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Spilanthes acmella: Pharmacognostic and Phytochemical studies

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ABSTRACT: Background: *Spilanthes acmella* (*Asteraceae*) is a well-known anti-toothache plant. It is traditionally used as diuretic, anti-inflammatory, analgesic, antioxidant and vasorelaxant. **Aim:** The research study was aimed to carry out macro and microscopic, pharmacognostic and phytochemical screening studies of *S. acmella* whole plant. **Methods:** The micro and macroscopic studies was done by Microscopy technique. The plant was extracted by Soxhlation method. The phytochemicals analysis was done by standard chemical analytical method. **Results:** The water extractive value has been found higher in root, where ethanol extractive value was higher in leaf. The *S. acmella* contained phytochemicals such are alkaloids, carbohydrates, tannins, amino acids and sesquiterpenes. **Conclusion:** It could be concluded that the pharmacognostic study of *S. acmella* will also serve as reference material in preparation of plant monograph. The research studies need to be extended for pharmacological and analytical investigations.

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INTRODUCTIONS:

Herbal medicines are called as phytomedicines as their various parts like plant's seeds, berries, root, leaves, bark or flowers of plant, are used for medicinal purposes. Globally, India is a central market for herbs based [1,2]. Herbal medicines, synonimically called as nutraceuticals, current market estimated at about $ 80-250 billion in USA and in Europe**.** The important drugs of plant are in use today were derived from starting molecules of plant origin. For the first time, clinically useful psychoactive drugs were fortuitously identified [3,4].

S. acmella is very beautiful stout herbs of 20 to 50 cm high with gold and red flowers inflorescences without

flower petals. The stems are prostrate, often reddish, hairless. This plant contain golden buds and leaves are opposite, petiolate, broadly ovate, acute at apex. Due to sensation quality, *S. acmella* has no odour [5,6].

Fig 1. *Spilanthes Acmella* plant.

S acmella is is known as toothache plant which reduces the pain associated with toothaches and can induce saliva secretion [7-9]. Literature survey revealed *S. acmella* possessed various pharmacological actions like antifungal, antipyretic, local anaesthetic, bioinsecticide, anticonvulsant, antioxidant, aphrodisiac, analgesic, pancreatic lipase inhibitor, antimicrobial, antinociception, diuretic, vasorelaxant, anti-human immunodeficiency virus, toothache relieve and anti-inflammatory effects [10-14].

The objectives of the study to carry out the Phytochemical and Pharmacognostic investigation of *S. acmella.*

MATERIALS AND METHODS:

The Dragondroff's and Fehling reagents were purchased from S.D. Fine Chemical, Mumbai. The solvents petroleum ether, Chloroform, and Methanol were purchased from HiMedia Laboratories Pvt. Ltd., Mumbai. All others chemicals, solvents and reagents were of analytical grade and procured from authorized dealer.

Collections and authentication of plants:

The whole plant of *S. acmella* was collected from rural areas of Moinabad, Hyderabad in the month of March and April. The plant was authenticated by qualified taxonomist of Botanical Survey of India, Deccan Regional Centre, Hyderabad, Telengana. The leaves, flower, fruits were collected, washed under running water, air dried under low intensity of sunlight for 48 h and later in hot air oven at low temperature for one week. The dried leaves and flowers were crushed for size reduction to a coarse powder using an electrical blender and stored for further study.

Macroscopic and microscopic analysis

The macroscopic and microscopic of the *S. acmella* plant were studied as per the standard prescribed procedure. The transverse sections of leaf, stem, roots and stomatal index were prepared and stained with Safranin and Fast green as per the procedure. Powder microscopy was performed according with the prescribed procedure. The microphotographs were taken by Bright field microscope with digital camera Canon Power shoot SD130015 for detailed studies [15-17].

Extraction of plants:

Powdered plant material (30 g) was extracted at room temperature with petroleum ether (40 to 60 ^{o}C) exhaustively and successively using Soxhlet apparatus for 12 h. The procedure was repeated with chloroform, methanol and water respectively with drying the marc before the next batch of extraction. Extracts were filtered using Whatman filter paper no. 2 and excess solvent was removed by distillation. The combined aqueous extract was however concentrated under reduced pressure using rotary evaporator. Concentrated extracts were dried at 40 ^{o}C temperature on a water bath. The dried extracts were collected and kept in sterile sample bottles at ambient temperature. Their yields and other physical properties were noted and recorded [18,19].

Pharmacognostical investigation:

The detailed and systematic pharmacognostical evaluation would give valuable information of the plant for the future studies. The various dried parts of *S. acmella* were subjected to physical evaluation studies like loss on drying, foreign organic matter content, moisture content, acid insoluble, water soluble and total ash values. It was also evaluated for water and ethanol soluble extractive values. All evaluations were done as per standard procedure of Indian Ayurvedic Pharmacopoeia [20-23].

Phytochemical investigation:

The water, petroleum ether, Chloroform, and Methanol extracts of plant *S. acmella* (Leaves and flowers) were subjected to phytochemicals screening for identification of plant constituents like alkaloids, glycosides, tannins, carbohydrates, flavonoids, sterols, mucilages and saponins. All screenings were done as per standard procedure of Indian Ayurvedic Pharmacopoeia [24-16].

RESULTS AND DISCUSSIONS:

Microscopy of Leaf (T.S.):

In T.S. of leaf, the microscopic analysis of *S. acmella* leaves revealed simple membranous leaves showed wavy uniseriate epidermal cells, bicellular or tricellular trichome with basal cell showing rough cuticle and anomocytic stomata (Fig 2. D). The ventral mesophyll was well organized showing two layers of palisade parenchyma and several layers of spongeous parenchyma. The cross-section of central rib shows a concave-convex profile and two vascular bundles are present in addition to conducting vessels (Fig 2. A).

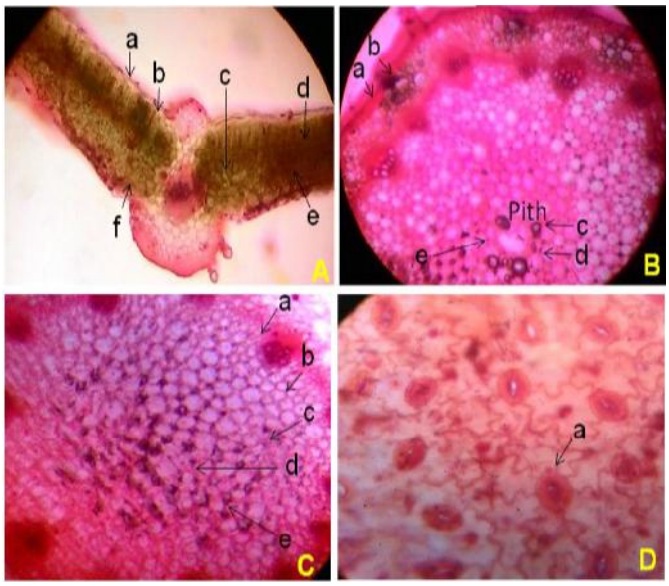

Fig 2. A. T.S. of Leaf: a-upper epidermis, b-cuticle, c-vascular bundles, d-palisade parenchyma, e-spongy parenchyma, f-lower epidermis. B. T.S. of Stem: a-epidermis, b-hypodermis, c-meta xylem, d-proto xylem, e-phloem. C.T.S. of Root: a-epidermis, b-cortex, c-pericycle, d-medulla, e-phloem. D. Surface view of leaf epidermis showing a-anomocytic stomata.

Microscopy of Stem (T.S.):

A transverse section of the stem was wavy in outline with trichomes and stomata were present. The outermost layer was epidermis, which was covered by a thick cuticle. After epidermis, hypodermis (sclerenchyma) was present; below of which cortex was present. Starch grains were simple, present in the cortical cells. Endodermis was single layered. Pericycle consist 1-2 layered, vascular bundles consist of metaxylem and phloem and pith is present in the center (Fig 2. B).

Microscopy of Root (T.S.):

A T.S. of root was circular in outer line and the outer most layers was thick walled epidermis interrupted by 1 to 2 celled, cortex parenchymatous 6 to 7 layered cell broad, tangentially elongated. Endodermis consist of single layered, next to it pericycle was present. Large Pith was present in the middle. Medullary rays were present. Vascular bundles were consisting of xylem and phloem. The phloem was well developed (Fig 2. C).

Organoleptic evaluations:

The organoleptic behavior of various *S. acmella* plant is given in Table 1. The taste was found to be pungency.

Physicochemical studies:

The physical evaluation data of leaves and roots of *S. acmella* is given in Table 2, which revealed that loss on drying was 6.87 (Leaf) and 7.87 % (Root), where as this plant contain least moisture content (Average of 3.7 %). The plant was containing very least foreign organic matter (0.18 %). The total ash value was found to be 7.3 % for leaf and 9.5 % for root. The water and ethanol soluble extractive was found to be good. The response of plant parts powder form to various chemical reagents was given in Table 3.

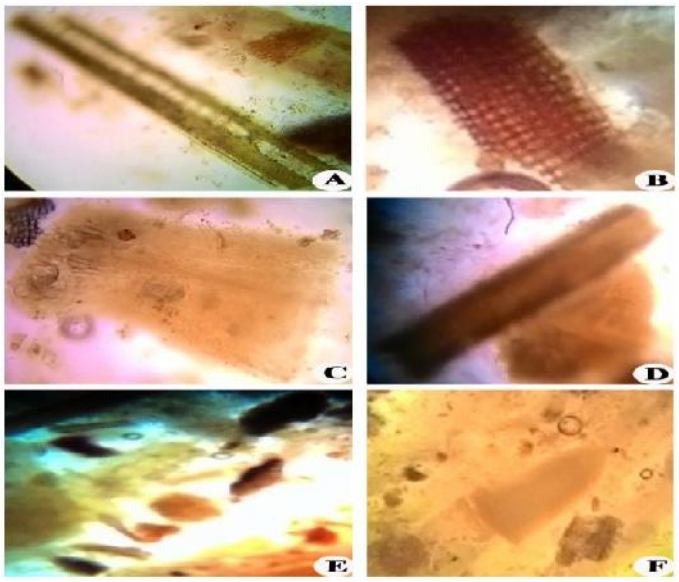

Fig 3. A. Tracheid fibers. B. Fragment of parenchyma. C. Phloem fibers D. Vessels with pitted wall. E. Oxalate crystals. F. Prismatic crystals.

Phytochemical investigation:

The percentage extract value of various solvent was in the order of methanol > chloroform > petroleum ether, as given in Table 4. The phytochemical screening data of leaf and flower of plant *S. acmella* is given in Table 5 and 6. The petroleum ether extract of leaf of *S. acmella* was found to contain flavonoids and terpenoids. The ethanol extract of leaf of *S. acmella* was found to contain alkaloids and tannins. The methanol extract of leaf of *S.*

acmella was found to contain alkaloids, glycosides, flavonoids and terpenoids. The petroleum ether extract of flower of *S. acmella* was found to contain flavonoids and terpenoids. The ethanol extract of flower of *S. acmella* was found to contain alkaloids. The methanol extract of flower of *S. acmella* was found to contain alkaloids, flavonoids and terpenoids.

Table 1. Organoleptic evaluation of various parts of *Spilanthes acmella* Murr.

Parts	Colour	Odour	Taste
Flower	Yellow red	Pleasant	Pungent
Fruit	Brown	Pungent	Pungent
Seed	Brown	Salty	Pungent
Leaves	Dark green	Pleasant	Pungent
Stem	Green to red	Odour less	Pungent
Root	Pale yellow	Foul	Pungent

Table 2. Physical Constants *Spilanthes acmella* plant parts.

Constant	Leaf	Root
Loss on drying	8.76	6.76
Foreign organic matter	0.13	0.23
Moisture content	4.32	3.10
Ash values		
Total ash	7.3	9.5
Acid insoluble ash	3.5	5.5
Water soluble ash	2.0	4.0
Extractive values		
Ethanol soluble extractive	21.2	16.5
Water soluble extractive	11.2	18.2

Table 3. Behaviour of whole plant powder with different chemical reagents.

Treatment	Color
Powder + HNO_3	Brick red
Powder + H_2SO_4	Brown
Powder + NH_3	Brick red
Powder + $FeCl_3$	Brown
Powder + NaOH	Dark yellow
Powder + Glacial acetic acid	Dark green

Stomata Index:

The stomata index value of plant *S. acmella* is given in Table 7. The stomata index for adaxial and abaxial surface of leaf was 140.6 and 181.2.

CONCLUSION:

Physicochemical studies finding possess total ash content has been higher in root and acid insoluble ash also higher in root; it may be due to the earth components. The water extractive value has been found higher in root; however ethanol extract has found to be higher in leaf. The qualitative phytochemical screening showed that the plant is a rich source of alkaloids, carbohydrates, tannins, amino acids and sesquiterpenes. The results of present study will also serve as reference material in preparation of monograph. The research studies need to be extended for pharmacological and analytical investigations.

Table 4. Percentage of extract.

Sample	Sample Weight	Extract weight	Extract (%)
Chloroform	25 g	10.8	43.2
Pet. Ether	25 g	3.6	14.4
Methanol	25 g	11.21	44.84

Table 5. Phytochemical analysis of the leaves of *Spilanthes acmella* Murr.

Phyto-Constituents	Pet. Ether Extract	Ethanol Extract	Methanol Extract
Alkloids	-	+	+
Glycosides	-	-	+
Tannins	-	+	-
Flavonoids	+	-	+
Terpenoids	+	-	+
Phlobatannis	-	-	-

Key: (-) =Negative, (+) = Positive.

Table 6. Phytochemical analysis of the flowers of *Spilanthes acmella* Murr.

Phyto-Constituents	Pet. Ether Extract	Ethanol Extract	Methanol Extract
Alkloids	-	+	+
Glycosides	-	-	-
Tannins	-	-	-
Flavonoids	+	-	+
Terpenoids	+	-	+
Phlobatannis	-	-	-

Key: (-) =Negative, (+) = Positive.

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laboratory facilities to complete this research work. Authors also wish to thank Botanical Survey of India, Deccan Regional Centre, Hyderabad, for authentication of Plant.

Table 7. Stomatal Index f leaves of *S. acmella*.

Trial	AdSL		AbSL	
	Margin	Middle	Margin	Middle
I	123	112	135	180
II	136	140	156	202
III	138	145	160	210
IV	145	156	165	217
V	148	163	167	220
Avg.	138	143.2	156.6	205.8
	140.6		181.2	

AdSL and AbSL are adaxial and abaxial surface of leaf.

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