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A PHARMACOGNOSTICAL AND *IN-VITRO* ANTI-ARTHRITIC STUDY ON FLOWERS AND FRUITS OF APAMARGA (*ACHYRANTHES ASPERA* LINN.)

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ABSTRACT

Apamarga (*Achyranthes aspera* Linn.) is one of the important medicinal plants belonging to Amaranthaceae family. The work is aimed to study the morphological, microscopic, pharmacognostic evaluation as well as extraction of flowers and fruits of *Achyranthes aspera* Linn. The color, taste, odor and powder nature were determined for the flowers and fruits of *Achyranthes aspera* Linn. Microscopic images of cross-section of flowers and fruits revealed useful diagnostic features. From the results of the study, it can be concluded that ethanolic extract of *A. aspera* Linn. possessed *in-vitro* anti-arthritic property. Hence, the plant can be used as a potent natural anti-arthritic agent.

Keywords: *Achyranthes aspera* Linn., Morphology, Microscopy, Pharmacognosy, Anti-arthritic study

1. INTRODUCTION

Herbal medicine is still the major source of medicine for around 75-80 percent of the world's population, primarily in poor nations, due to superior cultural acceptance, compatibility with the human body, and less adverse effects. The nature has provided medicinal substances for thousands of years, dating back to the dawn of humanity. Medicinal plants have been used to treat human ailments since the start of civilisation. More than 80,000 plant species have been discovered and utilised as medical herbs across the world, according to estimates **[1]**. Herbal medicine

is still the major source of medicine for around 75-80 percent of the world's population, primarily in poor nations, due to superior cultural acceptance, compatibility with the human body, and less adverse effects **[2]**.

Achyranthes aspera Linn. is one of several medicinal plant species with great therapeutic potential known as Prickly Chaff flower (English). The plant is a member of the Amaranthaceae family and is found as a weed across the world's tropical and subtropical climates. It is used to treat fever, wound healing, toothache, arthritis, gynaecological diseases, urinary disorders, bug and snake bites, abdominal tumours, stomach discomfort, and a variety of other maladies in many traditional healthcare systems. The plant is reported to be used as antimicrobial, larvicidal, antifertility, immunostimulant, hypoglycemic, hypolipidemic, anti-inflammatory, antioxidant, diuretic, cardiac stimulant, antihypertensive, anti-anasacra, analgesic, antipyretic, antinoiceptive, prothyrodic and antispasmodic activity **[2]**. Many research suggest that *Achyranthes aspera*'s aqueous solution has antibacterial action against *Bacillus typhosus*, *Streptococcus heamolyticus*, and *Staphylococcus aureus*, while its leaves' aqueous and alcoholic extracts have antibacterial activity against *E. coli* and *S. aureus*. Some research has been done on the antibacterial action of *Achyranthes aspera* extracts against pathogens **[3]**.

The present work is to determine the collection, herbarium preparation and storage of *Achyranthes aspera.* The aim is to study the morphological, microscopic, pharmacognostic evaluation, extraction of flowers and fruits, as well as to study *in-vitro* anti-arthritic study on ethanolic extract of *Achyranthes aspera*.

2. METHOD

2.1 Collection and authentication of plant Material

Plant materials *Achyranthes aspera* were collected from Chopda region of Jalgaon district (Maharashtra). The plants were authenticated by Botanical Survey of India, Western Regional Centre, Pune and herbarium was deposited in Department of Pharmacognosy, Smt. S. S. Patil College of Pharmacy, Chopda Dist. Jalgaon.

2.2 Organoleptic characters of flowers and fruits of *Achyranthes aspera*

The acquired plant raw material was identified and authenticated by researching their characteristics systematically using procedures provided in pharmacognosy literature. Sensory characteristics recorded colour, taste, odour, and powder nature **[4]**.

2.3 Microscopy study of flowers and fruits of *Achyranthes aspera*

2.3.1 Study of sections of flowers and fruits of *Achyranthes aspera*

Thin free hand transverse sections of fresh parts of *Achyranthes aspera* taken by maceration method. They were treated with phloroglucinol and hydrochloric acid (HCl) to identify different components. Photomicrographs were taken by using canon digital camera attached to Carl Zeiss Trinocular microscope **[5]**.

2.3.2 Study of powdered drug materials of flowers and fruits of *Achyranthes aspera*

Small quantity of flowers and fruits powder for studied first with distilled water then stained with phloroglucinol and concentrated HCl and microphotographs were taken. Microphotographs were taken by using canon digital camera attached to Carl Zeiss Trinocular microscope **[5]**.

2.4 Pharmacognostic study of flowers and fruits of *Achyranthes aspera*

2.4.1 Ash values

The residual substances that remain after the incineration of medicinal plant material is known as the ash content of crude drug. The total ash, water-soluble ash, and acid insoluble ash of the plant material were performed **[6]**.

2.4.1.1 Total ash value

The total ash was determined by incinerating 1 gm of accurately weighed air dried coarsely powdered drug in a tarred silica crucible which was previously ignited and cooled before weighing, at a temperature not exceeding 450˚C The ignition was repeated and the percentage of ash with reference to air-dried drug was calculated **[6]**.

$$\text{Total ash (\% w/w)} = \frac{\text{weight of ash}}{\text{weight of sample}} \times 100$$

2.4.1.2 Water soluble ash

With 25 ml of water, the whole ash was boiled for 5 minutes. The residue was washed with hot water, ignited for 15 min at a temperature not exceeding 450˚C, cooled and weighed. This weight was removed from the ash weight, and the weight difference reflects the water-soluble ash. The percentage of water-soluble ash was calculated with reference to air-dried drug **[6]**.

$$\text{Water soluble ash (\% w/w)} = \frac{\text{weight of water soluble ash}}{\text{weight of sample}} \times 100$$

2.4.1.3 Acid insoluble ash

The ash was heated for 5 minutes in 25 ml of dil. HCl before being filtered through ashless filter paper. The residue was washed with hot water, ignited, cooled in a desiccator and weighed. The proportion of acid insoluble ash was determined using air dried drug **[6]**.

$$\text{Acid insoluble ash (\% w/w)} = \frac{\text{weight of insoluble ash}}{\text{weight of sample}} \times 100$$

2.4.1.4 Sulphated ash

A silica crucible was heated to redness for 10 min, allowed to cool in desiccators and weighed. 1 g of substance was accurately

weighed and transferred to the crucible and weighed along with the contents accurately. It was ignited gently at first until the substance was thoroughly charred. Then the residue was cooled and moistened with 1 ml concentrated sulfuric acid, heated gently until white fumes are no longer evolved and ignited at 800 ± 25°C until all black particles have disappeared. The ignition was conducted in a place protected from air currents. The crucible was allowed to cool, and a few drops of concentrated sulfuric acid were added and heated. Ignited as before, allowed to cool, and weighed. The operation was repeated until two successive weighing does not differ by more than 0.5 mg. The percentage of sulphated ash concerning the air-dried drug was calculated **[7].**

$$\text{Sulphated ash (\% w/w)} = \frac{\text{weight of sulphated ash}}{\text{weight of sample}} \times 100$$

2.4.2 Extractive values

Extractive values are used to identify the quality and purity of the plant material. It determines the possible soluble phytochemicals in given solvent **[8]**.

2.4.2.1 Ethanol soluble extractive

5 gm of dried flowers and fruits' coarse powder of plants were macerated in a closed flask for 24 hours with 100 ml of 90% ethanol, shook regularly for 6 hours, and left to stand for 18 hours. Filtered promptly, taking care against ethanol loss. In a tarred flat bottomed shallow dish, 25 ml of the filtrate was evaporated to dryness. The residue was dried and weighed at 105 °C. The percentage of ethanol soluble extractive was estimated using air dried medication as a reference **[8]**.

2.4.2.2 Water soluble extractive

5 gm of flowers and fruits' coarse powder was weighed and dissolved in 100 ml of water in a stoppered flask, heated to 80 °C, agitated thoroughly, and left to stand for 10 minutes. It had been chilled. 2 gm kieselghur was added and filtered. 5ml of the filtrate was transferred to a tarred evaporating dish, where the solvent was evaporated over a water bath. Using air dried drug as a reference, the fraction of water-soluble extractive was calculated **[8]**.

2.4.3 Determination of crude fibre content

Approximately about 4 gm of finely powered crude drug was weighed and extracted with petroleum ether, initially at 35-38˚C and then at 52˚C. Dried material 2 gm was boiled with 200 ml of sulphuric acid for 30 minutes. Filtered the extracted material through a muslin cloth of pore size 0.841 mm and washed with warm water. The drug material was then simmered with 1.25%v/v sodium hydroxide (200 ml) for half an hour. The drug solution was strained again through muslin cloth of pore size 0.841 mm and washed with 25 ml of 1.25% H_2SO_4, water (50 ml) and ethanol

(25 ml) successively. The remnant after washing was transferred to a tarred crucible which is made up of silica, the weight of which was considered as the initial weight (W1). The residue was dried in a hot air oven (REMI-RDHO 50) for 2-3 hours at a temperature not exceeding 130°C. Utilizing a desiccator, the powder was cooled for half an hour and then the weight was determined immediately (W2). After this procedure, the residue was incinerated for half an hour at 600˚C in a muffle furnace. Cooled by desiccation and weighed again (W3). The percentage of crude fibre in the sample was estimated using the following formula **[9].**

$$\text{Crude fibre content} = \frac{[(W2-W1)-(W3-W1)]}{\text{Weight of sample taken}} \times 100$$

2.4.4 Determination of loss on drying

1 gm of the powdered leaves sample was weighed accurately and transferred to a tarred Petri dish. The petri dish was placed in a hot air oven (REMI-RDHO 50) and allowed to dry under a temperature of 105˚C for two hours. It was then removed from the oven. Utilizing a desiccator, the powder was cooled to room temperature and repeated the same procedure till a consistent weight was obtained. Loss on drying was quantified in psercentage weight by weight in comparison with air dried drug **[6]**.

$$\text{LOD } (\%\ w/w) = \frac{\text{Weight loss}}{\text{Weight of sample}} \times 100$$

2.5 *In-vitro* study of anti-arthritic activity

In-vitro activity was studied using inhibition of protein denaturation (IPD) method. The reaction mixture (0.5ml) consisted of 0.45 ml bovine serum albumin (5% aqueous solution) and 0.05 ml of *Achyranthes aspera* ethanolic extract at different concentration. The samples were incubated at 37°C for 30 min. After cooling the samples, 2.5 ml phosphate buffer saline (pH 6.3) was added to each tube. Turbidity was measured UV- spectrophotometrically for control test 0.05 ml distilled water was used instead of extracts while product control test lacked bovine serum albumin. The percentage inhibition of protein denaturation was calculated as follows: **[10]**.

$$\%\ Inhibition = \frac{\text{Absorbance of Control} - \text{Absorbance of Test}}{\text{Absorbance of Control}} \times 100$$

The results were compared with Diclofenac treated sample.

3. RESULTS AND DISCUSSION

3.1 Organoleptic characters of flowers and fruits of *Achyranthes aspera*

Color, taste, odor and powder nature were recorded by sensory characters. Results are tabulated in **Table 1**.

3.2 Microscopy study of flowers and fruits of *Achyranthes aspera*

3.2.1 Study of sections of flowers and fruits of *Achyranthes aspera*

Plate 6 shows representative photomicrographs of transverse section of

Achyranthes aspera flowers. Transverse section of the seed is shown in **Figure 1**. Longitudinal section of the fruits is shown in **Figure 2**.

3.2.2 Study of powdered drug materials of flowers and fruits of *Achyranthes aspera*

Plate 6 shows representative photomicrographs of transverse section of *Achyranthes aspera* flowers. Transverse section of the seed is shown in **Figure 1**. Longitudinal section of the fruits is shown in **Figure 2**.

3.3 Pharmacognostic study of flowers and fruits of *Achyranthes aspera*

3.3.1 Determination of ash value:

A high ash value is indicative of contamination, substitution or adulteration by minerals. The residue remaining after incineration of plant material is the total ash or ash value. In the present investigation, the total ash content of flowers and fruits of *A. aspera* is found to be 5.46±0.02% and 9.69±0.01%, resp. which is less than the maximum acceptable limit of total ash (14%) recommended by European Pharmacopoeia. The results of ash values for the flowers and fruits of *Achyranthes aspera* are given in **Table 2**.

3.3.2 Extractive value

Ethanol-soluble and water-soluble extractive values of ingredients and formulation are depicted in **Table 3**, which shows 2.3±0.01 ethanol-soluble extractive value and 5.99±0.02 water-soluble extractive value of flowers of *Achyranthes aspera*. In case of fruits of *Achyranthes aspera*, 5.14±0.05 alcohol soluble extractive value and 3.306±0.02 water soluble extractive value was obtained. A higher water-soluble extractive value indicates that water is a superior extraction solvent than ethanol for the formulation.

3.3.3 Determination of crude fibre content

Adulteration above 10% significantly increases the fibre content of the sample, which can be used as a measure of detecting adulteration. But crude fibre content of *Achyranthes aspera* flowers and fruits was less than 10%, which shows very less adulteration in sample. The crude fibre content is an indication of nutritive value of the plant **(Table 4)**.

3.3.4 Determination of loss on drying (LOD)

LOD is performed to estimate the moisture content present in a crude drug. High amount of moisture content present in crude drugs may result in contamination. The moisture promotes the degradation processes caused by enzymes, development of microorganisms, oxidation and hydrolysis reactions. This study recorded moisture content of 6.9% and 8.5% for flowers and fruits of *A. aspera*, resp. which

is deemed to be good as the water content in herbal drugs should not be greater than 14% Low moisture content is an indication that the drug is properly processed. The results of LOD for the flowers and fruits of *Achyranthes aspera* are given in **Table 5**. From result it was confirmed that *Achyranthes aspera* of flowers had less moisture content than fruits.

3.4 *In-vitro* study of anti-arthritic activity

In *in-vitro* anti-arthritic activity by Bovine Serum denaturation method at concentration of 100, 250 and 500 mcg/ml showed 92.4, 94.3, 97.08% inhibition of denaturation of bovine serum whereas, standard diclofenac at 100, 250 and 500 mcg/ml which showed 94.93, 96.83, 98.35% inhibition of denaturation of bovine serum **(Figure 3)**.

Table 1: Organoleptic characters of flowers and fruits of *Achyranthes aspera*

Sr. No.	Parameter	*Achyranthes aspera*	
		Flower	Fruit
1	Color	Whitish yellow	Whitish yellow
2	Odor	Specific	Specific
3	Powder nature	Coarse	Coarse
4	Taste	Astringent	Astringent

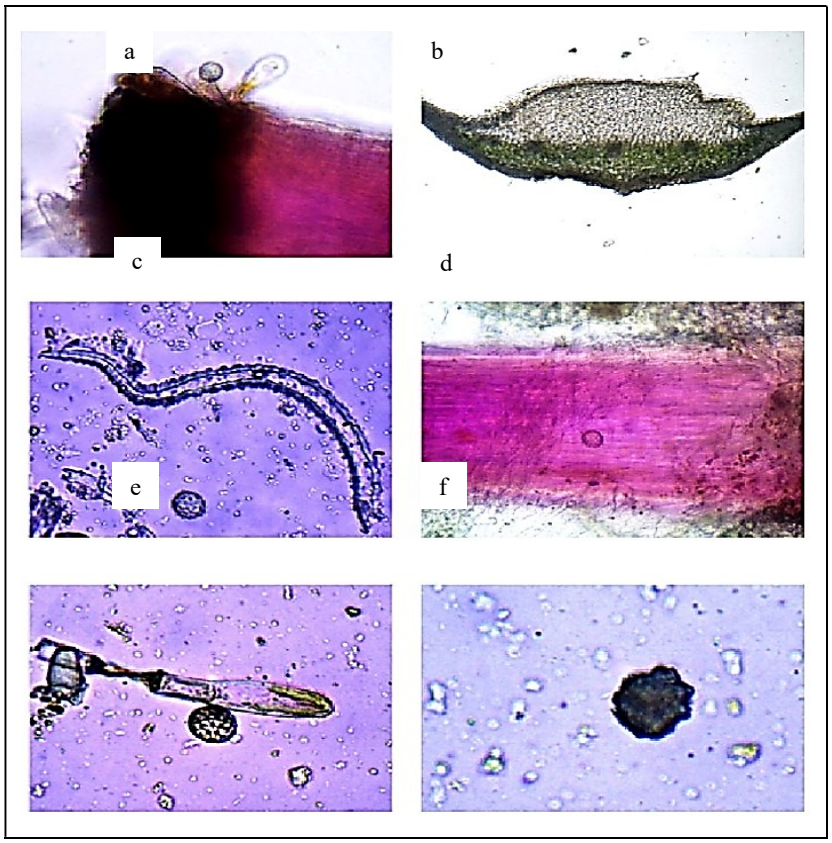

Figure 1: Transverse section of: a-*Achyranthes aspera*; b-Gynoecium along with pollen grain; c- Style with pollen grain; d- Fibres; e-Rosette Crystals; f-Grandular trichoms with prismatic crystals

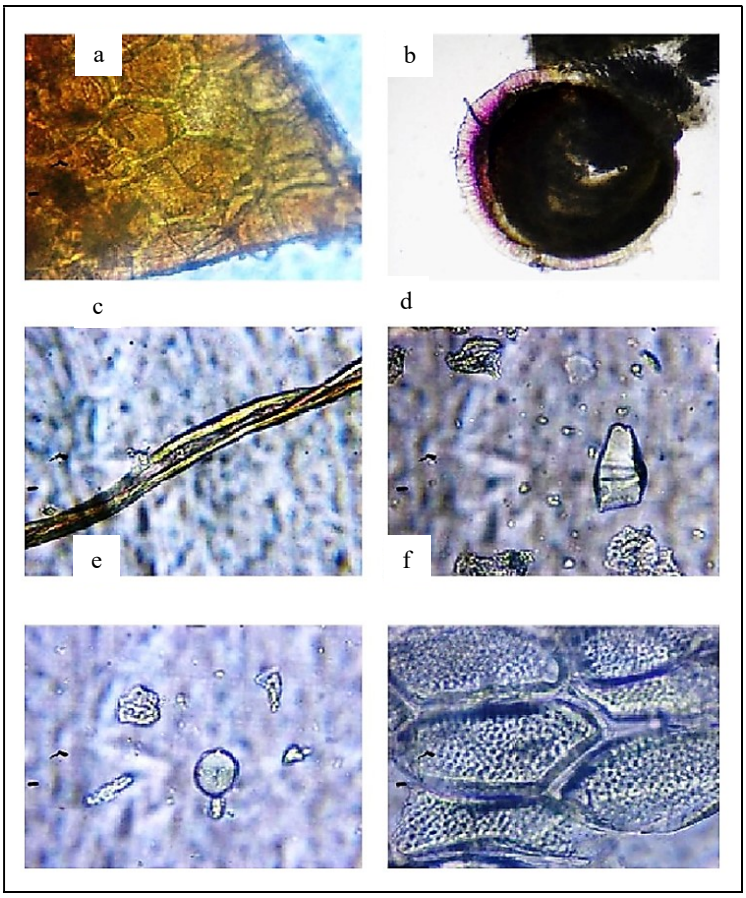

Figure 2: Transverse section of: a- Epicarp cells; b- Fibres with white luman; c- Seed; d- Prismatic crystal; e- Starch grain; f- Pitted Paranchyma

Table 2: Ash value of flowers and fruits of *Achyranthes aspera*

Sr. No.	Parameter	Plant Parts	
		Flowers	Fruits
1	Total ash	5.46±0.02	9.69±0.01
2	Acid insoluble ash	3.73±0.01	1.53±0.02
3	Water soluble ash	0.06485±0.01	0.10125±0.01
4	Sulphated ash	1.14±0.36	3.68±0.43

Table 3: Extractive value of flowers and fruits of *Achyranthes aspera*

Sr. No.	Solvent	Plant parts	
		Flowers	Fruits
1	Ethanolic extract	2.3±0.01	5.14±0.05
2	Water-soluble extract	5.99±0.02	3.306±0.02

Table 4: Crude fibre content of flowers and fruits of *Achyranthes aspera*

Sr. No.	Plant parts	Crude fibre content
1	*Achyranthes aspera* Flowers	1.14 ±0.01 %w/w
2	*Achyranthes aspera* Fruits	1.07 ±0.02 %w/w

Table 5: LOD of flowers and fruits of *Achyranthes aspera*

Sr. No.	Plant material	LOD %
1	*Achyranthes aspera* Flowers	6.9
2	*Achyranthes aspera* Fruit	8.5

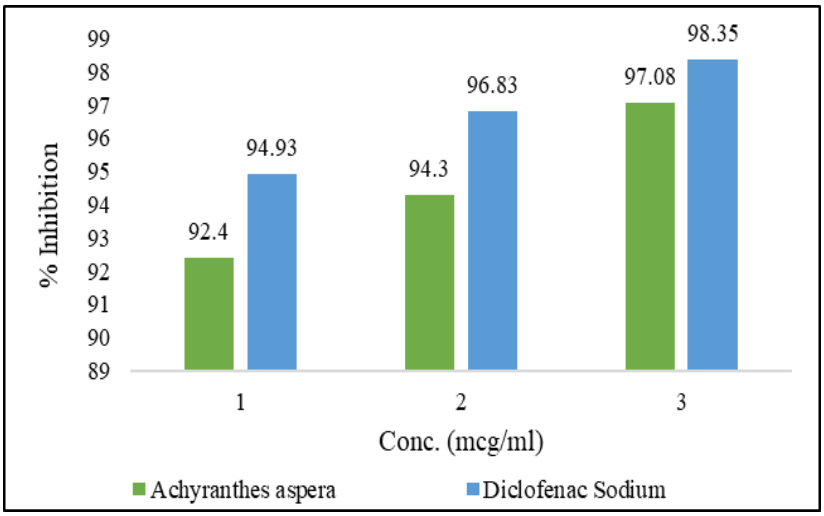

Figure 3: % Inhibition of *Achyranthes aspera* and Diclofenac Sodium

4. CONCLUSION

The data generated from this study would be helpful in the authentication of various constituents of fruits and flowers of *Achyranthes aspera* Linn. The microscopic features would prove useful for laying down pharmacopoeial standards. Morphology as well as various pharmacognostic aspects of different constituents were studied which will help in authentication and quality control. From the results of the study, it can be concluded that ethanolic extract of *A. aspera* Linn. possessed *in-vitro* anti-arthritic property. Hence, the plant can be used as a potent natural anti-arthritic agent.

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