), indicating its potential to interfere with multiple stages of the viral lifecycle. Avarol, structurally related to Avarone, also showed high affinity, particularly for CP (-6.9) and PR (-8.5). Hippospongide A stood out with an exceptionally strong binding to protease (-9.4 kcal/mol), nearly matching Darunavir's potency, along with good binding to RT (-7.1). Another notable candidate, 12-Chloro-11-hydroxydibromoisophakellin, exhibited strong binding to PR (-9.2). On the other end of the spectrum, Dysideanin A consistently displayed weak interactions with all three targets (RT and CP: -3.6, PR: -5.1), suggesting minimal therapeutic potential as an HIV inhibitor.

Pharmacokinetic profile

The drug-likeness and pharmacokinetic properties of the selected compounds were analyzed using in silico ADMET predictions, shown in Table 2. According to Lipinski's Rule of Five, 12 compounds showed no violations, indicating good potential for oral bioavailability. Only two compounds, Darunavir and Avarol, had one violation, and Lenacapavir had three violations, suggesting limited drug-like behavior. In terms of predicted oral bioavailability, 14 compounds scored high (≥0.55), with Spongia acid having the highest score (0.85), while Lenacapavir had the lowest (0.17). Regarding absorption, nine compounds showed high Caco-2 permeability (log Papp > 0.9),

suggesting efficient intestinal absorption. Compounds such as Avarone and Dysideanin B had particularly high permeability, whereas a few compounds like Stevensine and 12-Chloro-11-hydroxydibromoisophakellin showed low permeability. Blood-brain barrier (BBB) penetration was limited in most compounds, with only Avarone, Dysideanin B, and N-Methylmanzacidin C showing positive logBB values, indicating the potential to cross the BBB.

Metabolic predictions showed varied inhibition of cytochrome P450 (CYP) enzymes. Efavirenz, Lenacapavir, and Hippospongide A were found to inhibit multiple CYP enzymes, which could influence drug–drug interactions. Single CYP inhibition was observed in Avarol, Avarone, and Dysideanin B. Notably, seven compounds, including Darunavir and Spongia acid, showed no CYP inhibition, indicating a lower likelihood of metabolic interference. While specific elimination data, such as clearance rate, were not directly available, compounds with lower permeability and poor CYP interaction profiles may exhibit slower elimination.

In terms of toxicity, only two compounds, Avarone and Dysideanin B, were predicted to be AMES mutagenic. Hepatotoxicity was present in nine compounds, including Efavirenz and Darunavir, suggesting a risk for liver-related adverse effects. Most compounds were non-hepatotoxic. Only one compound, Debromohymenialdisine, showed potential hERG II inhibition, indicating low overall cardiotoxicity risk. Based on predicted acute toxicity (LD_{50}), Avarol and Avarone were identified as the most toxic, while Spongia acid was the least toxic compound among the set.

The docking results reveal significant insights into the inhibitory potential of marine phytoconstituents against key HIV targets, with detailed implications for antiretroviral drug development. Binding affinity, measured in kilocalories per mole (kcal/mol), quantifies how strongly a compound interacts with its target. Critically, more negative values indicate stronger binding, meaning the compound can more effectively disrupt the virus's lifecycle. For example, Darunavir's exceptional protease binding (-9.8 kcal/mol) makes it a clinical gold standard, as it tightly inhibits this essential HIV enzyme.

Reverse Transcriptase (RT) inhibition

Marine-derived compounds demonstrated significant potential in inhibiting HIV Reverse Transcriptase (RT), a critical enzyme responsible for converting viral RNA into DNA, a key step in viral replication (Sarafianos *et al*., 2009). The docking was targeted specifically at the RNase H domain of RT, which plays an essential catalytic role and requires coordination with divalent metal ions such as Mn^{2+} or Mg^{2+} (Neamati, 2011). Depending on the crystallization environment, water molecules or calcium ions may also be observed. The core catalytic residues involved in this domain include Asp443, Glu478, Asp498, and His539, which facilitate substrate binding and cleavage activity, as shown in Figure 2 (a,b,c).

Among the standard drugs, Lenacapavir exhibited the most favourable binding affinity (-7.7 kcal/mol), validating its potency. Notably, the marine-derived compounds Avarone (-7.0 kcal/mol) and Hippospongide A (-7.1 kcal/mol) outperformed Efavirenz (-7.2 kcal/mol), a widely used RT inhibitor in current antiretroviral therapy, as shown in Table 1. This suggests these natural products may possess comparable or superior binding potential, warranting further investigation. Avarone, in particular, demonstrated consistent multi-target affinity across RT, CP, and PR, indicating a structurally versatile molecule capable of fitting into diverse viral protein binding sites. This adaptability might stem from its planar quinonoid structure, which allows effective interactions within the RT active site, potentially coordinating with key catalytic residues and metal ions.

Capsid Protein (CP) inhibition

The HIV capsid protein (CP) plays a pivotal role in protecting the viral RNA genome and facilitating key stages of viral replication, including nuclear import and integration *(*Ding *et al.*, 2016). Inhibiting the capsid protein can disrupt viral assembly and maturation, making it an attractive therapeutic target. Specifically, the N-terminal domain (NTD) of the capsid protein is critical for oligomerization and structural stability, and thus a prime site for small-molecule inhibition.

In the present study, marine-derived compounds demonstrated superior binding affinity to the CP when compared to FDA-approved standards. Avarone exhibited the highest binding affinity among all tested compounds (-7.4 kcal/mol), followed by Avarol (-6.9 kcal/mol), both outperforming Lenacapavir (-6.8 kcal/mol), which is currently one of the most effective capsid-targeting, as shown in Table 1. The enhanced performance of Avarone and Avarol is likely attributable to their structural characteristics, particularly their hydrophobic quinone framework, which promotes stable interactions with the hydrophobic residues within the capsid's NTD domain. The 3D and 2D docked image of the hit molecules with the capsid protein is shown in Figure 3 (a,b,c). Notably, Avarone's strong and consistent binding to CP positions it as a leading candidate for capsid-targeted antiretroviral therapy. Its potential to interfere with capsid assembly or stability suggests a mechanism that could complement or enhance existing treatment regimens, particularly in the face of emerging drug resistance.

Protease (PR) inhibition

HIV protease (PR) is an essential viral enzyme responsible for cleaving the Gag and Gag-Pol polyproteins into functional structural proteins and enzymes (Fun *et al.*, 2012). This maturation step is crucial for producing infectious viral particles; thus, protease inhibition effectively halts the HIV lifecycle. The enzyme's active site typically includes conserved Asp25 and Asp29 residues from each monomer, forming a catalytic dyad that mediates substrate hydrolysis through acid-base catalysis.

In this docking analysis, marine-derived compounds demonstrated remarkable potential as protease inhibitors, with binding affinities that closely approached or even rivalled that of the FDA-approved benchmark, Darunavir (-9.8 kcal/mol). Among these, Hippospongide A showed a particularly high affinity (-9.4 kcal/mol), followed closely by 12-Chloro-11-hydroxydibromoisophakellin (-9.2 kcal/mol). The strong binding of Hippospongide A is especially noteworthy, as its interaction profile suggests potential engagement with PR's catalytic aspartates via hydrogen bonding or metal coordination, mirroring the interaction mechanisms of Darunavir.

Furthermore, Hippospongide A exhibited potent dual-target binding, with high affinity for both RT (-7.1 kcal/mol) and PR (-9.4 kcal/mol). This indicates a broader spectrum of inhibitory activity, offering the potential to disrupt multiple stages of the viral replication cycle, an important trait for reducing viral escape and resistance. The 2D and 3D binding pose depicted in Figure 4 (a,b,c). These findings underscore the promise of Hippospongide A and related marine scaffolds as templates for next-generation protease inhibitors, with possible multi-target capabilities that could be further explored through molecular dynamics and structural optimization.

ADMET profile

The in silico ADMET evaluation revealed that most of the selected marine and synthetic compounds exhibit promising oral drug-like characteristics. Fourteen out of fifteen compounds complied with Lipinski's Rule of Five, with minimal violations, which strongly correlated with high predicted oral bioavailability (≥0.55). Spongia acid stood

out as the most bioavailable compound (0.85), likely due to its optimal physicochemical properties, including balanced lipophilicity and molecular weight. In contrast, Lenacapavir showed poor bioavailability (0.17), which aligns with its three Lipinski violations and suggests limited oral efficacy. Compounds like Avarone and Dysideanin B exhibited high Caco-2 permeability, indicating favourable absorption profiles. However, a few compounds with low permeability, such as Stevensine, may require prodrug strategies or nano-formulation to enhance their oral bioavailability. Regarding distribution, only a few compounds, including Avarone and Dysideanin B, demonstrated significant blood-brain barrier (BBB) penetration, making them potential candidates for central nervous system (CNS) indications, albeit with a possible risk of neurotoxicity. Meanwhile, compounds like Lenacapavir and Darunavir were non-penetrant, making them more suited for peripheral therapeutic targets.

Metabolic profiling showed a wide range of CYP inhibition potential among the compounds. Multi-CYP inhibitors like Efavirenz and Lenacapavir pose a higher risk of drug–drug interactions, potentially requiring careful dose adjustments in clinical settings, especially when co-administered with drugs metabolized by CYP1A2, 2C9, or 3A4 enzymes. On the other hand, compounds such as Darunavir and Spongia acid did not inhibit any major CYP isoforms, indicating safer profiles in polypharmacy. Toxicity assessments raised concerns for several candidates. Hepatotoxicity was observed in nine compounds, consistent with clinical data for Efavirenz and Darunavir. Mutagenicity was predicted in Avarone and Dysideanin B, which could be addressed through structural modification to improve safety. Although most compounds showed minimal cardiotoxicity, Debromohymenialdisine's predicted hERG inhibition warrants further cardiac evaluation. Acute toxicity data highlighted Avarol and Avarone as potentially hazardous due to low LD_{50} values, while Spongia acid was again confirmed as a relatively safe compound with high LD_{50} and favourable ADMET properties.

Based on the cumulative ADMET profile, Spongia acid emerges as a high-priority candidate for further development, showing strong bioavailability, metabolic stability, and low toxicity, making it ideal for non-CNS applications. Avarol also presents a balanced profile with moderate permeability and minimal toxicity concerns. In contrast, compounds like Lenacapavir, despite their clinical relevance, demonstrated several ADMET liabilities, including poor absorption and high hepatotoxicity. Likewise, Avarone and Dysideanin B, though suitable for CNS delivery, may require optimization to reduce their mutagenic and toxic risks. Future research should focus on derivatizing high-risk but pharmacologically interesting compounds, such as Avarone and Dysideanin B, to retain therapeutic potential while minimizing safety concerns. In vivo validation of Spongia acid's pharmacokinetic and toxicity profiles, as well as mechanistic studies on hepatotoxicity in Efavirenz and Darunavir analogues, will be crucial steps in advancing these candidates toward clinical consideration.

Structural and therapeutic implications

Marine-derived compounds show strong promise as next-generation HIV inhibitors, particularly due to their ability to engage multiple viral targets with high affinity. While FDA-approved drugs tend to be more target-specific Lenacapavir, for instance, shows strong binding to RT (-7.7 kcal/mol) and CP (-6.8), but weaker interaction with PR (-7.9), and Darunavir binds well to PR (-9.8) but only moderately to RT and CP (around -6.3 and -5.5 respectively) marine candidates present a broader inhibition profile. Avarone stands out with consistently high binding scores across RT (-7.0), CP (-7.4), and PR (-8.7), thanks to its flexible quinonoid structure. Likewise, Hippospongide A combines strong PR (-9.4) and RT (-7.1) affinity through its

macrocyclic lactone framework, and Avarol shows comparable inhibition of CP (-6.9) and PR (-8.5), surpassing drugs like Efavirenz, which binds modestly to all three sites.

In addition to their binding efficiency, many marine compounds also show more favourable ADMET profiles. Spongia acid, for example, has the highest oral bioavailability (0.85), no signs of toxicity or CYP enzyme inhibition, and an excellent safety margin (LD_{50}: 2.46). Avarone and Dysideanin B exhibit good blood-brain barrier penetration (logBB: 0.26 and 0.43, respectively), offering potential to reach HIV reservoirs in the CNS, something current drugs fail to achieve. However, some compounds still need optimization. Avarone's mutagenic potential and Hippospongide A's mild CYP inhibition highlight areas for structural refinement. Also, low permeability observed in 12-Chloro-11-hydroxydibromoisophakellin suggests the need for advanced formulation strategies like nano-delivery. Overall, these findings suggest that marine-derived scaffolds not only expand the scope of HIV inhibition but also offer safer and more pharmacologically versatile candidates for future drug development.

CONCLUSION

Based on the *in-silico* study, Hippospongide A and Avarone emerged as the most promising leads against multi-drug-resistant HIV by combining high potency, complementary mechanisms, and distinct pharmacokinetic advantages. Hippospongide A provides dual inhibition of HIV reverse transcriptase and protease, surpassing standard drugs like Efavirenz in RT inhibition and rivaling Darunavir in protease targeting, with a favorable safety profile and low predicted toxicity. Avarone, meanwhile, demonstrates strong efficacy across all three major viral targets and uniquely penetrates the blood-brain barrier, positioning it to address HIV reservoirs in the CNS, an area where current treatments like Lenacapavir fall short. However, Avarone's mutagenic and hepatotoxic potential necessitates structural refinement. Together, these compounds present solutions to resistance and viral persistence challenges. As these findings are based on computational models, further experimental validation is required to confirm efficacy and safety in biological systems.

Abbreviation

2D= Two-Dimensional; 3D= Three-Dimensional; Å= Ångström (unit of molecular distance); ADMET= Absorption, Distribution, Metabolism, Excretion, and Toxicity; AIDS= Acquired Immunodeficiency Syndrome; AMES= Ames Mutagenicity Test; BBB= Blood-Brain Barrier; CAI= Capsid Inhibitor; Caco-2= Human Colorectal Adenocarcinoma Cell Line (used for permeability assay); CNS= Central Nervous System; CP= Capsid Protein; CYP= Cytochrome P450 Enzymes (general); CYP1A2= Cytochrome P450 Family 1 Subfamily A Member 2; CYP2C9= Cytochrome P450 Family 2 Subfamily C Member 9; CYP2C19= Cytochrome P450 Family 2 Subfamily C Member 19; CYP2D6= Cytochrome P450 Family 2 Subfamily D Member 6; CYP3A4= Cytochrome P450 Family 3 Subfamily A Member 4; ΔG= Gibbs Free Energy Change; DNA= Deoxyribonucleic Acid; FDA= Food and Drug Administration; Gag= Group-specific Antigen Polyprotein; HIV= Human Immunodeficiency Virus; hERG= Human Ether-à-go-go-Related Gene (cardiotoxicity marker); kcal/mol= Kilocalories per Mole (unit of binding energy); LD_{50} = Lethal Dose for 50% of the Test Population (acute toxicity index); logBB= Logarithm of Brain-to-Blood Concentration Ratio; logP= Logarithm of Partition Coefficient (lipophilicity); logPapp= Logarithm of Apparent Permeability Coefficient (Caco-2 assay); Mg^{2+} = Magnesium Ion; Mn^{2+} = Manganese Ion; MMFF94= Merck Molecular Force Field 94; NTD= N-terminal Domain (of capsid protein); PDB= Protein Data Bank; PDBQT= Protein Data Bank format with Partial Charge and Atom Type; PI= Protease Inhibitor; pkCSM=

Pharmacokinetics and Chemistry-based Signature Method; PR= Protease; PubChem= Public Chemical Database (NIH); RCSB= Research Collaboratory for Structural Bioinformatics; RNA= Ribonucleic Acid; RMSD= Root Mean Square Deviation; RT= Reverse Transcriptase; RTI= Reverse Transcriptase Inhibitor; SDF= Structure Data File; SwissADME= Swiss Absorption, Distribution, Metabolism, and Excretion Predictor; ΔG= Change in Gibbs Free Energy (Docking score)

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: Binding affinities (kcal/mol) of FDA-approved drugs and marine-derived compounds with HIV targets: reverse transcriptase (RT), capsid protein (CP), and protease (PR).

S.no	Compound name	Binding affinity		
		Reverse transcriptase	**Capsid protein**	**Protease**
	Standard			
1	Efavirenz	-7.2	-5.4	-7.5
2	Darunavir	-6.3	-5.5	-9.8
3	Lenacapavir	-7.7	-6.8	-7.9
	Marine phytoconstituent			
4	3-Bromohymenialdisine	-5.5	-5.7	-8.3
5	12-Chloro-11-hydroxydibromoisophakellin	-5.8	-5.6	-9.2
6	Avarol	-6.8	-6.9	-8.5
7	Avarone	-7	-7.4	-8.7
8	Debromohymenialdisine	-5.8	-5.7	-7.8
9	Dysideanin A	-3.6	-3.6	-5.1
10	Dysideanin B	-6.1	-4.6	-6.8
11	Furodysinin lactone	-5.9	-4.9	-7.9
12	Hippospongide A	-7.1	-6.6	-9.4
13	N-Methylmanzacidin C	-5.1	-4.8	-6.9
14	Spongia-13(16),14-dien-19-oic acid	-5.8	-5.2	-8.6
15	Stevensine	-5.4	-5.4	-7.7

Table 2: In silico ADMET profile of marine sponge-derived compounds and reference antiretroviral drugs.

Compound	Lipinski	Bioavailability	CYP Inhibition	Caco-2 (log Papp)	BBB (logBB)	AMES	Hepatotoxicity	hERG II Inhibition	LD50 (log mg/kg)
Efavirenz	0	0.55	CYP1A2,2C19,2C9	1.13	0.33	No	Yes	No	-0.26
Darunavir	1	0.55	-	0.76	-1.34	No	Yes	No	0.08
Lenacapavir	3	0.17	CYP2C19,2C9,3A4	0.81	-2.5	No	Yes	No	0.34
3-Bromohymenialdisine	0	0.55	-	0.04	-0.92	No	Yes	No	0.27
12-Chloro-11-hydroxydibromoisophakellin	0	0.55	-	-0.11	-2.47	No	No	No	0.15
Avarol	1	0.55	CYP1A2	1.3	-0.02	No	No	No	-0.58
Avarone	0	0.55	CYP2C19	1.56	0.26	Yes	Yes	No	-0.41
Debromohymenialdisine	0	0.55	-	-0.2	-0.52	No	Yes	Yes	0.72
Dysideanin A	0	0.55	-	0.87	-0.81	No	Yes	No	-0.19
Dysideanin B	0	0.55	CYP2D6	1.52	0.43	Yes	No	No	-0.54
Furodysinin lactone	0	0.55	-	1.28	0.33	No	No	No	0.01
Hippospongide A	0	0.55	CYP2C19,2C9	1.33	-0.05	No	No	No	-0.5
N-Methylmanzacidin C	0	0.55	-	-0.02	0.79	No	No	No	0.73
Spongia-13(16),14-dien-19-oic acid	0	0.85	-	1.44	0	No	No	No	2.46
Stevensine	0	0.55	-	-0.1	-1.31	No	Yes	No	0.66

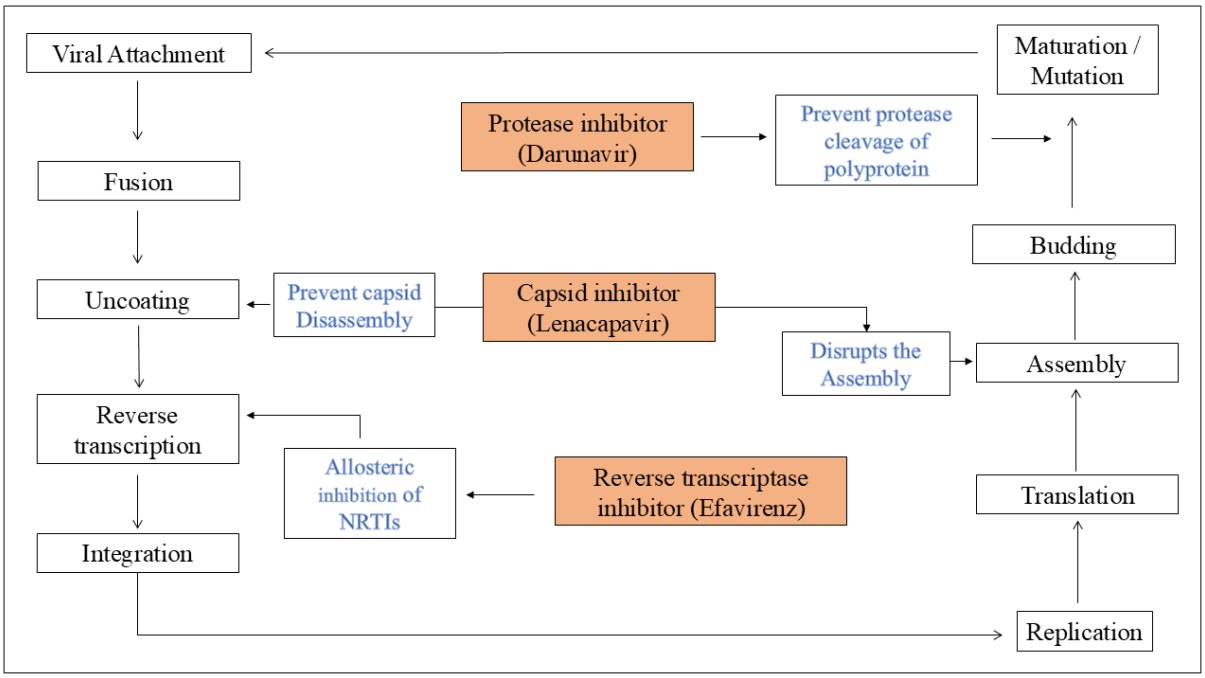

Figure 1: Classification of antiretroviral inhibitors targeting distinct stages of the HIV life cycle.

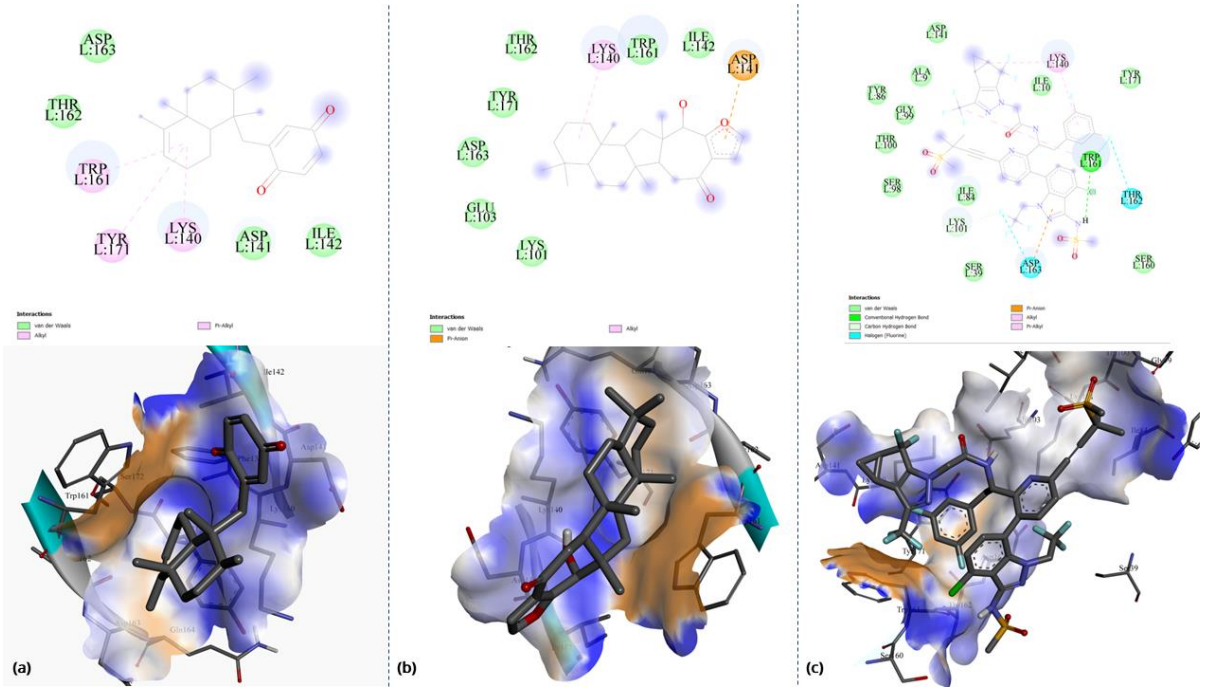

Figure 2: The best binding poses of (a) Avarone, (b) Hippospongide A, and (c) Efavirenz with the reverse transcriptase (3LP3)

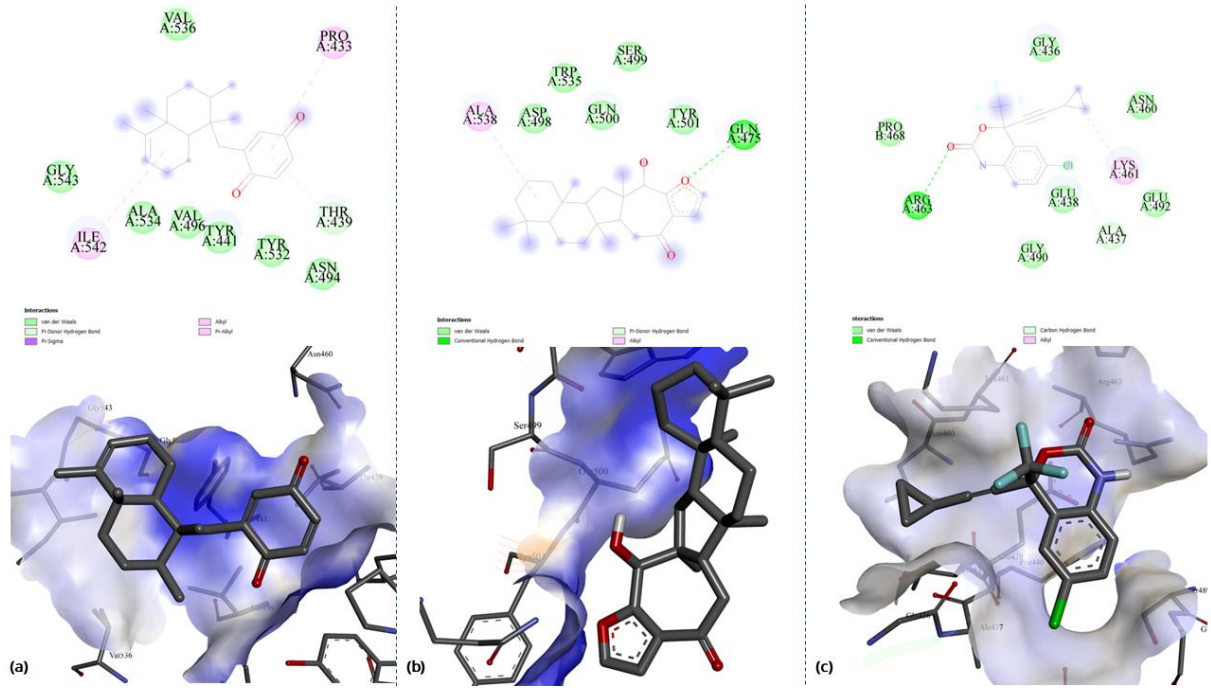

Figure 3: The best binding poses of (a) Avarone, (b) Hippospongide A, and (c) Lenacapavir with the Capsid Protein (1E6J)

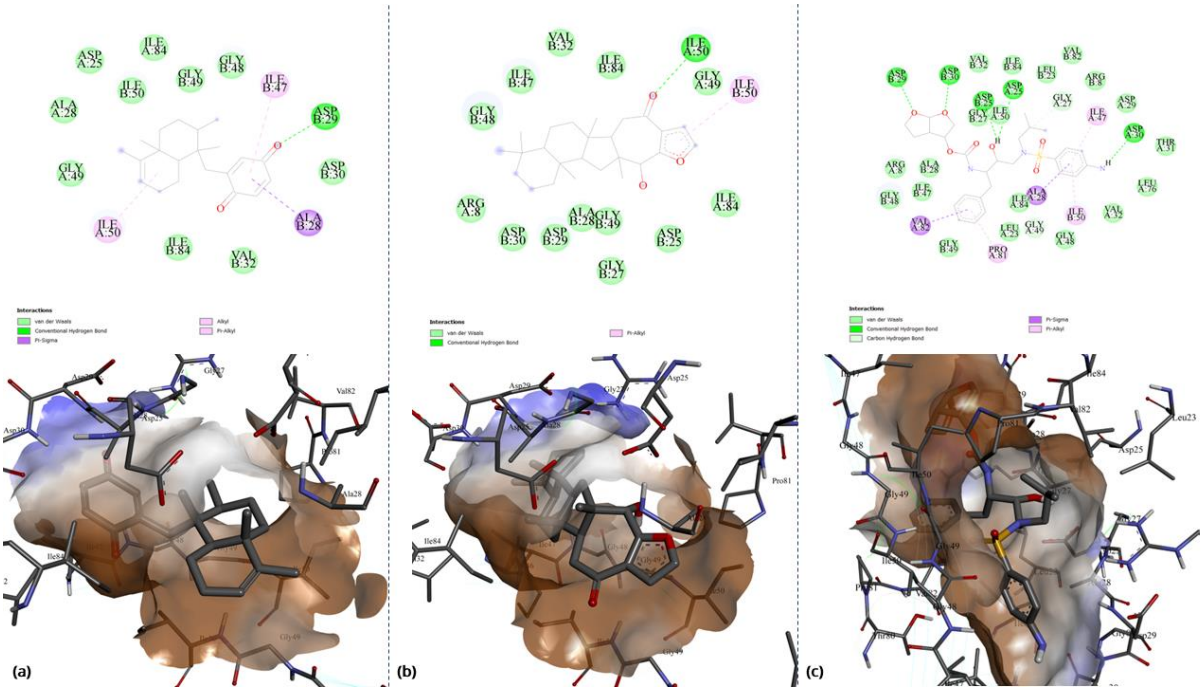

Figure 4: The best binding poses of (a) Avarone, (b) Hippospongide A, and (c) Darunavir with the enzyme Protease (1HVR)

Genetic variability in landraces of spring onion (*Allium chinense*) of Nagaland

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ABSTRACT

Spring onion (Allium chinense) is an important condiment crop in Nagaland known for its diverse landraces. The present study was undertaken to assess the genetic variability and interrelationships among yield and yield- contributing traits. Significant differences were recorded among 20 genotypes in all studied characters except for pseudostem length and pseudostem diameter. The GCV and PCV for yield per plot, bulb size, weight of whole cluster, number of cluster and bulb diameter were high indicating presence of sufficient variation for these traits. In the present investigation, high values of heritability and genetic advance were obtained for bulb size followed by weight of whole cluster and number of cluster. Correlation analysis revealed that the degree of genotypic correlation was higher than phenotypic correlation. The path analysis showed that the traits like number of leaves, number of cluster, weight of the whole cluster and dry matter content could serve as selection criteria in future breeding programs for improving yield potential in Allium chinense. The 20 genotypes of Allium chinense were grouped into 4 different clusters.Cluster I &III showed maximum inter cluster distance followed by cluster I & IV. Among different characters studied, contribution of days to 80% maturity was maximum towards divergence followed by bulb size.

Keywords: *Allium chinense,* correlation, D^2analysis, genetic variability, path analysis,

INTRODUCTION

Allium chinense (spring onion)is valued for its varied uses as food and medicinal properties. In India, this crop is mainly grown in northeastern states like Nagaland, Manipur, Arunachal Pradesh and Mizoram. *Allium chinense* is packed with many vitamins and minerals and the protein content on a fresh weight basis is 2.2%, fat 0.3%, carbohydrate 13.1% (Bah *et al.*, 2012). It contains high saponin, terpenes and alkanes, these bio-constituents can be further used in medicinal and therapeutic application (Rhetso *et al.*, 2020). *Allium chinense* is a much-known plant to all the locals in Nagaland and it has also grown well but due to the lack of research and scientific knowledge the potential of this crop is not yet discovered.

Genetic variability is important in a crop improvement program for effective selection. Hence, it is essential to assess the variability parameters for different characters in available germplasm. Genetic diversity measures are taken to quantify the overall genetic dissimilarity among different accessions, helping to identify distinct genetic lines. Therefore, present investigation was undertaken to evaluate genetic variability and diversity for bulb yield and other related traits among different genotypes as it provides foundation for selection of desirable genotypes

to be used in breeding programs (Amerullah *et al.,* 2021).

MATERIALS AND METHODS

The present investigation was conducted in Nagaland University at Medziphema campus, Nagaland during November –May in the year 2021-2022.The experimental site (Medziphema) is located at an altitude of 310 m above mean sea level, with geographical location of 25^0 45'43"N latitude and 93^0 33'04"E longitude. A total of 20 landraces of spring onion collected from various parts of Nagaland were used in the study and are presented in Table 1.The experiment was conducted using randomized complete block design (RCBD) with three replications. Within every replication, there were twenty plots with 30 plants per plot measuring 1m x 1m, spaced at intervals of 50cm. Plant spacing within rows was 15cm, while row spacing was 20cm.

The observations were taken on five randomly sampled plants in each plot on 15 quantitative parameters *viz,* leaf length, leaf diameter, pseudostem length, pseudostem diameter, bulb size, bulb height, bulb diameter, bulb width of neck, dry matter content, fresh weight, days to 80% maturity (of plant), weight of whole cluster (average of 10 clusters from mature plant), yield per plot. Analysis of variance was done as suggested by Panse and Sukhatme (1957).The coefficients of variation were calculated following the methods outlined by Burton and De Vane (1953). Heritability estimations were derived as per Allard (1960).

h^2_b (%) = σ^2_g/σ^2_px 100 where,h^2_b = Heritability in broad sense; σ^2_g = genotypic variance; σ^2_p = phenotypic variance

The potential genetic advance was worked out based on the approach by Johnson *et al.,* (1955).GA = k. σ_p. h^2_b where, k = selection differential at 5% selection intensity, the value of which is 2.06; σ_p= Phenotypic standard deviation;h^2_b = Heritability in broad sense

Correlation coefficients were determined as suggested by Al-Jibouri *et al.*(1958). Using Dewey and Lu (1959) method genotypic correlation coefficients were partitioned into direct and indirect effect. Genetic diversity was estimated using D^2 statistic (Mahalanobis, 1936). Clustering of genotypes was performed following Tocher's method (Rao, 1952). The data were analyzed using the INDOSTAT software.

RESULTS AND DISCUSSION

Analysis of variance was significant for all the characters except for pseudostem length and pseudostem diameter. Similar results were reported by Dehdari *et al.* (2001). The higher values of phenotypic coefficient of variation (PCV) with respect to the corresponding genotypic coefficient of variation (GCV) were recorded for all the traits (Table 2.) with low influence of environment. In the present study yield per plot, weight of the whole cluster, number of cluster and bulb diameter showed high GCV and PCV indicating genetic factors controlling these traits. Kohsa and Dhatt (2013) reported similar results. Heritability in broad sense includes both additive and non-additive gene effects (Hanson *et al.,* 1956). A high heritability indicates a significant role of genetic factors in controlling the traits. The high heritability was obtained for days to 80% maturity (98%) followed by bulb size (91%), number of leaves (73.4%). Similarly high genetic advance was obtained for bulb size followed by weight of whole cluster, number of cluster (Table 2.). High heritability and high genetic advance were observed for number of cluster, weight of whole cluster and bulb size indicating these variables are controlled by additive genes and selection would be worthwhile for future breeding program.

Similar observation was recorded by Parmar *et al.* (2018).

Correlation coefficient between nine characters was computed at genotypic and phenotypic levels and was tested at 5% and 1% levels of significance as presented in Table 3 and Table 4 respectively. In general the extent of genotypic correlation was greater than phenotypic correlation. The path analysis (Table 5) revealed that number of clusters contributed maximum positive direct effect on yield per plot followed by days to 80% maturity, bulb size, weight of the whole cluster, dry matter content, number of leaves and fresh weight. Among these traits bulb size and fresh weight had non- significant and negative correlation with yield, respectively. Number of leaves, number of cluster, weight of the whole cluster and dry matter content showed a strong positive correlation with yield and a positive direct effect, suggesting a genuine association between these traits. Thus, it is suggested that while selecting, emphasis should be given on these traits for yield improvement. Similar results were reported by Singh *et al.* (2018) and Rajshree Gayen *et al.* (2025). Characters like leaf diameter and number of clusters can be increased through indirect selection of bulb width. The estimated residual factor was 0.3024 which indicates that there might be a few more pertinent characters whose inclusion will give better understanding of causal relationships.

D^2 statistic is a powerful tool to measuring genetic diversity among genotypes. Based on the magnitude of D^2 values20 genotypes were grouped into 4 clusters(Table6).Cluster I and cluster II had the largest number of genotypes involving 8 genotypes each followed by cluster III and cluster IV with two genotypes each. Inter cluster distance (Table 7) was maximum between cluster I & cluster III (104.85) followed by cluster I and cluster IV (81.86) showing genetic diversity among genotypes. Cluster means (Table 8) for different characters showed substantial differences. In cluster I genotypes showed superior performance for leaf diameter, pseudostem diameter, bulb height, days to 80% maturity, bulb size. Cluster II recorded superior performance for leaf length, pseudostem diameter. Cluster III recorded superior performance for leaf length, pseudostem length, and number of cluster. Cluster IV recorded superior performance for bulb width of neck, weight of whole cluster, fresh weight, dry matter content, yield per plot. Some clusters exhibit higher values for certain traits which confirms genetic diversity. The contribution of different characters to the total genetic diversity(Table 9) indicated that days to 80% maturity contributed the maximum (93.68%) towards genetic divergence (this may be because of physiological traits contributing to days to maturity) followed by bulb size (2.68%). Rashid *et al*. (2012) reported similar observation.

CONCLUSION

In the present study yield per plot, weight of the whole cluster, number of cluster and bulb diameter showed high GCV and PCV indicating genetic factors controlling these traits. Characters like Number of leaves, number of cluster, weight of the whole cluster and dry matter content showed significant positive correlation and positive direct effect, hence while selection emphasis should be given to these characters.20 genotypes were grouped into four clusters based on D^2 values. Characters days to 80% maturity contributes the maximum (93.68%) towards genetic divergence followed by bulb size (2.68%).Based on mean yield performance, the genotype 14 (Morükyüm 3), genotype 15 (Khuovei 6) and genotype 17 (Khuovei 7) was found elite genotypes for cultivation.

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: Local name of the spring onion (*Allium chinense*) landraces and their place of collection.

Genotypes	Landraces name	Place of collection
1	Khuovei-1	Sochunoma
2	Khuovei-2	Chumukidima
3	Khuba -1	Jaluki
4	Aüshih	Mon (Yangchung)
5	Khuovei-3	Kidima
6	Khuovei-4	Visema
7	Khuovei-5	Chechama
8	Lasen -1	Wokha
9	Khuba-2	Peren
10	Khuva	Pfutsuru
11	Morukhium-2	Chimongner
12	Aüshih	Mon
13	Morükyüm-1	Kiphire (Seyochung)
14	Morukhium-3	Tuensang
15	Khuovei-6	Medziphema
16	Zhiyü	Nuiland (Shouba)
17	Khuovei-7	Kohima
18	Khuovei-8	Cakhabama
19	Lasen	Wokha
20	Alulasing	Mokokchung

Table 2:Estimates of genetic parameters for 15 characters in springonion (*Allium chinense*)

Character	Mean ± SE ±	Range	GCV	PCV	ECV	h^2 (Broad Sense)	Genetic Advance	Genetic Advance as % of Mean
No. of leaves	6.46±0.70	5.47-7.67	8.24	18.78	7.05	73.4	0.80	12.45
Leaf length (cm)	27.22±1.74	23.87-30.01	5.72	11.07	2.86	25.1	0.80	2.95
Leaf diameter (cm)	0.33±0.04	0.27-0.38	9.98	21.80	7.66	58.9	0.04	12.12
Pseudostem length (cm)	3.01±0.32	2.41-3.76	10.98	18.68	2.06	30.5	0.02	0.80
Pseudostem diameter (cm)	0.49±0.06	0.39-0.60	12.67	20.15	5.02	15.7	0.02	4.10
No. of cluster	9.82±1.49	7.40-20.60	26.35	30.51	6.45	75.1	4.63	47.22
Bulb height (cm)	2.21±0.21	1.69-2.49	10.57	17.14	3.70	12.3	0.06	2.67
Bulb diameter (cm)	1.53± 0.38	1.08-2.44	22.33	43.70	11.73	27.6	0.19	12.70
Bulb width (cm)	0.84± 0.14	0.63-1.08	13.49	15.65	10.02	55.19	0.24	15.34
Days to 80% Maturity	165.49± 0.13	153.4-173.67	3.97	4.13	2.97	98	13.51	8.17
Weight of whole cluster(g)	231.10±39.29	77.33-416	29.45	32.88	28.15	73.3	114.71	49.64
Fresh wt. (g)	21.83±3.60	15.80	18.00	28.59	7.19	16	1.29	5.92
Dry matter content (g)	7.11±1.23	5.15-11.19	19.02	29.94	7.93	17.4	0.48	6.81
Bulb size (cm)	4.68±0.46	1.33-7.00	16.96	32.61	11.11	91	2.86	61.13
Yield per plot (g)	495.92±180.71	1.25-833.33	48.45	63.11	31.93	43.4	215.01	43.36

Table 3: Estimates of genotypic correlation coefficient between different characters in spring onion (*Allium chinense*)

Characters	Leaf length (cm)	Leaf diameter (cm)	Pseudo stem length (cm)	Pseudostem diameter (cm)	No. of cluster	Bulb height (cm)	Bulb diameter (cm)	Bulb width (cm)	Days to 80% Maturity	Weight of whole cluster (g)	Fresh wt. (g)	Dry matter Content (g)	Bulb size (cm)	Yield per plot (g)
No. of leaves	0.679**	-0.803*	0.749**	0.605**	0.388*	0.262	0.434*	0.982**	-0.133	0.193	0.567**	0.009	-0.323	0.421*
Leaf length		-0.851**	0.334	0.409*	0.008	0.867**	0.996**	0.071	0.584**	-0.499*	0.400*	0.854**	0.449*	0.471*
Leaf diameter			0.623**	0.313	0.773**	-0.044	0.751**	0.098	-0.297	-0.007	-0.591**	-0.216	0.092	0.220
Pseudostem length				0.053	-0.163	0.803**	0.303	0.768**	0.493*	0.932**	0.958**	0.648**	0.011	-0.503*
Pseudostem diameter					-0.994**	-0.218	0.194	0.779**	0.273*	0.794**	0.603**	0.545**	0.052	0.475*
No. of cluster						0.062	-0.005	0.025	-0.680**	0.255	0.405*	0.237	-0.592**	0.766**
Bulb height							-0.300	0.275	0.895**	-0.520*	0.188	0.502*	0.672**	0.401*
Bulb diameter								0.958**	-0.170	-0.370	0.988**	0.220	0.661**	0.720**

Bulb width								0.153*	0.122	0.095	0.495*	0.315	0.401*
Days to 80% Maturity									-0.628**	0.210	0.106	0.325	0.346
Weight of whole cluster										0.527**	0.688**	-0.163	0.990**
Fresh wt.											0.955**	0.397*	-0.572**
Dry matter wt.												0.363	0.567**
Bulb size													0.336

Note : * * significance at 1% level, * significance at 5% level

Table 4: Estimates of phenotypic correlation coefficient between different characters in spring onion *(Allium chinense*)

Character	Leaf length (cm)	Leaf diameter (cm)	Pseudo stem length (cm)	Pseudo stem diameter (cm)	No. of cluster	Bulb height (cm)	Bulb diameter (cm)	Bulb Width of neck (cm)	Days to 80% Maturity	Weight of whole cluster (g)	Fresh wt. (g)	Dry matter content (g)	Bulb size (cm)	Yield per plot (g)
No. of leaves	0.075	0.295	0.13	0.208	-0.28	-0.074	-0.073	0.566**	0.113	-0.074	0.231	0.172	0.296	0.078
Leaf length		0.47	0.39	0.113	0.207	0.527**	0.039	-0.226	-0.298	0.372	0.246	0.171	-0.096	0.342
Leaf diameter			0.288	0.501*	-0.362	0.517**	0.298	0.075	0.222	0.147	0.314	0.179	0.053	0.137
Pseudo stem length				-0.052	-0.081	-0.048	0.278	0.201	-0.281	0.538**	0.304	0.289	-0.193	0.371
Pseudo stem diameter					-0.381*	0.11	-0.145	0.076	0.104	0.292	0.334	0.372	0.439*	0.083
No. of cluster						-0.182	-0.088	-0.383*	-0.591**	0.222	-0.532**	-0.505**	-0.499*	0.441*
Bulb height							0.011	-0.314	0.309	-0.151	0.162	0.069	0.287	0.006
Bulb diameter								0.043	0.094	0.215	-0.033	-0.068	-0.377	0.257
Bulb width									-0.022	0.100	0.494*	0.478*	0.314	0.341
Days to 80% Maturity										-0.537**	0.085	0.044	0.309	0.226
Weight of whole cluster											0.202	0.264	-0.127	0.475*
Fresh wt.												0.975**	0.498*	0.019
Dry matter content													0.508*	0.077
Bulb size														0.208

Note : * * significance at 1% level, * significance at 5% level.

Table- 5: Direct and indirect effect of different characters of genotypic level in spring onion (*Allium chinense*) on yield per plant.

	No. of leaves	Leaf length (cm)	Leaf diameter (cm)	Pseudo stem length (cm)	Pseudo stem diameter (cm)	No. of cluster	Bulb height (cm)	Bulb diameter (cm)	Bulb width (cm)	Days to 80% Maturity	Weight of whole cluster(g)	Fresh wt. (g)	Dry matter content (g)	Bulb size (cm)	r_g for Yiel per plot (g)
No. of leaves	1.335	-0.682	-1.071	2.333	0.807	0.517	0.348	0.579	-2.644	-0.177	0.257	-1.521	-1.346	-0.430	0.421
Leaf length	1.155	-2.261	1.924	12.060	-0.923	-0.017	-1.960	-2.252	11.465	-1.320	1.128	5.425	4.190	-1.014	0.471
Leaf diameter	5.513	5.847	-6.872	-4.284	-2.149	-5.312	0.301	-5.155	28.157	2.036	0.048	4.057	1.481	-0.632	0.220
Pseudo stem length	-0.030	0.092	-0.011	-0.017	0.053	0.003	-0.066	0.023	-0.307	0.025	-0.051	-0.034	-0.028	0.017	- -0.503
Pseudo stem diameter	-4.143	-2.797	-2.143	20.916	-6.852	6.807	1.492	-1.327	-25.890	-1.866	-5.434	-10.98	-10.580	-7.207	0.475
No. of cluster	5.618	0.111	11.202	-2.356	-14.397	14.492	-15.384	-0.071	43.833	-9.848	3.689	-20.35	-17.921	-8.570	0.766
Bulb height	-0.946	-3.137	0.159	-13.760	0.788	3.841	-3.619	1.085	-8.232	-3.237	1.878	-4.296	-1.816	-2.429	0.401
Bulb diameter	-0.979	-2.247	-1.693	2.938	-0.437	0.011	0.677	-2.256	4.415	0.382	0.835	-2.228	-2.751	-1.489	0.720
Bulb width	0.957	2.448	1.978	-8.578	-1.824	-1.460	-1.098	0.944	-0.483	-0.074	-0.059	3.908	3.618	1.117	0.401
Days to 80% Maturity	-1.651	7.258	-3.684	-18.588	3.385	-8.447	11.120	-2.106	1.895	12.431	-7.800	2.606	1.315	4.030	0.346
Weight of whole cluster	0.748	-1.940	-0.027	11.402	3.085	0.990	-2.019	-1.439	0.474	-2.441	3.890	2.046	2.675	-0.631	0.990
Fresh wt.	-0.761	-1.602	-0.394	1.307	1.070	-0.938	0.793	0.659	-5.404	0.140	0.351	0.668	0.638	0.932	-0.572
Dry matter content	-3.308	-6.076	-0.706	5.401	5.065	-4.054	1.645	3.998	-24.572	0.347	2.255	3.134	3.279	4.466	0.567
Bulb size	-3.928	5.456	1.119	-12.306	12.804	-7.198	8.169	8.036	-28.170	3.947	-1.975	17.001	16.583	12.172	0.336

Residual effect: 0.3024

Table 6: Clustering pattern of 20 genotypes of spring onion(*Allium chinense*) on the basis of genetic divergence

Cluster number	Number of genotypes	Genotypes
Cluster I	8	Khouvei-4, khouvei-5, Lasen-1, Khuba-2, Khuva, Aüshih, Morükyüm-1, Khouvei-8
Cluster II	8	Khuba-2,Aüshih, Morükyüm 2, Morükyüm-3, Khouvei-6, Zhiyü, Khouvei-7, Lasen
Cluster III	2	Khouvei-2, Alulasing
Cluster IV	2	Khouvei-1, Khouvei-3

Table 7: Average inter and intra cluster distance of 20 spring onion (*Allium chinense*)

Cluster number	Cluster I	Cluster II	Cluster III	Cluster IV
Cluster I	**11.29**	49.01	**104.15**	81.86
Cluster II		**10.26**	56.52	34.26
Cluster III			**9.01**	23.09
Cluster IV				**0**

Table 8: Cluster wise mean value of 20 genotypes of Spring onion (*Allium chinense*)

Characters/ cluster	No. of leaves	Leaf length (cm)	Leaf diameter (cm)	Pseudostem length (cm)	Pseudostem diameter (cm)	No. of cluster	Bulb height (cm)	Bulb diameter (cm)	Bulb width neck (cm)	Days to 80% Maturity	Weight of whole cluster (g)	Fresh wt. (g)	Dry matter content (g)	Bulb size (cm)	Yield per plot(g)
Cluster I	6.4	27.12	0.34	2.92	0.49	8.53	2.26	1.62	0.79	172.17	205.96	21.75	7.02	5.08	487.81
Cluster II	6.7	26.83	0.33	3.04	0.49	9.19	2.09	1.39	0.9	163.87	210.46	22.43	7.35	4.71	599.44
Cluster III	6.07	28.31	0.32	3.24	0.47	14.47	2.04	1.68	0.77	154.44	102.33	19.7	6.51	3.44	190.04
Cluster IV	6.2	27.86	0.32	2.72	0.47	11.13	2.14	1.37	0.83	158.13	263.67	24.08	7.74	5	650.37

Table 9: Contribution of each character towards divergence

Source	**Times Ranked 1st**	**Contribution %**	**Source**	**Times Ranked 1st**	**Contribution %**
No. of leaves	0	0.00%	Bulb width of neck (cm)	0	0.00%
Leaf length (cm)	9	1.00%	Days to 80% Maturity	178	93.68%
Leaf diameter (cm)	0	0.00%	Weight of whole cluster(g)	0	0.00%
Pseudostem length (cm)	0	0.00%	Fresh wt. (g)	0	0.00%
Pseudostem diameter (cm)	0	0.00%	Dry matter content (g)	3	1.58%
No. of cluster	1	0.53%	Bulb size (cm)	7	2.68%
Bulb height (cm)	0	0.00%	Yield per plot (g)	1	0.53%
Bulb diameter (cm)	0	0.00%			

SHORT COMMUNICATION

Determination of quality parameters of pulp and peel of different mango varieties under Chhattisgarh Plains zone

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ABSTRACT

The pulp represents the quality of any mango variety which determines its market price and demand but mango peel is also used as a by-product in the food industry, hence finding out its biochemical properties is a very important factor based on which we can use the pulp of that mango variety and then its by-product peel. The fruit quality parameters like total soluble solids (TSS), titratable acidity and total sugar content in the pulp and peel of ten mango varieties were evaluated and quantified. Pulp (%) was found to be highest in Chhattisgarh Swarnprabha (78.10%) and peel (%) was found to be lowest in Langra (10.79%) variety. Whereas in biochemical properties, total soluble solids (^{0}Brix) of pulp and peel were found to be highest in Chhattisgarh Gaurav variety (22.74°B) and (4.79°B), titratable acidity was found to be best in Chhattisgarh Achar (0.74%) and (0.21%) and total sugar was found to be best in Chhattisgarh Swarnprabha (17.71%) and (4.79%).

***Keywords**:* Mango variety, peel, physico-chemical characteristics, pulp

Mango fruit possesses high nutritional and therapeutic values and it has high demand both domestic and foreign markets. Plant-derived products are an important source of bioactive substances such as dietary fiber, vitamins, minerals, and various physic-chemicals such as polyphenols and carotenoids, which contribute to its potential health-promoting properties with amazing health benefits (Jain and Shankaran 2024). Pulp content in mango fruits is a very important factor that determines the quality of the variety. Mango is rich sources of many nutrients and phyto-chemicals such as carotenoids, vitamins, acids, polyphenols and prebiotic dietary fiber that have nutritional and medicinal effects (Rajasekaran and Soundarapandian 2024). Studies on fruit quality can be helpful in selecting better quality fruits. Mango peels, dried mango peel, amchur and mango shake are particularly sustainable sources of bioactive compounds with antibacterial, enzymatic, and antioxidant properties (Kučuk *et al.*, 2024). Nutrients present in pulp and peel of mango fruit vary among mango varieties, there is no such published report on biochemical properties present in pulp and peel of different mango varieties grown in plain region of Chhattisgarh. In this context, the present study was carried out to investigate the physico-chemical characteristics of pulp and peel of ten mango varieties grown in Chhattisgarh.

Fresh and healthy 10 varieties of mature mango fruits were taken from Horticulture Farm, Department of Fruit Science, College of Agriculture, IGKV, Raipur, Chhattisgarh during the year 2019-20 and 2020-21. Ten varieties were Chhattisgarh Swarnaprabha, Chhattisgarh Pawan, Chhattisgarh Achar,

Chhattisgarh Raj, Chhattisgarh Gaurav, Chhattisgarh Nandiraj, Dashehari, Langra, Mallika and Amrapali. Mango fruits were cleaned in 0.5% chlorine solution to remove impurities and foreign matter and then washed thoroughly with water. Then the fruits were dried under shade to remove excess water. Mango fruits were ripened at room temperature in the laboratory and after ripening the fruits were transferred to the laboratory of the department. The ripe mangoes were peeled from the fruits using a sterilized knife and carefully separated from the pulp. The peels were then dried using an oven dryer (50 ^{0}C) and the dried mango peels obtained were subsequently ground into fine powder. Mango peel powder was kept at room temperature (15–24^{0}C) in a sealed plastic bag that was 75µm thick. It was then analyzed for a number of physicochemical characteristics. The investigation was carried out following Randomized Block design; each variety was considered as a treatment, so there were 10 treatments. For each variety 9-mature mango fruits were collected of uniform size that was replicated three times with three fruits in each replication.

Total Soluble Solids (TSS) content of the fruits was estimated with the help of a hand refractrometer (0 to 32°Brix) and the values were corrected at 20°C. One drop of the fruit juice was placed on the glass disc of the refractrometer and TSS was observed and noted for different treatments. The glass disc was thoroughly cleaned after each operation and TSS was expressed as °Brix. The total titratable acidity was determined by titrating fruit juice against 0.1 N NaOH in the presence of phenolphthalein indicator. The appearance of light pink colour was taken as the end point. The result was expressed in terms of per cent acidity of the fruit pulp. Total sugar was estimated as per the method described by Lane and Eynon, A.O.A.C (1990). A quantity of 50 ml lead free filtrate was taken in a 100 ml volumetric flask to which 5 ml of concentrated HCl was added, mixed well and then kept for 24 hrs at room temperature. Acid was then neutralized with NaOH using a drop of phenolpthalein as an indicator till the pink colour persisted for at least few seconds. Then volume was made up to 100 ml. Total sugars were then estimated by taking this solution in a burette and titrating it against standard Fehling's solution mixture of A and B (1:1) using methylene blue indicator, taking brick red colour as the end point. The statistical analysis was carried out for each observed character under the study using MS-Excel, OPSTAT. The data generated from these investigations were analyzed as described by Gomez and Gomez (1984) by applying completely randomized design (CRD) with three times replicated. Data were subjected to analysis of variance (ANOVA) by using OPSTAT online software. The mean difference at 5 per cent level of significance (LOS). Critical difference (CD) at 5 per cent level of probably it was used for comparison among treatments.

The pooled data of two years of all parameters have been presented in the Table 1. Pulp per cent (%) was observed highest being Chhattisgarh Swarnprabha (78.10%) while the lowest was found in Mallika (60.45%). Peel per cent (%) was found to be lowest in mango cv. Langra (10.79 %) while it was highest in Mallika (20.26 %). The variation in pulp and peel percentage was found to vary considerably among varieties due to their genetic and climatic factors. Similar observations were also noted by Ahmed and Mohamed (2015); Sinha *et al.* (2020); Hada and Singh (2017a) and Jilani *et al.* (2010).

The highest total soluble solids in mango pulp and peel were found in Chhattisgarh Gaurav variety (22.47 ^{0}Brix) and (4.79 ^{0}Brix) and the lowest total soluble solids were observed in Chhattisgarh Achar (13.10 ^{0}Brix) and (3.80 ^{0}Brix) . Variation in TSS in varieties of mango pulp and peel was also noted by Kankhare *et al.*(2019) ; Trong *et al.* (2020) and Tanu *et al.* (2020) and they mentioned that this variation was due to the genetic structure as well as weather conditions of mango varieties.

Chhattisgarh Achar variety had the highest titratable acidity (0.74%) and (0.21%) and lowest found in Langra (0.19 %) & (0.16%). The similar trend was reported by some researchers like Kankhare *et al* (2019) and Shinde *et al.*(2015).

The total sugar content in was highest in both pulp & peel of mango variety Chhattisgarh Gaurav (17.71%) & **(**4.79 %) respectively and the lowest was found in Chhattisgarh Achar (10.04%) & (3.80 %). This finding confirms the results of Tanu *et al.* (2020); and Shinde *et al.*(2015) that total sugar content varied across pulp which may be a genetic character of the variety. The finding is also similar to Serna-Cock *et al.* (2016).

It can be conclude that among the different mango varieties grown in plain area of Chhattisgarh; we found that Chhattisgarh Swarnprabha showed highest pulp content (78.10 %) and while Langra had the maximum peel content (10.79 %). The TSS and Total sugar in peel were found to be highest Chhattisgarh Gaurav variety, while titratable acidity showed highest in the variety Chhattisgarh Achar. By knowing the biochemical properties of pulp and peel, value added products may be prepared accordingly.

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: Studies on mango pulp, peel content and their bio-chemical parameters viz., total soluble solids (°B), titrable acidity (%) and total sugar (%) of mango varieties

Varieties	Pulp (%)	Peel (%)	TSS of pulp (°B)	TSS of peel (°B)	Acidity of pulp (%)	Acidity of peel (%)	Total sugar of pulp (%)	Total sugar of peel (%)
Chhattisgarh Swarnaprabha	78.10	13.84	18.06	4.79	0.45	0.19	15.72	4.79
Chhattisgarh Pawan	67.10	18.54	16.41	4.11	0.34	0.17	15.64	4.11
Chhattisgarh Achar	77.58	11.12	13.10	3.80	0.74	0.21	10.04	3.80
Chhattisgarh Raj	73.23	10.81	17.00	4.01	0.43	0.16	14.24	4.01
Chhattisgarh Gaurav	76.20	11.88	22.47	4.79	0.46	0.17	17.71	4.79
Chhattisgarh Nandiraj	72.55	11.86	17.42	4.10	0.24	0.17	14.64	4.10
Dashehari	71.82	13.60	16.7	4.36	0.20	0.18	14.70	4.36
Langra	76.45	10.79	20.00	3.92	0.19	0.16	14.35	3.92
Mallika	60.45	20.26	17.04	4.18	0.26	0.17	13.72	4.18
Amrapali	75.56	12.58	18.16	4.16	0.20	0.19	14.68	4.16
SE(m) ±	**0.42**	**0.71**	**0.77**	**0.14**	**0.04**	**0.01**	**0.56**	**0.14**

SHORT COMMUNICATION

Mapping of physiognomic changes in steppe vegetation using geomatic tool: case of the Naâma region (South West Algeria)

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ABSTRACT

For several decades, the steppe region has been undergoing advanced degradation due to the combined effects of climatic and anthropogenic factors. This study was conducted to monitor the dynamics of the different steppe plant formations and to demonstrate the value of geomatics for spatial management and decision-making. Mapping and processing of Landsat-8 satellite images from 2018 made it possible to map and assess this evolution between 1978 and 2018. The results show a decline in the area occupied by the paraclimax steppe from 391240 ha (83.51%) in 1978 to 304056 ha (65%) in 2018. The psammophilous steppes also experienced a very significant increase, from 26410 ha in 1978 to 128838 ha in 2018. The regressive dynamics of the vegetation cover is accompanied by significant dune movements and a considerable reduction in the area of the matorrals of Juniperus phoenicea L and Stipa tenacissima L.

Keywords: Anthropogenic factors, dynamics, Landsat-8 satellite, matorrals, psammophilous steppes, steppe region,

The rapid loss of biodiversity is one of the most critical environmental challenges faced by the present generation (Ekanayake and Fernando, 2021). Climatic factors and human action are the main factors responsible for the decline in biodiversity (Mamadzhanov *et al*., 2024). The high plains of southern Oran are very fragile steppe rangelands where the ecological and socio-economic balance is compromised by the combined effects of severe climacic hazards dominated by drought and increasing anthropogenic pressure on natural resources. The steppe ecosystem is in a state of permanent deterioration so that urgent measures are needed to protect, conserve, regenerate, improve and manage the natural area (Khader *et al*., 2009, Amara *et al*., 2025).

Steppe spaces of the Naâma area are rather well representative of all steppe spaces of western Algeria. They are exploited as rangelands with a mean carrying capacity of 10 sheep/ ha although the possibilities are only of 0.9 ha / sheep (Mekhloufi *et al*., 2020). The regressive evolution of vegetation, characterized by the gradual replacement of para-climacic steppes with *Stipa tenacissima* L., *Artemisia herba-alba* Asso and *Lygeum spartum* L by degraded steppes with low productivity, dominated by thorny and toxic species such as *Atractylis*

serratuloides Sieber ex Cass., *Peganum harmala* L and *Noaea mucronata* (Forssk.) Asch. & Schweinf. The gradual evolution of the psammophilous formations is related to the dune movements that shape their environment.

In addition, diachronic analysis by remote sensing makes it possible to understand the evolution of the environment and the processes of regression or progression of vegetation over periods of up to tens of years. The objective of our research is to show how the comparison of satellite images between two different periods (1978 and 2018) makes it possible to assess the extent of the physiognomic changes that have occurred in the vegetation cover in the Naâma and Mecheria region and to locate the area's most affected and most threatened by the silting process and therefore the areas where priority action should be taken.

The choice of the period for studying physiognomic changes was not random. It was practically between 1970 and 2018 that the Algerian steppe experienced major changes in its vegetation cover following the accentuation of the phenomenon of desertification and overgrazing. After 2018 and until today, the situation of the steppe has not changed.

The study area is part of a pastoral region covering an area of 29514 km² (Hadjadj and Guerine, 2024). It is located between the Tellian and Saharian atlas in its western part, bordered to the north by the wilayates (provinces) of Tlemcen and Sidi bel Abbés, to the south by the wilaya of Bechar, to the east by the wilaya of El-Bayadh and to the west by the Kingdom of Morocco. The surface area of grazing land is 2 153740 ha, including 430000 ha of esparto grass cover and 514300 ha of forests and scrubland (Benguerai, 2011). The study area extends over two communes Mécheria and Naâma, covering an area of 486500 ha at altitudes ranging from 1128 to 2100 m.

Steppe soils are calcareous, characterised by a shallow depth, a very low organic matter content (less than 1%) which decreases with depth, while the level of limestone increases and hinders plant development. The texture is predominantly sandy, with poor structural stability and low water retention capacity, allowing only xeric vegetation adapted to these environmental conditions to develop (Benabdeli, 2000). In general, the study region is characterized by an average annual rainfall of less than 190 mm/year (period 1990-2022). This precipitation is irregular, low, and stormy. The average annual temperature is 19.5°C. The hottest month is July with a temperature of 30.5°C and the coldest is January with a temperature of 7.5°C. The Emberger pluviothermal quotient calculated for this period is 20.3, which allows the study area to be classified in the lower arid bioclimatic zone with cool winters. The dry season in the region extends almost throughout the year (Hadjadj and Guerine, 2024; Amara *et al*., 2025). The sirocco blows for an average of 36 days a year, concentrated in June and July (Louassa, 2010).

The perennial plant formations of the area are dominated by degradation facies of steppes with *Stipa tenacissima* L, *Lygeum spartum* L which constitute poor rangelands and relicts of chamaephytic steppes based on *Artemisia herba-alba* Asso as well as degradation facies based on *Noaea mucronata* (Forssk.) Asch. & Schweinf. and *Thymelea microphylla* Coss. The study area is marked by the presence of wooded steppes with *Pistacia atlantica* Desf. associated with formations of *Ziziphus lotus* (L.) Lam. The mountain area is characterized by the presence of pre-forest formations, these are sparse shrub formations of *Quercus ilex* subsp. *Ballota* (Desf.), *Pinus halepensis* Mill (artificial formations), *Juniperus phoenicea* L and *Juniperus oxycedrus* L (Hadjadj *et al*., 2020).

The municipality of Mecheria has approximately 109991 inhabitants in 2002 according to the planning and budget monitoring department, which represents almost 1/3 of the total population of the wilaya of Naâma. For the commune of Naâma, the population is estimated at around

28753 inhabitants in 2021, which represents nearly 9% of the total population of the wilaya of Naâma (Amara *et al*., 2025).

The proportion of the nomadic population in the two communes has decreased significantly, it is less than 5% (2.60% in Mecheria and 1.74% in Naâma) of the total population of the two communes. This decrease is due to the reduction of transhumance and sedentarization caused by rural housing programs, leading to overexploitation of the rangelands. Agro-pastoral activity remains a determining factor, pastoralism is the traditional productive activity of the steppe (Hadeid, 2008). The total agricultural area of the wilaya is 2203460 ha, of which the useful agricultural area represents only 1.14%, or 25019 ha, and the rest (2178441 ha) is made up of pastures. The pastoral livestock in this steppe area is estimated at around 1273094 heads, of which the predominant species is sheep (around 91.25% of the livestock) (Amara *et al*., 2025).

The method used to assess the dynamics of plant formations is based on the 1978 phytoecological map, vegetation surveys, a 2018 Landsat-8 satellite image and Geographic Information Systems. The approach consists of digitising the 1978 phytoecological map, supervised classification of the satellite image, field survey and finally a comparison between the two maps of plant groupings. The investigation area on the satellite image was delimited by entering the coordinates of the four corners of the map, so that the remaining part of the image corresponds exactly to the map.

There are three main stages in processing the satellite image: pre-processing, classification of the image using the Maximum Likelihood algorithm and production of the 2018 plant ecology map. Geometric correction of the satellite image and the 1978 plant ecology map in the same geographical reference frame (UTM, Zone 31 North).

In order to make the 1978 phytoecological map comparable to the one produced in 2018, the biotope parameters of the plant groups had to be taken into account based on the ranges of the main species characteristic of the environment, the field surveys of Gaddas (Gaddas, 2001) and the work carried out by (Khader *et al*., 2022) made this comparison possible. Vegetation dynamics were assessed by mapping the location of physiognomic vegetation units potentially containing the habitats of the various dominant species (para-climatic degradation, sandy areas, closed depressions, relief). Assessing the accuracy of the classification is very important for understanding the results obtained and using them for decision-making (Plourde and Congalton, 2003). The most common elements of accuracy assessment are overall accuracy, producer accuracy, user accuracy and the Kappa coefficient (κ). The scientific literature has provided meanings and calculation methods for these elements (Plourde and Congalton, 2003; Foody *et al*., 2003).

In our case, the supervised classification performed, the overall accuracy and the Kappa coefficient were calculated to give an idea of the quality of the classification. One hundred and fifty test and training sites covering the main plant formations in the region were undertaken through observations and plant-ecological surveys using the linear transect method. In order to assess the spatial dynamics of the various plant groups over a period of 40 years, the only fairly accurate map of plant formations in the area was used, the phytoecological map produced in 1978 by URBT (Biological and Terrestrial Research Unit).

In 1978, of the nine plant groups mapped, the perennial and para-climacic species identified covered an area of 391240 ha, *i.e*., 83.51 % of the total area of the study region. The steppe dominated by *Stipa tenacissima* L, with an estimated area of 173600 ha (37.06 %), the *Lygeum spartum* L steppe, with an area of 125900 ha (26.88 %, the clear *Artemisia herba-alba* Asso steppe

with 48210 ha or 10.29 %, the rest of the plant formations cover only 25.77 % including mixed steppes covering an area of 34810 ha (7.43 %) and matorrals on 32530 ha (6.94 %), the psamphyle group covered a total area of 26410 ha or 5.64%, 75.84% of which was *Thymelea microphylla* Coss. and 24.16 % *Retama raetam* (Forssk.) Webb., and finally the halophilic steppes of the closed depressions covered an area of 18320 ha (3.91 %) and the southernmost steppe formation dominated by *Arthrophytum scoparium* (Pomel) Iljin. occupied 87200 ha (1.86 %) and 87200 ha (1.86%) of the study area respectively (Table 1).

The use of 150 reference points for the validation resulted in an overall accuracy of 83.08 % for the classification of the TM 2018 image, with a Kappa Index of 0.8121. Reading of the statistics in Table 1 and Figure 2, which illustrate the results of the land cover areas for the 2018 image, shows that the largest area of land cover per entity in terms of surface area is occupied by *Stipa tenacissima* L with an estimated surface area of 109862 ha or 23.45 % of the total study area, followed by rangelands with *Lygeum spartum* L with an area of 106631 ha or 22.76 %, psomophytes with an area estimated at 93513 ha or 19.96% and formations with *Artemisia herba-alba* Asso with 46241 ha which represents 9.87 % of the study area, the rest of the plant formations cover only 25.77 % of which the *Retama raetam* (Forssk.) formation *and* the *Arthrophytum scoparium* (Pomel) Iljin. formation extend respectively over a surface area of 35325 ha or 7.54 % and 34 201ha or 7.30 % followed by the halophytes with a surface area of 18412 ha or 3.93% and the matorrals formations which occupy 17194 ha or 3.67 %. Finally the mixed formations which cover 7120 ha or 1.52 % of the total surface area of the study area.

A reading of the statistics in Table 2 shows clearly that the mixed steppe with *Stipa tenacissima* L and *Lygeum spartum* L declined by 63,738 ha, i.e. 3.61 % of its initial area, while the fairly dense mixed steppe with *Lygeum spartum* L and *Artemisia herba-alba* Asoo declined considerably during the study period, to 27 689 ha, i.e. 5.81 % compared with 1978, followed by the steppe with *Lygeum spartum* L and Matorrals of *Juniperus phoenicea* L and *Stipa tenacissima* L, which lost 19 269 ha or 4.11 % and 15 336 ha or 3.27 % respectively of their initial areas, and the open steppe with *Artemisia herba-alba* Asso declined by 1 969 ha or 0.42 %. On the other hand, the psammophilous groups with *Thymelea microphylla* Coss. and *Tamarix africana* Webb.have increased significantly, with an estimated 73 483ha or 15.78 %, as well as the psammophilous grouping with *Retama raetam* (Forssk.) on living dunes and the steppe with *Arthrophytum scoparium* (Pomel) Iljin., *Stipa tenacissima* L and *Launaea acanthoclada* M. which increased by 28 945 ha, or 6.18 % and 25 481ha or 6.18 % respectively and the halophilous groupings with *Salsola vermiculata* L. and *Atriplex halimus* L with 92 ha or 0.02 % of its initial area.

Usually, the following "scale" is used to interpret the obtained value of kappa κ obtained (Landis and Koch, 1977):

Kappa value (κ)	Assessment
< 0	Big disagreement
0,00 - 0,20	Very weak agreement
0,21 - 0,40	Weak agreement
0,41 - 0,60	Medium tuning
0,61 - 0,80	Satisfactory agreement
0,81 - 1,00	Excellent agreement

This is the reading grid proposed by Landis and Koch in 1977 from which it is clear that our first classifications present satisfactory agreements. However, an excellent agreement (kappa equal to 0.8121) is recorded for the classification of the 2018 Landsat image. The phytoecological map drawn up by URBT (1978) provides a fairly

accurate overview of the spatial distribution of the main plant formations in the study region. In 1978, esparto grass (*Stipa tenacissima* L*),* sparte (*Lygeum spartum* L) and white wormwood (*Artemisia herba-alba* Asso) played a dominant role in the landscape. In the 1970s and 1980s, these three species provided 80-90% plant cover (Aidoud *et al*, 2006). They formed the perennial floristic backdrop to the homogeneous orotopographical complex which is the high steppe plains of southern Oran. The steppe with *Lygeum spartum* L is much more heterogeneous and most often appears as a mosaic including various steppe stands. The matorrasl grouping dominated by *Juniperus phoenicea* L and *Stipa tenacissima* L is found on mountain ranges. Steppe formations characterised by the dominance of grasses (*Lygeums partum* L) or chamephytes (*Artemisia herba–alba* Asso) on glacis and foothills, plant formations characteristic of the salty substrates of sebkhas and dayas (*Atriplex halimus* L, *Salsola vermiculata* L.) and steppe formations characterised by the Saharian bioclimate dominated by *Remth* (*Arthrophytum scoparium* (Pomel) Iljin.), (Kaabeche, 2000). Finally, the *Retama raetam* (Forssk.) group colonizing sandy soils and occupies large areas close to inhabited areas and also the edges of living dunes forming dune strips.

Identifying and mapping the spatial distribution of the main plant formations in 2018 makes it possible to assess the dynamics of each formation. This map confirms the observations made in the field concerning a significant decline in some plant groupings, such as the steppe of *Stipa tenacissima* L and the *Lygeum spartum* L *steppe,* which only covered an area of 109 862 ha in 2018, i.e. a rate of 23.45 %, *Arthrophytum scoparium* (Pomel) Iljin. groups covered an area of 34,200 ha in 2018, groups composed of psamophilous species *Thymelea microphylla* Coss. covered an area of 93 500 ha and *Retama raetam* (Forssk.) on living dunes covered an area of 18,400 ha (Table 1).

The strong regression observed during this period is recorded at the level of the rather dense mixed steppe formations of *Lygeum spartum* L and *Artemisia herba-alba Asso*, the steppe with *Lygeum spartum* L, *Stipa tenacissima* L *and Lygeum spartum* L, and the clear steppe with *Artemisia herba-alba* Asso with an overall rate of 14.05 % or a surface of 112665 ha compared to the year 1978. This deterioration can be explained by the over-exploitation of plant resources by expanding sheep population, strong demographic pressure and a period of exceptional drought between 1970 and 1998, characterized by a reduction in rainfall (estimated by various authors between 17 and 22 %) and a lengthening of the drought period (from 1 to 2 months) (Aidoud *et al.*, 2006). This reduction in the annual rainfall in the high steppe plains has a definite impact on the fragility and decline of the esparto grass cover. In addition, the change in the lifestyle of livestock farmers, who have become semi-sedentary with agro-pastoral activities, the increase in the pastoral load on steppe rangelands and intensive illicit land ploughing accelerated by using mechanical tools in the steppe environment, in particular the tractor and the disc plough.

The 2000s were also marked by the launch of agricultural support programs for agro-pastoralists, who cleared the rangelands as they colonizing the deep, clay soils, which are more or less the most fertile. Overall, and despite its exceptional resistance to drought and grazing, several authors confirm that steppe cover, particularly white wormwood, is in serious decline, mainly due to human activity, especially the uprooting and clearing of this species (Benabdeli, 2000).

Several of authors (Aidoud *et al*., 2006), in particular, were moving in the same direction, sounding the alarm by highlighting the increasing decline of the *Stipa tenacissima* L steppes, despite the return of the rain, and questioning the irreversibility of the phenomenon. Benabdeli (2000) noted: "Under the combined effect of overgrazing and drought, the *Stipa tenacissima* L steppe

is in an advanced state of degradation, facilitating a process of desertification".

The degradation phase experienced by the *Stipa tenacissima* L formation since 1975, mainly as a result of anthropogenic pressure induced by clearing and development for rain-fed cereal growing, is becoming very worrying. This formation is finding it difficult to regenerate and dying back and disappearing at a worrying rate.

The current state of degradation of the *Juniperus phoenicea* L and *Stipa tenacissima* L scrublands shows overexploitation of woody vegetation. This is explained, on the one hand, by the need for firewood.

Psammophyllous rangelands have increased significantly, by 27.30 % compared with 1978, especially at micro-dune level, as a result of pastoral development work carried out as part of the fight against desertification, carried out by the High Commission for the Development of the Steppe (HCDS) which consist on the one hand of the mechanical and biological fixation of the dunes and the pastoral plantations based on *Atriplex canescens (*Pursh) Nutt.*,* on the other hand by the controlled managed rangelands protected areas of the rangelands in the places affected by the phenomenon of desertification generated during the 80s and 90s.

In the face of this alarming situation, there is an urgent need for a thorough assessment to promote a development and conservation policy based on concerted and participatory planning. This policy must be based on indigenous species. Operations such as the rangeland fencing, planting fodder shrubs and mechanical and biological dune fixation have yielded satisfactory results in the rehabilitation of degraded rangelands. To this end, there is an urgent need to promote these operations in the steppe rangeland development policy.

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: Spatial distribution of land use classes in 1978 and 2018

N°	Plant groups	Surface area (ha)	
		1978	2018
1	Matorrals of *Juniperus phoenicea* L and *Stipa tenacissima* L	32530	17194
2	Steppe with *Stipa tenacissima* L and *Lygeumspartum* L	173600	109862
3	Fairly densely mixed steppe with *Lygeum spartum* L and *Artemisia herba- alba* Asso	34810	7121
4	Open steppe with *Artemisia herba-alba* Asso	48210	46241
5	Steppe with *Lygeum spartum* L	125900	106631
6	Steppe with *Arthrophytum scoparium* (Pomel) Iljin., *Stipa tenacissima* L and *Launaea acanthoclada* M.	8720	34201
7	Halophilous communities with *Salsola vermiculata* L. and *Atriplex halimus* L	18320	18412
8	Psammophilous groups with *Thymelea microphylla* Coss. and *Tamarix africana* Webb.	20030	93513
9	Psammophilous group with *Retama raetam* (Forssk.) living dunes	6380	35325

Table 2: Changes in the surface area of land use units over the study period (1978 - 2018).

N°	Plant groups	Area (ha)		Difference	
		1978	2018	Area (ha)	%
1	Matorrals of *Juniperus phoenicea* L and *Stipa tenacissima* L	32530	17194	-15336	-3.27
2	Steppe with *Stipa tenacissima* L and *Lygeumspartum* L	173600	109862	-63738	-3.61
3	Fairly densely mixed steppe with *Lygeum spartum* L and *Artemisia herba- alba* Asoo	34810	7121	-27689	-5.91
4	Open steppe with *Artemisia herba-alba*	48210	46241	-1969	-0.42
5	Steppe with *Lygeum spartum* L	125900	106631	-19269	-4.11
6	Steppe with *Arthrophytum scoparium* (Pomel) Iljin., *Stipa tenacissima* L and *Launaea acanthoclada* M.	8720	34201	25481	5.44
7	Halophilous communities with *Salsola vermiculata* L. and *Atriplex halimus* L	18320	18412	92	0.02
8	Psammophilous groups with *Thymelea microphylla* Coss. and *Tamarix africana* Webb	20030	93513	73483	15.68
9	Psammophilous group with *Retama raetam* (Forssk.) living dunes	6380	35325	28945	6.18

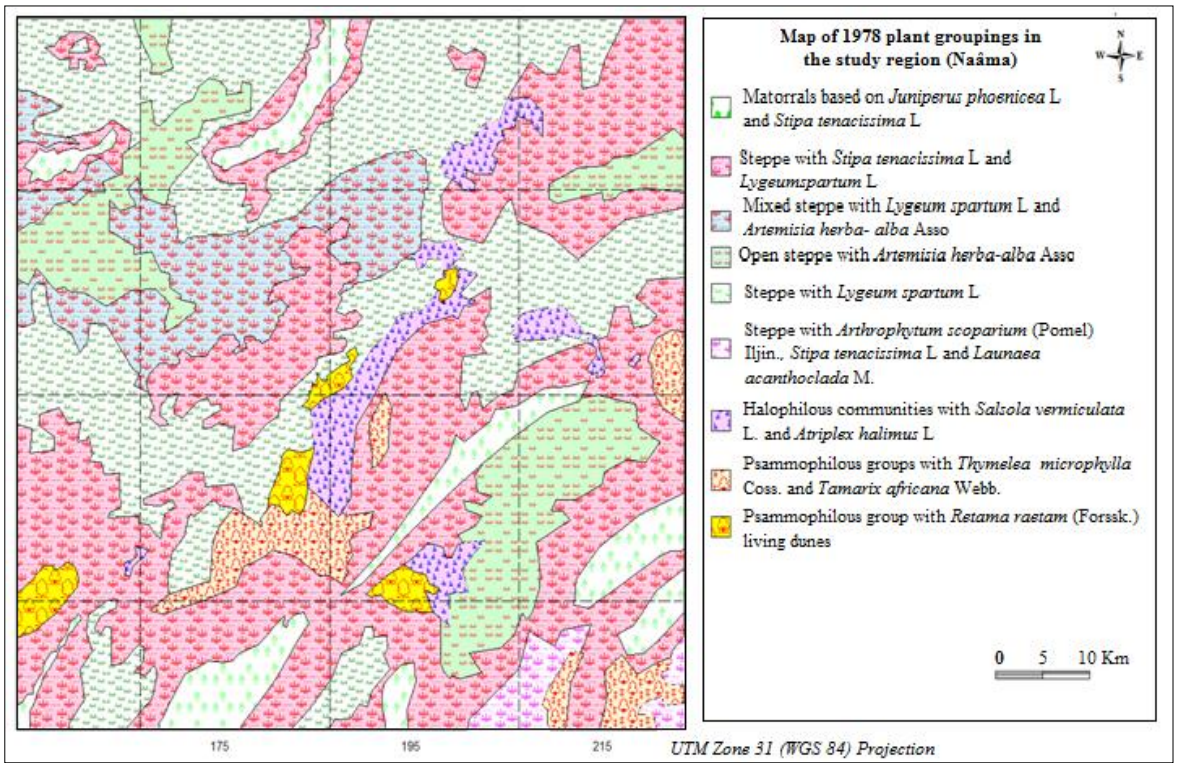

Figure 1: Map of 1978 plant groups in the study area

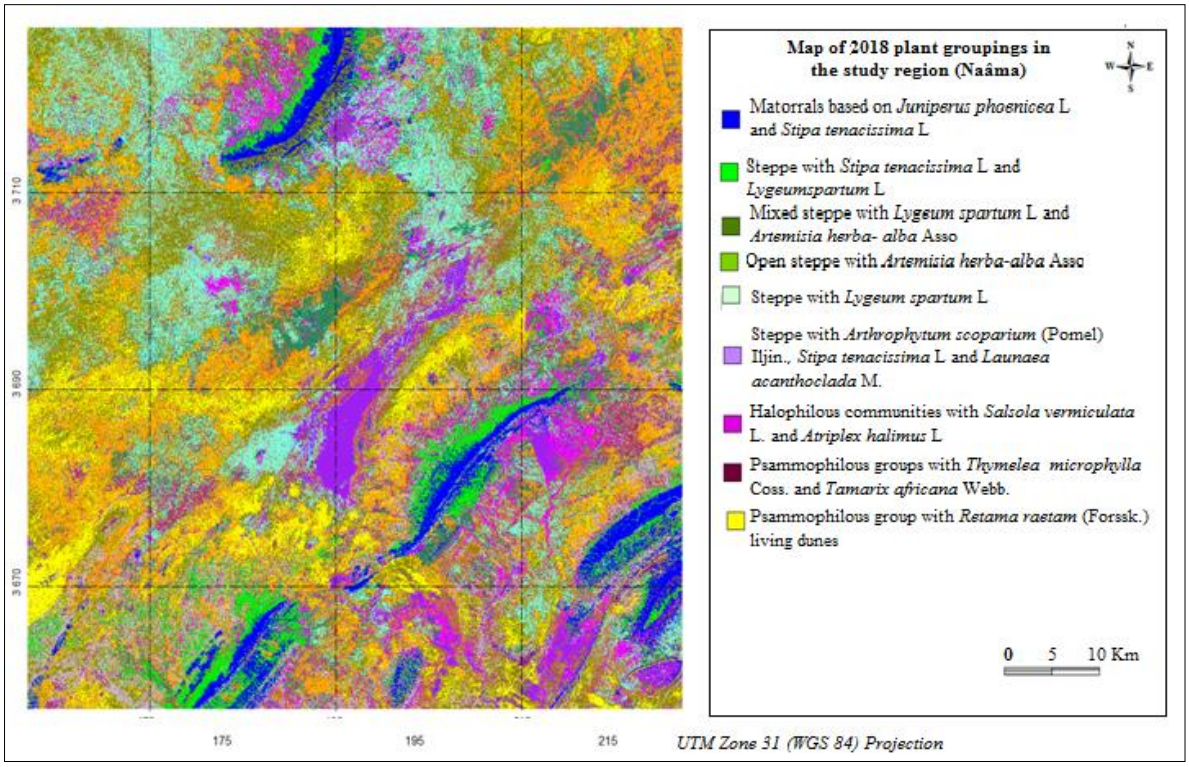

Figure 2: Map of 2018 plant groupings in the study region

SHORT COMMUNICATION

Utilization of vegetable extracts for biostimulation and enhancement of medicinal properties in *Aloe vera* cultivation

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ABSTRACT

The rising demand for natural and sustainable agricultural inputs has spurred interest in plant-based biostimulants to boost crop productivity and phytochemical quality. Aloe vera (Aloe barbadensis Miller), valued for its medicinal and commercial applications, owes its utility to bioactive compounds like polysaccharides, phenolics, and antioxidants. This study investigates the effect of aqueous and hydroalcoholic extracts from spinach (Spinacia oleracea), carrot (Daucus carota), and beetroot (Beta vulgaris) on the growth and phytochemical profile of Aloe vera. Over an 8-week greenhouse trial, plants received foliar and soil applications of these extracts. Treated plants showed significant gains in vegetative growth, chlorophyll levels, and leaf biomass versus controls. Biochemical analysis revealed increased phenolic content, flavonoids, and antioxidant activity, especially in beetroot- and carrot-treated groups. Notably, carrot extracts also led to higher polysaccharide yields, enhancing Aloe vera's therapeutic value. These findings suggest vegetable extracts act as natural elicitors, promoting secondary metabolite biosynthesis. As low-cost, eco-friendly biostimulants, they hold promise for improving the vitality and commercial quality of medicinal plants.

Keywords: Antioxidant activity, foliar application, polysaccharides, secondary metabolites, sustainable agriculture

Aloe vera is a perennial succulent belonging to the family Asphodelaceae, widely cultivated for its gel-rich leaves that are used in pharmaceuticals, nutraceuticals, cosmetics, and functional foods. The gel is composed primarily of water (~98–99%) but contains a diverse array of biologically active compounds that confer its medicinal value (Savvas and Ntatsi, 2015; Bhowmik, 2019). The phytochemical profile of *Aloe vera* is highly sensitive to environmental conditions, cultivation practices, and stress factors such as drought, salinity, and nutrient deficiency (Yakhin *et al*., 2017). These stresses often lead to suboptimal growth and reduced concentrations of valuable secondary metabolites. Biostimulants offer a sustainable, eco-friendly strategy to address these challenges. Defined as substances or microorganisms that stimulate natural processes to improve nutrient uptake, stress tolerance, and crop quality (du Jardin, 2015), they differ from fertilizers in that their primary action is physiological rather than purely nutritional. Plant-derived biostimulants, including vegetable extracts, are rich in amino acids, phenolics, phytohormone-like molecules, and organic acids, which can act as elicitors—triggering secondary metabolism and enhancing phytochemical accumulation (Ertani *et al*., 2014).

While previous studies have shown that seaweed extracts and compost teas improve biomass, chlorophyll content, and antioxidant activity in *Aloe vera* (Chow *et al.*, 2005; Yakhin *et al*., 2017), the potential of vegetable-derived extracts—especially from spinach (*Spinacia oleracea*), carrot (*Daucus carota*), and beetroot (*Beta vulgaris*)—remains underexplored. These vegetables are abundant in bioactive compounds such as betaines, betalains, carotenoids, phenolic acids, and nitrates, which could synergistically boost growth and enhance the medicinal quality of *Aloe vera*.

Therefore, this study evaluates the effects of aqueous extracts from spinach, carrot, and beetroot on the growth, physiology, and phytochemical profile of *Aloe vera*. By integrating detailed phytochemical analysis with agronomic assessment, it aims to demonstrate how targeted biostimulant applications can sustainably increase both yield and medicinal value, meeting the growing demand for high-quality *Aloe vera* products in the pharmaceutical and cosmetic sectors.

The trial was performed in spring 2025 within a climate-controlled greenhouse at the Landscaping Plants and Nursery Research Unit, affiliated with the Italian Council for Agricultural Research and Economics (CREA) in Pescia (PT), Italy. Aloe vera (*Aloe barbadensis Miller*) plants, approximately four months old and confirmed to be uniform and free from disease symptoms, were selected for the study. Each plant was potted in a 25 cm diameter plastic container filled with a sterilized growth medium comprising sandy loam soil blended with compost in a 3:1 proportion. Before the treatments commenced, the plants were allowed to acclimate for 14 days under ambient conditions with regular watering to ensure uniform physiological status. The experimental layout followed a completely randomized design (CRD) with four treatment groups and five replicates per treatment, totaling 20 pots. The groups were: T0 (Control): Distilled water only; T1: Aqueous spinach extract; T2: Aqueous carrot extract; T3: Aqueous beetroot extract. Each treatment involved both foliar spray and soil drenching, applied weekly for 8 consecutive weeks.

Organic fresh spinach (*Spinacia oleracea*), carrot (*Daucus carota*), and beetroot (*Beta vulgaris*) were obtained from a local farm. After washing and removing non-edible parts, each vegetable was chopped and homogenized using a blender with distilled water at a 1:10 (w/v) ratio. The homogenates were filtered using muslin cloth followed by Whatman No. 1 filter paper. The filtrates were considered 100% stock solutions. These were applied directly as aqueous extracts without any dilution. For foliar application, 100 ml of each extract was sprayed evenly on the leaves using a hand sprayer. For soil drenching, 200 ml per pot was poured around the plant base. Control plants received the same volume of distilled water. All extracts were freshly prepared 24 hours before application and stored at 4°C to prevent microbial degradation (Gómez-Merino and Trejo-Téllez, 2015).

After 8 weeks of treatment, morphological data were recorded. Parameters included: plant height (cm): measured from the soil surface to the tip of the tallest leaf using a measuring scale; number of leaves per plant; leaf length and width (cm): averaged from three randomly selected leaves per plant; fresh weight (g): harvested whole plant weight measured using a digital balance; dry weight (g): to assess dry biomass, plant samples were dried in a hot air oven at 65 °C for 72 hours and then weighed. All observations were made during morning hours to minimize the influence of daily temperature and light fluctuations on the measurements.

Fresh leaf tissue (0.5 g) was homogenized in 80% acetone, and the resulting extract was centrifuged at 10,000 rpm for 10 minutes to obtain a clear supernatant. Absorbance readings were taken at 645 nm and 663 nm with a UV–Visible spectrophotometer. Chlorophyll a, chlorophyll b, and total chlorophyll concentrations were calculated using Arnon's standard equations (Yakhin *et al*., 2017).

The Folin–Ciocalteu assay was used to estimate total phenolic content in dried *Aloe*

vera gel extract. After mixing with sodium carbonate and incubating at room temperature, absorbance was measured at 765 nm. Gallic acid served as the standard, and results were expressed as GAE per gram of dry extract (Saa *et al*., 2015).

Total flavonoids in *Aloe vera* extract were measured using an aluminum chloride-based colorimetric method. The mixture was incubated for 30 minutes, and absorbance was measured at 415 nm. Results were calculated as quercetin equivalents per gram of dry sample. Antioxidant Activity (DPPH Assay). The antioxidant activity of *Aloe vera* gel extract was assessed using the DPPH radical scavenging method. After mixing the extract with a methanolic DPPH solution, the reaction was kept in the dark at room temperature for 30 minutes (Chow *et al.*, 2005). Absorbance was then measured at 517 nm to calculate the scavenging efficiency by using following formula:

$$\text{Inhibition } (\%) = \left(\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) \times 100$$

The antioxidant activity of Aloe vera gel extracts was evaluated using the DPPH free radical scavenging method described by Chow et al. (2005) with minor modifications. Briefly, 1 ml of *Aloe vera* extract (1 mg ml^{-1}) was added to 3 ml of a freshly prepared 0.1 mM DPPH (2,2-diphenyl-1-picrylhydrazyl) solution in methanol. The mixture was vortexed and incubated for 30 min in the dark at room temperature. The absorbance was measured at 517 nm against a methanol blank using a UV–Vis spectrophotometer.

The percentage of DPPH radical scavenging activity was calculated using the formula:

$$\text{Antioxidant activity } (\%) = \frac{A_0 - A_1}{A_0} \times 100$$

where A_0 = absorbance of control (DPPH + methanol) and A_1 = absorbance of sample (DPPH + extract).

High-performance liquid chromatography (HPLC) with photodiode array detection at 254 nm following Keem *et al.* (2023). C18 reverse-phase column, mobile phase of methanol:water (70:30 v/v), flow rate 1 mL/min. HPLC quantification as per Souza *et al*., (2017), using detection at 430 nm.

Vitamin C estimated via 2,6-dichlorophenolindophenol titration (AOAC, 2016; Pegg and Eitenmiller, 2017); β-carotene measured spectrophotometrically at 450 nm after hexane extraction Raman *et al.* (2023).

To ensure consistency, all extractions, biochemical assays, and measurements were performed in triplicate. All instruments were calibrated prior to use. Blank controls and standards were included in every batch of biochemical assays.

Polysaccharide content in *Aloe vera* gel was measured using the phenol sulfuric acid method. After reaction with phenol-sulfuric acid and 30-minute incubation, absorbance was measured at 490 nm. Results were expressed as glucose equivalents per gram of dry sample (Saa *et al*., 2015).

Acemannan was quantified using the colorimetric periodic acid–Schiff (PAS) method with commercial acemannan standard (Sigma-Aldrich, USA) following Eberendu *et al.* (2005). Lyophilized gel samples were enzymatically digested with cellulase to release polysaccharides. Periodic acid oxidation followed by Schiff reagent reaction produced a pink chromophore measured at 550 nm.

One-way ANOVA was used to evaluate treatment effects, and Tukey's HSD test was applied for pairwise comparisons where significant differences were found ($p < 0.05$). Data are presented as mean ± standard deviation. Statistical analysis was conducted using SPSS or R, while GraphPad Prism and Excel were used for graph preparation.

Vegetable extracts significantly enhanced the vegetative growth of *Aloe vera* compared to the control group (Table 1). The highest plant height was recorded in the beetroot extract treatment (40.3 ± 1.6 cm), followed by carrot (38.1 ± 1.4 cm) and spinach (35.7 ± 1.5 cm). These increases in plant stature are consistent with previous reports indicating

that bioactive compounds in vegetable extracts can promote cell elongation and division (Calvo *et al*., 2014.).

Similarly, leaf number and fresh weight improved significantly. Plants treated with beetroot extract produced the most leaves (15.5 ± 0.6), indicating a strong biostimulatory effect, possibly due to the high betaine and nitrate content that supports nitrogen metabolism and shoot formation (Nardi *et al*., 2016). The fresh biomass gain in beetroot-treated plants (205.8 ± 6.5 g) was 41% higher than control, aligning with findings from Rouphael *et al.* (2018) on natural biostimulants enhancing biomass. Leaf morphological parameters followed similar trends to plant height and leaf number (Table 1). The longest leaves were recorded in the beetroot extract treatment (49.6 ± 2.2 cm), followed by carrot (46.8 ± 2.0 cm) and spinach extract (44.2 ± 2.1 cm). The control group had the shortest leaves (37.8 ± 1.8 cm). Likewise, leaf width increased significantly, from 5.8 ± 0.3 cm in the control to 7.3 ± 0.4 cm under beetroot treatment. These increases suggest that vegetable extract biostimulants enhanced cell expansion and water retention capacity in leaf tissues, promoting overall leaf growth. The improvement in laminar dimensions may be attributed to enhanced photosynthetic activity and nitrogen assimilation stimulated by compounds such as betaine and nitrate in beetroot and carrot extracts (Nardi *et al*., 2016). Similar findings were reported by Calvo *et al*. (2014) and Rouphael *et al*. (2018), where biostimulant applications enhanced leaf area and thickness in horticultural crops.

The dry weight of leaves was also significantly increased with all treatments (Table 1), further supporting the role of vegetable extracts in improving biomass accumulation through improved water and nutrient utilization (Rouphael *et al*., 2017).

Chlorophyll content showed a marked increase in all treatment groups **(**Table 2**).** The control group had the lowest chlorophyll content (1.25 ± 0.05 mg/g FW), while beetroot-treated plants recorded the highest (2.01 ± 0.08 mg/g FW). This suggests enhanced photosynthetic capacity due to biostimulant activity. These results are consistent with prior studies indicating that phenolic-rich extracts enhance photosynthetic pigment biosynthesis by reducing oxidative stress and improving enzymatic activity (Saa *et al*., 2015). The improved pigment concentration under beetroot treatment may be due to betacyanin-mediated photoprotection, stabilizing chlorophyll under greenhouse light fluctuations.

As shown in Table 2, total phenolic content was highest in beetroot-treated plants (11.2 ± 0.4 mg GAE/g DW), representing a 65% increase over control. Carrot and spinach treatments also significantly improved phenolic accumulation. This is indicative of the elicitor effect of bioactive molecules in vegetable extracts, which stimulate secondary metabolite pathways such as phenylpropanoid metabolism (Yakhin *et al*., 2017).

Antioxidant activity (measured by DPPH radical scavenging) showed similar trends, with the highest values observed under beetroot extract ($65.4 \pm 2.5\%$) **(**Table 2**).** This finding aligns with reports by Nardi *et al*. (2016), who observed significant increases in antioxidant defense under natural elicitor treatments in medicinal plants.

Additionally, the flavonoid content of the gel **(**Table 2) increased significantly under carrot and beetroot extract treatments, suggesting the upregulation of flavonoid biosynthesis genes such as CHS and FLS, commonly triggered under stress signaling and elicitation (Gómez-Merino and Trejo-Téllez, 2015).

The total flavonoid content of *Aloe vera* gel showed a pronounced increase following vegetable extract treatments (Table 2). The control group exhibited the lowest flavonoid concentration (3.25 ± 0.15 mg QE g^{-1} DW), while beetroot extract achieved the highest level (5.21 ± 0.22 mg QE g^{-1} DW), representing a 60% increase over control. Carrot and spinach extracts also resulted in significant enhancements. This improvement can be attributed to elicitor-like compounds such as phenolics, ca-

rotenoids, and nitrates that stimulate chalcone synthase (CHS) and flavonol synthase (FLS) gene expression, key enzymes in the flavonoid biosynthetic pathway (Yakhin *et al*., 2017).

The elevated flavonoid content corresponds with enhanced antioxidant activity, suggesting a coordinated activation of phenolic and flavonoid metabolism under biostimulant influence. Similar findings have been reported in medicinal plants treated with natural extracts, where elicitor compounds promote flavonoid accumulation as part of stress adaptation and defense priming (Gómez-Merino and Trejo-Téllez, 2015; Rouphael *et al*., 2018).

Polysaccharides, especially acemannan, are considered critical for the medicinal efficacy of *Aloe vera*. Table 2 shows a significant enhancement in polysaccharide concentration across all treatments. Beetroot extract resulted in the highest increase (194.3 ± 5.4 mg/g DW), supporting the hypothesis that certain vegetable extracts stimulate carbohydrate biosynthesis. Polysaccharide accumulation may be attributed to enhanced carbon assimilation, as well as hormonal stimulation of sugar metabolism. This biochemical enrichment reinforces the potential of vegetable extracts in improving both yield and functional value of *Aloe vera* gel.

Application of vegetable extracts significantly enhanced *Aloe vera*-specific bioactives in the gel and latex fractions.

Control plants recorded 5.8 ± 0.3 mg/g fresh gel, whereas beetroot extract treatment increased this to 8.9 ± 0.4 mg/g ($p < 0.05$) (Table 3). This 53% increase suggests upregulation of polysaccharide biosynthesis, likely due to carbohydrate precursors and elicitor compounds in the extract (Chow *et al*., 2005).

Highest concentrations were observed in spinach extract treatment (2.85 ± 0.09 mg/g dry latex), representing a 32% increase over control (Table 3). Elevated anthraquinone glycosides may reflect stress-related secondary metabolism triggered by biostimulants (Yakhin *et al*., 2017).

Carrot extract notably enhanced aloe-emodin content by 27% compared to control, potentially linked to phenolic precursors in carrot-derived biostimulants (Table 3) (Keem *et al*., 2023).

All treatments improved ascorbic acid content (Table 3), with beetroot extract yielding the highest value (23.4 ± 0.8 mg/100 g fresh gel), possibly due to its nitrate content promoting antioxidant biosynthesis (Gómez-Merino and Trejo-Téllez, 2015).

Spinach extract increased β-carotene content by 41%, consistent with its own carotenoid profile and potential priming effect on the terpenoid pathway (Table 3). These results demonstrate that vegetable-derived biostimulants can enhance *Aloe vera*'s unique medicinal compounds particularly acemannan and anthraquinones beyond the general phenolic/flavonoid profile (Saa *et al*., 2015).

Earlier studies using commercial biostimulants (seaweed extracts, humic substances) reported similar benefits for *Aloe vera* and other medicinal plants. However, this is among the first controlled studies using *vegetable-based* extracts, particularly beetroot and carrot, as direct plant growth stimulants and phytochemical enhancers. Comparable findings were recently observed in basil and lettuce treated with onion peel and tomato pulp extracts, which increased chlorophyll, polyphenols, and root length (Ertani *et al*., 2014; Halpern *et al*., 2015). This suggests a broader application of food byproducts and vegetable waste in sustainable agriculture.

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 vegetable-derived biostimulants on growth parameters of *Aloe vera*

Treatment	Plant Height (cm)	Leaf Number	Fresh Weight (g)	Leaf Length (cm)	Leaf Width (cm)	Leaves Dry Weight (g)
Control	28.4 ± 1.2 a	10.2 ± 0.6 a	145.3 ± 5.4 a	37.8 ± 1.8 a	5.8 ± 0.3 a	18.6 ± 0.9 a
Spinach Extract	35.7 ± 1.5 b	13.1 ± 0.5 b	178.6 ± 6.2 b	44.2 ± 2.1 b	6.6 ± 0.4 b	23.8 ± 1.0 b
Carrot Extract	38.1 ± 1.4 b	14.2 ± 0.7 c	190.1 ± 5.9 c	46.8 ± 2.0 c	6.9 ± 0.3 b	25.2 ± 1.1 c
Beetroot Extract	40.3 ± 1.6 c	15.5 ± 0.6 d	205.8 ± 6.5 d	49.6 ± 2.2 d	7.3 ± 0.4 c	27.1 ± 1.2 d
CD ($p \leq 0.05$)	1.728	0.694	8.372	2.234	0.382	1.052
CV (%)	12.59	15.50	14.69	4.76	4.95	4.89

Table 2: Effect of vegetable-derived biostimulants on biochemical parameters of *Aloe vera*

Treatment	Chlorophyll (mg/g FW)	Total Phenolics (mg GAE/g DW)	Total Flavonoids (mg QE g^{-1} DW)	Polysaccharide Content (mg g^{-1} DW)	Antioxidant Activity (%)
Control	1.25 ± 0.05 a	6.80 ± 0.40 a	3.25 ± 0.15 a	132.6 ± 4.8 a	41.20 ± 2.10 a
Spinach Extract	1.72 ± 0.07 b	9.10 ± 0.30 b	4.12 ± 0.18 b	168.2 ± 5.6 b	55.60 ± 2.30 b
Carrot Extract	1.85 ± 0.06 b	10.30 ± 0.50 c	4.85 ± 0.20 c	181.7 ± 5.9 c	59.80 ± 2.00 c
Beetroot Extract	2.01 ± 0.08 c	11.20 ± 0.40 d	5.21 ±0.22 d	194.3 ± 5.4 d	65.40 ± 2.50 d
CD ($p \leq 0.05$)	0.086	0.558	0.276	7.934	3.024
CV (%)	15.31	17.44	4.73	4.21	15.94

Table 3: Effect of vegetable extract biostimulants on Aloe vera–specific medicinal compounds

Treatment	Acemannan (mg g^{-1} fresh gel)	Aloin A + B (mg g^{-1} dry latex)	Aloe-emodin (mg g^{-1} dry latex)	Vitamin C (mg 100 g^{-1} fresh gel)	β-Carotene (µg g^{-1} fresh gel)
Control	5.8 ± 0.3 a	2.16 ± 0.07 a	1.12 ± 0.05 a	18.6 ± 0.7 a	7.4 ± 0.4 a
Spinach Extract	7.6 ± 0.4 b	2.85 ± 0.09 c	1.28 ± 0.06 b	21.8 ± 0.6 b	10.4 ± 0.5 c
Carrot Extract	8.1 ± 0.3 b	2.52 ± 0.08 b	1.42 ± 0.07 c	22.6 ± 0.9 b	9.5 ± 0.4 b
Beetroot Extract	8.9 ± 0.4 c	2.44 ± 0.10 b	1.33 ± 0.05 b	23.4 ± 0.8 c	9.8 ± 0.5 b
CD ($p \leq 0.05$)	0.47	0.12	0.09	1.04	0.63
CV (%)	5.21	4.56	5.74	4.18	6.02

SHORT COMMUNICATION

Physicochemical and analytical evaluation of *Moringa* gum

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ABSTRACT

Moringaoleifera (commonly referred to as the drumstick tree) is probably one of the most well-known trees in terms of medicinal and nutritional value. The Moringa gum is of interest as pharmaceutical excipient, based on its physicochemical properties and biocompatibility among the different components. The present research article aims to evaluate comprehensively some Physicochemical and analytical properties of Moringa gum, giving insight into its possible pharmaceutical applications. Research on Gum Organoleptic Properties and Features. This study examines the characterization of Moringaoleifera gum as a natural polymer. Moringa gum was extracted and purified according to standard protocols, followed by detailed physicochemical and thermal analyses. The gum was evaluated by using Fourier Transform Infrared Spectroscopy (FTIR) and Differential Scanning Calorimetry (DSC) Scanning electron microscopy (SEM), Thermal analysis and UV-spectrophotometric study. These results indicate that Moringa oleifera gum holds potential as a natural polymer for designing sustained release drug delivery systems.

Keywords: Analytical characterization, *Moringa* gum, natural gum, natural polysaccharides, physicochemical properties.

Moringa gum (MOG) is a hydrophilic exudate obtained from the stem of the *Moringa oleifera* tree (Singh *et al.*, 2021). Initially, the gum appears white, but it gradually turns reddish brown with prolonged exposure. *Moringa oleifera* belongs to family Moringaceae is indigenous to India. The plant is primarily grown for its leaves and seed pods, which are utilized in traditional medicine and as vegetables. The study explored the hypoglycemic potential of *Moringa oleifera* flower extract through *in vivo* and *in silico* approaches (Karim *et al.*, 2025). The plant gum exudes through injury or cut to plant stem that have formed typically within an inner secondary cortex of the cork cambium of the bark (Gupta *et al*., 2020). This gum ultimately solidifies and emerges from the bark in shapes resembling teardrops or stalactites when it comes into contact with the environment

Various experiments were conducted as per rules and regulations. Gum from *Moringa oleifera* was harvested. The exudates of *Moringa oleifera* were extracted from the wounds of the trees located in Gwalior, Madhya Pradesh. After complete drying, the collected gums were crushed and sieved through mesh number 80 before being stored in an airtight container. The powdered gums

were subsequently utilized for further research and analysis.

The organoleptic characteristics, such as color, flavor, aroma, and taste, were evaluated as per Umamaheshwari *et al.* (2021), pH was evaluated as per Kannan *et al*. (2025). Solubility, swelling index, loss on drying and ash value was determine as per Rongpipi *et al.* (2023)**.** Specific gravity (SG)**,** was measured in room temperature by using a SG bottle as per the procedure given by Yaumi *et al*. (2016). Water holding capacity evaluated as per Karaman *et al.* (2014), Bulk and tapping density, Angle of repose (Tan θ), Hausners index, Compressibility index (C%) are evaluated as per Hesse *et al.* (2021). Viscosity was evaluated as per Bal *et al.* (2020).

The morphological characteristics of MOG powder were analyzed utilizing a scanning electron microscope (ZEISS, EVO 18, China). The samples were analyzed as per Parlayici and Aras (2024.). A differential scanning calorimeter (Q10, TA Instruments, USA) was employed to conduct a differential scanning calorimetric analysis of MOG. The samples were analyzed as per Desoky *et al*. (2024) and Cuthbertson *et al.* (2024.) FTIR spectroscopy, utilizing Agilent technology, was employed to determine the functional groups the samples were analyzed as per Circelli *et al.* (2024) and Campanale *et al*. (2023.). An X-ray diffractometer (Miniflex 2, Rigaku, Japan) was employed to record the powder X-ray diffractograms of MOG the samples were analyzed as per Tamboli *et al.* (2024) and Son *et al.* (2020). The differential thermal analysis (DTA) and thermogravimetric analysis (TGA) of MOG was determined by Shimadzu (DTG-60H thermogravimeter) japan. The samples were analyzed as Desoky *et al.* (2024) and Cuthbertson *et al.* (2024.). The refined polysaccharide was dissolved in distilled water, enabling the use of UV-Vis analysis (Shimadzu UV-1800, Japan) for the characterization of the material (Shobana *et al*., 2022).

Scanning electron micrograph of *Moringa* gum has been given in Fig. 1. Analysis of the electron micrographs demonstrates that the *Moringa* gum particles are characterized by a polyhedral configuration (Rimpy *et al*., 2017). Moreover, the micrographs reveal the presence of surface cracks and pores in *Moringa* gum, along with a two-layered morphology typical of pure *Moringa*. It is noteworthy that the finely milled gum powder (purified) exhibits a considerably larger size. In terms of nanomaterials, this size range is deemed ideal (Ghosh *et al.,* 2021).

Differential scanning calorimetry (DSC) assesses heat absorption or release in relation to temperature, reflecting physical or chemical transformations within the sample (Fig. 2). The presence of an endothermic peak typically indicates the loss of water content from the compound. The DSC thermogram reveals that the glass transition occurs at 39.22°C, which is influenced by the material's intrinsic characteristics such as its structure, bonding, and molecular weight. Additionally, the endothermic peak for the polymer is observed at approximately 223°C, which is referred to as the enthalpy of transitions and is associated with the crystallinity of the material (Ranot *et al*., 2022).

In Figure 3 illustrates the FTIR spectra of MOG. The broad absorption band observed at 3391 cm^{-1} is attributed to the stretching of –OH groups. The peak at 1611 cm^{-1} corresponds to the C=O stretching of acetyl, while the peaks at 2928 cm^{-1} are likely associated with –CH stretching. Additionally, the peaks at 1516 cm^{-1} and 1438 cm^{-1} can be linked to the C=O stretching of the carboxylic acid in glucuronic acid. Furthermore, the peak at 1285 cm^{-1} may be attributed to –C-OH. MOG also exhibited bands characteristic of carbohydrates within the range of 1000–1200 cm^{-1} (Rimpy *et al*., 2017).

The diffractogram (Fig. 4) confirmed the amorphous characteristics of gum powder, as it exhibited no clear diffraction pattern, unlike the X-ray diffraction pattern of MOG. The X-ray diffraction spectra of MOG powder revealed an absence of identifiable diffraction peaks at (2θ) (Rimpy *et al*., 2017).

Thermal curves for enthalpy obtained through Differential Thermal Analysis (DTA) and mass changes measured by Thermogravimetric Analysis (TGA). The thermograms of the MOG samples are presented in Figure 5. In the case of pure MOG gum, a significant degradation was observed between 228°C and 324°C, resulting in a weight loss of 49%, indicative of polysaccharide decomposition. The TGA results for crude gum revealed a multistage degradation process, with an initial mass loss of 8% commencing at approximately 65°C, as illustrated in Figure 5A. The thermogram pattern of the compound indicated that no intermediates were likely formed during the decomposition process. A notable weight reduction of 49% occurred within the temperature range of 228°C to 324°C (Irfan *et al*., 2021).

The DTG curve indicated the precise temperatures at which exothermic and endothermic processes took place, specifically at 102.60 °C and 116.26 °C, respectively. This observation may provide insight into the degradation observed during the second stage (Fig. 5C). The absence of significant additional peaks in the first derivative curve implies that moisture loss may solely account for the subsequent weight reduction. The differential thermogram (DTA) revealed two distinct endothermic peaks and three exothermic peaks, which included two weak and one broad peak, as illustrated in the energy change (ΔμV) (Fig. 5C). The initial exothermic peak began to appear concurrently with the conclusion of the second endothermic peak, correlating with the weight loss noted in stage III (refer to Fig. 5C) (Irfan *et al.*, 2021).

DTA a significant and robust peak was observed in the thermograms of MOG, indicating an endothermic transition occurring at approximately 100 °C, followed by an exothermic transition in the range of 500 to 600 °C (Fig. 5B). In contrast, gum exhibited less pronounced endothermic and exothermic transitions at 100 to 300 °C and 500 to 600 °C, respectively (Fig. 5B). The degradation patterns of different gum polysaccharides vary due to their distinct structural and functional characteristics (Irfan *et al*., 2021).

UV-Vis spectrophotometry is a vital instrument in analytical chemistry. The observed absorption behavior is a result of "surface plasmon resonance," which is induced by the electromagnetic field. This method highlights the absorption characteristics of MOG, whereas the gum sample does not exhibit any peaks, as demonstrated in (Fig. 6).

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: Result observation of different characteristics of *Moringa* Gum

S. No.	Characteristics		Observation	Remarks
01	Color		Brownish black	----------
02	Odour		Characteristic	----------
03	Taste		Mucilageneous	----------
04	Smell		Characteristic	----------
05	Flavor		Characteristic	----------
06	Solubility		Sparingly soluble in water, practically insoluble in acetone, alcohol, chloroform and ether	Formed a viscous solution in water
07	pH		5.9 ±052	----------
08	Swelling %	Water	94±058	----------
		0.1N Hcl (1.2 pH)	85±0.45	
		Buffer (6.8pH)	94±0.37	
09	Loss on drying %		11.34±0.54	----------
10	Total ash% W/W		2.6±0.27	----------
11	Acid insoluble ash %W/W		0.56±0.29	----------
12	Melting point ^{0}C		57±2	----------
13	Specific gravity g/ml		0.979±0.03	(0.5% W/V)
14	Water holding capacity g		19±0.24	Per 100 g Gum
15	Water content %		1.50±0.06	(KF titration)
16	Pour density g/ml		0.72±0.07	----------
17	Tapped density g/ml		0.87±0.21	----------
18	Angle of repose ^{0}		30±0.04	Excellent
19	Hausner ratio		1.21±0.24	Fair
20	Cars index		17.24±0.18	Fair
21	Heat		No visual change	40^{0}C
22	Light		No visual change	Sun light
23	Humidity		Aggregation of gum in to lumps	----------
24	Particle size µ		32±1.11	----------
25	Viscosity	%W/V	Viscosity (CPS)	----------
		0.1	1.44±0.03	
		0.2	2.37±0.02	
		0.3	3.54±0.07	
		0.4	4.79±0.07	
		0.5	9.73±0.04	

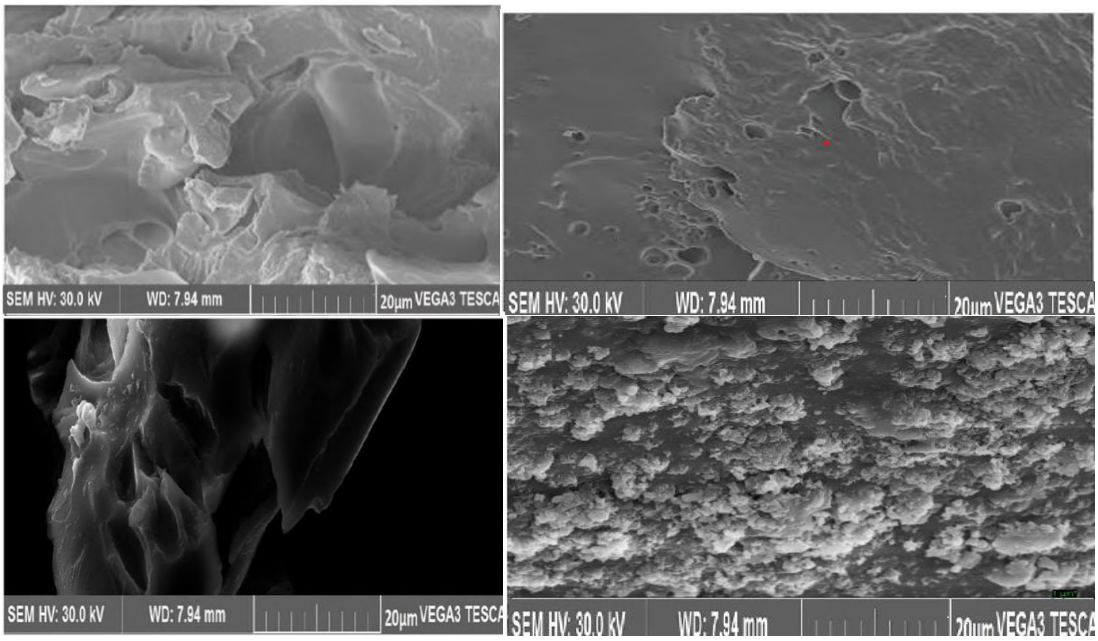

Fig. 1: SEM Study of *Moringa* Gum

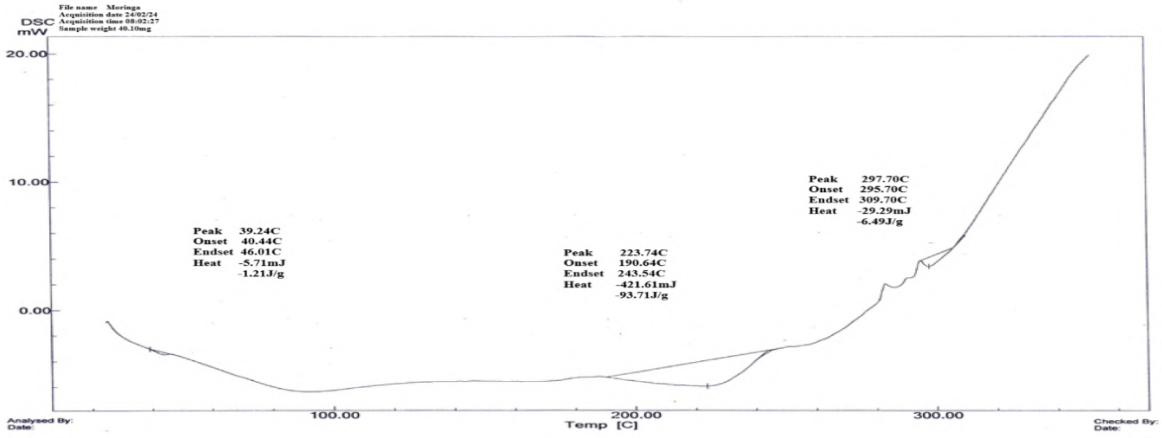

Fig. 2: DSC Study of *Moringa* Gum

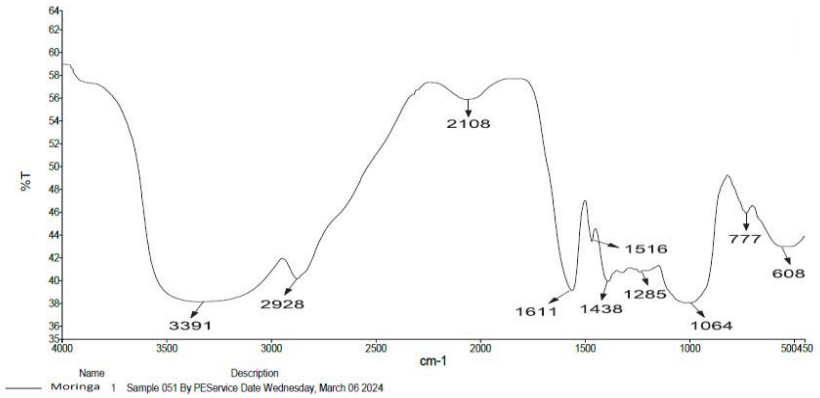

Fig. 3: FTIR Study of *Moringa* Gum

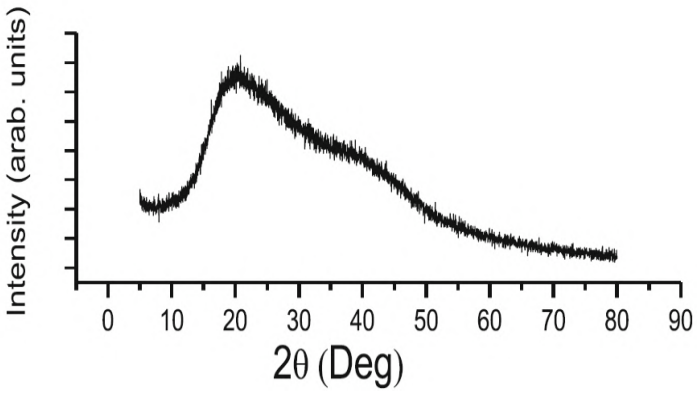

Fig. 4: X-Ray Study of *Moringa* Gum

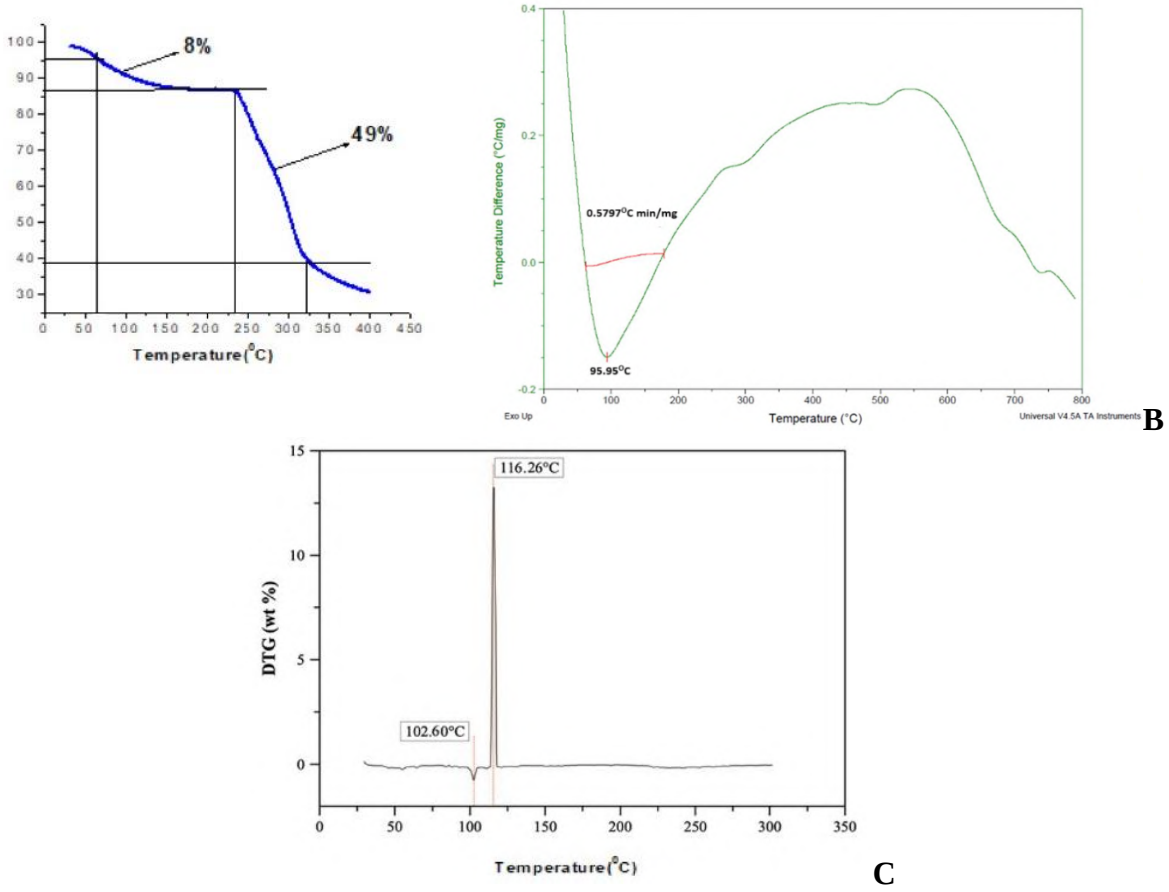

Fig. 5: Thermal Study of *Moringa* Gum

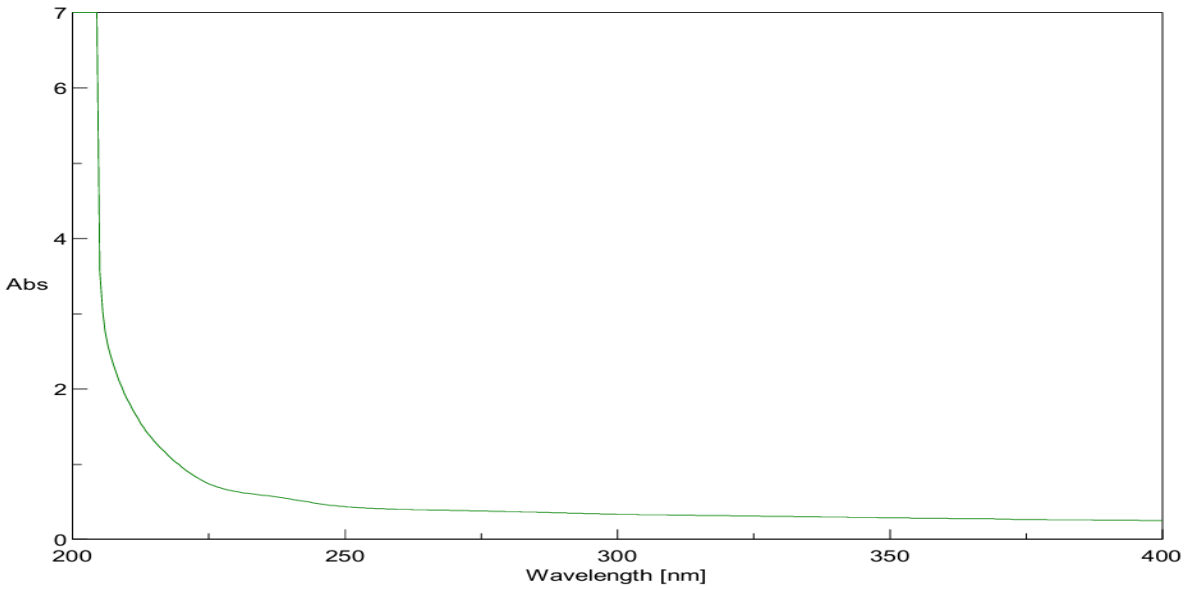

Fig. 6: UV Absorption Study of *Moringa* Gum

SHORT COMMUNICATION

Sensory evaluation and nutritive value estimation of food products developed from the edible blossoms of *Allium cepa*, *Carica papaya* and *Cucurbita maxima*

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ABSTRACT

Flowers are conceived of as a sort of "new vegetable" in the food chain and one of the most promising novelties for satisfying the growing need for food innovation both in terms of organoleptic and nutraceutical profiles. Keeping this in mind, this study involved the attempt to convert the edible flowers of Allium cepa, Carica papaya and Cucurbita maxima into ten different ethnic food products, namely, onion flower mustard pickle, onion flower chili vinegar pickle, onion flower chaat masala, pumpkin flower jam, pumpkin flower sweet and sour pickle, pumpkin flower powder phuluri, pumpkin flower powder laddoo, papaya flower sweet and sour pickle, papaya flower mustard pickle (sour) and papaya flower powder soup. An organoleptic study of the developed food products showed that papaya flower powder soup had the highest mean hedonic score for all the attributes with the highest overall acceptability. In contrast, the papaya flower mustard pickle had the lowest acceptability. According to the Hedonic R analysis, the sweet and sour pickle pumpkin flower and papaya flower powder soup had overall higher preference values than the other tested pickles and powders. The flower-based food products recorded low in fat and moderate in protein, carbohydrate and soluble fibre. The food products were found rich in β-carotene and ascorbic acid, and are good sources of calcium, potassium, phosphorous, manganese and copper. Overall, the prepared flower-based food products, ethnic to Indian cuisine, are nutrient-dense and are likely to be acceptable, affordable and sustainable potential nutritional sources.

Keywords: Edible flowers, food composition, food products, Hedonic R analysis, sensory evaluation, sustainable nutrition.

The growing scientific evidence of the human health benefits of using flowers as food has rapidly evolved in the last couple of years. Brightly coloured flowers have been used for hundreds of years to make teas and wines; they are also dried and used as medicines or herbs; others are crystallized and used as desserts and supplements to butters, jams, marinades and sauces (Raquel *et al.,* 2017). It was reported that the active components of the flowers, like the phytochemicals, can be extracted by different methods, including maceration, reflux extraction, percolation, Soxlet extraction,

and boiling, which can then be utilized for preparing herbal medicines (Kazakova *et al.*, 2024). The available literature encourages the consumption of edible flower-based food products; for example, the Mahua (*Madhuca longfolia*) laddoo is prepared locally by tribal women. Butterfly pea (*Clitoria ternatea*) and *Dahlia coccinea* are reportedly converted into Indian deserts. Pansy (*Viola* × *wittrockiana*) is also used in soups and desserts. *Bauhinia variegata* is used to make pickles, and *Rhododendron arboretum* is used to make chutney or a sweet and sour pickle (Kumari *et al.,* 2021).

Allium cepa umbel reportedly contains approximately 89% water, 9% carbohydrates, and 1% protein and has an energy value of 166 KJ. *Allium cepa* flowers, which are usually discarded after the stalk is removed, are also good sources of phenols and flavonoids (Halder *et al*.,2022). A study in the Philippines confirmed that the male papaya flower as a functional ingredient for herbal tea production, primarily owing to its appealing aroma. In Indonesia, buds of male papaya flowers, locally called 'Bunga pepaya' are consumed in local dishes (Halder *et al*.,2022). *Cucurbita maxima* flowers have been consumed locally as vegetables in Mexico and India since ancient times as salads, dressings, soups and main dishes. *Cucurbita maxima* flower extracts with a high quantity of flavonoids (17.2 mg QE/100 g) have previously shown significant free radicle scavenging activity (Carboni *et al*., 2025). This study formulated and evaluated the sensory attributes of ten newly formulated food products from the edible flowers of *Allium cepa*, *Carica papaya* and *Cucurbita maxima* that can act as supplementary foods by increasing the nutrient content of our regular diet if incorporated judiciously. The nutritive value per 100 g of the prepared ethnic dishes was also calculated. The flowers were chosen for their high nutritional content and easy accessibility.

Whole-flower samples of *Allium cepa*, *Carica papaya* and *Cucurbita maxima* were collected from local markets in West Bengal, India. The flowers were graded based on their cleanliness, firmness, maturity, colour, size, and shape and were free from insect infestation and mechanical injury. Only the selected flowers were soaked in water at room temperature for 2 minutes to reduce the surface microbial load. After this, the flowers were converted into pulp, powder, vinegar soaked and finely chopped for further processing.

For onion flower mustard pickle: ingredients used were Vinegar-soaked flowers-100g, amchur powder-10g, mustard powder-5g, salt- 3g, mustard oil-20g, five spices cumin, brown mustard, fenugreek, nigella and fennel) -3g, dry red chilli powder-2g. The vinegar-soaked flowers were taken in a bowl. The spices were all added one by one with the flowers and mixed thoroughly. The mixture was carefully poured into a sterilized, airtight glass jar, capped and kept untouched for 10 days. It was further used for sensory evaluation.

For onion flower chilli vinegar pickle: ingredients were used **as** vinegar soaked flowers -100g, ginger-5g medium whole, garlic cloves-5g, green chilli-5g, vinegar-50 ml, salt- to taste.
The vinegar-soaked flowers were taken in a bowl. The rest of the ingredients were added one by one into the flower and mixed thoroughly. The mixture was carefully transferred into a sterilized, airtight glass jar, capped and kept untouched for 10 days. It was further used for sensory evaluation.

For onion flower chaat masala (with powder): Onion flower powder-5g, amchur powder-3g, black pepper-2g, rock salt-5g w2ere used. To the onion flower powder, the spices were added and mixed thoroughly to obtain the chaat masala, kept in an airtight container.

For pumpkin flower jam: Fresh pumpkin flower pulp-200g, powdered sugar-

150g, pectin- 1.5g, lime- 5g whole, orange peel powder- 10g, sodium benzoate- 0.5g were taken. Pumpkin flower pulp was boiled on low flame by continuously stirring, powdered sugar was added to the mixture, pectin was added next, lime juice and orange peel powder went next and stirred continuously. At this point, it was checked for doneness. Upon reaching the desired consistency, the mixture was removed from flame and sodium benzoate was added. The jam was cooled, capped and stored for sensory evaluation in a sterilized glass jar.

For pumpkin flower sweet and sour pickle/chutney: Pumpkin flower chopped finely- 35g, ginger-finely chopped- 5g, garlic-finely chopped- 5g, dried chilli finely chopped whole-5g, vinegar- 15 ml, sugar- 35g were used. Finely chopped flowers were taken in a pan and sugar was added and set on low flame. It was continuously stirred. After the sugar melted, rest of the ingredients were added to the mix and stirred well on heat. The pickle was cooled, capped and stored for sensory evaluation in a sterilized airtight glass jar.

For pumpkin flower phuluri (with powder): Pumpkin flower powder-5g, besan-20g, green chilli-chopped-5g, ajwain-0.5g, salt-to taste, split Bengal gram dal-25g, pea lentils-25g, baking powder-0.25g, refined soya bean oil- for frying-50ml were taken. To the pumpkin powder, besan, green chilli chopped, ajwain, lentil pastes, salt, and baking powder were added. The mixture was made into small balls by hand and deep-fried. The phuluries were served hot immediately for the sensory evaluation. To the pumpkin powder, besan, green chilli chopped, ajwain, lentil pastes, salt, and baking powder were added. The mixture was made into small balls by hand and deep-fried. The phuluries were served hot immediately for the sensory evaluation.

For Pumpkin flower laddoo (with powder): Pumpkin flower powder-20g, besan-80g, Soyabean oil-50 ml, sugar powdered-100g, cardamon black- whole-2g, ghee- 1tbsp were taken. In a pan, the oil was warmed on low flame, elaichi was added to it, and when the aroma started coming out, besan was added and stirred well, next the pumpkin powder was added and continuously stirred. The pan was taken off the flame and the sugar were added. Ghee was added next and mixed well. The mixture was given the shape of small balls. The laddoos were allowed to cool and then used for sensory evaluation immediately.

For papaya flower sweet and sour pickle/chutney: raw mango-100g finely chopped, papaya flower-100g, sugar-60g, garlic chopped-16g, ginger chopped-7g were taken. To a pan, freshly chopped papaya flowers soaked in vinegar and the sugar was added first and put on low flame. As the sugar started melting rest of the ingredients were added to it and stirred continuously. On attaining the perfect consistency of a pickle, it was cooled, capped and stored for sensory evaluation in a sterilized airtight glass jar.

For papaya flower mustard pickle: Papaya flower- 100g, raw mango finely chopped-100g, mustard oil-25 ml, mustard powder-20g, red chilli powder- 10g, salt- 5g, turmeric powder-3g were taken. The flower petals soaked in vinegar were used for this pickle. The petals were mixed well with all the ingredients and left untouched for a week. The pickle was stored for sensory evaluation in a sterilized airtight glass jar.

For papaya flower soup (with powder): Papaya flower powder- 5g (Flower paste is microwaved in convection mode to obtain the powder), ready-to-cook vegetable soup powder sachet-10g, water-150 ml were taken. With the soup powder contents, the papaya flower powder was added and hot water was poured and stirred continuously to avoid any lump formation. Served hot in small cups for sensory evaluation.

Sensory analysis was carried out for the following attributes: a) Appearance b) Colour c) Aroma d) Taste e) Overall acceptability.

Total carbohydrate content was estimated spectrophotometrically by using glucose as a standard (Szklarek *et al*., 2022)**.** The protein content was determined by using the Lowry *et al.* (1951) method. Fat content was

determined using the Soxhlet extraction method according AOAC (2005). Total Dietary Fibre Assay Kit (FOSS-Tecator, Fibretec™ E1023, Hoganas, Sweden) according to AOAC (2005) was used to determine the soluble fibre content. Thiamine and riboflavin were estimated according to Okwu and Josiah (2016). Niacin content was calculated as per Sadasivam and Manickam (1996). 100mg of pure ascorbic acid dissolved in 100 ml of 6% HPO3 was used as the Ascorbic acid standard (1mg/ml) in the method given by Sadasivam and Manickam (1992), for ascorbic acid estimation. Total β-carotene was presented as mg/100g on a fresh weight (FW) basis by using the method by Kishore *et al.* (2025). Atomic absorption Spectrometer (ICP-OES; Model No: VDV-5110; Manufactured by: Agilent Technologies) was used for carrying out the mineral estimations by applying the official methods of the Association of Official Analytical Chemists (AOAC, 1990 and 2016).

The data gathered from the sensory evaluation were statistically analysed by using SPSS 17 software. The standard deviation of the overall acceptability and sensory attributes of the food products among the panellists were determined. R-Index analysis is a nonparametric analysis that calculates the size of the difference or similarity between products (Lee and Houtj 2009). R-Index analysis was used here as a detection and sensory difference test, along with hedonic scaling and for the measurement of consumer concepts.

The data presented in Table 1 shows that the flower-based food products prepared in this study are low in fat and can be consumed without increasing blood cholesterol levels. Food products such as pumpkin flower phuluri plants are deep fried in soya bean oil, whereas onion flower mustard pickle and Papaya flower mustard pickle contain added mustard oil. However, since the quantity of these items to be served will be much less than 100 g, the fat content will be reduced to a negligible amount.

As shown in Table 2, flower food products are rich in β-carotene. The ICMR's Recommended Dietary Allowances (RDA) for adult men and women for vitamin A are 1000 µg/d and 840 µg/d, respectively (Anon, 2023). The total ascorbic acid concentration of food products ranges from 2 mg to 116 mg/100 g, which is sufficient for meeting the RDA standards of 80 mg/d and 65 mg/d for adult men and women, respectively (Anon., 2023).

The food products are particularly rich in calcium, potassium and phosphorous, as shown in Table 3. Calcium is needed for growth and bone development, and calcium deficiency can lead to osteoporosis. Phosphorus is an essential element of the human body and is required for a wide range of processes, such as ATP synthesis, signal transduction and bone mineralization (Serna and Bergwitz 2020). The prepared food products are also good sources of manganese and copper, which may be sufficient for fulfilling the recommended dietary allowances. Although copper deficiency is less common in humans, low intakes may adversely affect cholesterol and glucose metabolism, blood pressure control, cardiovascular function and the immune system (Burkhead and Collins, 2022).

From Table 4 and Figure 1, based on five different hedonic attributes, we can derive that amongst all the products, papaya flower soup had the highest hedonic mean score for all the attributes and overall acceptability (8.43±0.72), while papaya flower mustard pickle had the lowest acceptability, with a mean hedonic score of 6.73±1.28.

R-index values were computed from the data when they were ranked in order of preference, as described in the matrix of Figure 2, indicating the degree of preference for one pickle over its adjacent pickle in the hedonic ranking. According to Table 5, equal overall preferences or 100% preferences for the five different pickles have not been reported. It can be concluded that, overall, product B (pumpkin flower sweet and sour

pickle) has the highest preference value compared to the other tested pickles.

As shown in Table 6, no similar overall preferences or 100% preferences were reported for the four different edible flower powder products. Thus, from the above analysis, it can be concluded that, compared with the other powder products, product B (pumpkin flower powder phuluri) has a relatively high preference for product D (papaya flower powder soup), which has the overall highest preference value.

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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: Approximate values of the macronutrient content of food products (per 100 g serving) prepared from *Allium cepa, Carica papaya* and *Cucurbita maxima* blossoms.

Food products (100 g)	Carbohydrate (g)	Protein	Fat	Soluble fibre	Energy (kcal)
Onion flower mustard pickle	2.18	2.05	22.22	1.21	216.9
Onion flower chilli vinegar pickle	5.12	1.34	0.25	1.40	28.00
Onion flower chaat masala	1.29	0.20	0.05	0.87	1.94
Pumpkin flower jam	5.00	0.27	0.15	1.71	26.43
Pumpkin flower sweet and sour pickle	8.19	2.47	0.25	1.64	44.89
Pumpkin flower phuluri (4 pieces)	35.6	15.6	38.16	4.04	548.00
Pumpkin flower laddoo (4 pieces)	10.34	3.65	11.17	1.83	114.53
Papaya flower sweet and sour pickle	15.11	1.92	0.15	5.34	18.35
Papaya flower mustard pickle	17.3	5.85	18.76	2.68	188.44

Table 2: Approximate micronutrient contents (vitamins) of food products (per 100 g of serving) prepared from *Allium cepa*, *Carica papaya* and *Cucurbita maxima* blossoms.

Serial No.	Food Products	Thiamine (B_1)	Riboflavin (B_2)	Niacin (B_3)	Total ascorbic acid	β-carotene
	(100 g)	(mg)				(µg)
1.	Onion flower mustard pickle	0.807	0.116	1.028	4	34.45
2.	Onion flower chilli vinegar pickle	0.250	0.121	0.608	16.17	30.6
3.	Onion flower chaat masala	0.230	0.090	0.430	4.34	15.74
4.	Pumpkin flower jam	0.163	0.015	0.162	50.96	2.81
5.	Pumpkin flower sweet and sour pickle	0.810	0.094	0.408	27.86	40.35
6.	Pumpkin flower phuluri	0.387	0.133	1.232	69.61	306.67
7.	Pumpkin flower laddoo	1.020	0.015	0.142	2.17	23.37
8.	Papaya flower sweet and sour pickle	0.203	0.071	0.479	116.05	83.46
9.	Papaya flower mustard pickle	0.327	0.184	1.846	113.5	234.54

Table 3: Approximate micronutrient contents (minerals and trace elements) of food products (per 100 g of serving) prepared from *Allium cepa, Carica papaya* and *Cucurbita maxima*.

Serial No.	Food Products	Calcium (Ca)	Potassium (K)	Iron (Fe)	Phosphorous (P)	Copper (Cu)	Manganese (Mn)
	(100 g)	(mg)					
1.	Onion flower mustard pickle	88.85	238.47	2.61	45.70	0.491	0.72
2.	Onion flower chili vinegar pickle	61.29	275.59	2.26	25.76	0.726	1.29
3.	Onion flower chaat masala	62.02	144.53	1.91	3.07	0.47	0.61
4.	Pumpkin flower jam	59.23	149.80	1.68	24.76	3.32	0.34
5.	Pumpkin flower sweet and sour pickle	60.54	250.03	2.14	63.81	3.48	0.83
6.	Pumpkin flower phuluri	77.25	625.13	6.59	230.45	3.76	0.98
7.	Pumpkin flower laddoo	58.99	734.09	8.09	276.87	3.32	0.49
8.	Papaya flower sweet and sour pickle	75.60	284.17	2.43	63.05	2.61	0.61
9.	Papaya flower mustard pickle	161.50	546.50	5.47	212.4	2.81	1.54

Table 4: Mean hedonic values of the food products as evaluated by panel members

Food Product Name		Sensory Properties				
		Colour	Aroma	Consistency	Taste	Overall Acceptability
Onion Flower	Mustard pickle	6.83±1.59	7.5±1.13	7.06±1.43	6.86±1.4	7.00±1.33
	Chilli vinegar pickle	6.73±1.22	7.66±1.58	6.46±1.97	7.53±1.77	7.6±1.37
	Chaat masala (with powder)	7.96±0.55	7.96±0.88	8.06±0.78	8.23±0.85	8.4±0.67
Pumpkin flower	Jam	7.16±1.46	6.5±1.3	7.4±1.27	7.96±0.88	7.6±0.95
	Sweet and sour pickle	6.73±1.43	6.83±1.34	6.6±1.54	6.9±1.53	7.03±1.09
	Phuluri (with powder)	7.8±0.69	7.53±0.93	7.83±0.69	8.03±0.98	8.26±0.69
	Laddoo (with powder)	7.03±0.8	7.46±1.13	7.7±0.87	7.6±1.03	7.73±0.94
Papaya flower	Sweet and sour pickle	7.5±1.3	6.76±1.38	7.3±1.11	7.06±1.38	7.16±1.23
	Mustard Pickle (sour)	7.8±0.8	7.73±0.9	7.16±0.94	7.13±0.89	6.73±1.28
	Soup (with powder)	8.26±0.78	8.03±0.76	8.3±0.7	8.3±0.66	8.43±0.72

The data are expressed as the mean ± SD (n = 30).

Table 5: Percentage of preference for one product over another for the different pickles as calculated from the hedonic R-Index response matrix for pickles

Preference	Percentage
Prefer A to B	35.3%
Prefer A to C	48.1%
Prefer A to D	48.6%
Prefer A to E	56.6%
Prefer B to A	64.6%
Prefer C to A	51.8%
Prefer D to A	51.3%
Prefer E to A	43.3%
Prefer B to C	64.0%
Prefer B to D	66.3%
Prefer B to E	71.4%
Prefer C to B	36.0%
Prefer D to B	33.6%
Prefer E to B	28.5%
Prefer C to E	59.0%
Prefer C to D	49.2%
Prefer E to C	41.0%
Prefer D to C	50.7%
Prefer D to E	60.0%
Prefer E to D	39.9%

Table 6: Percentage of preference of one product over another for the different powder products as calculated from the hedonic R-Index response matrix for powders

Preference	Percentage
Prefer A to B	34.5%
Prefer A to C	68.2%
Prefer A to D	47.8%
Prefer B to A	42.3%
Prefer C to A	31.7%
Prefer D to A	48.4%
Prefer B to D	41.1%
Prefer B to C	62.1%
Prefer D to B	55.5%
Prefer C to B	37.8%
Prefer C to D	31.2%
Prefer D to C	65.3%

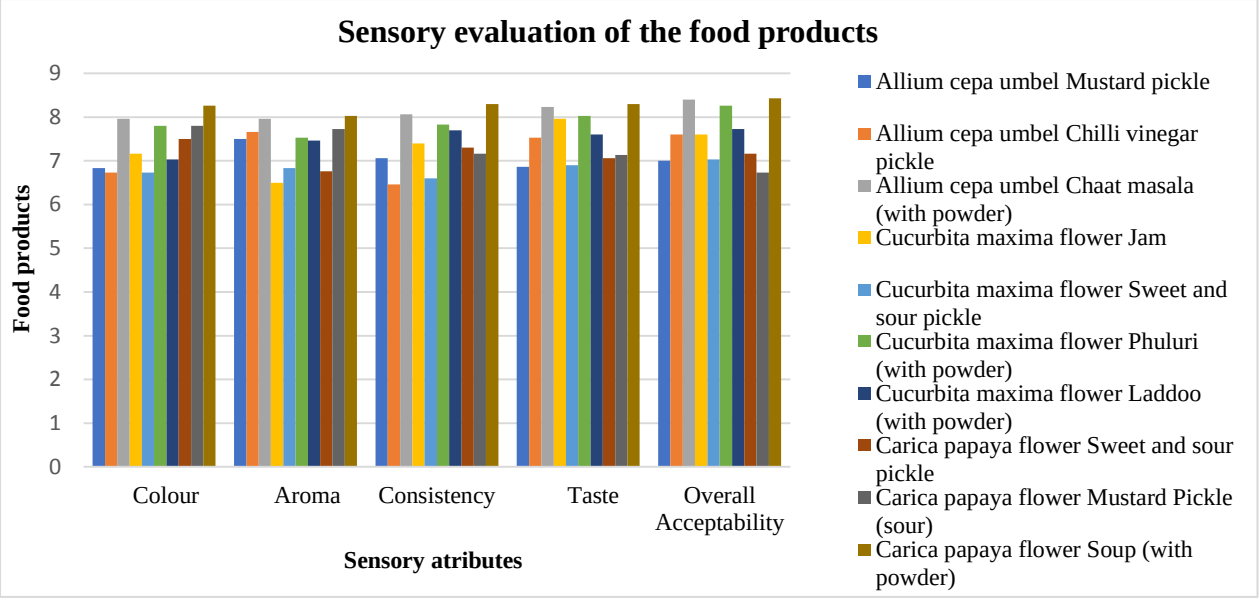

Figure 1: Graphical representation of the sensorial evaluation of food products prepared from *Allium cepa* umbel, *Carica papaya* flowers and *Cucurbita maxima* flowers.

	1st	2nd	3rd	4th	5th
Onion flower mustard pickle [A]	9	9	6	6	0
Onion flower chilli vinegar pickle [B]	16	8	4	2	0
Pumpkin flower sweet and sour pickle [C]	9	8	11	2	0
Papaya flower sweet and sour pickle [D]	5	17	5	3	0
Papaya flower mustard pickle [E]	6	8	10	5	1

Like more ← → Like less

Figure 2: Hedonic R-Index response matrix for pickles

	1st	2nd	3rd	4th
Onion flower powder chaat masala [A]	17	11	2	0
Pumpkin flower powder phuluri [B]	13	13	4	0
Pumpkin flower powder laddoo [C]	10	8	11	1
Papaya flower powder soup [D]	17	9	3	0

Like more ← → Like less

Figure 3: Hedonic R-Index response matrix for powder

Figure 4: Prepared Food products: 1. Onion flower mustard pickle 2. Onion flower chilli vinegar pickle 3. Onion flower chaat masala (with powder) 4. Pumpkin flower jam 5. Pumpkin flower sweet and sour pickle/chutney 6. Pumpkin flower laddoo (with powder) 7. Papaya flower sweet and sour pickle/chutney 8. Papaya flower mustard pickle 9. Papaya flower powder 10. Pumpkin flower phuluri (with powder)

SHORT COMMUNICATION

Nutritional enhancement of extruded vermicelli through incorporation of finger millet and carrot pomace

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ABSTRACT

This study focused on the development and characterization of nutrient-enriched cold-extruded vermicelli using composite flour blends incorporating finger millet, pulse flour, corn flour, semolina, and carrot pomace. Two formulations were developed: control (100% semolina), composite formulation V1 (40% finger millet, 20% corn flour, 20% red gram pulse flour, 10% semolina,10% carrot pomace). Cold extrusion was performed using a single-screw extruder. Vermicelli demonstrated superior nutritional, functional attributes. Proximate analysis revealed that vermicelli had significantly higher protein (17.05 g), dietary fiber (14.7 g), and iron (6.58 mg) compared to the control. The fatty acid profile showed increased monounsaturated (0.49 g) and polyunsaturated fatty acids (1.33 g), as compared to the control (0.2 g monounsaturated and 0.6 g polyunsaturated fatty acids), indicating a healthier lipid composition. The cooking quality of the vermicelli was improved, as evidenced by a higher water absorption capacity (3.47%), expansion ratio (2.62), and a low cooking loss (2.21%), which falls within the acceptable range. These results demonstrate the potential of incorporating finger millets and carrot pomace as functional ingredients in the development of health-oriented, ready-to-cook vermicelli, offering enhanced nutritional quality along with value-added functional benefits.

Keywords: Finger millet, carrot pomace, vermicelli, cold extrusion, composite flour

The increasing consumer demand for convenient, ready-to-cook foods with added health benefits has led to the reformulation of traditional products like vermicelli. Vermicelli is a type of traditional extruded product typically made from refined wheat flour and consumed widely in many Asian and Middle Eastern countries. It is thin, dried, and often roasted before use in both sweet and savory preparations. While conventional vermicelli is low in fiber and micronutrients, its nutritional value can be enhanced by incorporating composite flours from millets, pulses, or vegetables (Kulkarni *et al*., 2012).,Conventional vermicelli made from refined semolina lacks key elements like dietary fiber, essential amino acids, and micronutrients. Incorporation of functional ingredients offers a viable approach to enhance the nutritional profile of vermicelli (Kulkarni *et al*., 2012). Ragi, also known as finger millet (*Eleusine coracana*), is a nutrient-dense, gluten-free cereal that is well known for its health advantages. Because of its high calcium content, it is beneficial for bone health, especially in women, children,

and the elderly. Finger millet is high in calcium and also has a considerable quantity of protein and dietary fiber, which help with digestion, blood sugar balance, and muscular growth. Along with B-vitamins like thiamine, riboflavin, and niacin, it also offers vital minerals like iron, magnesium, phosphorus, potassium, and zinc (Devi *et al*., 2014). A by-product of the juice-making process, carrot pomace is rich in beta-carotene and dietary fiber, providing sustainability and nutritional advantages (Verma *et al.,* 2020). Functional ingredients like millets and vegetable pomace maintain their nutritional value when cold-extruded vermicelli is made without the use of heat (Gandhi *et al.,* 2022), this technique preserves bioactive substances like fiber and antioxidants while improving texture, nutrient density, and acceptability. The addition of finger millet and carrot pomace enhances vermicelli's health advantages and qualifies it for use in food formulations with added value (Shobana *et al*., 2013). Value addition is a successful tactic to boost the popularity of underutilized fruit and vegetable species, decrease post-harvest waste, and guarantee their year-round availability (Dissanayake *et al.*, 2023). Thus, the goal of the current study was to improve the nutritional value of extruded vermicelli by adding carrot pomace, red gram pulse flour, and finger millet corn flour.

Finger millet, corn flour, red gram pulse and semolina were procured from Agra markets. Red gram was prepared by cleaning and lightly roasting the dal to enhance flavor and digestibility. After cooling, it was finely ground using a mixer grinder and sieved to obtain uniform flour, then stored in an airtight container. A Kuvings B1700 juicer was used to extract the carrot pomace, which was then collected, dried in a hot air oven at 60℃ for four hours, powdered into a fine powder, and kept in an airtight container until it was needed again.

- Two formulations were developed: Control (100% semolina)
- Composite formulation Vermicelli (V1) (40% finger millet, 20% corn flour, 20% red gram flour, 10% semolina,10% carrot pomace) presented in Table 1.

The composite flour blends were prepared as per the formulation and mixed thoroughly for uniform distribution. A single-screw extruder (G.L. Extrusion Systems, 5 HP) equipped with a 2 mm circular die was then used to cold extrude these blends.The extrusion was carried out at ambient temperature without external heating, which helps retain heat-sensitive nutrients such as vitamins, antioxidants, and bioactive compounds (Gandhi *et al*., 2022).The extruded strands were cut to uniform lengths and air-dried under shade at room temperature for 6–8 hours to reduce moisture content and ensure better shelf stability. After drying, In order to preserve it for later use and analysis, the vermicelli was sealed in low-density polyethylene (LDPE) bags and kept at room temperature (Kumar and Sharma *et al*., 2020).

Protein, fat, carbohydrate, dietary fiber, amylose and amylopectin were analyzed using AOAC, (2000) methods. Mineral analysis was carried out using Atomic Absorption Spectroscopy (AAS) following the standard procedures outlined by AOAC (2000). Fatty acid profiles were determined by gas chromatography and HPLC, respectively. Cooking quality *i.e.,* Water absorption, cooking loss, and expansion ratio were determined using standard methods described by AACC (2000). Sensory Evaluation was conducted by 9 point hedonic scale. Statistical analysis was conducted using SPSS with significance at $p \leq 0.05$.

The data presented in Table 2, highlights the nutritional improvements observed in the

formulated vermicelli compared to the control. Protein content in the vermicelli was considerably higher (17.05 g) than the control (8.86 g), attributed to the inclusion of pulse flour and finger millet. This finding is supported by Rani *et al.* (2021), who reported increased protein levels in vermicelli enriched with legume flours due to their high lysine content. Dietary fibre content also increased significantly in the vermicelli (14.7 g) compared to the control (3.30 g), which aligns with Verma *et al.* (2020), who found that carrot pomace significantly enhances fibre content when added to cereal-based products.

Amylose content was higher in the vermicelli (13.3%) than in the control (10.3%), which may support improved glycaemic response; this is consistent with the results of Pradeep and Guha (2011), who observed elevated amylose levels in fibre-rich extruded products. Fat content was slightly reduced in the formulated vermicelli (1.0 g) relative to the control (1.4 g), in agreement with Sharma *et al.* (2015), who reported decreased fat retention in millet-based noodles. Carbohydrate levels remained similar in both samples (71.8 g in vermicelli and 72.3 g in control), indicating that functional ingredient addition had minimal impact on total carbohydrate content.

The data presented in Table 3**,** highlight the mineral content of vermicelli showed a substantial improvement over the control. Calcium content increased from 12.6 mg in the control to 24.2 mg in the formulated vermicelli, while iron content increase from 1.22 mg in the control 6.58 mg in formulated vermicelli. This increase is primarily due to the inclusion of finger millet, which is naturally rich in calcium and iron (Kumar and Sharma, 2020). Similar enhancements in mineral content through the use of millets have been reported by Shobana *et al.* (2013). The contribution of carrot pomace may have further improved the iron and calcium levels, consistent with findings by Verma *et al.* (2020) also reported increase in mineral content in extruded products enriched with fruit and vegetable residues.

The Fatty Acid Analytical technique used in the study is used to compare the fatty acid data shown in Table 4 between the profiles of the control and formulated vermicelli. The data reveal that the formulated Vermicelli contains significantly higher levels of beneficial unsaturated fatty acids. Specifically Mono unsaturated fatty acid increased from 0.2 g in the control to 0.49 g in vermicelli while polyunsaturated fatty acid showed an even more remarkable increase from 0.6 g in the control to 1.33 g in vermicelli. Although there was an increase in saturated fatty acid in formulated Vermicelli (0.47 g) compared to the control (0.2 g), the overall profile shows a favorable shift toward unsaturated fats. Unsaturated fatty acids, especially monounsaturated and polyunsaturated fats, are good for cardiovascular health because they lower low-density lipoprotein (LDL) cholesterol, promote anti-inflammatory reactions, and enhance lipid profiles in general. They are linked to a lower risk of metabolic diseases and heart disease when included in the diet Schwab *et al.* (2014),.These results are consistent with earlier studies, by Verma *et al.*, (2020) and Pradeep and Guha (2011), which emphasized the enhancement of fatty acid profiles through the incorporation of fiber-rich and millet-based ingredients. Therefore, the vermicelli demonstrates an improved lipid profile, supporting its development as a healthier, functional food product.

Data from the cooking quality table is shown in Table 5. Three important factors are compared between the control and vermicelli in the Cooking Quality: water absorption, cooking loss, and expansion ratio.The

Cooking Quality compares three key parameters water absorption, cooking loss, and expansion ratio between the control and vermicelli. The results indicated that vermicelli demonstrated enhanced cooking quality. Water absorption increased from 3.26% in the control to 3.47% in formulated vermicelli, suggesting improved hydration and rehydration ability during cooking, likely due to the presence of fibre-rich ingredients like carrot pomace and finger millet. Cooking loss, although slightly higher in vermicelli (2.21%) than in the control (1.83%), remained within acceptable limits, indicating that the product retained its structural integrity despite its elevated fibre content. The expansion ratio was also higher in vermicelli (2.62) compared to the control (2.18), reflecting better swelling capacity and increased volume post-cooking. These improvements align with findings by Devi *et al.* (2020) and Sharma *et al.* (2015), who reported that millet-based and vegetable pomace-enriched noodles exhibited superior cooking characteristics. The results confirm that incorporating functional ingredients into vermicelli formulations enhances cooking quality, making the final product more desirable in terms of texture, cooking stability, and consumer appeal. The improved cooking quality of vermicelli is attributed to the functional ingredients used. The higher water absorption is due to the presence of dietary fiber from carrot pomace and finger millet, which enhances hydration during cooking (Verma *et al.*, 2020). Slightly increased cooking loss may result from fiber disrupting the starch matrix, but it remains within acceptable limits, indicating structural stability (Devi *et al.*, 2020). The higher expansion ratio reflects better swelling, supported by the starch and amylose content in finger millet and pulses, contributing to a desirable texture and improved mouthfeel (Pradeep and Guha, 2011; Sharma *et al.*, 2015).

The sensory evaluation data shown in Table 6, the prepared Vermicelli sample was more palatable than the control sample in every sensory category. The sensory evaluation results indicated that formulated Vermicelli demonstrated better acceptability the control sample across all sensory attributes. Formulated Vermicelli received higher mean scores for appearance (7.45), colour (7.81), flavour (7.30), texture (7.60), aftertaste (6.95), and overall acceptability (7.85), compared to the control with respective scores of 6.45, 6.45, 6.90, 6.91, 6.35, and 6.75. These improvements can be attributed to the incorporation of functional ingredients such as finger millet and carrot pomace. Similar enhancements in sensory quality were reported by Devi *et al.* (2020), who found that vegetable pomace improved the color, flavor, and overall acceptability of noodles. Pradeep and Guha, (2011) also demonstrated improved sensory attributes in finger millet-based vermicelli. Furthermore, Banu *et al.* (2022) noted enhanced texture and appearance in extruded products developed with composite flours.

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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: Composite flour

Sample	Finger Millet	Corn Flour	Pulse Flour	Semolina	Carrot Pomace
Control	–	–	–	100 g	–
V1	40 g	20 g	20 g	10 g	10 g

Table 2: Nutritional analysis of vermicelli

Parameters	Control	Vermicelli	T-value
Protein (g)	8.86±0.12	17.05±0.3	*4.3
Fat (g)	1.4±0.05	1.0±0.1	*0.04
Carbohydrate (g)	72.3±0.6	71.8±0.5	*0.01
Dietary Fiber (g)	3.30±0.07	14.7±0.7	*4.3
Amylose (%)	10.3 ±0.1	13.3±0.8	*0.03

Mean SD is used to express values, while p < 0.05 indicates significance.

Table 3: Mineral Analysis of vermicelli

Mineral	Control	Vermicelli
Calcium(mg)	12.6±0.7	24.2±0.9
Iron (mg)	1.22±0.5	6.58±0.7

Values are expressed in mean SD and significant at $p \leq 0.05$

Table 4: Fatty acid analysis of vermicelli

Sample	SFA (g)	MUFA (g)	PUFA (g)
Control	0.20	0.20	0.60
Vermicelli	0.47	0.49	1.33

Mean SD is used to express values, while p < 0.05 indicates significance.

Table 5: Cooking quality of vermicelli

Parameters	Control	Vermicelli	T Value
Water Absorption (%)	3.26±0.8	3.47±0.9	*6.35
Cooking Loss (%)	1.83±0.7	2.21±0.7	*0.009
Expansion Ratio	2.18±0.7	2.62±0.3	*0.002

Mean SD is used to express values, while p < 0.05 indicates significance.

Table 6: Sensory evaluation of vermicelli

Attributes	Control	Vermicelli 1
Appearance	6.45 ± 0.70^{b}	7.45 ± 0.73^{a}
Colour	6.45 ± 0.12^{b}	7.81 ± 0.24^{a}
Flavour	6.90 ± 0.41^{ab}	7.30 ± 0.14^{a}
Texture	6.91 ± 0.20^{b}	7.60 ± 0.07^{a}
Aftertaste	6.35 ± 0.14^{b}	6.95 ± 0.12^{a}
Overall Acceptability	6.75 ± 0.14^{b}	7.85 ± 0.45^{a}
F-value	**9.38***	**21.94****

Mean SD is used to express values, while p < 0.05 indicates significance.

SHORT COMMUNICATION

Antioxidant potentiality of *Artemisia absinthium* from Handwara region of Jammu and Kashmir

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ABSTRACT

Artemisia absinthium, commonly known as wormwood, (Tyethwan) is well known medicinal plant for its extensive used in traditional ayurvedic medicine for treating hepatitis, wound healing and jaundice. The aim of this study was to conduct antioxidant potentiality of Artemisia absinthium from Handwara region of J&K. Phytochemical analysis demonstrated high content of phenolic (4.9925 mg GAE/g) and flavonoidal compounds (45.375 mg QE/g). The DPPH assay results depicted an IC50 value of 23.9742 µg/mL indicating significant free radical scavenging ability, comparable to standard antioxidants such as ascorbic acid. This study validates the traditional medicinal uses of A. absinthium and establishes its potential as a natural source of antioxidants. These findings suggest that A. absinthium could be further explored for therapeutic applications, contributing to the development of natural antioxidant formulations for health and well-being.

Keywords: Antioxidants, *Artemisia absinthium*, phytochemical analysis

Medicinal and aromatic plants continue to play important role in ensuring health security of the nation and world (Rathore 2025). The therapeutic potential of medicinal plants is attributed to their complex chemical composition, which include a wide range of bioactive compounds, including phenolics, terpenoids, and flavonoids. *Artemisia absinthium* is a member of the Asteraceae family, one of the most significant polymorphism taxa in the field of pharmacology. Most of the plants in this category are located in temperate regions of the northern hemisphere, but there are a few species that can also be found in southern hemisphere. Plants have tendency to produce numerous secondary metabolites that occur naturally and are significant in pharmacology. These necessary metabolites, which may carry essential oils, saponins, flavonoids, and glucosinolates (Watson *et al*., 2002), are primarily used to combat various illnesses like cancer, inflammation, bacterial, viral, and fugal related infections. *Artemisia absinthium* that originates from the temperate regions of Eurasia and Northern Africa is found in Kashmir also at the elevation of 2100 meters (Javed e*t al*., 2012). It has hairy, ribbed stems and silvery, pinnatifid leaves. The flower heads are heterogamous, with female ray florets and hermaphrodite disc florets, surrounded by long white hairs. The marketed drug appears as grayish-white fragments of broken leaves,

flower heads, and hairy twigs with ridged branches (Sharopov e*t al*., 2012).

The fresh and healthy plants of *Artemisia absinthium* were collected from Handwara region, an area between Baramulla and Kupwara zone of Union territory of Jammu and Kashmir an average elevation of 1,582m (5,190ft) above sea level for research purpose. The plant material was maintained and stored at Department of Plant Science, School of life sciences, Central University of Himachal Pradesh, Shahpur Campus (Himachal Pradesh).

For Phytochemical analysis Folin-ciocalteu reagent -FCR, Gallic acid (standard), Sodium carbonate, Methanol, distilled water, Quercetin, Aluminium chloride, DPPH (2,2--Diphenyl-1-picrylhydrazyl) Ascorbic acid, Sodium phosphate, Ammonium molybdate, Sulphuric acid, Sodium nitrite, Sodium hydroxide etc were used. The sample was collected from the forests of Galganzer. The sample was dried in shade and powder was obtained by grinding the sample with a mortar and pestle. The sample that had been pulverized and dried was macerated in methanol for 72 hours at room temperature (28 ± 2 ◦C) with periodic shaking. Once the extraction was completed it was filtered and was reextracted using the same procedure and solvent. The residue obtained was stored in refrigerator.

The total phenolic content in the methanolic extract of *A. absinthium* was determined following the method described by Bhat *et al.* (2018). In a test tube, 100 µL of the *A. absinthium* extract was mixed with 3 mL of Folin-Ciocalteu reagent that was diluted in the ratio of 1:9, shaken thoroughly, and left to stand for 10 minutes. Then, 7.5 g of Na_2CO_3 was added to the mixture

The technique called aluminium chloride colorimetric (Bhat e*t al*., 2018) was incorporated to evaluate total flavonoid content (TFC). 100 µl of plant extract was taken. 150 µl of sodium nitrate and 150 ml of 2 percent aluminium chloride solution were added, shaken well and allowed to settle for 6 min. Then added 1ml sodium hydroxide, the mixture was left to stand for 30 min at room temperature while being periodically shaken. Using a spectrophotometer, the mixture's absorbance was measured at 510 nm. The content of total flavonoids was expressed as mg of quercetin equivalents (QE) per g of the extract.

The free radical scavenging activity of the extracts were examined using 2,2-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging technique (Bhat e*t al*.,2018). Mixture was left for 30 min.The absorbance of solution was recorded at 517 nm by using spectrophotometer. Percentage inhibition was then computed using the following formula**:**

$$\%I = \frac{A^{Control} - A^{Sample}}{A^{control}}$$

Where A (control) denotes absorbance of the test compound and A (sample) represents absorbance of the control which contains all of the chemicals except the test compound. The IC50 value was calculated using the scavenging percentagae versus concentration. The average IC50 value was computed.

The total antioxidant capacity of *A.absinthium* extracts was measured by phosphomolybdate assay (Phillips e*t al*.,1994). 100 µl of the plant extract were mixed with 3 mL of the reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and then incubated at 95 ◦C for 90 min. After which the samples were left to cool down the absorbance of the mixture was measured at λ 695 nm using a UV spectrophotometer against a blank. After calculation, the extracts' overall antioxidant capacity was reported as milligrams of ascorbic acid equivalents (mg AAE/g) of dry weight.

To quantify the amount of phenol present in the *A. absinthium* extract the total Phenolic content test was done using Folin – Ciocalteu (FCR) method. The amount of phenolic content was evaluated using the standard curve equation: $y = 0.0185x +$

0.3746, $R^2 = 0.9671$, where y is the absorbance at 765nm and x is the total phenols in the *A. absinthium* extract (mg/ml). Total phenolic content of extract was 4.9925mgGAE/g. The results of the comparative analysis of phenolic content were quite lesser than the study conducted by (Bhat e*t al*.,2018) with values 24.31mgGAE/g.

To quantify the amount of flavonoid present in the *A. absinthium* extract the total flavanoid content test was performed using Alumunium chloride method. The amount of flavonoid content was estimated using the standard curve equation: $y = 0.0022x + 0.01066$, $R^2 = 0.9717$, where y is the absorbance at 510nm and x is the total phenols in the *A. absinthium* extract (mg/ml). Total Flavonoid content of extract was 45.375mgQE/g. The results of the comparative analysis of flavonoid content were quite agreeable to the study conducted by (Bhat e*t al*.,2018) with slight difference in values i.e. 39.52mgQE/g. Phenolics and flavonoids, directly contribute to the antioxidant capacity of plants (Sanket e*t al*.,2025).

The free radical scavenging activity of the *Artemisia absinthium* extracts were examined using 2,2-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging technique. DPPH is a free radical and have a deep violet colour, when it gets reduced its colour change to yellow and became DPPH-H.

The maximum per cent radical scavenging activity is obtained at 25µg/ml concentrations of methanol extract of *Artemisia* and lowest at 5µg/ml. With an IC50 value of 23.9742µg/ml (Figure 1). The results of the comparative analysis were quite higher than the study conducted by (Bhat e*t al*., 2018) with values IC50: 14.88µg/ml. The maximum per cent radical scavenging activity is obtained at 25µg/ml concentrations of Ascorbic acid (standard use) and lowest at 5µg/ml (Figure 2). The percent RSA increases as the concentration of the substance increases, indicating that the substance has a higher radical scavenging activity at higher concentrations. The IC50 value is 33.32 µg/ml, which is the concentration required to achieve 50 percent RSA.

For both the plant extract and ascorbic acid, the percent RSA increases with the concentration. At all concentrations (5, 10, 15, 20, and 25 µg/ml), the plant extract shows consistently high percent RSA, around 70 percent.. The percent RSA for ascorbic acid is significantly lower compared to the plant extract at all tested concentrations. At 5 µg/ml, ascorbic acid shows around 20 percent RSA. The percent RSA increases gradually, reaching around 35 percent at 25 µg/ml. The plant extract demonstrates a much higher antioxidant potential compared to ascorbic acid across all tested concentrations.

The phosphomolybdenum technique was used to test the spectrophotometric antioxidant capability of the *Artemisia absinthium* extracts, with a maximum absorption calculated at 695 nm (Figure 3). Ascorbic acid equivalents (AAE)/gram of dry weight plant material were used to represent the antioxidant capacity of the *A. absinthium* extracts. The reported total antioxidant capacity for AAE was 10.71425mg AAE/g. The results of the comparative analysis were quite higher than the study conducted by (Phillips e*t al*.,1994) with values3.57mg AAE/g.

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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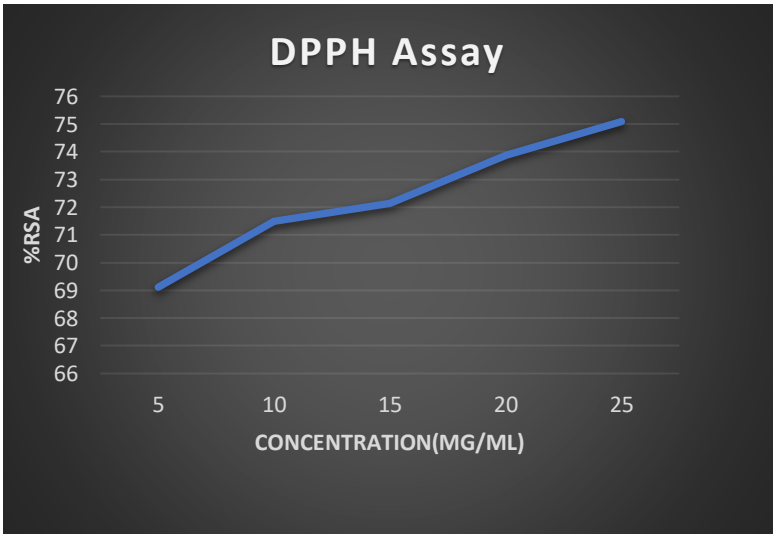

Figure 1: **Graph is plotted between concentration (µg/ml) on X axis and % Radical Scavenging Activity on Y axis**

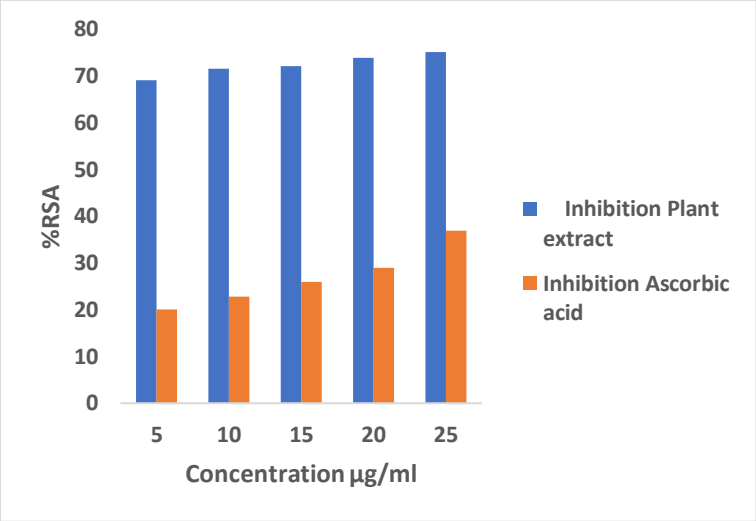

Figure 2: **Comparative analysis of DPPH Assay**

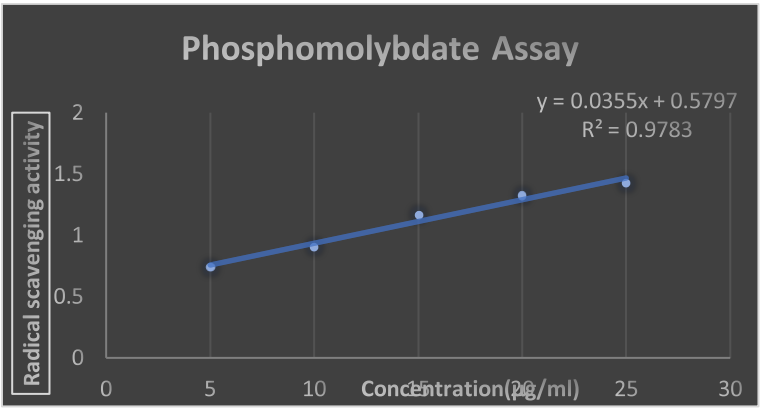

Figure 3: Graph is plotted between concentration (µg/ml) on X axis and % Radical Scavenging Activity on Y axis.

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Book: Bompard, J.M. and Schnell, R.J. 1997. Taxonomy and Systematics. In Litz, R.E. (ed). *The Mango. Botany, Production and Uses*. Wallingford: CABI publishing. pp.19-41.

Anonymous. 1979. *Mango varieties of West Bengal. Technical Bulletin No. 1.* Department of Horticulture, Faculty of agriculture, Bidhan Chandra Krishi Viswavidyalaya. Pp.52.

Chapter in book: Singh, Harminder, Thakur Anirudh and Jawandha, S. K. 2010a. Varietal improvement and production technologies in peach. *In. Temperate fruits in subtropics.* WS Dhillon (ed). Department of Horticulture, Punjab Agricultural University, Ludhiana pp 5-8.

Proceedings: Blake, M.A. 1932. The J.H. Hale as a parent in peach crosses. *Proc. Am. Soc. Hort. Sci.,* **29**:131-136.
Monet, R. 1979. Transmission génétique du caractère 'fruit doux' chez le pêcher. Incidence sur la selection pour la qualité. *In: Proceedings of Eucarpia Fruit Section Symposium. Tree Fruit Breeding.* INRA, Angers, France, pp. 273–276.

Bulletin: Gray, P. 1914. The compatibility of insecticides and fungicides. *Monthly bulletin of California,* July, 1914.

Annual meetings: Schenck, N.C. 1965. Compatibility of fungicides with insecticides and foliar nutrients. *57th annual Meeting of American Phytopathological Society,* 3-7 October, 1965.

Reports: Anonymous, 1971. Investigations of insects pests of sorghum and millets. *Final Technical report,* 1965-70, IARI, New Delhi, pp.157.

Annual report: Anonymous, 2010. *Annual Report for 2010-11*, Faculty of Horticulture, Bidhan Chandra Krishi Viswavidyalaya, Monhanpur, Nadia, West Bengal, India. Pp.80-85.

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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