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STANDARDIZATION, FINGERPRINTING AND QUALITY CONTROL OF *SPILANTHES ACMELLA* (L.) MURR.

Anil V. Dusane, Khushbu M. Boharaand and Shivani A. Dusane

Botany Dept.,Sir Parashurambhau College, Pune-30.
anildusane@gmail.com

Abstract:

Spilanthes acmella L. (Asteraceae) is a medicinally important plant commonly known as 'Akarkara'. *S. acmella* is a rich source of phytochemicals. In ayurvedic system of medicine *S.acmella* is used for the treatment of scabies, psoriasis, scurvey, toothache, infection of gums, paralysis of tongue and for stammering. Leaf of this plant contains valuable bioactive phytochemicals viz. alkamides (Spilanthol), isobutylamide derivatives, saponins, amino acids, tannins, sesquiterpens and alkaloids. This plant has been well documented for its uses as anti-flammatory, analgestic, antipyretic, anesthetic and immuno-stimulent. *S. acmella*has antimicrobial, fungicidal and insecticidal properties. In the present investigation exomorphic, anatomical, pharmacognostic, physico-chemical and chromatographic (fingerprinting) standards have been evolvedfor *S. acmella*. The parameters such as quantitative microscopy, fluorescence analysis, powder analysis and fingerprinting served as quality standards. The physico-chemical analysis of leaf podwer shown total ash, water soluble ash and acid insoluble ash values as 7.9%, 4.0% and 2.5%w/w respectively. While extractive values of leaf powder with water, ethanol and petroleum ether yielded 9.58%, 15.1% and 4.98%w/w respectively. The fluorescence studies of leaf revealed distinctive pattern. The characteristic TLC pattern of leaf extract using solvent systems, chloroform: methanol (3:7) served as the fingerprint for *S. acmella*.

Keywords: Ash values, finger printing, quality standards, pharmacognostic standards, physico-chemical studies, *Spillanthes acmella* and Thin Layer Chromatography.

Introduction

There is ever-increasing demand forherbal drugs in the domestic and international market as these drugs have negligible toxicity, compatibility to human system and high degree of biodegradation (Leng *et al*., 2011). *Spillanthes acmella* is an important medicinal plant ditributed in tropical and substropical regions around the world. It is a commonly known as 'Akarkar'. *S. acmella* is an annual hairy herb that grows up to 35-60 cm with yellow flowers. The whole plant or leaf or inflorescence is used as a drug. It is a rich source of therapeutic and medicinal constitutents (Manickam, 2014). *S. acmella*has many medicinal uses on health problems like desentry, rheumatism and tooathache (Oliver-Bever, 1986; Nakatani and Nagashima, 1992; Baruah and Laclercq, 1993). The leaf of this plant has therapeutic properties such as immuno-modulation, adaptogenic, diuretic, anti-inflammatory, analgestic, vasorelaxant, and antitoxicant(Yadav, 2012). Leaf of this plant mainly contains alkaloids, carbohydrates, tannins, steroids, carotenoids, sequiterpenes(Nagashima and Nobuji, 1991), essential oil (Shimada and Gomi,1995) and alkamides (Nakatani and Nagashima, 1992).

In view of the importance of *S. acmella* in folk and modern medicine, the exomorphic, anatomical, pharmacognostic, physico-chemical standards has been evolved for standerdization and ultimately for quality control.

Materials and Methods

Plant material: *S. acmella* (Asteraceae) was collected from Western ghats (Tamhini, Pune, Maharashtra). The healthy and fully matured leaves were taken for investigation.

Organoleptic characteristics: The characteristics viz. colour, odour and taste were recorded.

Microscopic studies: Free hand sections of leaf were taken and double staining (safranin and light green) carried out.Maceration studies and the preparation of permanent slides were made as per the standard procedure (Johanson, 1940). Starch grains were stained with iodine solution.

Powder analysis: Leaf powder was prepared by passing the grounded leaf through sieve mesh 60 (Sixty). Powdered drug was separately treated with phloroglucinol-hydrochloric acid (1:1) solution for observation of microscopical characteristics (Anonymous, 1998).

Pharmacognostic evaluation: The various quantitative microscopic characteristics of *S. acmella*leaf wereevaluated (Wallis, 2011).

Fluorescence studies: The fluorescence analysis was carried out as per the standard method (Chase and Pratt, 1949). The mountant medium viz. distilled water, 1 N NaOH, 1 N HCl, 1 N H_2SO_4 and 1 N HNO_3 were used and the fluorescence at ordinary light and at under 254nm and 366nm UV light recorded. The colour for fluorescence was confirmed from 'A

Mycological Colour Chart' of Rayner (1970). In the present investigation detailed fluorescence analysis of the powders and solvent extracts was carried out.

Physico-chemical analysis: This was carried out as per the WHO guidelines (Anonymous, 1998). The parameters viz. total ash, water-soluble ash and acid-insoluble ash values were determined. Water, ethanol and petroleum ether (60^0C-80^0C) soluble extractive values were determined. The leaf extractives were subjected to different phytochemical tests (Harborne, 1973).

Thin Layer Chromatography (TLC) studies: TLC studies were carried out as per the standard methods described by Stahl (1969). The final output of TLC were taken on commercially available precoated E. Merk plates. Water and ethanol leaf extracts were subjected to TLC. The solvent system Chloroform: methanol (3:7) was taken for finger printing. The spots were observed under UV 254 nm and 366 nm. Iodine vapour was used as a developer.

Results and discussion:

Macroscopic studies:

S. acmella is small, erect plant grows upto height 60cm. It is with gold and red flowers drugs is an important parameter in detecting adulteration, admixtures or improper handling of the drugs. The total ash is particularly important in the evaluation of purity of drugs. The ash values are of great significance in pharmacognostic studies. The extractive values are also specific for a particular plant species. These serves as important pharmacognostic parameter. The results of ash values and extractives of leaf have been tabulated in Table no. 3.

The physico-chemical analysis of leaf podwer shown total ash, water soluble ash and acid insoluble ash values as 7.9, 4.0 and 2.5%w/w respectively. The earlier workers recorded total ash, acid insoluble ash and water soluble ash 7.3%, 3.5%, 2.0% for leaf (Yadav, 2012). The extractive values of leaf powder with water, ethanol and petroleum ether yielded 9.58%, 15.1% and 4.98w/w respectively. The extractive values for ethanol and water has been recorded 21.2% and 11.2% (Yadav, 2012). However, high value (14.4%) have been recorded for petroleum ether extractive (Kavya and Pattar, 2015) than recorded in the present investigation.

Fluorescence analysis: It provides best clues for easy and rapid identification of this inflorescences. Leaves of this plant are opposite, petiolate, broadly ovate, narrowed at base, acute or obtuse at apex. Leaf has no specific odour but if is eaten it tastes salty to a strong pungent taste. It leaves a numb feeling in the mouth.

Microscopic studies of leaf:

T.S. of leaf shown wavy, uniseriate epidermal cells, 1-3 celled trichomes and anomocytic stomata. Mesophyll shown 1-2 layerd palisade and many layered spongy parenchyma. The cross-section of midrib shown a concave-convex profile and three vascular bundles were present (Fig.2).

Quantitative microscopy:

The quantitative microscopy is one of the important tools in the pharmacognostic evaluations. The quantitative microscopy

study is included as one of the standards in the Pharmacopoeias. The dimensions of stomata, stomatal index and diamensions of starch grains were recorded in table no.2.

Powder analysis: Leaf powderappeared green coloured. In the leaf powder cells of spongy parenchyma, palisade, vascular tissues and starch grains were observed.

Physico-chemical characteristics of leaf: The physical constant evaluation of the

plant species. The distinct pattern of fluorescence serves as criteria for identification. The leaf powder fluorescence has been recorded in Table no. 4. The fluorescence analysis of water, ethanol and petroleum ether has been recorded in table no.5

Preliminary phytochemical study: The activity of the particular drug is due to the presence of a particular phytochemical in appropriate concentration. In view of this, an extensive phytochemical and TLC studies have been carried out to detect the presence of various chemicals. The phytochemical and chromatographic studies is important in the standardization of the drugs also. The results of phytochemical tests have been recorded in table no.6.

The results of phytochemical tests revealed that the leaf contained saponins, flavanoids, tannins, alkaloids, steroids, sugars, glycosides, carbohydrates and proteins. The earlier workers recorded presence of tannins, alkaloids, steroids, sugars, glycosides, carbohydrates and proteins (Kavya and Pattar, 2015).In the presen investigation saponins, flavanoids, tannins, alkaloids, sugars, Terpenes, glycosides, carobhydrates and proteins have been detected.

TLC analysis: This technique has applications in standardization, determination of the ingredients of formulations and detection of adulterants or substitutes. TLC is also practiced as a qualitative tool for the study of admixtures. In the present investigation aqueous and ethanol extractives have been subjected to TLC studies using chloroform: methanol (3:7). The TLC studies have been tabulated in table no. 7.

The chromatographic studies are important for the fingerprinting of this plant species. The solvent system used was chloroform: methanol (3:7). The earlier researchers Rf values 0.47, 0.59 , 0.71, 0.77, 0.84 and 0.93 respectively for aqueous extract (leaf) in chloroform: methanol (3:7) solvent system (Manickam,2014).

In conclusion, Pharmacognostical evaluation of parameters like microscopy, physicochemical analysis, fluorescence analysis is necessary for standardization of herbals. These parameters also serves as standards of authetication of plant species. The macroscopic and microscopic evaluations are essential for the setting standards in this plant species. The results obtained for ash values have importance in quality control are used in detection of adulteration. The results of present study will also serve as reference material in preparation of monograph.

Table no.1. Organoleptic evaluation of *S. acmella*

Organoleptic characteristic	Leaf	Flower
Colour	Dark green	Yellow with red
Odour	Not specific	Not specific
Taste	Pungent	Pungent
Texture	Rough	Rough

Table no. 2. Quantitative microscopic studies of leaf S. acmella

Parameters	Dimensions
Stomata (Upper epidermis)	190µ (length)
Stomata (Lower epidermis)	180µ (length)
Stomatal index (Upper epidermis)	175
Stomatal index (Lower eipidermis)	138
Starch grains (Simple)	15X13 µ

Table no. 3. Physicochemical parameters *S. acmella* leaf

Sr. No	Physicochemical parameters	Leaf constants
1	Total ash	7.9 % (w/w)
2	Acid insoluble ash	4.0 % (w/w)
3	Water soluble ash	2.5 % (w/w)
4.	Water extractives	9.58%(w/w)
5.	Ethanol extractives	15.1% (w/w)
6.	Petroleum ether extractives	4.98 %(w/w)
7	Foreign organic matter	0.15%

Table no. 4. Fluorescence characteristics of *S. acmella* leaf powder

Sr. No.	Mountant medium	256nm	366nm	Natural day light
1	Dry powder	Mouse grey	Greenish grey	Pale mouse grey
2	P + D.W.	Olivaceous	Olivaceous grey	Grey olivaceous
3	P+1N HCl	Greenish black	Greenish black	Pale mouse grey
5	P+1N NaOH	Greenish grey	Olivaceous black	Greenish grey
6	P+1N H_2SO_4	Dull green	Greenish grey	Grey olivaceous
7	P+1N HNO_3	Greenish grey	Green	Grey olivaceous

Table no.5 Fluorescence characteristics of *S. acmella* leaf extracts

Sr. No.	Extracts	254nm	366nm	Natural day light
1	Water	Dark Green	Dull green	Greenish grey
2	Petroleum ether	Grey olivaceous	Greenish grey	Grey olivaceous
3	Ethanol	Green	Grey olivaceous	Grey olivaceous

Table no.6. Preliminary phytochemical studies of *S. acmella* leaf**.**

Sr. No.	Chemical	P.E	Et OH	D.W.
1	Saponins	-	+	+
2	Flavanoids	+	+	+
3	Tannins	-	+	+
4	Alkaloids	+	+	+
5	Steroids	+	+	-
6	Sugars	-	+	+
7	Terpenes	+	-	-
8	Glycosides	+	+	-
9	Carbohydrates	-	+	-
10	Proteins	-	+	-

P.E.= petroleum ether, EtOH= ethanol, D.W. = distilled water.

Table no.7. TLC Finger print of S. *acmella* leaf water extract

No. of spots	Rf value	254nm	366 nm	Iodine developer
1	0.68	Intense blue	Faint blue	Yellow
3	0.75	Intense blue	Faint blue	Yellow
4	0.83	Yellow	-	Yellow

Solvent system- chloroform: methanol (3:7)
Plates were observed under CAMAG UV cabinet
*Mean of 10 observations.

1.a

1.b

1.c

1.d

Figure.1a. Habit of *S. acmella,* 1.b Twig, 1c. Leaf, 1d. Inflorescence

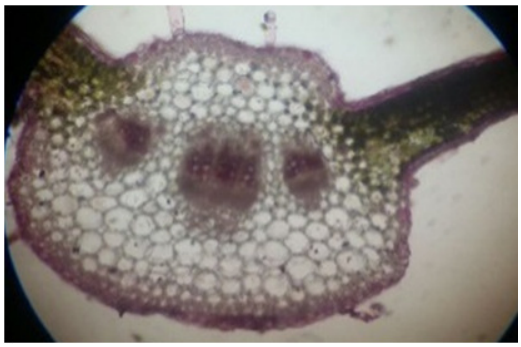

Figure.2 Leaf T.S. of *S. acmella* (X100)

Figure. 3. Water extract (chloroform: methanol, 3:7). Developer-Iodine for TLC plates.

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