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Pharmacognostic Studies of Apamarga Beeja and Patra (*Achyranthes aspera* Linn.)

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ABSTRACT

Apamarga is one of the commonly used herbs in India. It is also extensively used in the Indian systems of medicines i.e. Ayurveda in the treatment of many diseases like arsha, dadru, kandu, shula etc. The scientific name of Apamarga is Achyranthes aspera Linn., belongs to the family Amaranthaceae. Morphologically seed was pale green while seed was pale brown. Microscopically leaf showed epidermis and collenchymal cells. In present study the detailed pharmacognostic study of Achyranthes aspera Linn. seed and leaf were carried out to lay down the standards which could be useful in future experimental studies. The study includes macroscopy, microscopy, preliminary phytochemical screening and physico-chemical evaluation.

KEYWORDS : *Achyranthes aspera* Linn., Pharmacognosy, Microscopy

Introduction:

Herbal medicines are promising choice over modern synthetic drugs. They show minimum or no side effects and are considered to be safe. Generally herbal formulations involve use of fresh or dried plant parts. Correct knowledge of such crude drugs is very important aspect in preparation, safety, and efficacy of herbal products. Pharmacognosy is a simple and reliable tool by which complete information of the crude drug can be studied. [1] *Apamarga* is extensively used in the Indian systems of medicines i.e. *Ayurveda* in the treatment of many diseases like *arsha, dadru, kandu, shula* etc. [2] The scientific name of *Apamarga* is *Achyranthes aspera* Linn., belongs to the familyAmaranthaceae. [3]*Achyranthes aspera* Linn. is commonly known as uttarani (in kannada). Seed and leaf were selected for the present pharmacognosy study.

Achyranthes aspera Linn. is found throughout tropical Asia, Africa, Australia and America. An abundant weed in dry places and wastelands, from the seashore to 2,100 m high. Stem: erect, 0.5-2.0 m in high, base woody, angular or ribbed, simple or branched, often tinged with pink colour; nodes bulged. Leaves: opposite, petiolate, ovate-elliptic-obovate-rounded, in various sizes, apex usually rounded, finely or softly pubescent on both sides. Flowers: In an auxiliary or terminal spike, which is more than 50 cm in long, greenish white, bracteate and bracteolate? Perianth: lobes 4-6, glabrous, shining, ovate-oblong and pointed. Stamens: 5 in number, staminodesare truncate, fimbriate, ovary oblong, sub-Compressed and ovule solitary. Fruit: easily disarticulates, oblong or ovoid and utricle. Seeds: inverse, testa coriaceous, embryo annular and surrounded by floury albumin. Inflorescences: elongated or condensed. Spikes: (heads), racemes, or thyroid structures of varying complexity. Bracteoles membranous or scarious. [4]

In present study pharmacognostic standards of the *Apamarga* seed and leaf were studied. These standards are of upmost importance not only in finding out genuinity, but also in detection of adulterants in marketed drug.

Materials and Methods:

The leaves & seeds of *Apamarga* were collected in Hubli and Belgaum, Karnataka, where it is abundantly available. The leaves and seeds of *Apamarga* were authenticated from Botanical survey of India Pune. Aqueous & alcoholic extractions of leaves & seed samples of *Apamarga* were carried out in central laboratory B.M.K. *Ayurveda* Mahavidyalaya Belgaum, Karnataka.

Macroscopic Characters:

The leaf and seed were examined by naked eye to observe the morphological characters**.**

Microscopic: [5]

Leaf:

A young fresh leaflet of *Achyranthes aspera* Linn. was selected for section cutting 2 x 2 cm part of leaflet passing through midrib, from the middle of the leaflet is taken. The piece if leaflet was embedded in sample block of potato pith, for that 2 cm x 3 cm block of potato pith is prepared and a vertical cut up to 2/3rd height was made at the center of that pith. Cut piece of leaflet was inserted in that slit of pith and the extra portion of leaf was cut off. The sections of surface were taken out by moving the razor blade back and forth. The obtained sections were stained with safranin by standard technique of staining. A fine T. S. was selected for observation and washed under tap water and mounted on a slide to observe the different features under microscope.

Seed:

A young fresh persistent bracteole of *Achyranthes aspera* Linn. was selected for section cutting. The bracteole was removed & seed was collected. Cut piece of seed was inserted in that slit of pith and the extra portion of seed was cut off. The sections of surface were taken out by moving the razor blade. The obtained sections were stained with safranin by standard technique of staining. A fine T. S. was selected for observation and washed under tap water and mounted on a slide to observe the different features under microscope. Phytochemical studies and HPTLC were carried as per standard methods.

Results:

The drug was authenticated by a botanical expert and then pharmacognostic study of the drug samples i.e. macroscopic and microscopic were carried out to study its morphology, internal structures and their contents in detail.

Morphological characters:

The plant *Achyranthes aspera* Linn. is a small herb; 0.5-1 meter high irregular trunk, stem and branches covered with thin grey bark & thick at the end. Leaves: small, elliptic or ovate, 6-8cm long, 5-8cm broad, soft & hairy. The leaves are whitish on the dorsal side. Flowers: greenish white in colour & appear in inflorescence. Fruits: thin, elliptic & greyish. Simple dry indehiscent caryopsis. Seed: sub-cylindric, truncate at the apex, round at the base, endospermic, brown (Table 1)

Seed morphology:
Colour: Pale yellow to brown
Shape: Oval /oblong
Size: 1 to 4 mm, 1mm-width
Odour: Odourless
Taste: Acrid

Microscopic study:
T. S. of *Achyranthes aspera* Linn. leaf through midrib:

T. S. at the midrib shows a single layered epidermis composed of thick walled cubical cells. The upper epidermal layer is followed by 1-2 rows of hypodermis which in turn continuous with collenchymatous cells. A large number of collenchyma cells are present and it shows 12-15 xylem elements, surrounded with transcurrent parenchyma. T.S. of seed shows following characters.

1) Epidermis
2) Endosperm: Hard cell, thick walled
3) Embryo: With vascular bundle.

Table No: 1 Macroscopic Character:

Characters	Leaf	Seed
Colour	Pale green both side	PaleBrown
Upper surface	Hairy	---
Lower surface	Tomentose& pale green.	---
Venation	Reticulate	---
Size	The middle leaflet largest in size 8 to 10 cm long, 4 to 5 cm long Petiole at the base	4mm-length, 1mm-width
Margin	Entire slightly wavy	---
Shape	Ovate shape	Sub-cylindrical
Touch	Slightly hairy smooth	Smooth

PHYSICO-CHEMICAL STUDY:
Then the physico-chemical screening was done i.e. Ash value, Acid insoluble ash, alcohol and water soluble extractives values were determined. The ash values of all samples were within normal ranges as provided by herbal pharmacopoeia. This represents the inorganic salts present in drug like carbonates, bicarbonates etc. In this procedure the carbon is removed by heating at low temperature (Tables 2-9).

Table No. 2*:* Total ash value of *Patra:* [6]

Sr.no	Reference	Observed value	Standard Values
1.	*Ayurveda* Pharmacopeia	15.55%	Not more than 17%

Table No.3: Acid Insoluble Ash of *patra*

Sr.no	Reference	Observed value	Standard Values
2.	*Ayurveda* Pharmacopeia	4.23%	Not more than 5%

Table No. 4:Totalash value of *Beeja*

Sr.no	Reference	Observed value	Standard Values
3.	*Ayurveda* Pharmacopeia	4.74%	Not more than 17%

Table No.5: Acid Insoluble Ash of *Beeja*

Sr.no	Reference	Observed value	Standard Values
4.	*Ayurveda* Pharmacopeia	0.563%	Not more than 5%

Table No.6: Water Soluble Extractive Value of *Beeja*

Sr.no	Reference	Observed value	Standard Values
5.	*Ayurveda* Pharmacopeia	13.28%	Not less than 12%

Table No7: Water Soluble Extractive Value of *Patra*

Sr.no	Reference	Observed value	Standard Values
6.	*Ayurveda* Pharmacopeia	14.6 %	Not less than 12%

Table No.8:Alcohol (Ethanol) Soluble Extractive Value of *Beeja*

S.no	Reference	Observed value	Standard Values
07	*Ayurveda* Pharmacopia	2.28%	Not less than 2%

Table No 9:Alcohol (Ethanol) Soluble Extractive Value of *Patra*

S.no	Reference	Observed value	Standard Values
08	*Ayurveda* Pharmacopeia	3.3 %	Not less than 2%

Table No10: Yield of Extracts
A) *Patra*

S.No.	Solvent	Weight of Sample in gms	Weight of extract obtained (w/w)	Yield in gm %
1.	Ethanol	45	10.097	22.4
2	Water	100	24	24

B) *Beeja*

Sl.No.	Solvent	Weight of Sample in gms	Weight of extract obtained (gms w/w)	Yield in gm %
1.	Ethanol	45	8.024	17.83
2	Water	100	19	19

Acid insoluble ash values were also nearby standard reading. It's done to rule out presence of excess / foreign particle (i.e. other than the drug part) i.e. sand or silica etc, which may not be absorbed in acid media in body and may give rise to complications. In alcohol soluble and water-soluble extractive values are types of analytical pilot study done. To check the soluble chemical compound in the drug, and to get an idea regarding the yield of extract, this can be acquired by doing extraction on larger scales. By this the organic / inorganic compounds, which are needed for a particular therapeutic use can be processed by using a particular solvent / solvent system. Ethanol and Water-soluble extracts in that water yield is maximum yield in both the parts of the plant i.e. leaves and seed; when compared to yields given by ethanol it. In Water Extractive Value of leaf is 24% and Ethanol is 22.4 %. Whereas the extractive values of seed obtained with water is 19% and with ethanol it is 17.83%. Then the extraction procedures were carried out by using solvents like Ethanol. The procedure adopted was soxhelet extraction and cold maceration procedures. Ethanol was selected; since it is standard during the new drug extraction. The ethanol for this study was prepared in laboratory by distilling rectified spirit. Then the procured Ethanol's percentage (95%) was clarified by feasible laboratory method. (Table no 10)Then preliminary phyto-chemical screening was done, that reveals the presence of alkaloids, tannins, phenolic compound and alkaloid in leaves where extracts of seeds indicated the presence of carbohydrates, glycoside, tannins and phenolic compounds and alkaloids.(Table 11)

Table No: 11 Preliminary Phyto-Chemicals Screening: [7]

S. No	Tests	Patra		Beeja	
		Ethanol	Aqueous	Ethanol	Aqueous
1	Test for carbohydrates Molish's test	- ve	- ve	+ ve	+ ve
2	Test for reducing sugars Fehling's test	- ve	- ve	+ ve	- ve
3	Test for monosaccharides Barfoed's test	- ve	- ve	- ve	- ve

4	Test for hexose sugars Cobalt chloride test	- ve	- ve	- ve	- ve
5	Test for non-reducing polysaccharides (starch) Iodine test for starch	+ ve	+ ve	+ ve	+ ve
6	Test for proteins Biuret's test	- ve	- ve	- ve	- ve
7	Test for aminoacids Ninhydrin test	- ve	- ve	- ve	- ve
8	Test for steroids Salkwoski reaction	- ve	- ve	- ve	- ve
9	Test for glycosides				
	a) Test for cardiac glycosides	- ve	- ve	- ve	- ve
	b) Test for saponin glycosides	- ve	- ve	+ ve	+ ve
10	Test for Flavonoids				
	a) Shinoda test	- ve	- ve	- ve	- ve
	b) Lead acetate solution test	- ve	- ve	- ve	- ve
	c) NaOH + acid	- ve	- ve	- ve	- ve
11	Test for tannins & phenolic compounds				
	a) 5% $FeCl_3$ solution	+ ve	+ ve	+ ve	+ ve
	b) Lead acetate solution	+ ve	+ ve	+ ve	+ ve
	c) Acetic acid solution.	+ ve	+ ve	+ ve	+ ve
12	Test for alkaloids				
	Wagner's test	+ ve	- ve	+ ve	- ve
	Mayer's test	+ ve	- ve	+ ve	- ve
	Hager's test	+ ve	- ve	+ ve	- ve

Qualitative analysis of the plant extracts was done by some instrumental techniques i.e. T.L.C. and H.P.T.L.C. By this test in observation, it showed presence of various compounds that were detected by their Rf values. These Rf values can be directly or indirectly help for checking adulteration in raw or compound drug.

In T.L.C 5 gm of extract of samples was diluted to 25 ml of respective solvents and used for T.L.C. bands were observed at 254 nm and at 366 nm The Rf values found were common in Ethanol extracts and water extracts, i.e. 0.27, 0.39, 0.41, 0.58 and 0.87.(Table 12,13)

Table No 12: TLC/HPTLC Details:

Solvent System (Mobile Phase)
Chloroform: Ethyl Acetate: Formic Acid
5 : 4 : 1
Plate: preparative
Size: 10x10
Stationary phase: Silica gel GF 254

HPTLC Analysis:

Table No.13 UV Analysis of ethanol and aqueous extract of apamarga

Plant name	Amount of extraction plate (µg)	@ UV 254 nm	@ UV 366 nm
Apamarga Patra (Achyranthes aspera Linn. Leaves)			
a) Ethanol extract	500µg	0.27, 0.87	0.27, 0.34, 0.81, 0.89
b) Aqueous extract	500µg	0.27, 0.87	0.18, 0.27, 0.34, 0.36, 0.47. 0.87
Apamarga Beeja (Achyranthes aspera Linn. Seeds)			
a) Ethanol extract	500µg	0.39, 0.58	0.18, 0.69, 0.87
b) Aqueous extract	500µg	0.41, 0.58, 0.87	0.75, 0.87

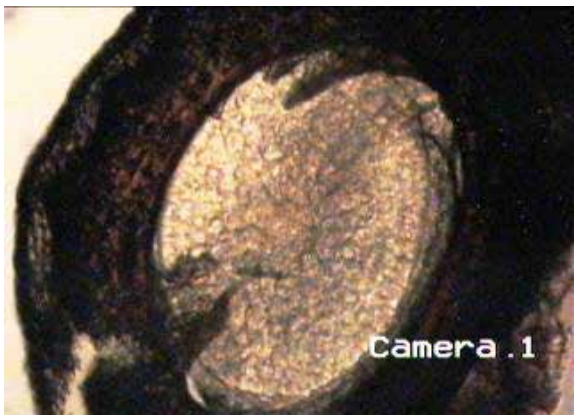
Remarks:

After derivatisation with the iodine there were 15 prominent bands were visible and the most Common compound which occurs in all the extracts are of Rf value 0.27, 0.39, 0.41, 0.58 and 0.87. In chromatographic profile, (finger print), it is necessary to record the absorbance spectra of the entire fraction. When comparing three extracts in finger printing approach it is necessary that Rf and peak area / heat of reference and sample should be matched. Secondly the spectra also must match.

(Image no: 1 *Apamarga*whole plant): [8]

(Image no: 2 *Apamarga*seed husk): [9]

(Image no: 3 T.S. of *Apamarga* Leaf)



(Image no: 4 T.S. of *Apamarga* Seed)

Discussion and Results:

Morphological and anatomical studies of the *Apamarga* leaf and seed powder will enable to identify the crude drug. Extraction process showed more active ingredients extracted in the water, compared to alcohol extraction. It indicates water soluble components are more in the *Apamarga*. The information obtained from preliminary phytochemical screening will be useful in finding out the genuinity of the drug. Phytochemically the seed and leaf were found to contain alkaloids, carbohydrates, glycosides, tannins etc. Ash value, extractive values can be used as reliable aid for detecting adulteration. The physico-chemical results i.e. ash value, acid insoluble ash, alcohol and water-soluble extractive values are within limits of Ayurvedic pharmacopoeia.

Apamarga Leaves and seed yield was more in Ethanol than the aqueous extract. The preliminary phyto-chemical screening of Ethanol and water extracts show the presence of alkaloids, flavonoids and traces of Glycosides. TLC and HPTLC possess some common Rf values found in Ethanol extract samples which represents similar compounds in both extracts. Thus, one part can be substituted in absence of the other. Here Rf values which are similar, matches with the peak areas denoted in the graph and spectrum also.

Conclusion:

The physico-chemical results i.e. ash value, acid insoluble ash, alcohol and water-soluble extractive values are within limits of Ayurvedic pharmacopoeia. *Apamarga* Leaves and seed yield was more in Ethanol than the aqueous extract. The preliminary phyto-chemical screening of Ethanol and water extracts show the presence of alkaloids, Flavonoids and traces of Glycosides. TLC, HPTLC possess some common Rf values found in Ethanol, extract samples which represents similar compounds in both extracts. Thus, one part can be substituted in absence of the other. Here Rf values which are similar, matches with the peak areas denoted in the graph and spectrum also.

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