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PHARMACOGNOSTICAL AND PHYTOCHEMICAL EVALUATION OF APAMARGA (*Achyranthes aspera* Linn.)

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ABSTRACT

Apamarga is well familiar since *Vedic kala* to the present era. It is used in various ailments as quoted by various lexicographic texts. In spite of having a confirmed botanical identity of *Apamarga* (*Achyranthes aspera* Linn*)* this plant is trapped as common weed throughout India. Any plant which is used medicinally requires detail study prior to its use because the therapeutic efficacy is absolutely depends on the quality of the plant drug used. Before using a drug it is very much essential to carry out its detailed pharmacognosy study and phytochemical study, as it is helpful for correct identification and provides clue for medicinal properties. Here in this paper macroscopic & microscopic study of root, stem, leaf and phytochemical analysis of *Apamarga* (*Achyranthes aspera* Linn*)* has been studied in detail to identify the genuine drug.

Keywords: *Achyranthes aspera* Linn, microscopy of root, stem and leaf phytochemical screening

INTRODUCTION

Apamarga is reputed as a best remedy for *Shirovirechana*.[1] It is useful in cough, asthma, bronchitis, dyspepsia, flatulence, colic, painful inflammations, opthalmopathy, skin diseases, cardiac disorders and renal and vesicle calculi.[2] It forms a chief ingredient of several important formulations like, *Apamargkshaar*[3], *Apamargkshaar Taila*,[4]and *Shankha Vati*[5] etc.

Apamarga and *Rakta Apamarga* are two varieties mentioned in *Bhavaprakash Nighanu* with different synonyms and properties. *Achyranthes aspera* Linn is accepted source of *Apamarga*.[6]

As per WHO norms, botanical standards are the proposed as a protocol for the diagnosis of the herbal drug. The phytochemical studies of drugs done by making use of various parameters help in standardizing the drug and authenticate it. It is expected an imminent need for a well coordinated research plan touching phytochemical study of drug like physiochemical analysis, HPTLC etc. The present study puts forth a set of anatomical parameter of root, stem, leaf and which can be employed to distinguish the original drug as mention in the classical *Ayurvedic* drugs from the other adulterants. This study throws light on the need to properly identify the plant species with their useful parts to achieve standardization of drug and *Ayurvedic* formulations.

MATERIALS AND METHODS

Pharmacognosy Study

Fresh green full-grown and healthy (*Achyranthes aspera* Linn.) plant was collected from its natural habitat. The plant was washed in pure water to remove all the impurities and was photographed. The leaf, root and stem are separated by cutting with a sharp blade. For stem and root cylindrical portion of almost straight and of sufficient length to hold the sample was selected. For leaf lamina, using a sharp blade, part of the leaf passing through the midrib was cut. Enough number of sections were taken. The sections were carefully transferred to a petridish containing water using a fine painting brush for selection of good sections. After staining, and mounting process the photographs of the sections were taken using digital camera.

Phytochemical analysis

Physio-chemical analysis Total ash[7], Acid Insoluble Ash[8], Water Insoluble Ash[9], Moisture Content[10], Volatile oil[11], Fiber Content[12],Tannin Content[13] were determined by using official methods. Results were mentioned in Table No. 1

HPTLC Accurately weighed 100mg of *Apamarga (Achyranthes aspera* Linn.) powder were refluxed with 100ml of methanol for 1hr separately and filtered using whatmann filter paper and made up to 100ml to get methanol extract at 1mg/ml. The stationary phase used was silica gel G 60 F. The mobile phase selected was Toluene: Ethylacetate: Diethylamine (7:2:1).The samples were applied at 6µl and 8µl. The plate was developed and dried for five minutes and was visualized under UV under 366nm, 254nm Fig No. 1

Qualitative Analysis of Crude Drug Table 2

Alkaloids

Dragendroff's test

To 0.5ml of alcoholic extract of *Apamarga* (*Achyranthes aspera* Linn) powder taken in a test tube, 2.0 ml of hydrochloric acid solution was added to it. To this acidic medium, 1.0ml of Dragendroff's reagent was added. An orange – red precipitate produced immediately indicates the presence of alkaloid.

Meyer's test

To 10 ml of the solution of alcoholic extract of *Apamarga* (*Achyranthes aspera* Linn*)* powder taken in a test tube, a few drops of Meyer's reagent was added. Formation of white or pale precipitate indicates the presence of alkaloid.

Flavonoids

To 0.5ml of the solution of alcoholic extract of *Apamarga* (*Achyranthes aspera* Linn) powder taken in a test tube, 5-10 drops of Dilute hydrochloric acid and a small piece of magnesium were added and the solution was boiled for few minutes. Presence of reddish pink colour indicates flavanoids.

Saponins

To 5ml of the solution of aqueous extract of *Apamarga* (*Achyranthes aspera* Linn*)* powder taken in a test tube, 1 - 3 drops of sodium bicarbonate solution was added. The mixture was shaken vigorously and kept for 3 minutes. A honey comb like froth formation in test tube indicates the presence of saponins.

Carbohydrates

Fehling's test

To 2ml of aqueous extract of *Apamarga* (*Achyranthes aspera* Linn) powder taken in a test tube, a mixture of equal parts of Fehling's solution A and B were added. The test tube was then boiled for few minutes. Formation of red or brick precipitate indicates the presence of carbohydrates.

Benedict's test

To 0.5ml of aqueous extract of *Apamarga* (*Achyranthes aspera* Linn*)* powder taken in a test tube, 5ml of Benedict's reagent was added and boiled for 5minutes. Formation of bluish green colour in test tube indicates the presence of carbohydrates.

Proteins

Ninhydrin test

To 1ml of the solution of aqueous extract of *Apamarga* (*Achyranthes aspera* Linn) powder taken in test tubes, 5ml of ninhydrin solution was added and heated in a boiling water bath for 2-3 minutes. Formation of blue or purple colour indicates the presence of proteins.

Phenols

Ferric Chloride test

To 1.0 ml of the solution of the alcoholic extract of *Apamarga* (*Achyranthes aspera* Linn) powder in a test tube, 2.0ml of distilled water was added followed by addition of a few drops of 10% aqueous ferric chloride solution. Formation of blue or green colour indicates the presence of phenols.

Lead acetate test

1.0 ml of the solution of the alcoholic extract of *Apamarga* (*Achyranthes aspera* Linn) powder taken in a test tube. 5ml distilled water was added followed by few drops of 1% aqueous solution of lead acetate. The formation of yellow precipitate in test tubes indicates the presence of phenols.

Steroids

To 2.0ml of the solution of chloroform extract of *Apamarga* (*Achyranthes aspera* Linn) powder taken in a test tube, 1.0ml of concentrated Sulphuric acid was added carefully along the sides of the test tube. A red colour was produced in the chloroform layer indicates the presence of steroids.

Tannins

Ferric chloride test

To 1-2ml of the solution of aqueous extract of *Apamarga* (*Achyranthes aspera* Linn) powder taken in a test tube, a few drops of 5% aqueous ferric chloride solution were added. A bluish black colour formed which disappeared on addition of diluted Sulphuric acid, forming a yellow brown precipitate indicates the presence of tannins.

Lead acetate test

To 5.0ml of the solution of the aqueous extract of *Apamarga* (*Achyranthes aspera* Linn) powder taken in a test tube, few drops of 1% solution of lead acetate was added. Formation of a yellow or red precipitate indicates the presence of tannins.

OBSERVATIONS AND RESULTS

Pharmacognosy Study

Macroscopic Features[16]

Root: Tap root cylindrical slightly ribbed upto 1.0 cm in thickness, rough due to presence of some root scars; secondary and tertiary roots present; yellowish-brown coloured.

Stem: Stem of the plant is erect, stiff, rather herbaceous, sometimes slightly woody at the base, not much branched; substrate and furrowed. Branches are more or less terete quadrangular, thickened above the nodes striated and pubescent.

Leaf: Leaves are simple, short-petioled, opposite, exstipulate, somewhat thick, membranous to leathery, velvety tomentose to pubescent, soft above ; variable in shape, orbicular – obovate, or elliptic abruptly atenuated at the base, very obtuse or shortly acuminate at tip, up to 10 cms, long by 7.5 cms broad and with the margin entire but, slightly wavy.

Microscopic Features

Histological Characters[17]

T.S. of Root: Fig No. 2

Periderm: Mature root shows 3-8 layered rectangular, tangentially elongated, thin-walled cork cells.

Cortex: Secondary cortex consisting of 6-9 layers, oval to rectangular, parenchymatous cells having a few scattered single or groups of stone cells. Small prismatic crystals of calcium oxalate are present in cortical region

Stele: Cortical area is followed by 4-6 discontinuous rings of anomalous secondary thickening composed of vascular tissues; small patches of sieve tubes distinct in phloem parenchyma, demarcating the xylem rings; xylem composed of usual elements. Vessels are simple and pitted. Medullary rays are 1-3 cells wide and small prismatic crystals of calcium oxalate are present numerously in medullary rays.

T.S of Stem: Fig No. 3

Transverse section of mature stem shows lignified, thin-walled cork cells and pericycle, discontinuous ring of lignified fibres. Young stem shows 6-10 prominent ridges, which diminish downwards up to the base where it becomes almost cylindrical.

Epidermis: Epidermis is single layered and covered by thick cuticle. Cuticle is having uniseriate, 2-5 celled, covering trichomes and they are glandular with globular head, 3-4 celled stalk

Cortex: 6-10 layered cortex is composed of parenchymatous cells, most of them containing rosette crystals of calcium oxalate. In the ridges cortex is collenchymatous. Here vascular bundles lie facing each ridge capped by pericyclic fibres

Vascular tissues: Vascular tissues show anomalous secondary growth having 4-6 incomplete rings of xylem and phloem; secondary phloem consisting of usual elements form incomplete rings.

Cambium: Cambial strip present between secondary xylem and phloem; secondary xylem consisting of usual elements, fibres being absent; vessels are annular, spiral, scalariform and pitted, fibres pitted, elongated and lignified

Pith: Pith is wide, consisting of oval to polygonal, parenchymatous cells; two medullary bundles, either separate throughout or found in some cases, present in pith.

T.S of Leaf : Fig No. 4

The transverse section of leaf passing through the midrib is broadly convex on the lower side and slightly grooved or flat on the upper side.

Epidermis: The dorsal and ventral surfaces are covered with single layered, rectangular cells of lower and upper epidermis respectively. Both the epidermal layers are covered with thick cuticle, traversed with stomata and bears simple and glandular trichomes of the same type as found on stem. Epidermis followed by 4-5 layered collenchyma on upper side and 2-3 layered on lower side

Mesophyll: Leaf lamina is occupied with mesophyll which is differentiated into palisade and spongy tissue. Both the surfaces of lamina contain 2-4 layers of palisade tissue. Palisade cells forms a thick parenchyma layer that is larger, slightly elongated in upper, while smaller and rectangular in lower surface. These layers are separated by spongy cells. Spongy parenchyma is 3-5 layers thick, more or less isodiametic parenchymatous cells. Large rosette crystals of calcium oxalate distributed in palisade and spongy parenchyma cells.

Vascular bundle: A vascular bundle is seen in the midrib. Here the ground tissue is 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.

Phytochemial Results

Table 1: Physico- chemical analysis of *Apamarga* (*Achyranthes aspera* Linn)

Sl no	**Experiments**	*Apamarga* [*Achyranthes aspera* Linn.]
1	Total ash	11.45%
2	Acid Insoluble Ash	5.5%
3	Water Insoluble Ash	6%
4	Moisture Content	9%
5	Volatile oil	1%
6	Fiber Content	34%
7	Tannin Content	0.11%

Table 2: Qualitative analysis of *Apamarga* (*Achyranthes aspera Linn*)

Experiment	*Apamarga* [*Achyranthes aspera* Linn.]
1) Alkaloids	
a. Dragendroff's test	Present
b. Meyer's test	Present
2) Flavanoids	Present
3) Saponins	Present
4) Carbohydrates	
a. Fehling's test	Present

b. Benedict's test	Present
5) Proteins	Absent
6) Phenols	
a. Ferric chloride test	Absent
b. Lead acetate test	Absent
7) Steroids	Absent
8) Tannins	
a. Ferric chloride test	Present
b. Lead acetate test	Present

DISCUSSION

Apamarga is one of the important drugs used in the various indigenous medicines and formulations of Ayurveda. Detailed pharmacognostical study of *Achyranthes aspera* Linn was decided to undertake to bring out the salient diagnostic features which would help in crude drug identification as well as standardization of the quality and purity of the drug in crude form. The following anatomical features are suggested to diagnose root, stem and leaf

Root: Cork cells are 3-8 layered, rectangular, tangentially elongated, thin-walled; cortex consisting of 6-9 layers, oval to rectangular, parenchymatous cells having a single or groups of stone cells

Stem: Epidermis is single layered and covered by thick cuticle having glandular trichomes; cortex is composed of 6-10 layered parenchymatous cells, most of them containing rosette crystals of calcium oxalate; in the ridges cortex is collenchymatous; Pith is wide, consisting of oval to polygonal, parenchymatous cells

Leaf: Both the epidermal layers are covered with thick cuticle, traversed with stomata and bears simple and glandular trichomes.

While observing the physiochemical analysis, total ash represents the inorganic salts in the drug. Thus ash value is a general criterion to ascertain the purity of any drug. Here a total ash value was found as 11.45%. Acid insoluble ash gives the percentage of sand and impurities that remains insoluble in dil.HCl and it was found to be 5.5%. Lower the value higher the purity of the drug. Water insoluble ash mainly gives the percentage of organic matter present in the ash of the drug. It was found to be 6%. Moisture content was found to be 9% and the less amount of moisture indicates the proper drying of the materials. Volatile oil content was observed 1%. Fiber content was found to be 34%.Tannin content was found as 0.11%. The following HPTLC fingerprint profiles are suggested to diagnose *Achyranthes aspera Linn* methonolic extract under UV366nm, it can be identify by the spot at Rf 0.74, Rf 0.89. The different extractive solution of crude drug powder of *Apamarga* showed the presence of alkaloids, saponin, carbohydrate, phenol, steroid, tannin.

CONCLUSION

This study on macroscopic and microscopic features of *Achyranthes aspera* Linn revealed set of anatomical parameters which may facilitate identification of genuine drug. Preliminary phytochemical study was also carried out and their details are mentioned along with the results, observation obtained in the experiments. These parameters help in standardizing the drug and give us an idea of phytochemistry of plant.

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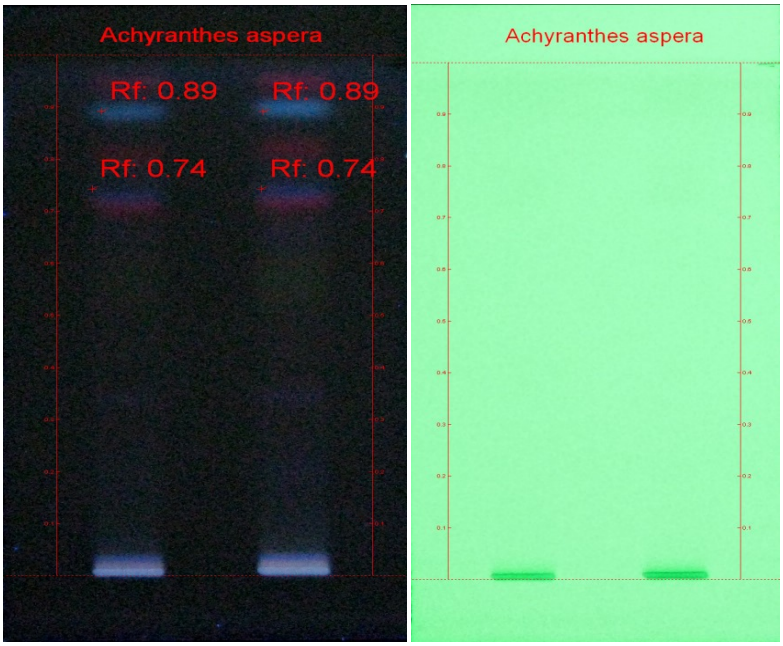

366nm **254nm**

Fig. 1: HPTLC of powdered *Apamarga* (*Achyranthes aspera* Linn.)

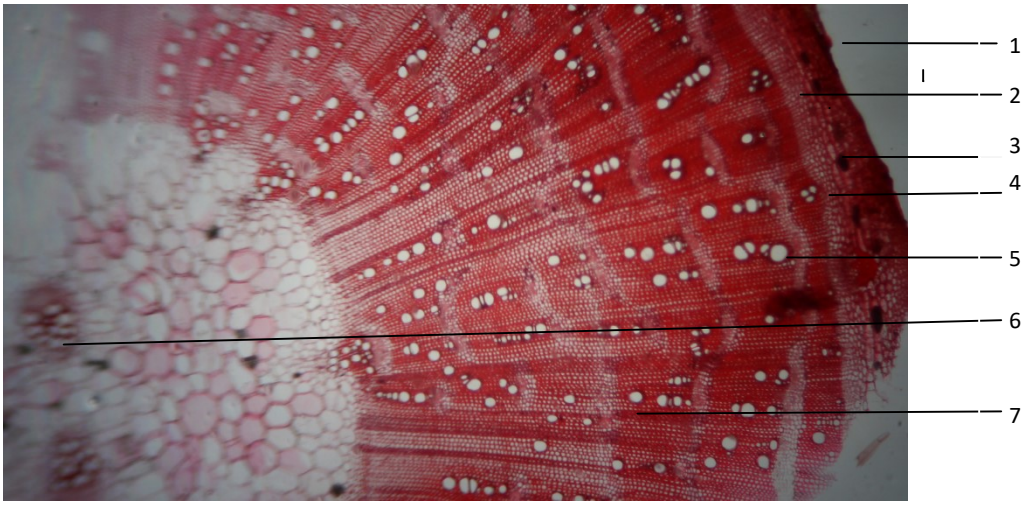

Fig. 2 T. S. of root of *Achyranthes aspera* Linn.

1. Periderm, 2. Secondary cortex, 3. Stone cells, 4. Phloem, 5. Xylem, 6. Vascular bundle, 7. Medullary ray

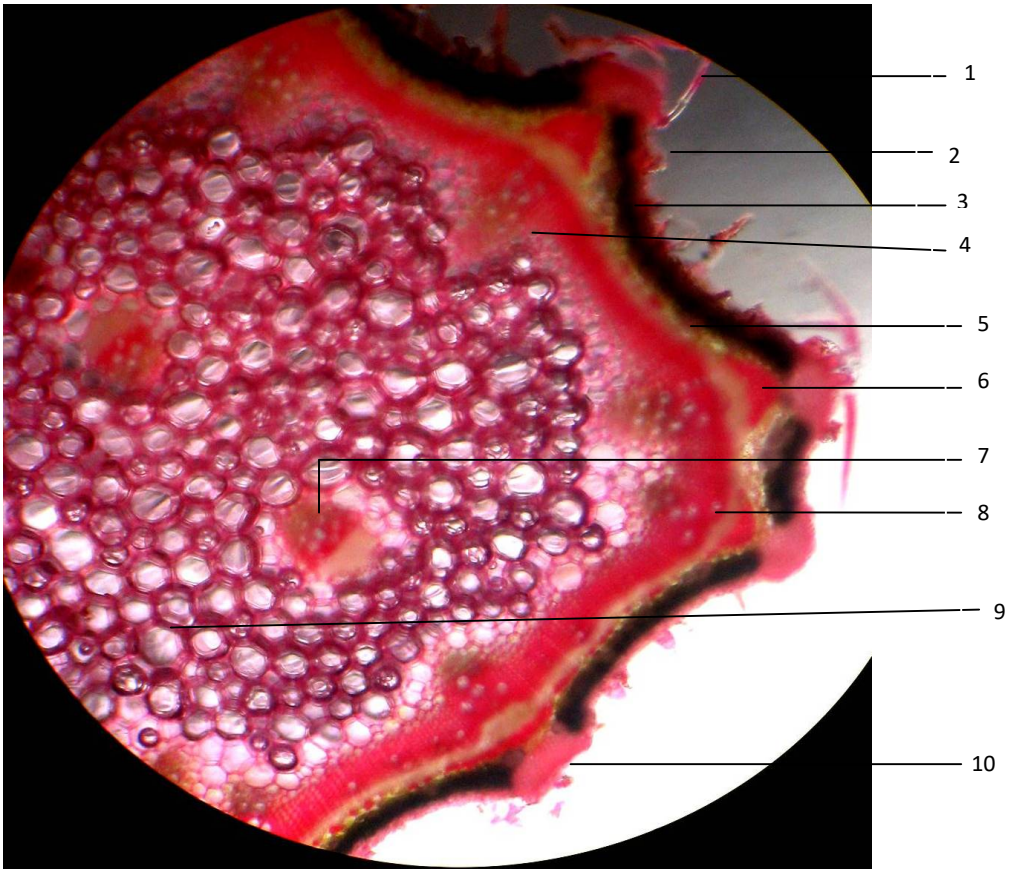

Fig. 3 T. S. of stem of *Achyranthes aspera* Linn.

1. Trichomes 2. Epidermis 3. Chlorenchyma 4. Xylem 5. Parenchyma 6. Lignified cells 7. Vascular bundle 8. Phloem 9. Pith 10. Collenchyma

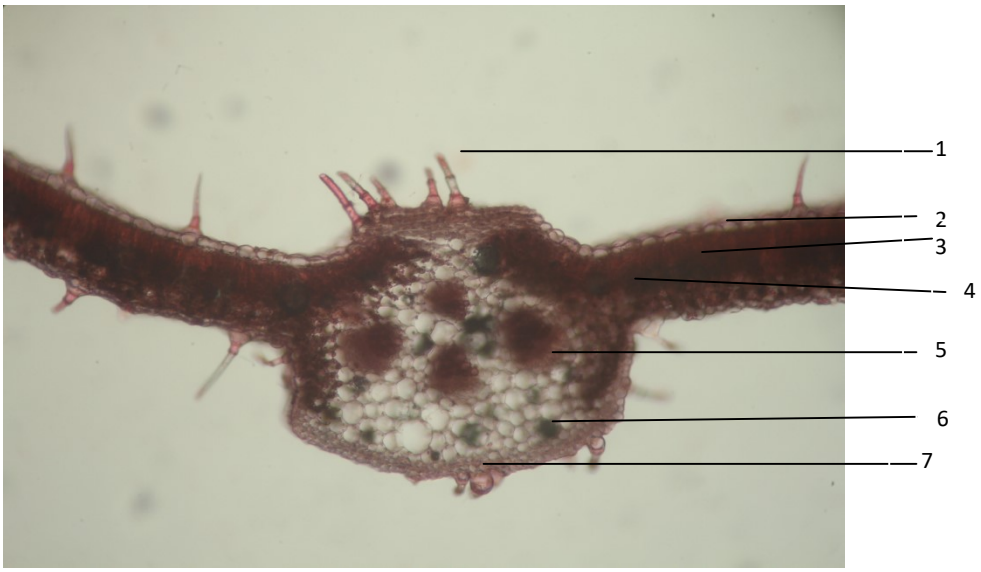

Fig. 4: T. S. of Leaf of *Achyranthes aspera* Linn.

1. Trichomes 2. Upper epidermis 3. Palisade tissue 4. Spongy tissue 5. Vascular bundle 6. Stone cells Collenchyma.

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