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INVITED ARTICLE

Spilanthes acmella- an important medicinal plant

A. Sabitha Rani*, Hajera Sana, G. Sulakshana, E.Shravya Puri and M. Keerti

Department of Botany, University College for Women,
Koti, Hyderabad-95, Telangana, India.

**Email:sabitaammana@yahoo.com*

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ABSTRACT

Spilanthes acmella Murr., commonly known as toothache plant, an important medicinal plant belonging to family Asteraceae. It has been reported to posses various biological activities like antipyretic, antidiuretic, antiinflammatory, antioxidant, immunomodulatory, hepatoprotective, anticancer and antitoothache etc. The plant has been found to produce important secondary metabolites like spilanthol, scopoletin, myrecene, á amyrin, â amyrin etc. Among all, the bioactive chemical component is spilanthol, an alkamide which is present in roots and all aerial parts of the plant. Spilanthol has high industrial demand for its use in pharmaceutical, cosmetic and toothpaste industry. S.acmella is quickly getting depleted from its natural habitat, because of its wider applications for commercial use. The plant is not meeting the industrial demand due to less commercial cultivation. In this context, the present review will throw light on its medicinal importance and pharmacological applications, cultivation practices and mass propagation through tissue culture techniques.

Key Words: *Spilanthes acmella*, phytochemical constituents, traditional and medicinal uses, cultivation

INTRODUCTION

Spilanthes acmella Murr., commonly known as toothache plant or Paracress or Eyeball plant is an important medicinal plant belonging to family Asteraceae. It has been reported to posses various biological activities like antipyretic, antidiuretic, antiinflammatory, antioxidant, immunomodulatory, hepatoprotective, anticancer and anti-toothache etc. The plant has been found to produce important secondary metabolites like spilanthol, scopoletin, myrecene, á amyrin, â amyrin etc. Among all, the bioactive chemical component is spilanthol, an alkamide which is present in roots and all aerial parts of the plant. Spilanthol has high industrial demand for its use in pharmaceutical, cosmetic and toothpaste industry. *S.acmella* is one of such important medicinal plants that quickly getting depleted from its natural habitat, because of its wider applications for commercial use. The plant is not meeting the industrial demand due to less commercial cultivation. The other major limiting factor in large scale propagation of *S.acmella* is low germination and viability of the seed (Pati *et al.,* 2006).

MEDICINAL PROPERTIES

Spilanthes acmella Murr. is an important medicinal plant, popularly known as toothache plant which reduces the pain associated with toothaches and induce saliva secretion. For centuries *S. acmella* has been widely cultivated for horticultural, medicinal, insecticidal, and culinary purposes and application for this purpose is still widespread in different parts of the world. Whole plant of *S. acmella* is rich in secondary metabolites, which impart a plethora of medicinal uses to the plant. Different parts of this plant possess multiple pharmacological activities, which include antimicrobial, antipyretic, local anaesthetic, bio-insecticide, anticonvulsant, antioxidant, aphrodisiac, analgesic, diuretic, toothache relieve and anti-inflammatory effects (Dubey *et al.,* 2013).

ACTIVE PRINCIPLE AND PHYTOCHEMICAL CONSTITUENTS

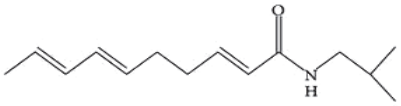
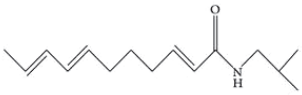
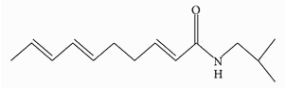
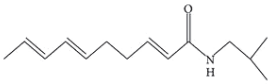
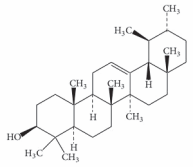
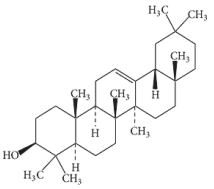
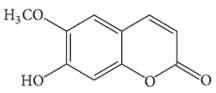
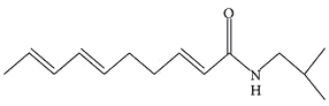
The medicinal properties of *S. acmella* are mainly due to the presence of a wide array of compounds with varying structural patterns, such as alkylamides (spilanthol), phenolics (ferulic acid and vanillic acid), coumarin (scopoletin) and triterpenoids, like â-sitostenone and stigmasterol (Prachayasittikul *et al*., 2009). Of these, the most abundant principle is Spilanthol, an antiseptic alkylamide, (2E, 6Z, 8E)-deca-2,6,8-trienoic acid N-isobutyl amide The analgesic activity of spilanthol has been attributed to an increased

gamma-aminobutyric acid (GABA) release in the temporal cerebral cortex. Spilanthol is bitter, strong pungent taste and produce local anaesthetic effects.

The flower head and root part of the plant have been reported to be the rich source of active principles. Antioxidant, Butylatedhydroxytoluene (BHT) and fatty acids (n-Hexadecanoic acid and tetradecanoic acid) have been obtained from extracts of flower heads. The leaves contain alkaloids, carbohydrates, pungent amides, tannins, steroids, carotenoids, essential oil, amino acids etc. (Savadi *et al.,* 2010). Besides the alkamides, pungent nonvolatile sesquiterpenoids have been found, such as polygodial and eudesmanolide II. Essential oils were isolated from the flowers of *S. acmella*, which contain limonenes, â-caryophyllene, Z-â-ocimene, ã-cadinen, thymol, germacrene D, Triterpenoids and myrecene (Dubey *et al.,* 2013).

The various chemical constituents of *Spilanthes acmella* and their structures are summarised in Table 1.

Table 1: Chemical structures of important secondary metabolites of *Spilanthes acmella*.

Names	Structures
Spilanthol	
deca-2E,7Z,9E-trienoic acid isobutylamide	
β-Sitosterol	
Vanillic acid	
α-Amyrin	
α-Amyrin	
Scopoletin	
Limonene	

TRADITIONAL USES

S.acmella is a well-known anti-toothache plant and is used in traditional medicine for many purposes. The different plant parts of *S.acmella* like flowers heads, leaves, roots, stem and other aerial parts have been used in various health care systems (Prachayasittikul *et al.,* 2013). The important traditional uses and applications of different parts of *Spilanthes acmella* in different healthcare systems are provided in the Table 2.

Table 2: Traditional uses and applications of different parts of *Spilanthes acmella* plant

Health Care	Treatment	Plant part Used
Medical	Rheumatism, fever Diuretics Flu, cough, rabies diseases, Tuberculosis, antimalarials, Antibacterial	Leaves, flowers
	Antifungals, skin diseases Immunomodulatory Antiscorbutic Local anesthetics Digestive , Obesity control (lipase inhibitor)	Leaves
	Snakebite	Whole plant
Dental	Toothache	Leaves, Flower
	Toothpaste	Leaves
	Pridontal disease	Flower heads, Roots
Beauty care cosmetics	Fast acting muscle relaxantAntiwrinkle	Whole plant

In the tropics and subtropics, this plant is widely used in traditional medicine. The major use in medicine is for toothache where the fresh flower head and leaves are chewed or placed in tooth cavities to relieve pain. Traditionally, *Spilanthes* plants are used to treat stammering in children, fungal skin diseases and remedy for snakebite.

In India, juice of inflorescence of *S. acmella* is used to treat mouth ulcers (Pushpangadan and Atal, 1986). Ethiopian traditional healers use the crushed aerial parts in a paste dressing for external injuries (Teklehaymanot *et al*., 2007). In Nigeria and Sri Lanka, *S. acmella* is used as a sialagogue (Jayaweer *et al*., 1981).

PHARMACOLOGICAL APPLICATIONS

Different parts of *Spilanthes acmella* plant shows various pharmacological activities. *S.acmella* leaves and flowers extract exhibits antimalarial, antiseptic, anti-bacterial properties. The flower heads of *S.acmella* can be chewed to relieve toothache and also as an analgesic (Leng *et al.,* 2011). Ayurvedic system of medicine, flower heads and roots are used in treatment of scabies, psoriasis, scurvy, infections of gums. The leaves are used as immune-modulatory, anti-scorbitic, ailagogine and digestive. Spilanthol, the most active antiseptic alkaloid extracted from this plant, is found effective against blood parasites (Yadav and Singh, 2010). The other bioactive compounds, scopoletin (coumarin) and ferulic acid (phenolics) found in this plant are reported to be of immense pharmacological interests (Prachayasittikul *et al*., 2009). Scopoletin is a phytoalexin, its production mainly seen upon pathogenic infection. It is considered as an important defence agent against bacteria and fungi (Smith, 1996). It has attracted the most attention because of its use in cardiovascular disease, antitumor and anti-thyroid treatment. In addition to this, scopoletin also possesses antioxidant, antimicrobial, anti-inflammatory, antipyretic and hepatoprotective properties. Ferulic acid is most highly regarded for its antioxidant property. Additionally, it exhibited a wide range of therapeutic effects against cancer, diabetes, cardiovascular and neurodegenerative diseases (Singh and Chaturvedi, 2015). The important pharmacological actions of *S.acmella* have been summarized in Table 3 and are listed below.

Table 3: Summary of pharmacological actions of *Spilanthes acmella.*

SL. no.	Pharmacological activity	Parts of plant used	Experimental models	Animals used
1	Local anaesthetic	Whole plant	Intracutaneous wheal in guinea pigs and plexus anaesthesia in frog	Guinea pig, frog
2	Antipyretic activity	Whole plant	Yeast induced pyrexia	Albino rats
3	Anti-inflammatory activity	Whole plant, leaves	Carrageenan induced paw oedema	Albino rats
4	Analgesic activity	Whole plant	Tail flick method, acetic acid induced abdominal constriction	Albino rats
5	Diuretic activity	Flowers (cold water extract), whole plant	Induction of dieresis using cold water extract	Albino rats
6	Vasorelaxant activity	Flowers	Partially endothelium induced nitric oxide and PGI_2	Albino rats
7	Antioxidant activity	Leaves & whole plant	DPPH Assay, TBARs and SOD method	*Invitro,* no animal
8	Antimalarial & larvicidal activity	Spilanthol extracted from whole plant	—	Eggs & pupae of vector
9	Aphrodisiac activity	Whole plant	—	Male rats
10	Antinociceptive activity	Whole plant	Acetic acid induced writhing	Mice
11	Immunomodulatory activity	Whole plant	—	Rats
12	Bioinsecticidal	Whole plant, leaves	—	—
13	Convulsant	Whole plant	Electroencephalogram (EEG) analysis	Albino rat

ANAESTHETIC ACTIVITY

The local anaesthetic activity of *Spilanthes acmella* has been carried out using two different animal models: (i) intracutaneous application in guinea pigs using nupercaine as standard (suitable for determining degree of anaesthesia) and (ii) plexus anaesthesia in frog using cocaine as standard (used for determining onset of anaesthesia) (Chakraborthy *et al.,* 2002). The mean onset of local anaesthetic action was very potent which could be attributed to the presence of alkylamides.

ANTIPYRETIC EFFECTS

Chakraborty *et al.* (2010) studied the antipyretic activity of *Spilanthes acmella* which was carried out by yeast induced method as yeast is commonly used for the induction of pyrexia. The antipyretic activity of *Spilanthes acmella* demonstrated in the study is attributed to the presence of flavonoids which are predominant inhibitors of either cyclo-oxygenase or lipo-oxygenase.

ANTI-INFLAMMATORY AND ANALGESIC ACTIVITY

The antiinflammatory activity of *Spilanthes acmella* has been carried out by the researchers using carrageenan induced hind paw oedema (Chakraborty *et al*., 2010). The extract was found to produce considerable dose-dependent inhibition of paw oedema which was less than the standard drug. They also demonstrated the analgesic activity of *Spilanthes acmella* using acetic acid induced abdominal constriction and tail flick method. The aqueous extract produced better results as compared to tail flick method which meant that the plant can be explored as peripherally acting

analgesic. The activity was attributed to the presence of flavonoids which are potent inhibitors of prostaglandins at later stages of acute inflammation.

ANTIBACTERIAL ACTIVITY

The different fractions were isolated from crude ethyl acetate extract of *S. acmella* and were studied against 27 strains of microorganisms (Prachayasittikul *et al*., 2009). The results showed that fraction E3 completely inhibited the growth of *Corynebacterium diphtheriae* with MIC value of 128 µg/mL. The antibacterial activity is also reported from the flower head extract of *S.acmella* (Sabitha and Murty, 2005).

ANTIFUNGAL ACTIVITY

The effect of different concentrations of *Spilanthes acmella* flower head extract against four different fungi: *Aspergillus niger, Aspergillus parasiticus, Fusarium oxysporum, and Fusarium moniliformi* was evaluated by Sabitha and Murty (2006). All the concentrations of the test solution inhibited the fungal species with varying degree of sensitivity. The maximum zone of inhibition was found at highest concentration (2 mg/l) and increased proportionally with the dose. Among the test organisms, high inhibition zones were observed in *F. oxysporium and F. moniliformis* followed by *A. niger and A. parasiticus.*

DIURETIC EFFECT

The diuretic potential of *Spilanthes acmella* whole plant as well as fresh flowers, extracted using cold water extract method showed strong diuretic activity when given orally in a single dose (Ratnasoorya *et al.,* 2004). The dieresis induced by the *Spilanthes acmella* flowers was found to be strong with intensity similar to that of furosemide and accompanied by marked increases in both urinary Na+ and K+ levels. The onset of the diuretic action of the aqueous extract was extremely rapid, and it also had a fairly long duration of action.

PANCREATIC LIPASE INHIBITION

Ethanolic extracts of the flowers of *Spilanthes acmella* are demonstrated to inhibit pancreatic lipase activity (40% at 2 mg/mL concentration *in vitro*) (Ekanem *et al*., 2007).

VASORELAXANT AND ANTIOXIDANT ACTIVITY

The plant extracts elicited vasorelaxations via partially endothelium induced nitric oxide and prostaglandin-I2 in a dose-dependent manner (Hossain *et al.,* 2012). Significantly, the ethyl acetate extract exhibited immediate vasorelaxation in nanogram levels and is the most potent antioxidant in the diphenylpicryl hydrazine assay. The chloroform extract displays the highest vasorelaxation with the highest antioxidant concentration. Antioxidant potential of leaves of *Spilanthes acmella* was also studied recently by the researchers and they found that the potent antioxidant activity in the crude ethanol extract of the leaves of the plant was attributed to the presence of tannins, flavonoids and phenolic compounds **(**Hajera *et al.,* 2014**).**

ANTIMALARIAL AND LARVICIDAL EFFECTS

Spilanthol is more effective even at low doses against eggs and pupae. In pupae, it seems to work on nervous system as evident by abnormal movement like jerks, spinning and uncoordinated muscular activity. This suggested that the drug disturbed the nerve conduction somewhere. The mortality of pupae in short span of time upon exposure to the drug also indicated that spilanthol greatly disturbs the ongoing processes of histolysis and histogenesis. Many researchers also reported spilanthol as a potent larvicidal agent (Sabitha *et al.,* 2005).

APHRODISIAC ACTION (INTERACTION WITH TESTOSTERONE AND SEXUALITY).

Aphrodisiac effect of the plant extract has been studied in male rats by Sharma *et al*. (2011). They stated that mount latency, intromission latency, ejaculation frequency and postejaculatory interval were increased in a dose-dependent manner after oral administration of extract.

IMMUNOMODULATORY ACTIVITY

Hexane and chloroform extracts of *Spilanthes acmella* were found to suppress nitric oxide production in stimulated macrophages at 80 mcg/mL by 72% and 85%, respectively (Wu *et al.,* 2008). Isolated spilanthol demonstrated dose-

dependent prevention of macrophage activation with 60% and 20% production of nitric oxide at 90 and 360 5ØßM concentrations, respectively. These inhibitory properties were accompanied by less nitric oxide synthetase and cyclooxygenase-2 mRNA and protein content, less cytokine production from macrsophages, and less nF-kB activation in the nucleus.

BIOINSECTICIDE AND CONVULSANT ACTIVITY

Several insecticidal compounds have been reported in *Spilanthes acmella* (Ramsewak *et al.,* 1999). Extract of *S. acmella* plant in rats was reported to induce full convulsions accompanied by typical electrographic seizures in the electroencephalogram (Mondal *et al.,* 1998).

INSECTICIDAL TOXICITY OF SPILANTHOL

Extract of Spilanthol from the flower heads of *Spilanthes acmella* was found to be active against *P. xylostella* (Sharma *et al*., 2012). The extracts from *Spilanthes* were most toxic against different mosquito species (i.e., Anopheles, Culex, and Aedes). The insecticidal property was attributed to spilanthol and alkamides. Besides, non-volatile sesquiterpenoids, saponins were also reported (Krishnaswamy *et al.,* 1975). Ethanol extract of flower heads of *Spilanthes* has shown a potent ovicidal, insecticidal and pupacidal activity at dose of 7.5 ppm concentration against Anopheles, Culex, and *Aedes* mosquito (Saraf and Dixit., 2002). The hexane extract of dried flower buds of *Spilanthes acmella* (3 N-isobutylamides: spilanthol, undeca-2E,7Z,9E-trienoic acid isobutylamide and undeca2E-en-8,10-diynoic acid isobutylamide) was found active against *Aedes aegypti* larvae. Ethanolic extracts of *Spilanthes acmella* (whole plants) were screened against early 4th instar larvae of *Culex quinquefasciatus* (Pitasawat *et al*., 1998). Spilanthol was shown to be toxic against adults of *P. americana*. It is one of the most potent compound when compared with conventional insecticides such as carbaryl, lindane, and bioresmethrin (Sharma *et al*., 2012). The *S. acmella* flower head extract also found to be effective in controlling the *Spodoptera litura,* an polyphagous, serious agriculture pest (Sabitha and Murty, 2009).

MUSCLE RELAXANT

The plant's extract is an active component used in beauty care cosmetics as a fast acting muscle relaxant to accelerate repair of functional wrinkles (Belfer, 2007). The *S. acmella* extract was also used for stimulating, reorganizing and strengthening the collagen network in anti-age applications (Schubnel, 2007).

ANTICANCER ACTIVITY

Spilanthol has been demonstrated to inhibit nitric oxide (NO) production in a murine macrophage cell line, to efficiently down regulate the production of inflammatory mediators interleukin (IL)-1â, IL-6 and tumor necrosis factor (TNF-á), and to attenuate the expression of cyclooxygenase-2 (COX-2) and inducible NO synthase (iNOS) (Wu *et al.,* 2008). Other investigations have also confirmed the down regulation of some pro-inflammatory cytokines by bioactive alkylamides under various experimental conditions (Cech *et al*., 2006). These findings suggest that spilanthol can be a useful inhibitor of inflammatory mediators and is a potential new lead compound for COX-2 selective non-steroidal anti-inflammatory drugs (NSAIDs).

CULTIVATION

Spilanthes acmella can be grown as an annual in most climates. It is frost-sensitive but perennial in warmer climates. Commercial *Spilanthes* plantations have been established to address the need for sustainable supplies of standardized, high quality raw materials. *S. acmella* grows well in full sun to partial shade reaching a height of 12 to 15 inches with a spread of 24 to 30 inches. It prefers rich, moist, well-drained soil with a pH of 6.1 to 6.5. It is easily established started from seeds directly sown in the garden or indoors pots. Seed should be sown in flats. *Spilanthes* can also propagate through stem cuttings. It needs regular watering and thrives well in high humidity in well-drained soils.

The optimal temperature for germinating the seeds is 20-24°C (68-75°F). It is important to sow the seeds by burrowing them to about only 1/4 inch

***Spilanthes acmella* Plant**

deep as they require light to stimulate germination. Germination takes approximately 1-2 weeks. For the best germination results, it is recommended to grow indoors in sterilized potting soil. A black earth and peat moss mixture works well. Always keep soil moist but never soggy. Once the seedlings have at least 2 sets of leaves they can be transplanted when the danger of frost has passed.

For utilizing the leaves and flowers of *Spilanthes acmella*, the whole plant can be harvested by cutting the plant to about 6". It will grow back and can be harvested again during the season. For harvesting the roots of the plant, the entire plant is plucked out and the roots are cut and separated from the plant. The harvested plant parts can be shade dried and stored in a dry place to avoid moisture and contamination.

PROPAGATION

Spilanthes acmella seeds have low rates of germination and moreover, propagation by seeds is also limited because of the highly heterozygous nature of the plant due to protandry, which prevents self-pollination (Reddy *et al.,* 2004). Field gene banks offer easy access to conserved material but they have risk of destruction by natural calamities, pests and diseases. Hence, *in vitro* conservation through plant tissue culture is the safest and efficient alternative for medicinal plant conservation. Tissue culture offers an opportunity to utilize plant cell, tissue or organ by growing them *in vitro* to get large number of plants and desired medicinal metabolites. *In S. acmella,* there are few reports on successful micropropagation through various explants.

MICROPROAGATION THROUGH HYPOCOTYL EXPLANTS

Saritha *et al.* (2002) were the first to report the successful tissue culture of *Spilanthes*. They reported multiple shoot proliferation from hypocotyl explants of 1-week-old seedlings on MS medium supplemented with BAP (2.2 mM) and NAA (0.54 mM). About 95% of the *in vitro* developed shoots rooted on half strength (½) MS medium containing IBA (4.9 mM).

MICROPROPAGATION THROUGH AXILLARY SHOOT PROLIFERATION

Haw and Keng (2003) attempted *in vitro* clonal propagation of *Spilanthes* by axillary shoot proliferation. The aseptic axillary buds formed multiple shoots within five weeks when cultured on MS medium supplemented with BAP (8.8 mM) and NAA (0.54 mM). However, the study lack crucial information on percent culture response, the rate of proliferation in recurrent cycles of shoot multiplication, frequency of rooting and transplantation was not attempted. *S. acmella* was *in vitro* multiplied using axillary buds as explants on MS medium supplemented with various

concentrations of BAP and NAA (Nelofar *et al*., 2015). In a similar study, shoot induction was observed from axillary and apical meristems as explants on MS medium supplemented with various auxins and cytokinins individually and in various combinations (Hajera and Sabitha Rani, 2017).

MICROPROPAGATION THROUGH NODAL SEGMENTS

Multiple shoots were induced from nodal explants on media supplemented with BAP and IAA (Yadav and Singh 2010). A report (Singh and Chaturvedi, 2010) on systematic clonal propagation by nodal segment culture is published whereby, detailed description on *in vitro* shoot multiplication, rooting and hardening are described. In this study, nodal explants of *S. acmella* bearing two opposite axillary buds were cultured on MS basal medium, supplemented with BAP and high rate of shoot multiplication was observed. A single shoot with long internodes was developed from axillary buds in 100% cultures when NAA (1.0 or 5.0 mM) was added to BAP containing medium.

Singh *et al*. (2009) established *in vitro* propagation system of *Spilanthes* using nodal segment transverse thin cell layer (tTCL) culture system. MS medium fortified with BAP (5.0 mM) was optimal for shoot regeneration from tTCL. On this medium, the explants inoculated in the upright orientation exhibited a high frequency (97%) of shoot regeneration from the edge of the explants, and the highest number of shoots (an average of 31.5) per explant.

DIRECT REGENERATION THROUGH LEAF EXPLANTS

Saritha and Naidu (2008) reported shoot regeneration from leaf explants. Maximum number of shoots per explants was recorded on MS medium containing BAP (13.2 mM) and IAA (5.7 mM). An anatomical study confirmed that shoot regeneration was via direct organogenesis. Micropropagation of *Spilanthes* by leaf-disc culture was also reported by Pandey and Agrarwal (2009). They obtained green and compact callus on MS medium supplemented with BAP (10.0 mM) and NAA (1.0 mM) in 15 days. Shoots were rooted on ½ MS + IBA (0.1 mM) within 2 weeks. The plantlets were successfully hardened and established in soil where they flowered and set viable seeds. Direct shoot regeneration and callus production was also observed from the leaf explants, supplemented with different concentrations of IAA (Tanwer *et al*., 2010). Recently, Singh and Chaturvedi (2012a) reported morphogenesis from leaf disc cultures. They cultured leaf-disc explants of 5 mm size on a range of media. At its optimal concentration of 5.0 mM, BAP showed highest percentage (100%) of shoot organogenesis with an average of 3.5 adventitious shoots, directly from the explants, without an intervening callus phase. In comparison to BAP alone and BAP + NAA, addition of IAA to MS + BAP medium enhanced the number of shoot-buds per explant significantly.

CROP IMPROVEMENT INCLUDING BIOTECHNOLOGY

In a previous study, an effective method for rapid and large scale multiplication of the plant was developed through tissue culture with a protocol for effective organic farming to boost the vigour and other quantitative traits of the plant.

HAIRY ROOT INDUCTION IN *SPILANTHES ACMELLA*

Hairy root cultures offer a promise for high production of valuable secondary metabolites used as pharmaceuticals, pigments and flavors. Genetically transformed hairy roots obtained by infection of plants with *Agrobacterium rhizogenes* are suitable source for production of bioactive molecules due to their genetic stability and fast growth in culture media devoid of growth hormones (Shanks and Morgan, 1999). Integration of plasmid into host plant genome is stable which accounts for genetic stability of transformed root cultures.

Research on hairy root production in the genus *Spilanthes* is still in its infancy. There is one report on the production of hairy roots of *Spilanthes paniculata* by infecting the cotyledons and hypocotyl segments with *A. rhizogenes* strains MTCC 2364 and MTCC 532 (Sheela *et al.,* 2008). In case of *A. rhizogenes* MTCC 532, the best frequency explant infection percent for hypocotyl and cotyledon explants were 75% and 76%. The values for *A. rhizogenes* MTCC 2364 were 78% and 76%.

A significant observation has been made in a recent study (Hajera Sana, 2018) in which hairy roots were induced from nodal segments and leaves of *S.acmella* by transfecting with *A.rhizogenes* MTCC 532. The hairy roots were multiplied and their spilanthol content was quantified using HPLC. This study reported the presence of high amount spilanthol in hairy roots (0.134%) compared to the other types of roots i.e *in vitro* produced roots (0.066%) and roots from field grown plants (0.056%). Hence hairy root induction can be employed as an alternative and sustainable source for spilanthol production, which holds immense potential for pharmaceutical applications.

UTILIZATION

Spilanthes acmella plant parts are predominantly used as extracts in personal care products, traditional medicines, pharmaceutical and culinary areas. There is a significant advances in all aspects of *Spilanthes* research and an increasing number of commercial *Spilanthes* products have appeared in the market place as personal care products, health care products and for culinary use. Most people find the spilanthol-induced tingling of the tongue unpleasant, but when cooked, the plants lose their strong flavor and may be used as a green leafy vegetable. For culinary purposes, a small amount of shredded fresh leaves adds unique flavors to salads. In addition, both fresh and cooked leaves are used in dishes such as stews and soups.

For medicinal use, worldwide the flower heads are used either fresh or dried and powdered, but the use of roots and leaves has been recommended as well. Infusions and decoctions are prepared from the aerial parts or roots and administered either orally or topically as compresses or baths.

PLANT EXTRACTION PROCEDURE

To extract the active principles and useful chemical compounds from *S.acmella* plant parts, the common techniques employed are basically maceration, infusion, percolation, digestion, decoction. The aqueous-alcoholic extraction are done by fermentation, counter current extraction, microwave assisted extraction, phytonic extraction (with hydrofluorocarbon solvents), ultrasound extraction (sonication), hot continuous extraction (Soxhlet), supercritical fluid extraction etc. The spilanthol can be extracted from *S. acmella* using simple maceration, supercritical fluid extraction, solid phase extraction and microwave assisted methods (Dias *et al*., 2012; Costa *et al*., 2014). In most of the studies hexane, ethanol, methanol and hydroethanolic solvents were used for spilanthol extraction.

ANALYTICAL TECHNIQUES FOR QUANTITATIVE AND QUALITATIVE ESTIMATION OF SPILANTHOL AND OTHER CHEMICAL COMPONENTS FROM *S.ACMELLA*

For spilanthol detection and quantification, so far, various analytical techniques such as High-Performance Liquid Chromatography (HPLC), Nuclear Magnetic Resonance (NMR), Gas Chromatography- Mass spectrometry (GC-MS) and Liquid Chromatography-Mass spectrometry were used. The NMR (Nuclear Magnetic Resonance) and High pressure liquid chromatography-Mass spectrometry (HPLCMS) were employed to determine structure of spilanthol in extracts of *S.acmella* (Nakatani and Nagashima (1992). Bae *et al*. (2010) used high pressure liquid chromatography-electrospray ionization-mass spectrometry (HPLC-ESI-MS) for rapid identification and quantification of spilanthol from *S. acmella*. Mbeunkui *et al.,* (2011) identified *Spilanthes* alkylamide by electrospray ionization-ion trap-time of flight mass spectrometry (ESI-IT-TOFMS) and validated by 1H-and13C-NMR analysis. Leng *et al*., (2011) employed GC-MS to detect spilanthol present in mother plant, flower heads and *in vitro* plantlets of *S. acmella.* Recently, Singh and Chaturvedi, (2012b) used HPLC and then MS I for identification and quantification of spilanthol present in *in vivo* and *in vitro* plants. Centrifugal partition chromatography (CPC) is another technique used for quantitative isolation of N-alkylamides from *S. acmella* methanolic flower extract.

IN VITRO PRODUCTION OF SECONDARY METABOLITES FROM *S.ACMELLA*

In *Spilanthe*s, so far, only two reports are available on *in vitro* metabolite production. First of all, Singh and Chaturvedi (2010) reported scopoletin accumulation in *in vitro* nodal segment

derived plant. They have developed a novel HPLC method with fluorescence detector for the quantitative estimation of scopoletin in *S.acmella*. The results of this study showed that the scopoletin content of the nodal segment derived plants was 0.104 mg/g DW of leaves which was comparable to that of the mother plant (0.101 mg/g DW of leaves). Scopoletin biosynthesis was induced in several plant species upon infection by different pathogens (Matros and Mock, 2004) and played an important role in defence mechanism against bacteria and fungi (Smith, 1996). However, no quantification studies were performed in either of these reports. This is the first report on detection and quantification of scopoletin in *S. acmella.* The study revealed that even the uninfected leaves of *Spilanthes* could accumulate the scopoletin. The same authors, after two year, observed spilanthol production from leaf of leaf disc derived plants. Interestingly, they noticed significantly higher spilanthol production (3294.3 mg/g DW) from leaf disc derived plants than from field grown plants. In the same study, callus cultures established from leaf disc accumulated low amount of spilanthol (998.03mg/g DW) (Singh and Chaturvedi 2012).The study confirms the earlier reports which suggested that differentiated (organized and dedifferentiated) cells and specialized organs generally produce most secondary products compared to dedifferentiated (unorganized) cells in cultures.

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