Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2014; 6(1); 122-127

ISSN: 0975-4873

Research Article

Pharmacognostic and Phytochemical Studies for the Establishment of Quality Parameters of Leaf of *Achyranthes aspera* Linn.

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Available online: 1st March 2014

ABSTRACT

Achyranthes aspera Linn., Fam. Amranthaceae (Apamarga) is extensively used herb in the Indian systems of medicine. This plant removes the vitiated doshas from the body hence the name Apamarga. Large numbers of phytochemical constituents have been isolated from the plant which possesses activities like antiperiodic, diuretic, purgative, laxative, antiasthmatic, hepatoprotective, and anti-allergic. Standardization of this drug is the key factor in regulating the therapeutic efficacy. The current work was undertaken to standardize the leaf material using simple pharmacognostic techniques, detailed microscopic evaluation and to develop a HPTLC fingerprint profile of leaf of *Achyranthes aspera* Linn. The present investigation deals with physico-chemical study, use of light microcopy and SEM studies of the plant for the establishment of quality parameters. The present work can serve as a useful tool in the identification, authentication and standardization of the plant material.

Keywords: HPTLC fingerprint, microscopy, Pharmacognostic studies, *Achyranthes aspera* Linn.

INTRODUCTION

Herbal drugs play an important role in health care programs especially in developing countries. Ancient Indian literature incorporates a remarkably broad definition of medicinal plants and considers 'all' plant parts to be potential sources of medicinal substances[1].

It is highly important to ensure quality and purity of herbal medicines in order to maximize the efficacy and minimize adverse side effects. Correct identification and quality assurance of the starting materials is an essential prerequisite to ensure reproducible quality of herbal medicine. Simple pharmacognostic techniques used in standardization of plant material include its morphological, anatomical and biochemical characteristics. [2]

Achyranthes aspera Linn. (Fam. Amranthaceae) is an erect or procumbent, annual or perennial herb, 1-2m in height, often with a woody base, commonly found as a weed of waysides, on roadsides[3-5]. Although it has many medicinal properties, it is particularly used spermicidal[6], antipyretic[7] and as a cardiovascular agent[8].

It is used by traditional healers for the treatment of fever, dysentery and diabetes[9]. Leaf decoction for cardiovascular toxicity has been reported[10], and the ethanol crude extract showed high larvicidal activity on the tick larvae against *Boophilis microplus*[11]. The ethanolic extract of the leaves and stem of the plant inhibited the growth of *Bacillus subtilis* and *Staphylococcus aureus* bacterial strains[12]. Roots are used as astringents to wounds, in abdominal tumor and stomach pain[13]. Leaf extracts were reported to posses thyroid stimulating, antiperoxidative and antifungal activity properties[14-15]. The aqueous and methyl alcohol extracts of the plant also decreased blood glucose levels in normal and alloxan diabetic rabbits[16]. It is reported to contain alkaloids, flavanoids, saponins, steroids and terpenoids. The water soluble alkaloid achyranthine isolated from *Achyranthes aspera* Linn. possess anti-inflammatory activity[17]. The plant is a good source of trace elements and each element has its individual impact in the structural and functional integrity of the living cells and organisms [18]

Very little systematic studies have been reported for microscopic study especially SEM studies and development of chemical fingerprint by HPTLC of leaf of *Achyranthes aspera* Linn. hence this work is an effort to establish microscopic and chemical standardization of *Achyranthes aspera* Linn. leaves. However no scientific standard parameters are available to determine the quality and genuineness of the drug. In the present study an attempt was made to standardize the drug using pharmacognostic studies, light microscopy, scanning electron microscopy (SEM) and HPTLC fingerprint. Analysis was done for different extracts of *Achyranthes aspera* Linn. from leaf; which can further lead to provide a beneficial information towards the quality of the drug and also standardization of the drug.

MATERIALS AND METHODS

Collection of plant material: Whole plant parts of *Achyranthus aspera* Linn. were collected in the month of August- September 2013 from Vasai region of Thane district. The plants were identified at Blatter's herbarium, St. Xavier's College, Mumbai. The accession number for *Achyranthes aspera* L. is 62490.

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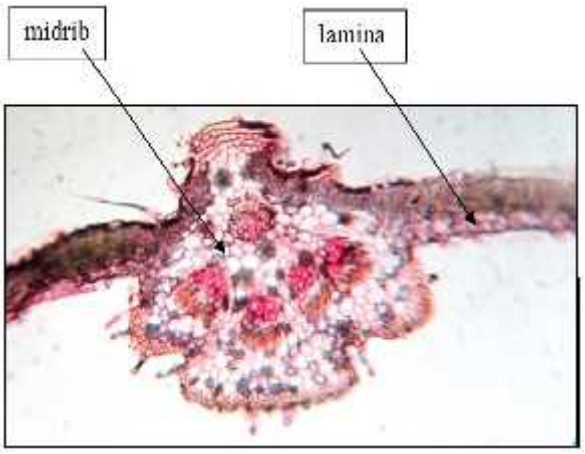

Fig. 1: T.S. of leaf of Achyranthes aspera Linn. with midrib and lamina. Crystals of calcium oxalate and trichomes.

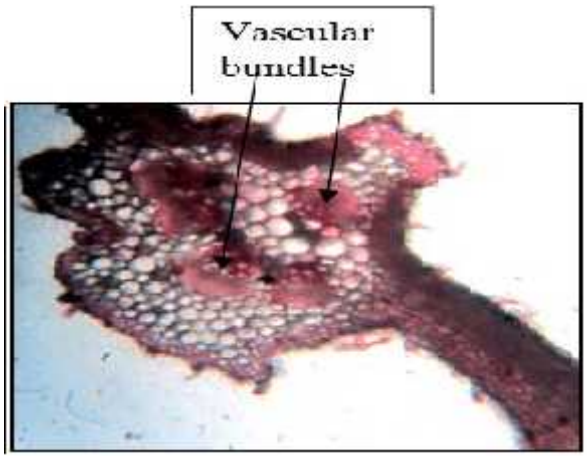

Fig. 2: T.S. of leaf of Achyranthes aspera Linn. with midrib showing vascular bundles.

Fig. 3: Leaf surface of Achyranthes aspera Linn. with many trichomes.

Fig. 4: Leaf surface of Achyranthes aspera Linn. with many anomocytic stomata.

Fig.5: A trichome on leaf surface of Achyranthes aspera Linn.

After confirmation of its botanical identity the leaves were subjected for morphological, physicochemical and HPTLC finger print studies.

Pharmacognostic studies:

Physicochemical parameters: Physicochemical parameters such as ash and extractive values were determined according to the methods described in Unani Pharmacopoeia of India, 2008[19].

Fluorescence analysis: Fluorescence analysis was carried out as per the method described by Trease and Evans[20].

Light Microscopy: For the anatomical analysis by LM, tangential thin hand sections of the fresh leaf was prepared by conventional micro techniques[21-22].

The sections were then fixed, dehydrated and stained. For permanent mounts Canada balsam, diluted with a small portion of xylene was used as an adhesive. Upon drying of the mountant, the slide was then examined under a microscope.

Scanning electron microscopy (SEM): In our work on SEM, the material was completely dried by keeping it in oven at 40^{o}C for 8 to 10 hrs. Then it was mounted directly on stubs using double-side adhesive tape and sputtered with a thin layer of gold in a JEOL JSM-1200 Fine Coater. The electron micrographs were obtained in a JEOL JSM-T220 scanning electron microscope at 15 kV, with an integrated digital image acquisition system.

HPTLC Fingerprint: The HPTLC analyses were performed on aluminium plates pre-coated with silica gel $60F_{254}$ (Merk, Germany). 5 µl of each extract were applied on the plate of 10 X 10 cm as bands of 10 mm width of each with the help of CAMAG linomat IV sample applicator. The plates were developed in a CAMAG twin-trough chamber previously equilibrated with a mobile phase for 20 minutes. The plate was developed up to 8 cm, air dried and documented at wavelength of 254 & 366 nm. Then the plates were derivatized with respective chemical reagents and heated at 105 ^{0}C on hot plate till the development of colour of bands and observed under white light and UV light at 366 nm and 254 nm.

Preparation of extract of the drug sample for HPTLC: The leaves of *Achyranthus aspera* Linn. were separated, washed thoroughly in distilled water and cut into small pieces. They were shade dried at room temperature. Dried leaf pieces were uniformly grinded using mechanical grinder to make fine powder. 0.5 g of powdered sample of leaf was sonicated with 5 ml. each of the 6 different solvents (Petroleum Ether, Chloroform, Acetone, Ethanol, Methanol and distilled water) for 10 minutes followed by filtration. Sonicated leaf extract was screened for non polar compounds and essential oil [23]. For compound determination 5µl. of each extract is applied as bands on different HPTLC plates of 10 X 10 cm dimension with

silica gel 60 f_{254} as stationary phase. Toluene, ethyl acetate (95:5) was used as mobile systems and bands were visualised at white light and uv light at 254 nm and 366 nm.

RESULTS

Pharmacognostic Studies

Organoleptic parameters: The leaf of *Achyranthes aspera* Linn. is dark green in color, with characteristic taste and with distinct odour.

Morphology: Leaves are simple, usually thick, subsessile, exstipulate, opposite, decussate, wavy margin, elliptic-obovate, petiolate and slightly acuminate. Petiole is very short and texture is pubescent due to the presence of thick coat of long simple hairs (trichomes).

Powder characteristics: Leaves are dark green in colour containing large number of uniseriate trichomes, shows fragments of elongated, rectangular, thin-walled epidermal cells, rosette and prismatic crystals of calcium oxalate, anomocytic stomata.

Physicochemical Characters: The physicochemical parameters of the drug such as total ash, water-soluble ash, acid insoluble ash, alcohol soluble matter and water-soluble matter (% w/w) were tabulated in table 1.

Fluorescence analysis of powdered drug: The flourescence analysis of the powdered drug upon treatment with different reagents and observation in UV short and long wavelength (254 & 366 nm.) regions and also in visible light showed corresponding colours in the solution as described in table 2 to 4.

Fluorescence analysis of powdered drug extracts in different solvents: The fluorescence analysis of powdered drug extracts carried out in different solvents and observed in UV short and long wavelength regions and also in visible light shown corresponding colours in the solution as described in the table 5.

Light Microscopy

T.S. of Leaf

Midrib: Shows a single layered epidermis, on both surfaces; epidermis followed by 4-5 layered collenchyma on upper side and 2-3 layered on lower side; ground tissue consisting of thin-walled, parenchymatous cells having a number of vascular bundles; each vascular bundle shows below the xylem vessels, thin layers of cambium, followed by phloem and a pericycle represented by 2-3 layers of thick-walled, non-lignified cells; rosette crystals of calcium oxalate found scattered in ground tissues.

Lamina: Shows single layered, tangentially elongated epidermis cells covered with thick cuticle having covering trichomes which are similar to those of stem found on both surfaces; mesophyll differentiated into palisade and spongy parenchyma; palisade 2-4 layered of thick parenchyma larger, slightly elongated in upper, while smaller and rectangular in lower surface; spongy parenchyma 3-5 layers thick, more or less isodimetric parenchymatous cells; idioblast containing large rosette crystals of calcium oxalate distributed in palisade and spongy parenchyma cells; stomata anisocytic and anomocytic in both surface; stomatal index 4.5-9.0 on upper surface, 9.0-20.0 on lower surface; palisade ratio 7.0-11; vein islet number 7-13 per sq. mm. (Figure 1-5)

Scanning electron microscopy: SEM analysis of leaves of herbal drug, *Achyranthes aspera* Linn. showed following features in its leaf powder:

Leaf surface with vein islets and stomata which are anisocytic (Plate no. 1), rosette crystals of calcium oxalate found on left side. Starch grains scattered (Plate no.2 and 3). Leaf surface with vein islets and stomata which are anisocytic, rosette crystals of calcium oxalate found on left side. Starch grains scattered (Plate no.4). Broken leaf surfaces, large no. of trichomes, rosette crystals of calcium oxalate, leaf surface with vein islets, stomata, non-glandular trichomes and prismatic crystals of calcium oxalate (Plate no.5 and 6).

HPTLC studies: Sonicated leaf extract which were screened for non polar compounds and essential oil

Plate 1: Leaf - Stomata

Plate 2: Leaf - Stomata

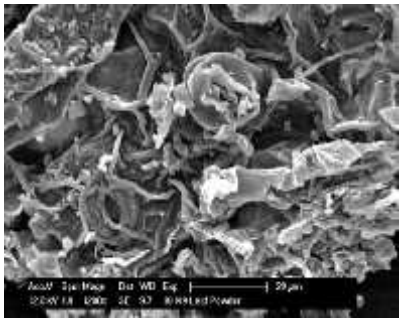

Plate 3: Stomata, starch grains

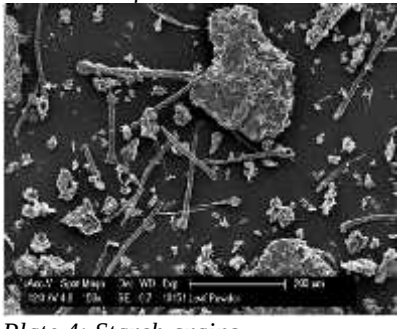

Plate 4: Starch grains

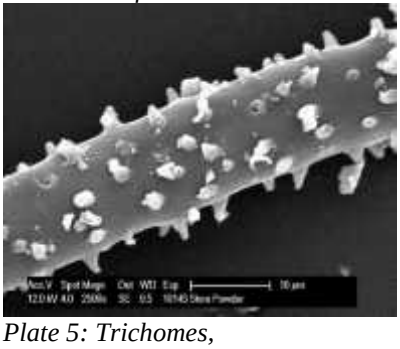

Plate 5: Trichomes,

Plate 6: prismatic crystals of calcium oxalate

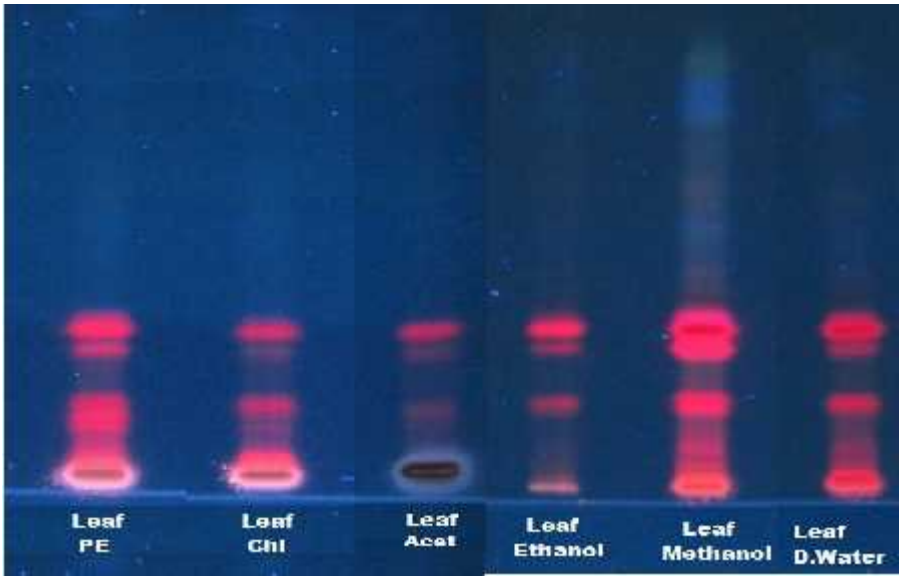

Plate 7: HPTLC chromatogram

Table 1: The physicochemical parameters of the powdered leaf

Sr. No.	Parameters	% content in leaf of *Achyranthes aspera* Linn.
1	Foreign organic matter	0.63
2	Total ash	9.0
3	Acid insoluble ash	1.2
4	Water soluble ash	4.7
5	Sulphated ash	6.3
6	Loss on drying	6.1
7	Crude fibre content	25.8

Table 2: Fluorescence characters of leaf powder of *Achyranthes aspera* Linn.

Sr. No.	Drug & Reagents	UV Light		Visible light
		Short (254 nm)	Long (366 nm)	
1	Leaf Powder as such	Black	Green	Dark green
2	Leaf Powder + 1N HCl	Black	Dark green	Light brown
3	Leaf Powder + Ammonia	Black	Dark green	Darkest green
4	Leaf Powder + Iodine	Darkest green	Black	Darkest green
5	Leaf Powder + Conc. HNO_3	Brown	Green	Reddish brown
6	Leaf Powder + dil. HNO_3	Green	Dark green	Yellowish brown
7	Leaf Powder + 1M H_2SO_4	Black	Blackish green	Dark green
8	Leaf Powder + 5% $FeCl_3$	Green	Black	Dark green
9	Leaf Powder + HNO_3 + 25% Ammonia	Dark green	Green	Yellowish green
10	Leaf Powder + 50% HNO_3	Yellowish green	Dark green	Brown
11	Leaf Powder + $K_2Cr_2O_7$	Black	Dark green	Dark green

showed best separation of bands at 366 nm without derivatization. Toluene, ethyl acetate (95:5) was used as mobile systems and bands were best visualised at 366 nm.

DISCUSSION

SEM is a powerful method for the investigation of surface structures of herbal medicines. This technique provides a large depth of field, which means, the area of the sample that can be viewed in focus at the same time is actually quite large.

Establishing standards is an integral part of establishing the correct identity and quality of a crude drug. Before any drug can be included in the pharmacopoeia, these standards must be established. The majority of the information on the identity, purity and quality of the plant material can be obtained from its macroscopy, microscopy and physico–chemical parameters.

SEM has also the advantage that the range of magnification is relatively wide allowing the investigator to easily focus in on an area of interest on a specimen that was initially scanned at a lower magnification. It produces a higher resolution compared to that possible using a light microscope, and the images obtained are three-dimensional and consequently this technique has been extensively used to investigate the surface topology of a wide variety of plant materials and can play a vital role in authentication of entire botanicals and those in fragmentized or in powder form. Furthermore, the tri-

Table 3: Fluorescence characters of the leaf extracts of *Achyranthes aspera* Linn. in different solvents.

Sr. No.	Solvent	UV Light		Visible light
		Short (254 nm)	Long (366 nm)	
1	Pet ether	Black	Green	Dark green
2	Chloroform	Dark green	Black	Black
3	Acetone	Black	Green	Dark green
4	Ethanol	Black	Dark green	Dark green
5	Methanol	Dark green	Black	Black
6	Water	Dark green	Green	Dark green

dimensional view images may be to an investigator it easier to interpret SEM images.

An HPTLC fingerprint is suitable for rapid and simple authentication. The HPTLC fingerprint developed may serve as a supplement chromatographic data and the information thus generated may be explored further as a tool for standardization.

CONCLUSION

The present work summarizes some important botanical microscopic characters of the leaf powder of herbal drug *Achyranthes aspera* Linn. These quality standards might be incorporated in quality control monographs for establishing the correct identity and quality of the crude drug.

The botanical control by microscopic examinations, using histological identification can be used as a rapid and inexpensive identification technique, but requires highly trained individuals and a limited standard references libraries are available for comparison.

HPTLC analysis revealed a better separation of individual secondary metabolites [24]. The plant can be used to discover bioactive products that may serves leads for the development of the new pharmaceuticals that address hither to unmet therapeutic needs. These plant derived bioactive compounds in addition of being developed directly as drugs can also served as prototype drug molecules known as "Lead Compounds" and as pharmacological probes to help better understand biochemical and physiological mechanisms [25].

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