A REVIEW ON PHYTOPHARMACOLOGY AND MICROPROPAGATION OF *SPILANTHES ACMELLA*

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Summary

Spilanthes acmella (Compositae) commonly known as Akarkara, mostly found in Indian subcontinent. Whole parts of the plant are rich in secondary metabolites, which impart mariculous medicinal uses to the plant. The bioactive constituents isolated from *S. acmella* are spilanthol, isobutylamides, triterpenoids, butylated hydroxytoluene, polygodial and eudesmanolide etc. The plant has found applications in pharmaceuticals as an antitoothache formulation for pain relief, swelling and gum infections, periodontosis and in mouthwashes. The extracts of different parts of plant used as anti-insecticidal, antimicrobial, antimalarial, analgesic, antipyretic, diuretic, antioxidant and cytotoxicity activities. Employment of techniques such as cell and tissue culture would provide means of rapid propagation and conservation of the plant species and, from the point of view of phytochemistry, give scope for enhancement of the quality and quantity of the bioactive secondary metabolites occurring in the plant.

Introduction

Spilanthes acmella L. belongs to the genus *Spilanthes*, family Asteraceae (Compositae). It is an herb found all around the world and widely distributed throughout the tropics and subtropics. It is native to the tropics of Brazil, and is grown as an ornamental (and as a medicinal) in various parts of the world. Commonly it is known as Toothache plant or Paracress or Eyeball plant. The name Eyeball plant should be obvious to anyone who is familiar with the plant's flowers, which are yellow and gradually turn to dark red in the center. The active constituent spilanthol chiefly present in leaves and flower heads, and produce analgesic activity used to numb toothache. The whole plants can be used in the treatment of dysentery and rheumatism[1]. A decoction of the plant can be taken internally as a diuretic and able to resolve stones in the bladder, while a decoction of the roots can be used as a purgative. It is also used as a defensive medicine for scurvy and stimulates digestion. Besides these medicinal uses, the flower heads have been used as a spice for appetizers by the Japanese. This review discuss the investigation made by various workers related to its medicinal uses, chemical constituents, pharmacological activities, micropropagation and other aspects considering this plant since years to till date.

Plant details

Distribution: This plant is widely distributed in the tropical and sub-tropical regions including America, North Australia, Africa, Malaya, Borneo, India and Sri Lanka. In India, it is confined to South and central part of India, and Jharkhand[2].

Plant description: *Spilanthes acmella* is synonymous with *spilanthes oleracea*. The genus *Spilanthes* contains 35 tropical species, of which three of them are reported from India. *Spilanthes acmella* is very beautiful, erect or ascending stout herbs, 20-50 cm high and can be grown as an annual in most climates. It is frost-sensitive but perennial in warmer climates. A small, erect plant, it grows quickly and sends up gold and red flower inflorescences. It can be grown in the ground or as a potted. 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. *Spilanthes acmella* has no flower petals, but instead, exhibits golden "buds" with a rust-red center. Leaves are opposite, petiolate, broadly ovate, narrowed at base, acute or obtuse at apex, flowering and fruiting in March-April[3].

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[4].

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 complaints[5]. It has also used in remedy of rheumatism and fever[6,7]. It has strong diuretic activity and the ability to dissolve urinary calculi[2]. It also exhibits antimalarial, antiseptic, anti-bacterial properties[5]. The leaves are used as immunomodulatory, adaptogenic, toothpaste, lithotriptic, antiscorbitic, ailagogine and digestive[3]. 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[2]. The flower heads of *S. acmella* can be chewed to relieve toothache and also as a haemostatic and analgesic[1]. Ayurvedic system of medicine, flower heads and roots are used in treatment of scabies, psoriasis, scurvy, infections of gums[8], periodontosis[9], paralysis of tongue and remedy for stammering in children and in mouthwashes[10]. *S. acmella* also possessed excellent anti-microbial activities against red halophillic cocci from salt cured fish[5]. Its extract is an active component used in beauty care cosmetics as a fast acting muscle relaxant to accelerate repair of functional wrinkles[11]. The plant extract was also used for stimulating, reorganizing and strengthening the collagen network in anti-age applications, e.g. in antiwrinkle cream formulations[12,13]. Spilanthol used as insecticide it shows potent ovicidal, larvicidal and pupicidal activity[14]. An Indian tribe used *S. acmella* to treat fungal skin conditions, such as athlete's foot, ringworm and nail infections [15].

Phytochemistry

Chemical analysis shows that *Spilanthes acmella* contain major pungent compound, spilanthol (N-isobutyl-2E, 6Z, 8E-decatrienamide) which is naturally occurring insecticide, and Butylated Hydroxytoluene**[**1]. Antioxidant, butylated hydroxytoluene (BHT) and fatty acids (n-Hexadecanoic acid and tetradecanoic acid) could be obtained from extracts of mother plant of flower heads[1]. The leaves contain alkaloids, carbohydrates, pungent amide tannins, steroids, carotenoids, essential oil, amino acid etc[3]. Besides the alkamides, pungent nonvolatile sesquiterpenoids have been found, such as polygodial and eudesmanolide II[4]**.** The pungent flavour of *Spilanthes acmella* is due to an unsaturated alkamid, spilanthol which present in its highest concentration (1%) in the flowers[4]. Essential oil 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[4]. The crude ethyl acetate of *S. acmella* was purified by chromatographic methods to give 3-acetylaleuritolic acid 1, vanillic acid 2 and-sitostenone 3[16].

Pharmacological studies

Anti-inflammatory and Analgesic: Aqueous extract of ariel 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[17]. Spilanthol shows 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[18,19].
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 is mediated supra-spinally accompanied with sedation[20].

Diuretic: 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[21]. Ethanol extract of leaves of *S. acmella* also significantly increased the urinary output (by 223%) and electrolytic excretion of Na^+ (by 136%) and K^+ (by 172%)[22].

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[23].

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 immunostimulant effect[24].

Antioxidant activity: The antioxidant activity of methanol extract of stem and leaves of *S. acmella* were measured using DPPH and superoxide radical scavenging assays[25]. 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[24]. 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[25]. In DPPH radical scavenging activity was found maximum in leaf and minimum in in root. Callus showed significant DPPH radical scavenging activity[25].

Biological activities

Antifungal activity: Different concentrations of *S. acmella* flower head extract (dried flower heads extracted with petroleum ether) were evaluated for antifungal activity (0.1 to 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[26].

Antibacterial activity: The different fractions were isolated from crude ethyl acetate extract of *S. acmella* and were studied against 27 strains of microorganisms. The results showed that fraction E3 completely inhibited the growth of *Corynebacterium diphtheriae* with MIC value of 128 µg/mL[16].

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 micropropagation 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 also successfully micropropagated through leaf, axillary bud and shoot tip.

Micropropagation through leaf: *Spilanthes acemella* has successfully been cultured *in vitro* 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 [25].

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[5].

Micropropagation through shoot tips: *S. acmella* was also micropropagated using shoot tip and nodal segments as explants for multiple shoot proliferation. Maximum multiplication (7.0 shoots) per explant was observed on MS medium supplemented with 2.0 mg/L BA and 0.1 mg/L NAA. Rooting was achieved using MS medium containing Indole-3-butyric acid[27].

Conclusion

The plant holds great promise as a commonly available medicinal plant and it is indeed no surprise that the plant is referred to in the Indian traditional circles. From the available literature on various aspects of the plant -traditional to biochemical and ethnobotanical to pharmacological and micropropagation however there many gaps which need to be filled by concurrent researchers in different disciplines. One must make the best use of the naturally available resources which provide valuable raw material for advanced research.

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