ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Spilanthes acmella an endangered medicinal plant - its Traditional, Phytochemical and Therapeutic properties – An overview

Nelofar Gulam Nabi[3], Tareq A. Wani[2,] Mukta Shrivastava[3], Abid wani[4] and Syed Naseer Shah[1]

1. Plant Tissue Culture Division, Indian Institute of Integrative Medicine (CSIR), Canal Road Jammu 180001, Jammu India.
2. Genetic Resources and Agrotechnology Division, Indian Institute of Integrative Medicine (CSIR), Canal Road Jammu 180001, Jammu India.
3. Govt. M.L.B P.G. College Bhopal (M.P) India.
4. Govt.S.S.L.Jain college vidishya (M.P) India.

Manuscript Info

Manuscript History:

Received: 25 November 2015
Final Accepted: 22 December 2015
Published Online: January 2016

Key words:

Spilanthes acmella, metabolites, bioactivities,conservation structure-activity, tissue culture.

Corresponding Author

Nelofar Gulam Nabi

nelofarnabi2@gmail.com

Abstract

Spilanthes acmella, belonging to family Asteraceae, is a well known genus comprising of over 60 species that are widely distributed in tropical and subtropical regions of the world. With high medicinal usage, it has been recognized an important medicinal plant with an increasing high demand worldwide. It is commonly known as antitoothache plant. From its traditional uses in health care and food, extensive phytochemical studies have been reported. Different types of bioactive compounds have been isolated from the plant from time to time. It has been reported very effective in various ailments including, anti-pyretic, anti-diuretic, anti-inflammatory, anti-oxidant, immunomodulatory, hepatoprotective, anti-cancer, antiAIDS and anti-toothache. The aim of the present review was to highlight the ethanobotanical knowledge of the plant used for ayurvedic preparations in relation to its use as therapeutic agent. Present reviewe also describes the endangered status of the plant and its possible conservation strategies through invitro regeneration practices. The data and information of this review can be utilized in drawing strategies for rational and more scientific use of medicinal plants in general and *Spilanthes acmella* in a particular way that can be extended for future scientific investigation in different aspects. The present review therefore aims to compile up to date and comprehensive information of *Spilanthes acmella* on its traditional, phytochemical, theraptic uses and its large-scale production.

INTRODUCTION

The World Health Organization (WHO) has estimated that about 80 % of the population in developing countries are unable to afford drugs and rely on traditional medicines especially those that are plant-based (Elumalai *et al.*, 2012).The countries include India (Jain *et a*l.., 2006; Little, 2004), Sri Lanka (Ediriweera 2007), Bangladesh (Rahmatullah *et al*, 2010), China and Japan (Little, 2004) and Thailand (Phongpaichit *et al.*, 2005; Sawangjaroen *et al*, 2006). The practice of botanical healing slowly disappeared from western countries with the introduction and advent of science and technology (Tyagi and Delanty, 2003). However, the uses of traditional medicine dramatically increased in Europe and North America in the last 50 years (Tyagi and Delanty, 2003). Herbal medicines have been utilized for many purposes, particularly in medical care as antiasthmatics (86.79 %), antirheumatics (62 %) (Jain *et*

al.., 2006), diuretics (60.22 %) (Kumar *et al*. 2010; Vanamala *et al*, 2012), antiinflammation (29.62 %), anticancer (9.75 %), antidiabetics (8.33 %), antimicrobials, antifungals, antioxidants, antiallergy, analgesics, antiobesity and antihypertention. Recently, health foods, herbs as well as dietary supplements enriched with medicinal ingredients such as antioxidants and bioactive metabolites have drawn considerable attention worldwide, especially herbs that are used as food and traditional medicine (Tyagi and Delanty, 2003). Our concern centers on medicinal plants bearing bioactive compounds, which are employed as therapeutics and health care (Abascal and Yarnell, 2010). *Spilanthes acmella* commonly known as "Akarkara", belonging to family Asteraceae, holds an important place in Indian and global scenario owing to its medicinal properties. In dental care it has been employed as anticariogenic (Ferrazzano *et al.*, 2011), analgesic (Abascal and Yarnell, 2010; Kroll, 1995), local anesthetic (Abascal and Yarnell, 2010), wound healing agents (Abascal and Yarnell, 2001, 2010; Jagan Rao *et al*.., 2012), antiinflammation (Abascal and Yarnell 2001, 2010) and recurrent aphthous stomatitis treatment (Abascal and Yarnell 2010). It has also been used for beauty care (Artaria *et al.*, 2011; Demarne and Passaro, (2009) and as health food along with curcumin (*Curcuma longa* Linn.) (Kohli *et al*.., 2005), ginger (*Zingiber officinale*) (Kubra and Rao, 2011), lemon grass (*Cymbopogon citrates* Stapf) (Nanasombat and Teckchuen, 2009), green shallot (*Allium cepa* var. aggregatum) (Rabinowitch and Kamenetsky, 2002), garlic (*Allium sativum* L.) (Borek, 2010), holy basil (*Ocimum sanctum* Linn.) (Singh *et al*.., 1996), sweet basil (*Ocimum basilicum* L.) (Lee *et al*.., 2005), hairy basil (*Ocimum basilicum* L.f. var. citratum Back.) (Chanwitheesuk *et al.*, 2005) and kitchenmint (*Mentha cordifolia* Opiz.) (Özbek and Dadali, 2007). It stimulates wound healing, protects the individual from cold and flu (Anonymous, 1989). Several preparations of *S. acmella* like Declatone neck antiwrinkle cream, Sinus support formula "intensify" and "Spilanthes supreme" – an antiviral formula are commercially available in market. Vajikaran drugs are specially recommended to people suffering from sexual insufficiency and people in advanced age losing interest in sexual act or failing in sexual performance (Sharma *et al.*, 2010). Besides having many specific drugs for enhancing sexual functions, the most commonly used is akarkara. Different plants are being referred to as akarkara, but the most prominent one is *Spilanthes acmella*. The akarkara plants are empirically used as powerfull aphrodisiac in traditional medicine practice in cases of sexual dis ability or depressed desire. *Spilanthes acmella* has long been used in the traditional Indian systems of medicine for the treatment of various sexual inadequacies and is claimed to improve sexual functions in man.

S. acmella has been reported as a highly endangered plant species (Sharma & Shahzad 2013). Conventional propagation methods of *S. acmella* are not ample for its mass propagation. The major constrains for its large multiplication are its poor vegetative propagation and low rate of seed germination (Rios-Chavez *et al*. 2003). Despite the multiple uses of *S. acmella*, no appreciable biotechnological advances have been made for this species to exploit or enhance its utility. No conclusive tissue culture reports exist till date, and all the biochemical studies carried out so far have been reported on plants growing in wild. As we are aware that major limitation to the commercial use of potential metabolites is their very scarce supply from field grown plants due to their seasonal growth, genetic, geographical, climatic variations, and insect and pathogen attack. Establishment of in vitro cultures helps to nullify the effect of seasonal variation and favours the facilitated yield and consistent production of active compounds, irrespective of seasons and regions. In this context, micropropagation is an important and beneficial tissue culture technique that can be effectively used for mass propagation of genetically uniform plants as well as for its conservation. The cell culture technique is complimentary and may provide competitive metabolite production systems when compared to whole plant extraction. Although a few reports are available on tissue cultures (Saritha *et al.*, 2002; Haw and Keng, 2003; Saritha and Naidu, 2008; Pandey and Agrawal, 2009; Singh *et al*., 2009a, 2009b), conditions for micropropagation were not optimized in either of these reports. Moreover, none of the reports have verified the ploidy stability of *in vitro* derived plantlets, which is the most important aspect for commercial application of any micropropagation protocol.

In this direction present effort was undertaken to account *Spilanthes acmella* as a plant of great interest owing to its known reputation as an antitoothache plant and other incredible medicinal usages. This review focuses on the general background, therapeutic uses, bioactive compounds and large-scale production of this plant.

Plant details

Distribution

Spilanthes acmella is widely distributed in the tropical and sub-tropical regions of the world including America, North Australia, Africa, Malaya, Borneo, India and Sri Lanka. In India, it is confined to South and central part of India (Yadav *et al.,* 2010)

Plant description

Spilanthes acmella belonging to family Asteraceae is a genus comprising of over 60 species that are widely distributed in tropical and subtropical regions of the world, such as Africa, America, Borneo, India, Sri Lanka and Asia (Sahuet *et al*. 2011; Tiwari *et al*., 2011). *S. acmella* is native to Brazil and is cultivated throughout the year as ornamental or medicinal plant. It is an annual or short-lived herb that is 40-60 centimeters tall. It is grown in damp areas (Tiwari *et al*.., 2011; Wongsawatkul *et al*.., 2008) and has low rate of seed germination and pitiable vegetative propagation (Tiwari *et al*.., 2011). Its flowers and leaves have pungent taste and when touched it is accompanied by tingling sensation and numbness (Wongsawatkul *et al*.., 2008) 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. The plant species has been commonly used as a folk remedy since time immemorial for various ailements like toothache, rheumatism and fever (Wongsawatkul *et al*.., 2008), as fresh vegetable (Tiwari *et al*.., 2011) as well as spice for Japanese appetizer (Leng *et al.,* 2011). *Spilanthes acmellais* is synonym with *Spilanthes oleracea*. *Spilanthes acmella* is a very beautiful, erect or ascending stout herb, and can be grown as an annual plant in most climates of the world. Being frost-sensitive it acts as a perennial in warmer climates. A small, erect herb grows swiftly and flourishs with gold and red flowal inflorescences. It can be grown in the ground or as a potted herb. A rich soil with compost is suitable and maintains a temperature of about 70 °F. Stems are glandular hairy with pungent taste. The whole plant is acrid in taste. It has striking cone-like flowers.. Leaves are opposite, petiolate, broadly ovate, narrowed at base, acute or obtuse at apex, flowering and fruiting in March-April, (Savadi *et al,*.2010). *Spilanthes acmella* L. is commonly known as Toothache plant and its various synonyms are *Bidens acmella*, *Bidenso cymifolia*, *Pyrethrum acmella*, *Spilanthes ocymifolia*, *Verbesina acmella*, and *Blainvillea acmella*.

Fig.1 Figure depicting the plant in flowering stage, a single flower, a plant in its natural habitat, crude powder form and seeds of the plant *Spilanthes acmella.*

Traditional uses

The whole plant parts (e.g. flowers, leaves, roots, stems and aerial parts) have been used in health care (Leng *et al*, 2004; Ospina De Nigrinis *et al.,* 1986; Purabi and Kalita, 2005; Research, 1976; Rios-Chavez *et al.,* 2003; Senthilkumar *et al.,* 2007; Tiwari and Kakkar, 1990) and food (Barman *et al.,* 2009; Boonen *et al..,* 2010; Wu *et al.,* 2008). Particularly, *S. acmella* or *S. oleracea* (paracress or eyeball plant), is a well-known antitoothache plant (Sahu *et al.,* 2011) and has been used as traditional medicine for many purposes. So far, various Thai medicinal plants have been used for the remedy of toothache as well as in other dental applications.

The important medicinal properties of the plant *S. acmella* are given in tabulated form as given.

Healthcare	Treatment	Plant extract	References
Medical			
	Rheumatism, fever Diuretics Flu, cough, rabies diseases, Tuberculosis, antimalarials, Antibacterials	leaves, flowers	Bunyapraphatsara and Chokechareunporn, 1999; Farnsworth and Bunyapraphatsara, 1992 Yadav and Singh, 2010 Haw and Keng, 2003
	Antifungal, skin diseases	leaves	Tiwari *et al*., 2011 Sahu *et al*., 2011
	Immunomodulatory		Leng *et al*., 2011; Sahu *et al*., 2011
	Antiscorbutic Local anesthetics Digestive		Tiwari *et al.*, 2011 Leng *et al.*, 2011; Sahu *et al*., 2011
	Obesity control (lipase inhibitor)	flowers	Yuliana *et al*., 2011
	Snake bite	whole plant	Tiwari *et al*., 2011
Dental	Toothache	leaves, flower	Haw and Keng, 2003; Tiwari *et al.*, 2011
	Toothpaste	leaves	Savadi *et al*., 2010
	Periodontal disease	flower heads, roots	Abascal and Yarnell, 2001; Sahu *et al*., 2011; Shimada and Gomi, 1995
	Recurrent aphthous stomatitis	leaves	Abascal and Yarnell, 2010
Beauty care cosmetics	Fast acting muscle relaxant Anti wrinkle	whole plant	Belfer, 2007 Demarne and Passaro, 2009; Schubnel, 2007

Table 1. Some of the important medicinal properties of *Spilanthes acmella*.

Sensory quality

Spilanthes acmella has no particular odour, but when eaten it has an interesting flavour that slowly develops from pleasant and salty to a strong, tickling burning pungency that leaves back a numb feeling in the mouth. Biting into a flower head of *Spilanthes acmella* is an adventure long remembered.

It has been well documented for its uses as a spice, antiseptic, anti-bacterial, antifungal, antimalarial and as a remedy for toothache, flu, cough and tuberculosis (Ang Boon Hawand Chan Lai Keng, 2003). Traditionally this plant is used in the treatment of dysentery, rheumatism, as a snake bite remedy, to treat stammering in children and many other diseases The whole aerial parts, flower heads and roots yield a compound known as spilanthol amide which has a saliva inducing effect (Shefali Arora *et al*.., 2011) and is a powerful insecticide and local anesthetic (Kishan Lal Tiwari *et al*.., 2011). In addition to spilanthol, *S.acmella* is an important source of highly valuable bioactive compounds such as phenolics, coumarin (scopoletin) and triterpenoids (Supaluk Prachayasittikul *et al*.., 2009). Considering data from the literature, it could be demonstrated that *S. acmella* possesses diverse bioactive properties and immense utilization in medicine, health care, cosmetics and as health supplements. As a health food, it is enriched with high therapeutic value with high potential for further development.

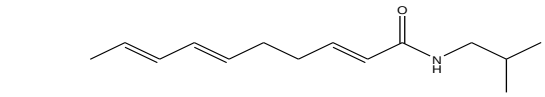
Medicinal uses

An extract of the leaves and flowers is traditionally used for the remedy of toothache because of anesthetic properties, stomatitis, flu, cough, rabies diseases and tuberculosis and throat mplaints. It has also used in remedy of rheumatism and fever. It has strong diuretic activity and the ability to dissolve urinary calculi. It also exhibits antimalarial, antiseptic, anti-bacterial properties. The leaves are used as immunomodulatory, adaptogenic, toothpaste, lithotriptic, antiscorbitic, lagogine and digestive. Spilanthol, the most active antiseptic alkaloid extracted from this plant, is found effective at extremely low concentrations against blood parasites, and indeed is a poison to most invertebrates while remaining harmless to warm-blooded creatures. The flower heads of *Spilanthes acmella* can be chewed to relieve toothache and also as a haemostatic and analgesic. In Ayurvedic system of medicine, flower heads and roots are used in treatment of scabies, psoriasis, scurvy, infection of gums, periodontosis, paralysis of tongue and remedy for stammering in children and in mouthwashes. *Spilanthes acmella* also possessed excellent anti-microbial activities against red halophillic coccifrom salt cured fish. Its extract is an active component used in beauty care cosmetics as a fast acting muscle relaxant to accelerate repair of functional wrinkles. The plant extract was also used for stimulating, reorganizing and strengthening the collagen network in anti-age applications, e.g. in anti wrinkle cream formulations. Spilanthol used as insecticide it shows potent ovicidal, larvicidal and pupicidal activity. An Indian tribe uses *S. acmella* to treat fungal skin conditions, such as athletes' foot, ringworm and nail infections. A dermal health compound, oral health tonic, and fungus fighter compound marketed by HerbPharm, USA, contains organically grown S. acmella Murray and is recommended for skin care, oral health, and antifungal uses**.**

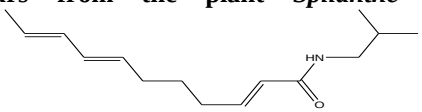
Phytochemistry

Chemical analysis shows that *Spilanthes acmella* contain major pungent compounds, spilanthol (N-isobutyl-2E, 6Z, 8E-decatrienamide) which is naturally occurring insecticide, and Butylated Hydroxytoluene (Leng *et al*.., 2011). Antioxidant, Butylated hydroxytoluene (BHT) and fatty acids (n-Hexadecanoic acid and tetradecanoic acid) could be obtained from extracts of mother plant of flower heads (Leng *et al*.., 2011).The leaves contain alkaloids ,carbohydrates, pungent amide 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. The pungent flavour of *Spilanthes acmella* is due to an unsaturated alkamid, spilanthol which is present in its highest concentration (1%) in the flowers. Essential oils were isolated from the flower of *S. acmella*, whose main constituents were limonene, β-caryophyllene, Z-β-ocimene, γ-cadinen, thymol, germacrene D and myrcene. The crude ethyl acetate of *S. acmella* was purified by chromatographic methods to give acetylaleuritolic acid, vanillic acid and sitostenone.

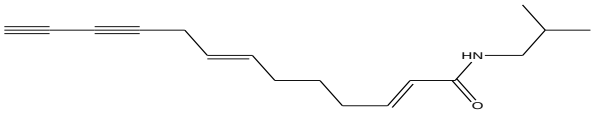
Some isolated compounds and their sitructurs from the plant *Spilanthe acemella* are as



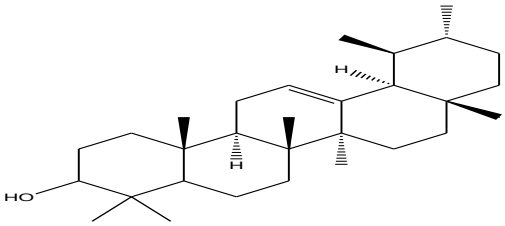
(1) N-Isobutyl-2,6,8-decatrienamide



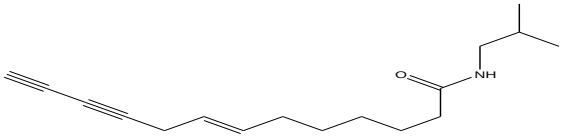
(2) 2,7,9-Undecatrienoic acid isobutylamide



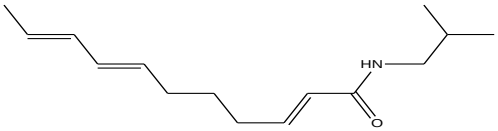
(3) N-(2-Methylpropyl)-2,7-tridecadiene-10,12-diynamide, 9CI



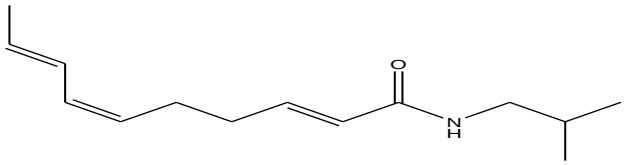
(4) 12-Ursen-3-ol



(5) N-Isobutyl-7-tridecene-10,12-diynamide



(6) 2,7,9-Undecatrienoic acid isobutylamide



(7) (2E,6Z,8E)-N-Isobutyl-2,6,8-decatrienamide

Antibacterial activity

The crude ethyl acetate extract of *S. acmella* showed promising antibacterial activities against 27 strains of microorganisms studied. Amongst the all fractions, the fraction E3 completely inhibited the growth of *Corynebacterium diphtheria* with MIC valueof128µg/mL (Sahu *et al.*, 2011).

Anti-inflammatory and Analgesic

Aqueous extracts of aeriel part of *S. acmella*, in experimental animal models showed dose-dependent inhibition of paw edema and increased pain threshold indicating significant anti-inflammatory and analgesic properties (Chakraborty *et. al*., 2010.) Spilanthol showed signficant antiinflammatory activity on lipopolysaccharide-activated murine macrophage model RAW 264.7, partly from inactivation of NF-KAPPA B which negatively regulates production of pro-inflammatory mediators (Wu *et. al*., 2008). Different doses of aqueous extract of fresh flowers were orally administered to male rats and their analgesic potential was detemined at different post treatment periods by using hot plate and tail flick tests. The analgesic activity was mediated supra-spinally accompanied with sedation (Peiris *et. a*l., 2002).

Larvicidal activity

Spilanthol, a major constituent of ethanolic extract of flower heads of *Spilanthes acmella* is having potent ovicidal, larvicidal and pupicidal activity. Maximum7.5 ppm concentration caused 100% motility of eggs, larvae and pupae of Anopheles, Culex and Aedes mosquito. Spilanthol is more effective even at low doses against eggs and pupae. In pupae, it seems to work on nervous system which was evident by abnormal movement like jerks, spinning and uncoordinated muscular activity suggesting thereby that it disturbs nerve conduction (D.K. Saraf *et. al.*, 2002). Pendse *et al*., (1946) found the ethanolic extract of *Spilanthes acmella* as one tenth active as compared to DDT

against Anopheles larva. The flower tops and aerial parts have been found to be toxic to mosquito larvae and Periplanata.The compound Spilanthol has been identified as having larvicidal activity (Kadir *et. al*., 1989).

Diuretic activity

The cold-water extract of flower of *S. acmella*, showed a marked increase in urine output, also marked increase in urinary Na+ and K+ levels and reduction of urine osmolarity suggesting that it is mainly acting as a loop diuretic activity. It may also inhibit ADH release and/or action. Ethanol extract of leaves of *S. acmella* also significantly increased the urinary output (by 223%) and electrolytic excretion of Na+ (by136%) and K+ (by 172%) (Yadav *et al.,* 2011).

Insecticidal activity

The crude seed extract of methanol, hexane, and deltamethrin of *S. acmella* showed significant insecticidal activity against Plutella xylostella. (Anuradha *et al* 2012)

Hepatoprotective and antioxidant activity

The different extracts were effective hepatoprotactive and antioxidants as demonstrated from flower extracts against paracetamol-induced liver damage. The study was conducted in 36 male Wistar rats of either sex, and six groups were established. While the first group was maintained as normal control (NC, distilled water), Groups 2 to 6 were administered 3 g/kg Paracetamol (PAR) for 2 day, 100 mg/kg Silymarin (SMR), 500 mg/kg Methanolic extract (MESP), Petroleum ether extract (PEESP), Ethyl acetate extract of *S. paniculata*(EAESP) suspended in 0.5% tween 80 plus PAR, respectively. PAR was administered in the same schedule as in group 2, the treatment with silymarin extracts was given for 10 days orally. It was observed that PAR significantly increased serum Alanine transaminase (ALT), Aspartate transaminase (AST), Alkaline phosphatase (ALP) activity liver MDA levels ($P<0.01$) and significantly decreased liver Glutathione (GSH) , catalase (CAT), superoxide dismutase (SOD) activity ($P<0.01$), when compared with the normal control group (NC). On the other hand, statistically significant ($P<0.01$) changes were observed in the biochemical parameters of the group which was administered SMR, PEESP and EAESP. Compared with the pathological changes observed in the liver in the form of congested sinusoids and centrilobular necrosis, in the group which was administered paracetamol alone (PAR), lesions were determined to be less severe particularly in the group (PEESP and EAESP). The study shows that administration of PEESP and EAESP offered a therapeutic potential for the treatment of hepatotoxicity induced by paracetamol via regulation of endogenous antioxidant system in liver (Ayaz *et.al*. 2013).

Vasorelaxant activity

The chloroform and ethylacetate extract of *S. acmella* on phenylephrine exerts maximal vasorelaxation in a dose-dependent manner, although less than acetylcholine-induced nitric oxide (NO) vasorelaxtion. Chloroform extract showed the highest vasorelaxation and antioxidant activity (Wongsawatkul *et.al*. 2008).

Immunomodulatory activity

The ethanol extract of leaves showed significant activation of macrophages and enhanced their function as compared to control, suggesting the herb as a potential natural drug for immune stimulant effect (Sahu *et al.* 2011).

Aphordasic activity

Ethanolic flower head extract of *Spilanthes acmella* shows sexual stimulation in Wistar albino male rats (Sharma *et al* 2011).

Antioxidant activity:

The antioxidant activity of methanol extract of stem and leaves of *S. acmella* were measured using DPPH and superoxide radical scavenging assays (Tanweer *et.al*, 2010).The result showed the methanol extract of stem *of S. acmella* to have the highest superoxide radical scavenging activity while leaves showed maximum DPPH scavenging activity. In superoxide radical scavenging assay, highest radical scavenging activity was observed in stem and callus, while minimum superoxide radical scavenging activity was found in roots (Tanweer *et.al.* 2010). In DPPH radical scavenging activity was found maximum in leaf and minimum in root. Callus showed significant DPPH radical scavenging activity (Tanweer *et.al*. 2010)

Antifungal activity

Different concentrations of *S. acmella* flower head extract (dried flower heads extracted with petroleum ether) were evaluated for antifungal activity (0.1to 2.0 mg). The diameter of inhibition zones ranged from 0.1 to 2.3 cm with the increase in concentration of test solution. In all the organisms, the maximum zone of inhibition was observed at 2000 mg concentration (Rani *et al.,* 2006)

***In vitro* micro propagation**

From the above discussion, it is clear that *S. acmella* is a promising medicinal herb due to its wide ranges of medicinal and pharmocolgical properties. Due to increasingly demanded and diminutive supply world wide as a plant-derived medicine (Tiwari *et al.*, 2011) there is not any established protocol for its mass multiplication. It has been recognized as one of the most important medicinal plants world wide (Singh and Chaturvedi, 2012). However, *S. acmella* has been documented as an endangered plant species due to the low rate of seed germination and poor vegetative propagation (Rios-Chavez *et. al*., 2003), including limited availability of information of the biosynthetic pathway of alkamides (Tiwari *et. al*., 2011). To increase the supply of *S. acmella*, invitro micro propagation has been recently proposed to be a reliable and routine approach for large-scale production (Sahu *et. al.*, 2011; Tiwari *et. al.*, 2011). The method is a useful tool for rapid cultivation of *S. acmella* which provides high yield and consistent production or quality of bioactive metabolites irrespective of seasons and regions (Singh and Chaturvedi, 2012) as well as conservation of genetic fidelity, long term storage and cost effectiveness (Sahu *et. al*., 2011).So far, a number of studies has been reported for successful invitro micro propagation of *S. acmella* through leaf, axillary bud, and shoot tip (Sahuet. *al.*, 2011). The content of spilanthol was found to be higher than the mother plant or those that are field grown (Singh and Chaturvedi, 2012). Importantly, the produced spilanthol (*in vitro*) showed strong (100 %) antilarvicidal activity against malaria and filarial vectors (Pandey and Agrawal, 2009). The methods employed different culture media, mostly using Murashige and Skoog media (MS) in combination with other plant growth regulators.

Micropropagation:

Micropropagation has become a reliable and routine approach for large-scale rapid plant multiplication, which is based on plant cell, tissue and organ culture on well defined tissue culture media under aseptic conditions. A lot of research efforts are being made to develop and refine micro propagation methods and culture media for large-scale plant multiplication of several number of plant species. Micropropagation has superiority over conventional method of propagation because of high multiplication rate and disease free plants. But, field performance of these tissue cultured plants depends on the selection of the initial material, media composition, growth regulators, cultivar and environmental factors. Some well developed in vitro techniques are currently available to help growers to meet the demand of the spices and pharmaceutical industry. For large-scale in vitro plant production the important attributes are the quality, cost effectiveness, maintenance of genetic fidelity, and long-term storage. *S. acemella* has been successfully micropropagated through leaf, axillary bud and shoot tip.

Micropropagation through axillary buds

S. acmella was successfully micropropagated using axillary buds as explants. The aseptic axillary buds formed multiple shoots within 5 weeks when cultured on MS medium supplemented with 2.0, 4.0, 6.0 and 8.0 mg benzyladenine (BA/l). The addition of IBA as low as 2 mg/l into the MS medium containing BA had no significant effect on the multiple shoot formation (Savadi *et. al.*, 2003).

Micropropagation through Nodal segments

Spilanthes acmella was successfully propagated using nodal segments as explants**.** Multiple shoots induced on Murashige and Skoog medium supplemented with various auxins and cytokinins individually and in various combinations. MS medium fortified with 1.0 mg/l BAP was found to be effective individually. The medium with 3.0 mg/l BAP + 1.0 mg/l IAA responded better as compared to other combinations (Kuldeep yadav *et al* 2010).

Micropropagation through leaf

Spilanthes acemella has been successfully cultured under invitro conditions through leaf. Leaf explants showed maximum callus formation on 2, 4-D at the concentration 6.78 µM/liter. Callus was fragile and yellowish green colored. However, IAA showed direct root induction from leaf and nodal explants at all the concentrations used (Tanwer *et. al.*, 2010).

Micropropagation through axillary and apical meristems

Shoot induction on Murashige and Skoog medium supplemented with various auxins and cytokinins individually and in various combinations has been achieved by using axillary and apical meristems as explants.MS medium fortified with 1.0 mg/l BAP and 0.1 mg/l NAA was found to be effective for multiple shoot induction by using axillary buds and apical meristems as explant in *S. acmella* (Nelofar *et. al.,* 2015).

Conclusion

As traditional medicine progresses in leaps and bounds and is gaining popularity worldwide, it is imperative to train the medical and scientific establishments that there are some features which are unique to phytotherapy, and which contribute to both efficacy and safety. Market interest in *Spilanthes acmella* products in the areas of pharmaceutical, cosmetic, and food industries has an excellent future, even as additional studies are required to recognize the impending relevances and properties which may explain their mechanisms of action. In order to determine the real potential of these products and to develop new technologies, greater understanding is needed to produce superior patient acquiescence. Lacking the correct understanding of the species, all of the phytochemical and pharmacological studies will be troublesome. The unique morphological diversity of the species results in a challenging taxonomy, hence accurate botanical identification is important to achieve authentic biological and phytochemical outcomes. More pharmacological validations are required to support some of the traditional claims. The biological activity determinations have only targeted alkamides, mainly spilanthol and related amides in this species. In order to validate the wide range of traditional uses of this species, other metabolites should be investigated. Biological activity which is due the maryiad of compounds prent in the species should be studied, validated, and established. Pharmacological and chemical studies are still necessary for several indications of this species. Its use as an analgesic deserves clinical investigation. Due to its endangard status in nature due to poor seed germination and absence of vegetative reproduction an efficient invitro regeneration protocol should be developed for its conservation and large scale multiplication. Moreover biotechnological interventions for the enhancement of important phytochemical constituents are need of the hour.

Acknowledgeme

NGN is grateful to MPCST and IIIM-CSIR JAMMU, for supporting financially and morally to carry out this work**.** All the authors are grateful to Director IIIM Jammu for providing necessary facilities.

References

Abascal K, Yarnell E. Herbs for treating periodontal disease. Alternat Complementary Ther 2001;7:216-20.

Abascal K, Yarnell E. Treatment for recurrent aphthous stomatitis. Alternat Complement Ther 2010;16:100-6.

Anonymous. The wealth of India: a dictionary of Indian raw materials and industrial products, Vol. 10. Publication and Information Directorate, CSIR, New Delhi, India, 1989; 11–12.

Ang Boon Haw and Chan Lai Keng. 2003.Micropropagation of *Spilanthes acmella* L., a bio-insecticide plant through proliferation of multiple shoots J. Appl. Hort.; 5(2):65-68.

Anuradha Sharma, Vishal Kumar, Rameshwar Singh Rattan, Neeraj Kumar, Bikram Singh , Insecticidal Toxicity of Spilanthol from *Spilanthes acmella* Murr. against Plutella xylostella L. American Journal of Plant Sciences, 2012, 3, 1568-1572

Artaria C, Maramaldi G, Bonfigli A, Rigano L, Appendino G. Lifting properties of the alkamide fraction from the fruit husks of *Zanthoxylum bungeanum*. Int J Cosmetic Sci 2011;33:328-33

Borek C. Garlic and aging: current knowledge and future considerations. In: Watson RR, Preedy RR (eds.): Bioactive foods in promoting health (pp 221-34). San Diego, CA: Academic Press, 2010.

Barman S, Sahu N, Deka S, Dutta S, Das S. Antiinflammatory and analgesic activity of leaves of *Spilanthes acmella* (ELSA) in experimental animal models. Pharmacologyonline 2009;1:1027-
Belfer WA. Cosmetic compositions comprising peptides and *Acmella oleracea* extract to accelerate repair of functional wrinkles US Pat. 2007; 2007048245.

Bunyapraphatsara N, Chokechareunporn O. Tradition medicinal plants. Bangkok: Prachachon, 1999.

Chakraborty A, Devi BRK, Sanjebam R, Khumbong S, Thokchom IS. Priliminary studies on local anesthetic and antipyretic activities of *Spilanthes acmella* Murr. in experimental animals models. Indian Journal of Pharmacology 2010; 42(5): 277-279.

Chakraborty A, Devi BRK, Rita S, Sharatchandra K, Singh TI. Preliminary studies on antiinflammatory and analgesic activities of *Spilanthes acmella* Murr. In experimental animal models. Indian J Pharmacol 2004;36:148-50.

Chanwitheesuk A, Teerawutgulrag A,Rakariyatham N. Screening of antioxidant activity and antioxidant compounds of some edible plants of Thailand. FoodChem 2005;92:491-7.

Dermarne F, PassDaro G.Use of an *Acmella oleracea* extract for the botulinum toxinlike effect thereof in an anti wrinkle cosmetic composition. US patent 2009;US 7,531193 B2.

D.K. Saraf and V.K. Dixit *Spilanthes acmella* Murr. : Study on Its Extract Spilanthol as Larvicidal Compound Asian J. Exp. Sci., Vol. 16, No. 1&2, 2002, 9-19

Ediriweera ERHSS. A review on medicinal uses of weeds in Sri Lanka. Trop Agric Res Ext 2007;10:11-6

Elumalai A, Pendem N, Eswaraiah MC, Naresh V. An updated annual review on antipyretic medicinal plants (Jan-Dec 2011). Int J Univers Pharm Life Sci 2012;2: 207-15.

Ferrazzano G, Amato I, Ingenito A, Zarrelli A, Pinto G, Pollio A. Plant polyphenols and their anticariogenic properties: A review. Molecules 2011;16:1486-507.

Haw AB, Keng CL. Micropropagation of *Spilanthes acmella* L., a bio-insecticide plant, through proliferation of multiple shoots. J. Appl. Hort. 2003; 5(2): 65-68.

Jain JB, Kumane SC, Bhattacharya S. Medicinal flora of Madhya Pradesh and Chattisgarh-A review. Indian J Tradit Knowl 2006;5:237-42.

Kadir H. A., Zakaria M. B., Kechil A.A. and Azirum M. S. (1989): Toxicity and electrophysiological effects of *Spilanthes acmella* (Murr.). Pestic. Sci. **25,** 329-335.

Kishan Lal Tiwari, Shailesh Kumar Jadhav, Veenu Joshi.2011. An updated review on medicinal herb genus *Spilanthes.* J Chin Integr Med.;9(11):1170-1178.

Kumar BNS, Swamy BMV, Swamy A, Murali A. A review on natural diuretics. Res J Pharm Biol Chem Sci 2010;1:615- 34.

Kohli K, Ali J, Ansari MJ, Raheman Z. Curcumin: A natural anti-inflammatory agent. Indian J Pharmacol 2005;37:141-7.

Kubra IR, Rao LJM. An impression on current developments in the technology, chemistry, and biological activities of ginger (Zingiber officinale Roscoe). Crit Rev Food Sci Nutr 2011;52:651-88.

Kuldeep Yadav and Narender Singh Micropropagation of *Spilanthes acmella* Murr. – An Important\ Medicinal Plant [Nature and Science 2010;8(9):5-11]. (ISSN: 1545-0740).

Lee S-J, Umano K, Shibamoto T, Lee KG. Identification of volatile components in basil (*Ocimum basilicum* L.) and thyme leaves (Thymus vulgaris L.) and their antioxidant properties. Food Chem 2005;91:131-7.
Leng TC, Ping NS, Lim BP, Keng CL. Detection of bioactive compounds from *Spilanthes acmella* (L.) plants and its various in vitro culture products. J Med Plant Res 2011;5:371-8.

Little JW. Complementary and alternative medicine: Impact on dentistry. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2004;98:137-45.

Pandey V, Agrawal V. Efficient micropropagation protocol of *Spilanthes acmella* L. possessing strong antimalarial activity. In Vitro Cell Dev Biol Plant 2009;45:491- 9

Pendse G. S., Gokhale V. G., Phalnikar N. L. and Bhide B. V. (1946) : Investigation on new plant larvicides with special references to *Spilanthes acmella*. J. Univ. Bombay Sect. A., 15, Pt. **3,** Sci. 20, 26-30.

Nanasombat S, Teckchuen N. Antimicrobial, antioxidant and anticancer activities of Thai local vegetables. J Med Plants Res 2009;3:443-9.

Nelofar Gulam Nabi and Mukta Shrivastava, *Spilanthes acmella* Murr. An important endangered medicinal plant and its conservation through Tissue culture techniques Indian J. Applied & Pure Bio. Vol. 30(2), 159-163 (2015).

Özbek B, Dadali G. Thin-layer drying characteristics and modelling of mint leaves undergoing microwave treatment. J Food Eng 2007;83:541-9

Peiris KPP, Silva GKJ, Ratnasooriya WD. Analgesic activity of water extract of *Spilanthes acmella* flowers on rats. Journal of Tropical Medicinal Plants 2002; 2(2): 120-126.

Phongpaichit S, Subhadhirasakul S, Wattanapiromsakul C. Antifungal activities of extracts from Thai medicinal plants against opportunistic fungal pathogens associated with AIDS patients. Mycoses 2005;48:333-8.

Rabinowitch HD, Kamenetsky R. Shallot (Allium cepa, Aggregratum group). In: Rabinowitch HD, Currah L (eds.). Allium crop science: recent advances (pp 409-10). New York: CABI Publishing, 2002.

Rahmatullah M, Rahman MA, Hossan MS, Taufiq-Ur-Rahman M, Jahan R, Mollik MAH. A pharmacological and phytochemical evaluation of medicinal plants used by the Harbang clan of the Tripura tribal community of Mirsharai area, Chittagong district, Bangladesh. J Alternat Complement Med 2010;16:769-85.

Rani SA, Murty SU. Antifungal potential of flower head extract of *Spilanthes acmella.* African Journal of Biomedical Research 2006; 9: 67-69.

Rios-Chavez P, Ramirez-Chavez E, Armenta-Salinas C, Molina-Torres J. *Acmella radicans* var. radicans: In vitro culture establishment and alkamide content. In Vitro Cell Dev Biol Plant 2003;39:37-41.

Saritha KV, Naidu CV. High frequency plant regeneration and in vitro flowering of regenerated plantlets of *Spilanthes acmella* Murr – An important threatened bio-insecticide medicinal plant, Acta Horticulturae 756: 183-198

Saritha, K.V., Prakash, E., Rama murthy, N., Naidu, C.V., 2002. Micropropagation of *Spilanthes acmella* Murr. Biol. Plant. 45, 581–584.

Savadi RV, yadav R, yadav N. Study on immunomodulatory activity of ethanolic extract *Spilanthes acmella* Murr. Leaves. Indian journal of natural Products and Resources 2010; 1(2): 204-207**.**

Schubnel L. A different approach to lifting efficacy based on a natural active ingredient. SOFW J 2007;133.

Sharma S & Shahzad A. 2013. Efficient microporpagation of *Spilanthes acmella* (L.) Murr.: a threatened medicinal herb. British Biotechnology Journal 3: 405-415.

Sharma, V., Thakur, M., Chauhan, N.S., Dixit, V.K., 2010. Effect of petroleum ether extract of Anacyclus pyrethrum DC on sexual behaviour in male rats. J. Chin. Integr. Med. 8, 767-773.

Singh M, Chaturvedi R. Evaluation of nutrient uptake and physical parameters on cell biomass growth and production of spilanthol in suspension cultures of *Spilanthes acmella* Murr. Bioprocess Biosyst Eng 2012a;35:943-951.

Singh, S.K., Rai, M.K., Asthana, P., Pandey, S., Jaiswal, V.S., Jaiswal, U., 2009a. Plant regeneration from alginate-encapsulated shoot tips of *Spilanthes acmella* (L.) AMurr., a medicinally important and herbal pesticidal plant species. Acta Physiol. Plant. 31, 649–653.

Singh, S.K., Rai, M.K., Asthana, P., Sahoo, L., 2009b. An improved micropropagation of *Spilanthes acmella* L. through transverse thin cell layer culture. Acta Physiol. Plant. 31, 693–698.

Singh S, Majumdar DK, Rehan HMS. Evaluation of antiinflammatory potential of fixed oil of *Ocimum sanctum* (Holybasil) and its possible mechanism of action. J Ethnopharmacol 1996;54: 19-26

Sahu J, Jain K, Jain B, Sahu RK. A review on phytopharmacology and micro propagation of *Spilanthes acmella*. Pharmacologyonline newslett 2011;2:1105-10.

Shefali Arora, Saurabh Vijay and Deepak Kumar.2011. Phytochemical and antimicrobial studies on the leaves of *Spilanthes acmella*. J. Chem. Pharm. Res.; 3(5):145-150.

Shimada T, Gomi T. Spilanthol-rich essential oils for manufacturing toothpastes or other oral compositions. JP Pat. 1995; JP Pat. 07090294.

Supaluk Prachayasittikul, Saowapa Suphapong, Apilak Worachartcheewan, Ratana Lawung, Somsak Ruchirawat and Virapong Prachayasittikul 2009 Bioactive metabolites from *Spilanthes acmella* Murr.Molecules;4(12);850-867.

Syed Ayaz Ali1, Shukla Mahanand1, Subur W. Kha 2013 Hepatoprotective and antioxidant activity of *Spilanthes paniculata* flower extracts on liver damage induced by paracetamol in rats Vol. 6(42), pp. 2905-2911,

Tanwer BS, Choudhary RK, Vijayvergia R. In vitro and in vivo Comparative Study of Primary Metabolites and Antioxidant Activity in *Spilanthes acmella* Murr. International Journal of Biotechnology and Biochemistry 2010; 6 (5): 819– 825.

Tiwari H, Kakkar A. Phytochemical examination of *Spilanthes acmella* Murr. J Indian Chem Soc 1990;67:784-5.

Tiwari KL, Jadhav SK, Joshi V. An updated review on medicinal herb genus *Spilanthes*. Chin J Integr Med 2011;9: 1170-8.

Tyagi A, Delanty N. Herbal remedies, dietary supplements, and seizures. Epilepsia 2003;44:228-35.

Vikas Sharma, Jente Boonen, Nagendra S. Chauhan, Mayank Thakur, Bart De Spiegeleer, V.K. Dixit *Spilanthes acmella* ethanolic flower extract: LC–MS alkylamide profiling and its effects on sexual behavior in male rats Volume 18, Issue 13, 15 October 2011

Wongsawatkul O, Prachayasittikul S, Isarankura-Na-Ayudhya C, Satayavivad J, Ruchirawat S, Prachayasittikul V. Vasorelaxant and antioxidant activities of *Spilanthes acmella* Murr. Int J Mol Sci 2008;9:2724-44.

Wu LC, Fan NC, Lin MH, Chu IR, Huang SJ, Hu CY et al. Anti-inflammatory effect of spilanthol from *Spilanthes acmella* on murine macrophage by down-regulating LPS-induced inflammatory mediators. J Agric Food Chem 2008; 56:2341-9.

Yadav K, Singh N. Micropropagation of *Spilanthes acmella* Murr. – An Important Medicinal Plant. Nature and Science 2010; 8(9): 5-11.

Yadav R, Kharya MD, Yadav N, Savadi R. Diuretic activity of *Spilanthes acmella* Murr. Leaves extract in rats. IJRPC 2011; 1(1): 57-61.

Yuliana ND, Jahangir M, Korthout H, Choi YH, Kim HK, Verpoorte R. Comprehensive review on herbal medicine for energy intake suppression. Obes Rev 2011;12: 499-514.