



E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2017; 6(5): 394-398
Received: 17-07-2017
Accepted: 18-08-2017

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Phytochemical & Pharmacognostical Profile of *Achyranthes aspera*

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Abstract

The plant *Achyranthes aspera* is widely used in Ayurvedic system of medicine as Antidiabetic, Antiasthmatic, diuretic, Antiviral, Anticarcinogenic, toothbrush, appetizer and cure gastric disorders. Roots are used in treatment of snake bites. The present study deals with the Pharmacognostical & Phytochemical study of the plant "*Achyranthes aspera*" for its identification and to distinguish it from the co-existing weeds and adulteration. In the present investigation, microscopical characters are evaluated and different parameters are applied for the physico-chemical studies include evaluation of colour, consistency of different extracts, extractive value, ash value, moisture content, fluorescence analysis and also qualitative phytochemical screening was performed. Since there is no proper information regarding this plant, our efforts were devoted to study the pharmacognostical and phytochemical properties of this plant. Thus, present study revealed the plant extract contains different chemical constituents like alkaloid, phenolic compound, saponin, flavonoid, proteins & amino acids. So this study was undertaken for proper identification, collection and investigation of the plant.

Key Words: *Achyranthes aspera*, Microscopic Charactersistics, Powdered drug & Extract, Phytochemical screening, Fluorescence analysis.

1. Introduction

Various species of *Achyranthes* are used medicinally in India, China and Bangladesh whereas *Achyranthes aspera* [synonym- apamarga, chirchira, chaff flower. Family- Amaranthaceae] is naturalized throughout the hot and moist parts of India^[17]. The leaves used for Gonorrhoea, Bowel complaint, stomachic, piles, skin eruptions^[1], diuretic^[2], Antiperiodic^[3], Antimicrobial^[4], Anti-inflammatory^[5], Hypoglycemic effect^[6] and Anticarcinogenic effects^[7]. Roots are used in pneumonia, as an astringent to bowels, menstrual disorders, stomachic^[8] and reported to have antifertility activity^[9]. Chemically, *Achyranthes aspera* contains Triterpenoid saponins posses oleanolic acid (0.54%), aglycone A,B,C and D, ecdysterone, long chain alcohol, 17-penta triacontanol, water soluble base betaine and enzyme level are isolated. Two long chain compound isolated from shoots have been characterized as 27-cyclohexylheptacosan-7-ol and 16-hydroxy-26-methylheptacosan-2-on by chemical a spectral investigations^[10]. A fresh leaves are used very effectively for the treatment of snake bites, dog bites and diarrhoea in folk medicines¹¹. Herbal drugs have a great growth potential in the global market because of their wide biological activities and purported higher safety margin then the synthetic drugs and lesser costs. India has a reach history of using plants for medicinal purposes. Pharmacognosy is a simple and reliable tool, by which complete information of the crude drug can be obtained^[1-4]. In Pharmacognosy, to overcome quality problems of herbal drugs, it is almost predictable to standardize the drugs for their rational therapeutic use. A disease cannot be managed comprehensively until the delivery of genuine samples of drug is ensured^[5]. Therefore, in the present study, the preliminary phytochemical screening of the plant "*Achyranthes aspera*" was carried out. The parameters applied in the present study makes valuable test to check the quality of drug.

2. Materials & Methods

The plant *Achyranthes aspera* was collected from the dumpy field of Ambikapur from the revenue district of Sarguja, in the state of Chhattisgarh. Then subsequently for more conformation the herbarium sheet of plant was authenticated by the Ex-Director (Farm Forestry), Dr. M. L. Naik of University Teaching Department, Sarguja University, Ambikapur, Sarguja (C.G.) with the reference no. SUA/UTD/17/39. Few authentic samples were preserved in our department for future reference.

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a. Pharmacognostical Studies

Morphological studies were done with simple microscope. The shape, size, apex, surface, base, margin, venation, colour, odour, taste of leaf, flower, seed, petiole, root were determined. Microscopically, studies were done by preparing a thin hand section of the leaf part, petiole part, root part and stem part. Microscopical studies were carried out as per the standard method [15]. Thin section of about 10-12µm thick were prepared and cleared with 5% NaOH and stained with phloroglucinol and hydrochloric acid and mounted in glycerine and observed under microscope. The powdered drug was separately treated with phloroglucinol-HCl solution and mounted in glycerine for microscopical evaluation [16]. (Figure no.2,3,4,5) Leaf constants such as stomatal index, vein islet number, vein termination number and palisade ratio were determined by using fresh leaves of the plant. Standard procedures were followed for all the evaluations [17]. All the chemicals and solvents used in experiment were of analytical

grade. (Table. 1)

Table 1: Quantitative Microscopy of Leaves of *Achyranthes aspera*.

S. No.	Leaf Constants	Value
1	Stomatal Index	16.73
2	Vein-islet number	19
3	Vein termination number	12
4	Palisade ratio	49

b. Fluorescence Analysis of powdered Drug under Ultra-violet Light

The plant of *Achyranthes aspera* were collected and dried in the shade and then pulverized in a grinder. Powdered drug was screened for fluorescence characteristics with and without chemical treatment. The observations pertaining to their colour in day light and under ultra-violet light (short & long) were recorded. (Table 2) [18].

Table 2: Fluorescence Characteristics of Powdered drug with different reagents.

S. No.	Reagent + Drug	Colour of powder at Day light	UV Light Short	UV Light Long
1	Untreated Powder	Buff Green	Green	Black
2	Powder + Nitric Acid	Brown	Green	Black
3	Powder + Saturated Picric Acid	Yellowish green	Light Green	Black
4	Powder + 1N HCl	Brown	Dark Green	Black
5	Powder + Glacial acetic Acid	Reddish Brown	Brown	Dark Green
6	Powder + Conc. H ₂ SO ₄	Deep Brown	Greenish Black	Black
7	Powder + 1N NaOH	Yellowish Brown	Dark Brown	Black
8	Powder + Ferric Chloride	Yellowish Green	Dark Green	Black
9.	Powder + Iodine	Reddish Brown	Black	Black

c. Physiological Parameters

Physicochemical parameters of the powdered crude drug such as loss on drying, total ash, acid-insoluble ash, water soluble ash, alcohol soluble extractive value, water soluble extractive value for the plant *Achyranthes aspera* were performed according to the standard methods [19-22] and recorded in the table. (Table 3)

Table 3: Physiological Parameters of *Achyranthes aspera*.

S. No.	Parameters	Mean Percentage (w/w)
1	Loss on Drying	7.7
2	Total ash Value	4.1
3	Acid-insoluble ash Value	1.7
4	Water soluble ash Value	2.6
5	Alcohol soluble extractive Value	6.9
6	Water soluble extractive Value	9.3

d. Phytochemical Screening

The powder of the air dried entire plant *Achyranthes aspera*, weighing about 100gm was successively extracted in Soxhlet apparatus with the solvents of increasing polarity such as petroleum ether (60° – 80°C), chloroform, acetone, methanol & water. The extracts were dried using rotary evaporator and percentage extractive value was determined. The dry extracts were screened for the presence of various phytoconstituents / secondary metabolites responsible for the therapeutic values of the drug like presence of alkaloids, glycosides, carbohydrate, tannins – phenolic compounds, proteins & amino acids, gums & mucilage, flavours & flavonoides, saponins and steroids & Sterols etc [13, 14, 23, 24]. The resulting data were recorded in the table. (Table 4)

Table 4: Preliminary Phytochemical Screening of plant *Achyranthes aspera*.

S. No	Phytochemical Test	Petroleum ether extract	Chloroform extract	Ethyl acetate extract	Methanolic extract	Water extract
I	Test for Alkaloids					
a	Mayer's Test	–	+	–	+	+
b	Wagner's Test	–	+	+	+	+
c	Hager's Test	+	+	+	+	+
d	Dragendroff's Test	–	–	–	–	–
II	Test for Carbohydrates and Glycosides					
a.	Molisch's Test	–	–	–	–	–
b.	Fehling's Test	–	–	+	+	–
c.	Barfoed's Test	–	–	–	–	–
d.	Benedict's Test	–	–	–	–	–
e.	Borntrager's Test	–	–	–	–	–
f.	Legal's Test	–	–	–	–	–
III	Test for Saponins					
	Foam Test	+	+	+	+	+

IV Test for Proteins and Amino acids						
a.	Millon's Test	-	-	+	+	+
b.	Biuret Test	-	-	-	-	-
c.	Ninhydrin Test	-	-	+	+	-
V Test for Phytosterols						
a.	Liebermann Burchard's Test	-	+	+	+	-
VI Test for Gum and Mucilages						
a.	Alcohols 95% Test	-	-	-	-	-
VII Tests for Phenolic Compounds and Flavonoids						
a.	Ferric chloride Test	-	+	-	+	-
c.	Lead acetate Test	+	+	-	+	+
d.	Alkaline Test	+	+	-	+	+

- Negative; + positive

e. Successive Extraction with Various Solvents

The plant was collected and dried in the shade and then pulverized in a grinder. The powdered drug was utilized for extraction. Material was passed through 120 meshes to remove fine powders and coarse powder was used for extraction. Successive extraction was carried out with Soxhlet using different solvents like Petroleum ether (60⁰ – 80⁰C), Chloroform, Acetone, Methanol & Water as per their polarity

successively. The extract was dried using rotary evaporator and was kept in a dessicator till experimentation. Obtained extract was weighed and percentage yield was calculated in terms of air-dried powdered crude [19-22]. The percentage yield of different extracts of powdered drug of *Achyranthes aspera* and their colour, consistency were reported in the table. (Table 5).

Table 5: Extractives values of different extracts of *Achyranthes aspera* powdered drug.

S. No.	Type of Extract	Colour	Odour	Consistency	Extractive Value (w/w)
1	Pet-Ether Extract(60 ⁰ – 80 ⁰ C)	Buff Green	Characteristic	Greasy	0.63 %
2	Chloroform Extract	Dark Green	Characteristic	Sticky	1.07%
3	Acetone Extract	Brown	Characteristic	Sticky	1.61%
4	Methanolic Extract	Deep Brown	Characteristic	Greasy	2.37%
5	Water extract	Black	Characteristic	Greasy	4.84%

f. Fluorescence Analysis of drug extract under Ultra-violet Light

Extract of *Achyranthes aspera* was screened for fluorescence analysis. The observations pertaining to their colour in day light and under ultra-violet light (short wave length & long wave length) were recorded [25]. (Table 6)

Table 6: Fluorescence Characteristics of Different Extract of *Achyranthes aspera*.

S. No.	Types of Extract	Day Light	UV Light Short	UV Light Long
1	Petroleum Ether Extract	Light Green	Green	Dark Green
2	Chloroform Extract	Green	Dark Green	Black
3	Acetone Extract	Brown	Deep Brown	Black
4	Methanolic Extract	Brown	Deep Brown	Black
5	Water Extract	Black	Black	Black



Fig 1: *Achyranthes aspera* Entire Plant.

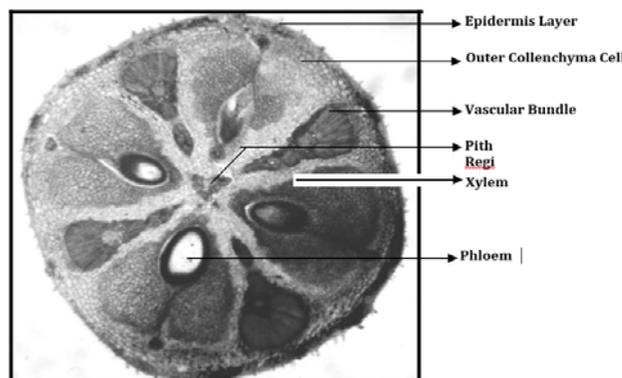


Fig 2: T.S. Petiole of *Achyranthes aspera*

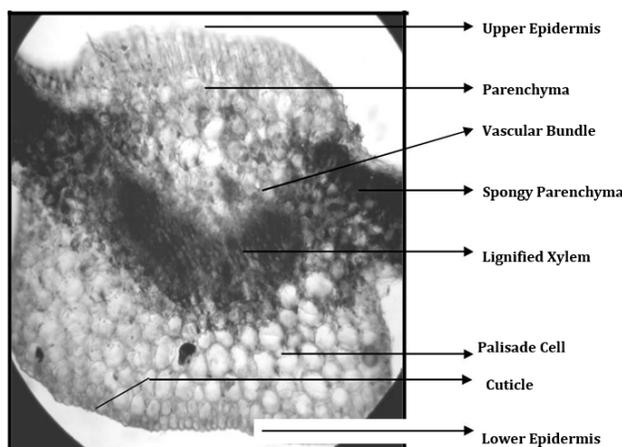


Fig 3: T.S. Leaf of *Achyranthes aspera*.

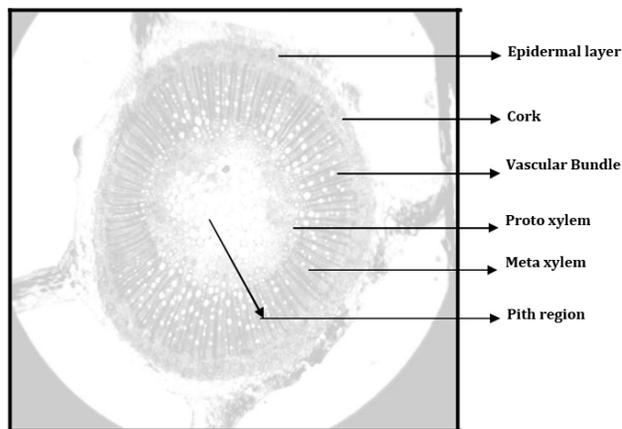


Fig. 4: T.S. Stem of *Achyranthes aspera*.

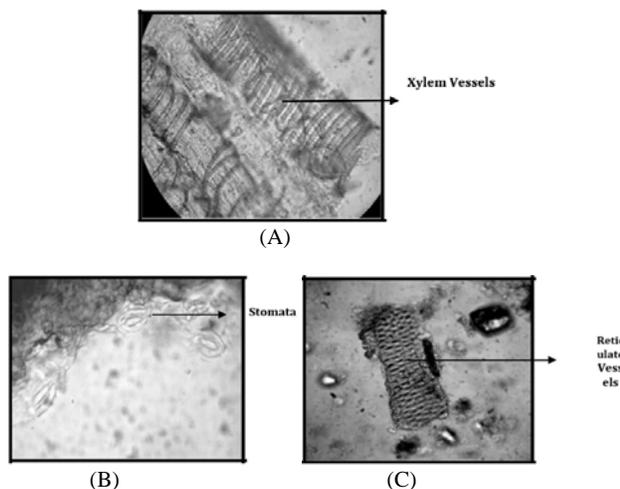


Fig 5: Powder Characteristics of different parts of *Achyranthes aspera*

3. Results and Discussion

The herbal drugs are currently being used in the treatment of various diseases without standardization. The quantitative determination of some pharmacognostical parameters is useful for setting standards for crude drugs. The results of the investigations could serve as a basis for proper identification, collection and investigation of the plant.

By performing the fluorescence characteristic of the powder drug with different chemical reagents, change in colour of the drug with fluorescence was observed when seen in short UV light.

The moisture content was 7.7% which was not so high as to facilitate bacterial growth. The other physicochemical parameters which ascertain the quality, purity and also helps in evaluation of the crude drug. The total ash value of the plant materials indicated the amount of minerals and earthy materials attach to the plant materials. Analytical results showed total ash value, acid insoluble ash value and water soluble ash value which were determined to be not more than 4.1% w/w, 1.7% w/w & 2.6% w/w respectively. While study of extractive value, water soluble extractive value indicated the presence of sugars, acid & inorganic compounds and was found to be 9.3% where as alcohol soluble extractive value indicated the presence of polar constituents like phenols, alkaloids, steroids, glycosides & flavonoids and was found to be 6.9%.

The powder plant material was extracted with a series of solvent in there increasing order of polarity i.e. petroleum-

ether, chloroform, acetone, methanol and water by soxhlet apparatus to isolate all kinds of phytoconstituents in plant material. Then colour, consistency, and extractive value of the extracts were evaluated. The methanolic and water extract have more extractive value (2.37% & 4.84%) and petroleum ether have less percentage (0.63%) of the extractive value.

All the extracts were subjected to various chemical test for preliminary identification of various phyto-constituents. The extracts were observed to contain alkaloids, saponins, flavonoids, steroids in petroleum ether, chloroform, acetone and methanolic extract.

Further finding reveled that extract of *Achyranthes aspera* contains some fluorescence compound which gives colour fluorescence at short wave length.

4. Conclusion

The pharmacognostic standards for the entire plant of *Achyranthes aspera*, are laid down for the first time in this study. The information obtained from preliminary phytochemical screening will be useful in finding out the genuinity of the drug. Ash values, extractive values, fluorescence analysis can be used as reliable aid for detecting adulteration. These simple but reliable standards will be useful to a lay person in using the drug as a home remedy. Also the manufacturers can utilize them for identification and selection of the raw material for drug production.

5. Acknowledgement

The Authors are grateful to Dr. M. L. Naik, Ex-Director, University Teaching Department, Sarguja University, Ambikapur, (C.G.) for his consistent advice & fruitful expertise knowledge.

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