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PRELIMINARY PHARMACOGNOSTICAL AND PHYTOCHEMICAL ANALYSIS OF FRUIT OF *APAMARGA (ACHYRANTHES ASPERA LINN.)*

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ABSTRACT

Introduction: Drug standardization is needed for identification, purity, safety, strength and effectiveness of drug. So, it becomes very important to perform the Preliminary Pharmacognostical and phytochemical analysis of the drug to conduct further experiments. *Apamarga* is a drug which is used since time immemorial for conditions like in *Kandu, Vrana, Visha* etc.[1] Fruit of *Apamarga* is used in the *Atyagni* condition.[2] It has got pharmacological actions like Diuretic, Spasmolytic, Hypoglycemic, Antimicrobial, Antibiotic, Antifungal, Anti-Implantation, Abortifacient, Hypotensive, Purgative.[3] **Objective:** To determine the Preliminary Pharmacognostical and phytochemical analysis of fruit of *Apamarga (Achyranthes aspera Linn*). **Materials and method:** Collection of fruits of *Apamarga* (*Achyranthes aspera* Linn) was done at its natural habitat of Udupi district and Pharmacognostical, phytochemical analysis was done according to standard methods. **Result and Discussion:** Macroscopical parameters showed One seeded, cylindrical, oblong in shape, utricle with truncate apex, 3mm in length and about 1.5mm in thickness, shiny brown, taste is mealy sweet. Odor was not characteristic. TS of fruit contained seed, having spool shaped perisperm loaded with starch grains, The Cotyledon was embedded with aleurone grains, fixed oil globules and vascular bundles. The foreign matter was nil in the crude drug. The total ash indicating total inorganic content was found to be 6.215 ± 0.01. Acid insoluble part of total ash, which indicates silica, was found to be 0.19 ± 0.01. Loss on

drying indicating moisture and other volatile matter was determined to be 1.79 ± 0.01. Water soluble secondary metabolites was found to be 2.79 ± 0.01. Study has shown the presence of Alkaloid, Steroid, Carbohydrate, Tannin, Flavonoids, Coumarins and Quinone. **Conclusion:** Assessment of phytochemicals and physicochemical constant values of this study would be used as reference standards for the research scholars to further perform animal experiments and clinical trials.

KEYWORDS: Macroscopy, Microscopy *Apamarga,* Analytical study.

INTRODUCTION

In Ayurveda we get the reference of *Dravya Pareeksha* where the Acharya has told certain parameters for *Dravya Sangrahana* and *Dravya Samrakshana* which becomes very important for any of the *yoga* preparation and their *Prayoga* over *Vyadhi*. Presently in modern science, the standardization of the crude drug becomes very important to finalize the genuine drug which is done through the qualitative and quantitative standards that includes microscopic, macroscopic, pharmacopeial standards and Chromatography parameters.

The drug *Achyranthes aspera Linn*. belongs to Amaranthaceae family[4,5] which is widely distributed throughout India, China, Japan, Burma, Sindh, etc.[6] It is an annual herb height about 0.3 to 0.9mts. Leaf which is Simple, subsessile, exstipulate, opposite, decussate, wavy margin, obovate, slightly acuminate, slightly acuminate and pubescent due to the presence of thick coat of long simple hairs. Flowers arranged in inflorescence of long spikes, Greenish white, numerous. Fruit is an indehiscent dry utricle enclosed within persistent, perianth and bracteoles and Seed is Sub-cylindric, trumeate at the apex, round at the base endospermic, brown color.[7,8,9] *Achyranthes aspera* is highly esteemed by traditional healers and used in treatment of asthma, bleeding, in facilitating delivery, boils, bronchitis, cold, cough, colic, debility, dog bite, dysentery, ear complications, headache, leucoderma, pneumonia, renal complications, scorpion bite, snake bite and skin diseases.[10] In Ayurveda it is identified as *Apamarga* which is used in *Kandu, Vrina, Visha* etc.

MATERIALS AND METHODS

Macroscopy

The external features of the test samples namely the fruit were documented using Canon IXUS digital camera. The macroscopic features were compared to local flora for authentication.

Microscopy

The sample fruit was preserved in fixative solution. The fixative used was FAA (Formalin-5ml + Acetic acid-5ml + 70% Ethyl alcohol-90ml). It was left in FAA for more than 48 hours. The preserved fruits were cut into thin transverse section using a sharp blade and the sections were stained with saffranine and mounted in glycerin. Transverse sections were photographed using Zeiss AXIO trinocular microscope attached with Zeiss AxioCam camera under bright field light. Magnifications of the figures are indicated by the scale-bars.

Powder microscopy

Pinch of *Apamarga* fruit powder previously sieved is put on the slide and mounted in glycerine and powder characters are observed under the Zeiss AXIO trinocular microscope attached with Zeiss Axio Cam camera under bright field light.

Preliminary pharmagnostical analysis[11]

Physicochemical Analysis

1. **Loss on drying at 105^{o}C**- 10 g of sample was placed in tared evaporating dish. It was dried at 105˚C for 5 hours in hot air oven and weighed. The drying was continued until difference between two successive weights was not more than 0.01 after cooling in desiccator. Percentage of moisture was calculated with reference to weight of the sample.
2. **Total Ash**-2 g of sample was incinerated in a tarred platinum crucible at temperature not exceeding 450˚C until carbon free ash is obtained. Percentage of ash was calculated with reference to weight of the sample.
3. **Acid insoluble Ash**-To the crucible containing the total ash, 25ml of dilute HCl was added and boiled. The insoluble matter was collected on an ashless filter paper (Whatman No.41) and washed with hot water until the filtrate became neutral. The filter paper containing the insoluble matter was transferred to the original crucible, dried on a hot plate and ignited to a constant weight. The residue was allowed to cool in a suitable desiccator for 30 mins and was weighed without any delay. The content of acid insoluble ash was calculated with reference to the air-dried drug.
4. **Water-soluble ash-** The ash was boiled for 5 min with 25 ml of water; insoluble matter was collected on an ashless filter paper, washed with hot water, and ignited for 15 min at a temperature not exceeding 450˚C. The weight of the insoluble matter was subtracted from the weight of the ash. The differences in weight represents the water-soluble ash with reference to the air-dried sample.

5. **Alcohol-soluble extractive-** Accurately weighed 4 g of the sample was taken in a glass Stoppard flask. 100 ml of distilled Alcohol (approximately 95%) was added and shaken occasionally for 6 hours. It was allowed to stand for 18 hours and rapidly filtered taking care not to lose any solvent. 25ml of the filtrate was pipetted out in a pre-weighed 100 ml beaker and evaporated to dryness on a water bath. It was kept in hot air oven at 105°C for 6 hours, cooled in a desiccator for 30 minutes and weighed. The percentage of alcohol soluble extractive matter of the sample was calculated. The experiment was repeated twice and the average value was taken.
6. **Water- soluble extractive-** Accurately weighed 4g of the sample was taken in a glass Stoppard flask. 100 ml of distilled water was added and shaken occasionally for 6 hours. It was allowed to stand for 18 hours and rapidly filtered taking care not to lose any solvent. 25ml of the filtrate was pipetted out in a pre-weighed 100 ml beaker and evaporated to dryness on a water bath. It was kept in hot air oven at 105°C for 6 hours, cooled in a desiccator and weighed. The percentage of water-soluble extractive matter of the sample was calculated. The experiment was repeated twice and the average value was taken.

Preliminary phytochemical Analysis

Extracts: - Aqueous extract, Alcoholic extract, Chloroform fraction, Ethyl acetate was taken.

Tests for alkaloids

- ***Dragendroff's test:*** To a few ml of the extract sample added with alcohol, a few drops of acetic acid and Dragendroff's reagent were added and shaken well. An orange red precipitate formed indicates the presence of alkaloids.
- ***Wagners's test:*** To a few ml of the extract added with acetic acid, a few drops of Wagner's reagent was added. A reddish-brown precipitate formed indicates the presence of alkaloids.
- ***Mayer's test:*** To a few a few ml of the extract sample added with acetic acid, a few drops of Mayer's reagent was added. A dull white precipitate formed indicates the presence of alkaloids.
- ***Hager's test:*** To a few ml of the extract sample added with in acetic acid, 3 ml of Hager's reagent was added, the formation of yellow precipitate indicates the presence of alkaloids.

Tests for carbohydrates

- ***Molisch's test:*** To a few ml of the extract sample added with 1 ml of α-naphthol solution, conc. sulphuric acid was added along the sides of test tube. A violet colour formed at the junction of the two liquids indicates the presence of carbohydrates.
- ***Fehling's test:*** A few ml of the extract sample was mixed with equal quantities of Fehling's solution A and B and the mixture was warmed on a water bath. The formation of a brick red precipitate indicates the presence of carbohydrates.
- ***Benedict's test:*** To 5 ml of Benedict's reagent, few ml of the Kashaya was added, boiled for two minutes and cooled. Formation of a red precipitate indicates the presence of carbohydrates.

Test for steroids

- ***Liebermann-Burchard test:*** Few ml of the extract was dissolved in chloroform, 1 ml of acetic acid and 1 ml of acetic anhydride was added, then heated on a water bath and cooled. Few drops of conc. Sulphuric acid were added along the sides of the test tube. Appearance of bluish green colour indicated the presence of steroids.
- ***Salkowski test:*** The extract was added with chloroform and equal volume of conc. Sulphuric acid was added. Formation of bluish red to cherry red colour in chloroform layer and green fluorescence in the acid layer indicates the presence of steroids.

Test for saponins

To a few ml of the extract, distilled water was added and shaken. Stable froth formation indicates the presence of saponin.

Test for tannins

To a few ml of the extract, a few drops of dilute solution of ferric chloride was added formation of dark blue colour shows the presence of tannins.

Test for flavonoids

Shinoda's test: To few ml of the extract added with alcohol, a few magnesium turnings and few drops of conc. hydrochloric acid were added and heated on a water bath. Formation of red to pink colour indicates the presence of flavonoids.

Test for phenol

To the few ml of the extract added with alcohol, two drops of alcoholic ferric chloride was added. Formation of blue-to-blue black indicates the presence of phenol.

Test for coumarins

To the few ml of the extract added with alcohol, a few drops of 2 N sodium hydroxide solution was added. Dark yellow colour formation indicates the presence of coumarins.

Test for triterpenoids

Few ml of the extract was warmed with bits of tin metal and few drops of thionyl chloride. Formation of pink colour indicates the presence of triterpenoids.

Test for carboxylic acid

Extract was treated with sodium bicarbonate. Brisk effervescence indicates the presence of carboxylic acid.

Test for resin

Few ml of the extract was mixed with water and acetone. Turbidity indicates the presence of resins.

Test for quinone

A few ml of the Kashaya sample was treated with 0.5% of sodium hydroxide. Deep coloration like pink, purple or red indicates the presence of quinone.

RESULT

Macroscopy

One seeded, cylindrical, oblong in shape, utricle with truncate apex, 3mm in length and about 1.5mm in thickness, shiny brown, taste is mealy sweet. Odor is not characteristic.

Fig. 1: Macroscopy of fruit of Apamarga (Achyranthes aspera Linn).

Microscopy

TS of fruit containing seed passing through the center of the seed shows centrally located wide, spool shaped perisperm loaded with starch grains, the radical and cotyledons are located at its two opposite ends underneath the groove; encircled by narrow outer testa.

Detailed TS shows an outer layer of thick walled cells of epidermis of testa with sinuously running vertical walls, covered with thin cuticle; two rows of tangentially running broad lumened cells being located underneath this and then a narrow rectangular cells of pigment layer; tegmen lying underneath this consists of two rows of narrow, irregular tangentially running cells of parenchyma, a wide parenchymatous zone of perisperm consisting of angular, tangentially running thin walled cells getting increased in size proportionately towards its inner sides, loaded compactly with starch grains and covered with a squarish thick walled cells of epidermis.

The Cotyledon consists of an upper and lower epidermis covered with thin cuticle, the cells of the lower one being bigger in size and shows one row of palisade cells underneath it; the remaining mesophyll cells are, wide lumened, rectangular small sized cells, piled up one above the other and embedded with aleurone grains, fixed oil globules and vascular bundles.

Powder microscopy

Powder microscopic characters observed when the fruit was crushed are masses of starch grains that are agglomerated, Perisperm embedded with starch, epidermal cells of cotyledon, fibers with forked tips, Pitted vessels, thick-walled parenchyma cells, Glandular trichomes, pericyclic fibers overlapping with underneath parenchyma, Trichomes, pollens, Rosette crystals etc.

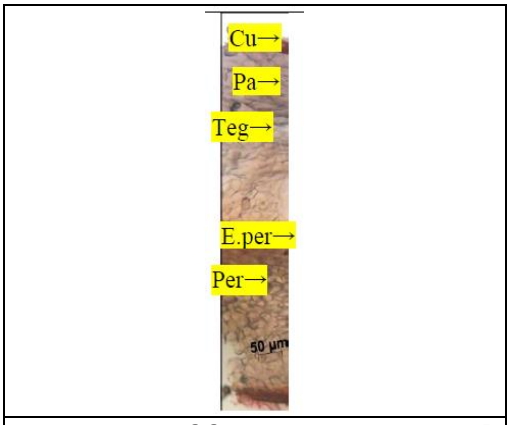


Fig 2a. T.S of fruit containing Seed

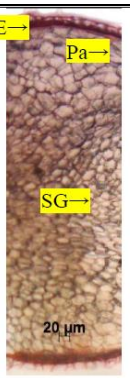


Fig 2b.A portion Enlarged

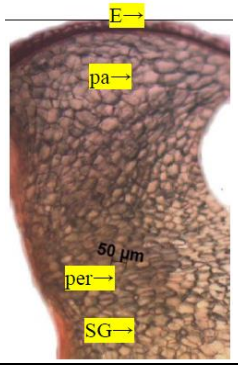


Fig 2c. Perisperm

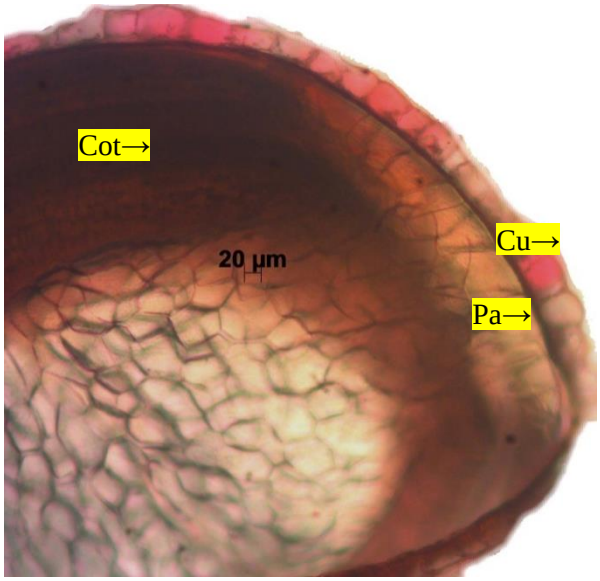


Fig 2d. T.S of seed containing cotyledon

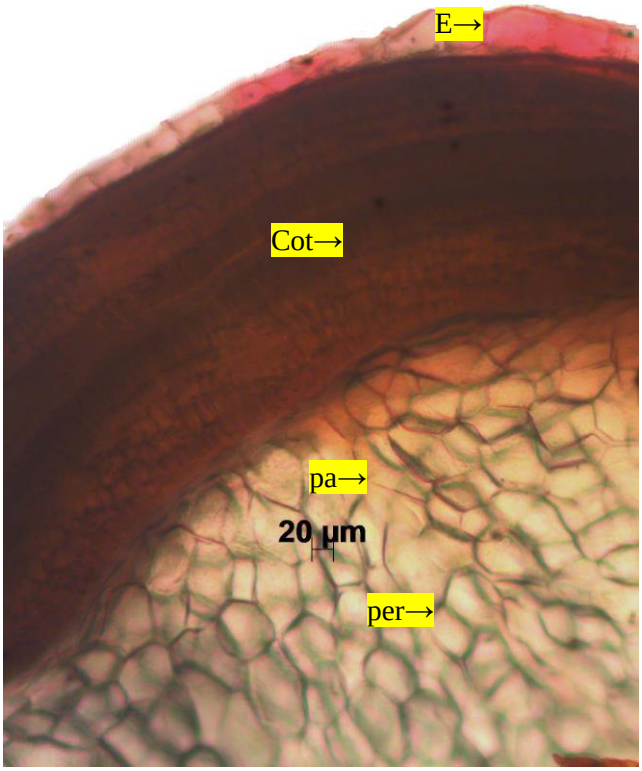
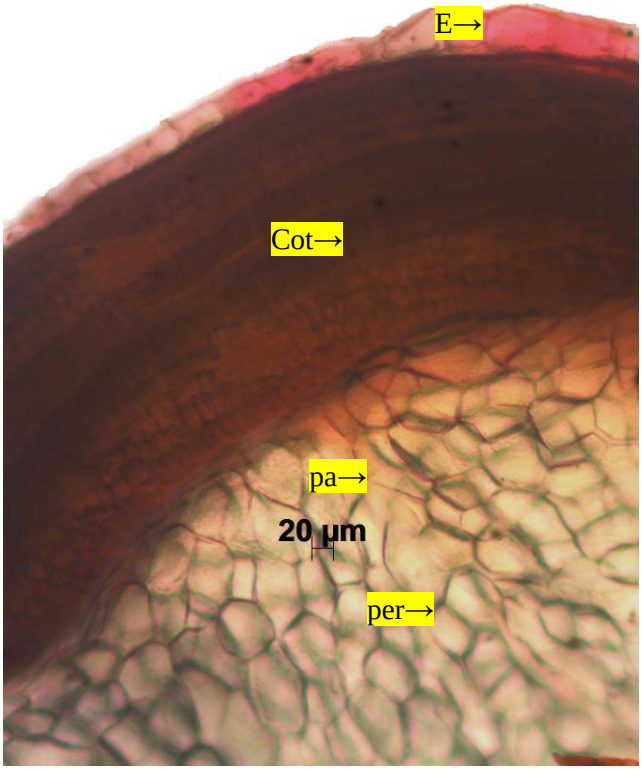


Fig 2e. Epidermis, Cotyledon, Perisperm



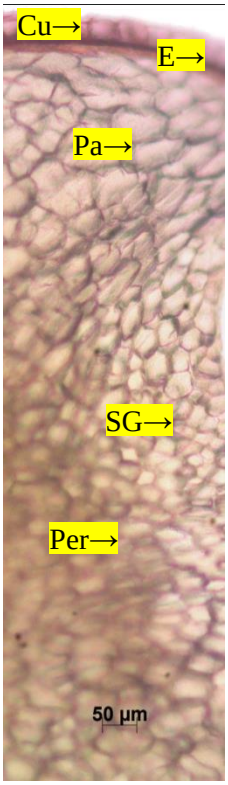


Fig 2f. Epidermis and cotyledon

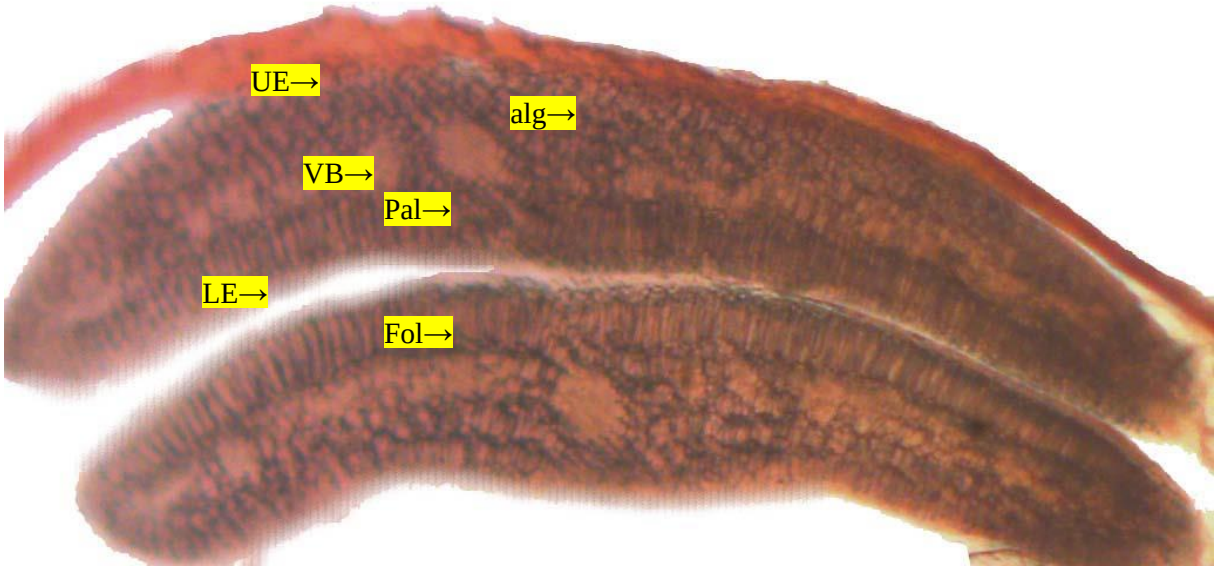


Fig 2g. Cotyledons

Cu – cuticle; E – epidermis; E.per – epidermis of perisperm; Pa – parenchyma; per – perisperm; SG – starch grains; Teg – tegmen.Cot – cotyledon; Fol- follicle; LE – lower epidermis; Pal – palisade; UE – upper epidermis; VB- vascular bundle.

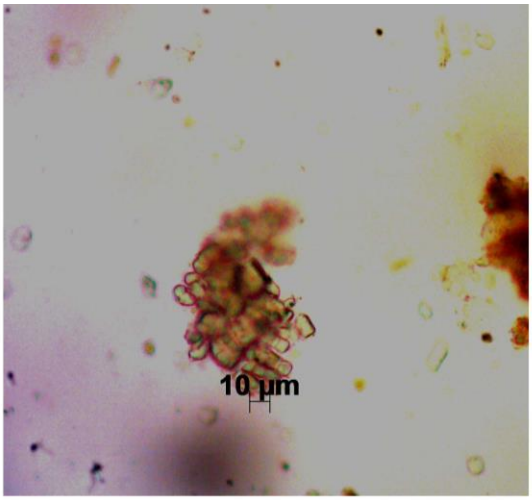


Fig. 3.1. Masses of Starch grains.

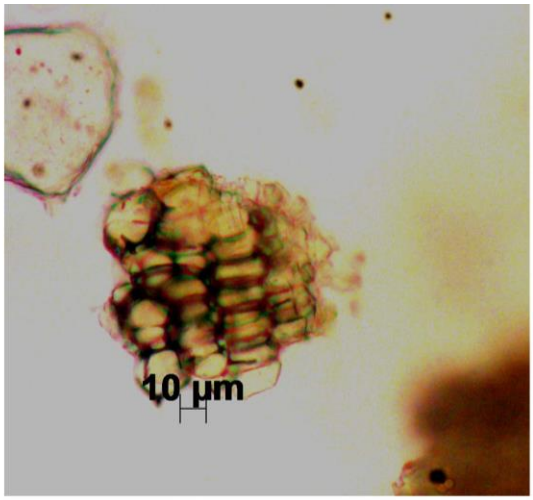


Fig. 3.2: Parenchyma cells thick walled.

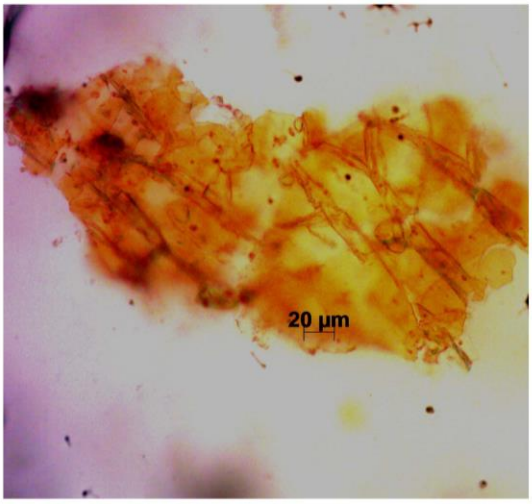


Fig. 3.3: Cork in surface view.

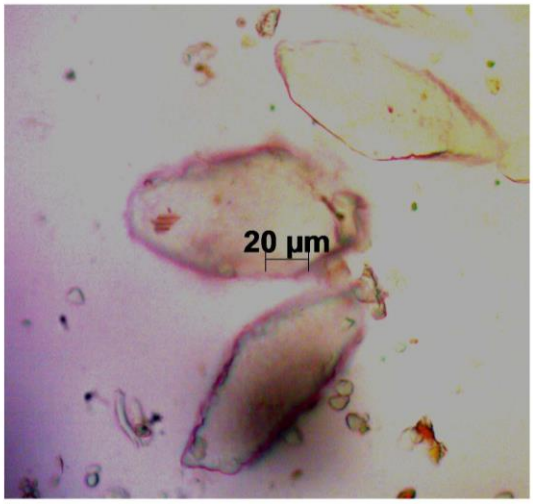


Fig. 3.4: Perisperm embedded with starch.

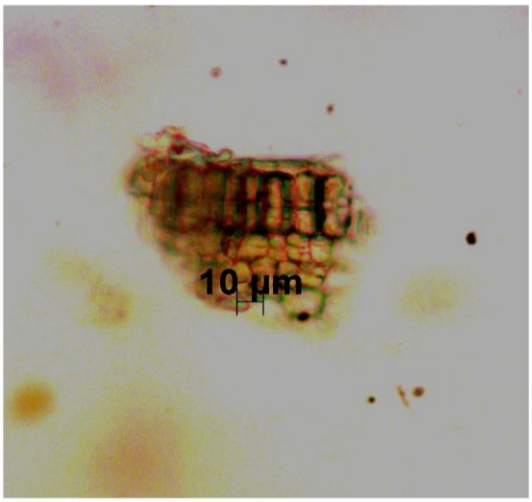


Fig. 3.5: T.S of Cotyledon.

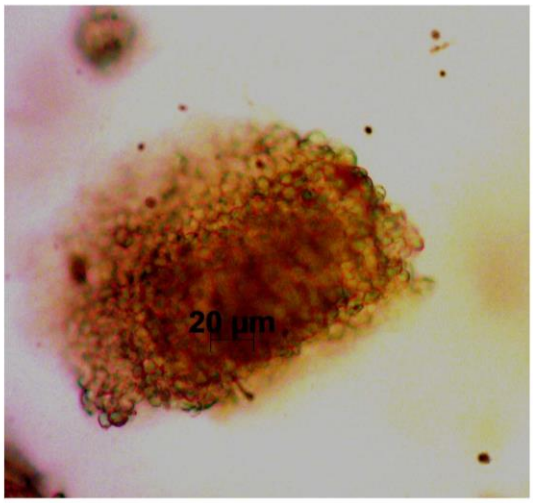


Fig. 3.6: Epidermis of Cotyledons.

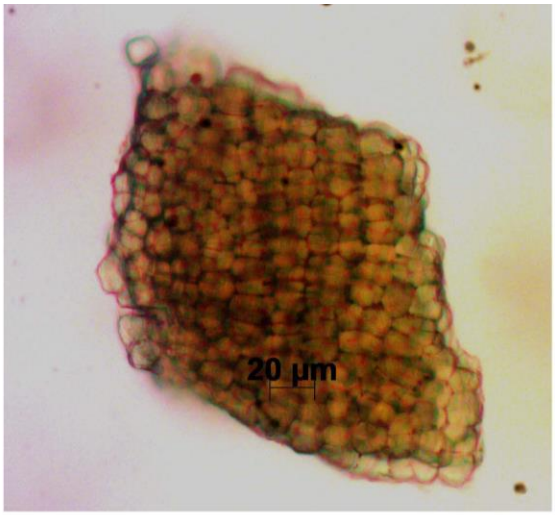


Fig. 3.7: Epidermis of cotyledons.

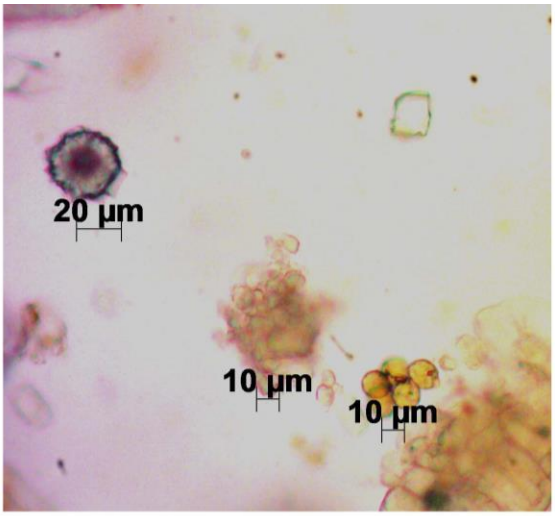


Fig. 3.8: Rosette crystals, Pollen grains.

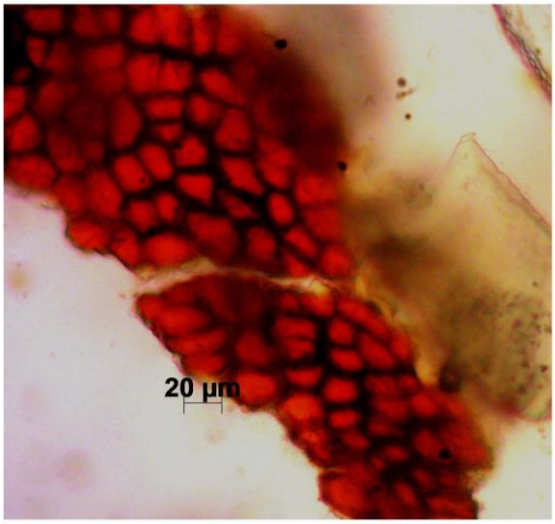


Fig. 3.9: Cork in surface view.

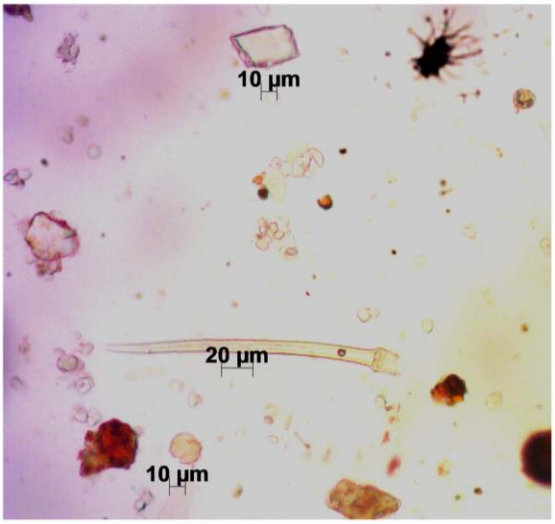


Fig. 3.10: Trichomes, Pollens, prismatic crystals.

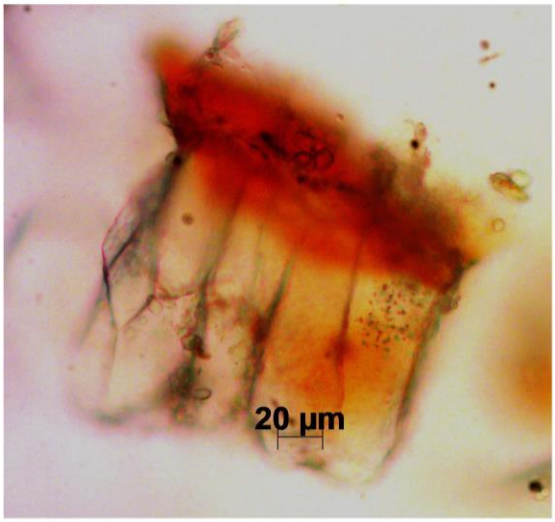


Fig. 3.11: Transversely cut cotyledons.

Fig. 3.12: Glandular trichomes.

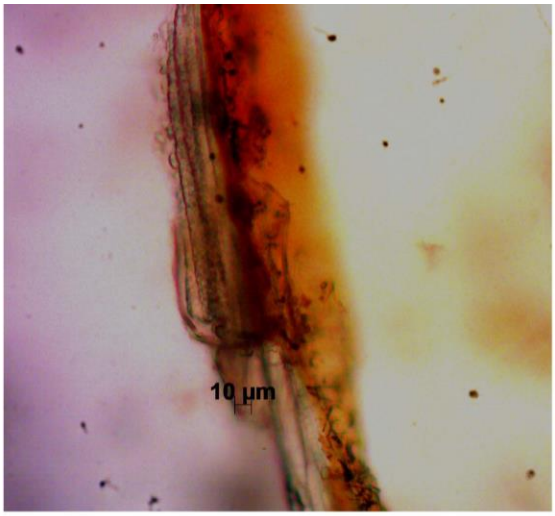


Fig. 3.13: Xylem fibres.

Fig. 3.14: Prisms of oxalate.

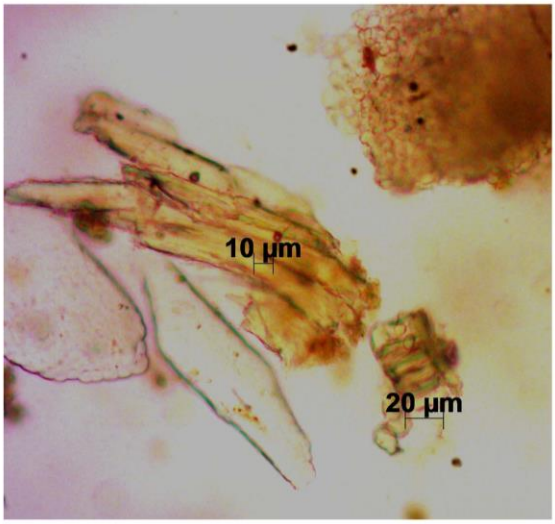


Fig. 3.15: Fibres with forked tip.

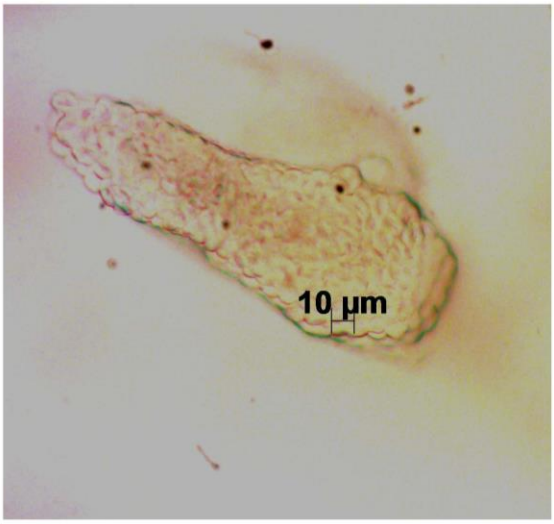


Fig. 3.16: Pitted vessel.

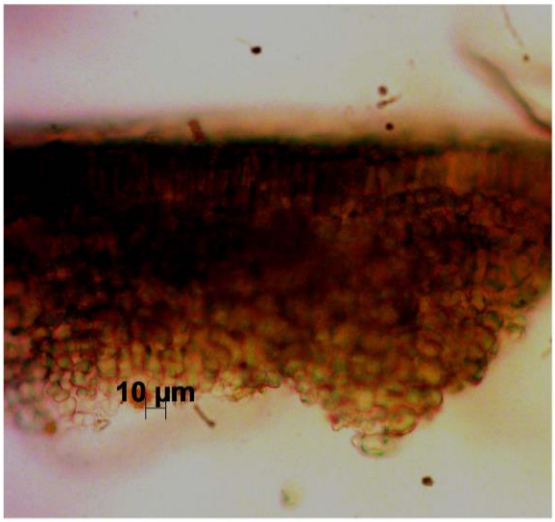


Fig 3.17. TS of cotyledon.

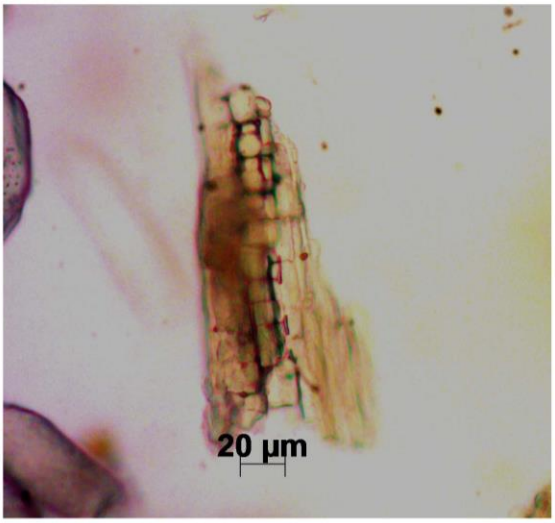


Fig 3.18. Pericyclic fibre overlapping with underneath parenchyma.

Figure 3: Powder Microscopy of fruit of *Achyranthus aspera.*

Physico chemical analysis

Table 1: Results of physicochemical parameters of *Achyranthes aspera* fruit.

Parameter	Results n = 3 %w/w Avg ± SEM
Loss on drying	1.79 ± 0.01
Total Ash	6.215 ± 0.01
Acid Insoluble Ash	0.19 ± 0.01
Water soluble Ash	2.79 ± 0.01
Alcohol soluble extractive value	3.14 ± 0.01
Water soluble extractive value	13.5 ± 0.48

Phytochemical Analysis

Table 2: Results of preliminary phytochemical screening of *Achyranthes aspera* fruit.

Test	*Inference*			
	Aqueous extract	*Alcoholic extract*	*Chloroform fraction*	*Ethyl acetate*
Alkaloid	+	+	-	+
Steroid	-	-	+	-
Carbohydrate	+	+	-	+
Tannin	+	+	+	+
Flavonoids	-	+	-	-
Saponins	-	-	-	-
Terpenoid	-	-	-	-
Coumarins	+	+	-	-
Phenols	-	-	-	-
Carboxylic acid	-	-	-	-
Amino acids	-	-	-	-
Resin	-	-	-	-
Quinone	+	+	-	-

DISCUSSION

The standardization of *Achyranthes aspera* Linn fruit with special reference to macroscopic, microscopic, physio chemical, preliminary phytochemical analysis was done in this study. *Apamarga* fruit One seeded, cylindrical, oblong in shape, shiny brown, taste is mealy sweet and with no characteristic Odor. Microscopy of the fruit showed the presence of aleurone grains, fixed oil globules and vascular bundles embedded in the cotyledon. Powder microscopic depicted starch grains, Perisperm embedded with starch, epidermal cells of cotyledon, fibers with forked tips, Pitted vessels, thick-walled parenchyma cells, Glandular trichomes, pericyclic fibers overlapping with underneath parenchyma, Trichomes, pollens, Rosette crystals etc.

The foreign matter was nil as sample drug was collected directly from the field personally. The total ash indicating total inorganic content was found to be 6.215 ± 0.01. Acid insoluble

part of total ash, which indicates silica, was found to be 0.19 ± 0.01. Loss on drying indicating moisture and other volatile matter was determined to be 1.79 ± 0.01. Water soluble secondary metabolites was found to be 2.79 ± 0.01.

The study of plant chemistry is nothing but phytochemical study. Here total of 4 extracts were studied namely, Aqueous extract, Alcoholic extract, Chloroform fraction and Ethyl acetate. Study has shown the presence of Alkaloid, Steroid, Carbohydrate, Tannin, Flavonoids, Coumarins and Quinone. The alkaloid and carbohydrate were present in all extract except Chloroform fraction. Tannin was also present in all extract. Steroid was found to be present only in Chloroform extract. Flavonoids in Alcohol extract and Quinone in both Aqueous and alcohol extract. The presence of these phytochemical will certainly exert its clinical efficacy.

Every part of Apamarga has been used in one or the other diseases as mentioned in Samhitas and their efficacy has been proved through researches too. The fruit of Apamarga is mentioned for *Atyagni* condition and it is possessed with *Madhura rasa* and *vipaka*.[12] Hence the study is carried out to prove it with pharmacognostical and phytochemical evidences.

CONCLUSION

The preliminary pharmacognostical and physicochemical constant is unique contribution of this research study through which the drug is standardized. This identification of phytochemicals and physicochemical constant values of this study would be used as reference standards for the research scholars. The phytochemical parameters help in the determination of the pharmacological activities. Standardization of drug help in the further carrying of animal experiments and clinical trail where new drug development can be done.

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