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ORIGINAL RESEARCH ARTICLE- EXPERIMENTAL STUDY

CHARACTERISATION OF WHOLE PLANT OF APAMARGA (*ACHYRANTHUS ASPERA*) VIS-A-VIS PHYSICO-CHEMICAL PROPERTIES, HPTLC AND POWDER MICROSCOPY

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ABSTRACT

Context: Physico-chemical analysis of drug *Apamarga* (*Achyranthus Aspera*) to know the genuine drug. **Aims:** Evaluation of Physico-chemical parameters and to develop analytical profile of drug *Apamarga* (*Achyranthus Aspera*). **Methods and Material**: As the *Apamarga* is grown everywhere like a weed and easily available also possess various medicinal properties was collected from specific source in partially dried form and dried completely, made in to powder form and various analysis like Organoleptic characters, Physico-chemical, HPTLC (High performance thin layer chromatography) & powder microscopy carried out. **Results**: The colour of powder was greenish brown, with bitter taste, the results obtained from analytical study shows that it contain 11.65% of moisture even after complete drying and pH 6.5 shows mild acidic in nature, and Ash value 9.75%, HPTLC photo documentation and R_f, (Retention factor) at 254nm, 366nm and 620nm after post Derivatization different peaks and R_f values at different nanometers was recorded and R_f value – 0.63 (purple) after post Derivatization – suggested the presence of oleanolic acid. Whereas powder microscopy explains about the presence of Pith parenchyma, Parenchyma with starch, Sclereids, seed testa etc. **Conclusion:** As the *Apamarga* is grown everywhere very commonly and possess various medicinal properties also can be used in different dosage forms and useful in wide range of therapeutic applications, hence the authentic *Apamarga* can be inferred by above said organoleptic characters and analytical parameters.

Keywords: *Apamarga, Achyranthus Aspera Analytical parameters, Physico-chemical, Powder microscopy, HPTLC*

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INTRODUCTION

Apamarga (*Achyranthes aspera*) is a species of plant in the *Amaranthaceae* family [1]. It has synonyms like *Adhahshalya*, *Apamarga* (plant acts as a hindrance to passers*), Kharamanjari* and *Avakpuspi* (refer to the arrangements in regular rows of flowers and fruits, the presence of sharp points on them and that the latter are downwardly pointed), *shaikharika* [2] (it grows abundently in higher attitudes), *Kihini* (Cures the eruptive conditions like vrana etc. or its touch may produce eruptions), *Mayuraka* (resemblance to the crest or comb on a Peacock's head), *Sikhari*(has flowers at the top), *Pratyakpuspi* (leaves of the plant are borne at right angle to the stem), *Markata* (Leaves have close apprised hairs beneath) [2, 3]. It is distributed throughout the tropical world can be found in many places growing very commonly. It possesses various medicinal properties and not only used as single drug but also, can be used in different dosage forms and useful in wide range of therapeutic applications. It's widely used as medicinal purposes especially as diuretics and even used for obstetrics and gynaecology purpose and it is having symptomatic healing effect in malaria fever [4].

Analytical study is the application of a process or a series of processes in order to identify and quantify a substance, the components of a solution, or the determination of the structures of chemical compounds and elements. Having ascertained the nature of the constituents of a given sample, the analyst determines how much of each component or of specified components are present. Such determinations lie within the realm of quantitative analysis. Each and every drug substance has its own physical and chemical characteristics which help for separating it from other closely related drug. Hence, Physico-chemical analysis provides the objective parameters to fix up the standards for quality of raw drugs.

Chemical constituents

Achyranthes aspera contains Oleanolic acid glycosides; ecdysterone, ecdysone, betaine, tritriacontanone, pentatriacontan-17-ol,27-cyclohexylheptacosan7-ol, 16-hydroxy-26 methylheptacosan -2-one, 4methylheptatriacont-1-en-10-ol, etracontanol, βsitosterol, pentatriacontane, pentatriacontan-6one, hexatriacontane, triacontane, hentriacontane, octacosan-10-one, triacosan-4-one; lauric, myristic, palmitic, stearic, oleic, linoleic, arachidic and behenic acids[5].

MATERIALS AND METHODS

The materials are collected from the various classical books of Ayurveda and the panchanga (whole plant) of *Apamarga* (*Achyranthus Aspera*), is collected in partially dried form from Hassan district of Karnataka

and dried in sunlight, made in to powder form and done various Analytical parameters like Organoleptic characters, Physico-chemical, HPTLC (High performance thin layer chromatography) & powder microscopy in S.D.M. centre for research in Ayurveda and allied sciences Udupi, Karnataka**.**

Methodology:

Preparation of Apamarga powder: The collected Apamarga is dried in sunlight after complete drying it is crushed with the help of khalwa yantra (Mortar and pestle) and then it is put in mixer grinder and filtered through sieves and stored in air tight container for analysis.

Methods adopted for Analytical study [6]:

1. Organoleptic characters

Organoleptic characters of the Raw Drug (Apamarga) and Apamarga kshara were noted using sensory organs.

2. Loss on drying at 105°C

10 g of sample was placed in tare 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.

3. Total Ash

Two gram of sample was incinerated in a tare 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.

4. Acid insoluble Ash

To the crucible containing total ash, add 25ml of dilute HCl and boil. Collect the insoluble matter on ashless filter paper (Whatmann 41) and wash with hot water until the filtrate is neutral. Transfer the filter paper containing the insoluble matter to the original crucible, dry on a hot plate and ignite to constant weight. Allow the residue to cool in suitable desiccator for 30 mins and weigh without delay. Calculate the content of acid insoluble ash with reference to the air dried drug.

5. Water soluble ash

Boil the ash for 5 min with 25 ml of water; collect insoluble matter on an ashless filter paper, wash with hot water, and ignite for 15 min at a temperature not exceeding 450˚C. Subtract the weight of the insoluble matter from the weight of the ash; the difference in weight represents the water soluble ash with reference to the air-dried sample.

6. Refractive index

Placed a drop of water on the prism and adjusted the drive knob in such a way that the boundary line intersects the separatrix exactly at the centre. Noted the reading. Distilled water has a refractive index of 1.3325 at 25˚C. The difference between the reading

and1.3325 gives the error of the instrument. If the reading is less than 1.3325, the error is minus (-) then the correction is plus (+) if the reading is more, the error is plus (+) and the correction is minus (-). Refractive index of oil is determined using 1 drop of the sample. The correction if any should be applied to the measured reading to get the accurate refractive index. Refractive index of the test samples were measured at 28˚C.

7. Specific gravity

Cleaned a specific gravity bottle by shaking with acetone and then with ether. Dried the bottle and noted the weight. Cool the sample solution to room temperature. Carefully filled the specific gravity bottle with the test liquid, inserted the stopper and removed the surplus liquid. Note the weight. Repeat, the procedure using distilled water in place of sample solution.

8. Determination of pH

Preparation of buffer solutions:

Standard buffer solution: Dissolved one tablet of pH 4, 7 and 9.2 in 100 ml of distilled water.

Determination of pH: 1 ml of sample was taken and mark up to 10 ml with distilled water, stirred well and filtered. The filtrate was used for the experiment. Instrument was switched on. 30 minutes time was given for warming pH meter. The pH 4 solution was first introduced and the pH adjusted by using the knob to 4.02 for room temperature 30°C. The pH 7 solution was introduced and the pH meter adjusted to 7 by using the knob. Introduced the pH 9.2 solution and checked the pH reading without adjusting the knob. Then the sample solution was introduced and reading was noted. Repeated the test four times and the average reading were taken as result.

9. HPTLC (High performance thin layer chromatography)

Sample preparation

1gm of sample was dissolved in 10.0ml of alcohol, warmed on a water bath and filtered. 4, 8 and 12µl of the above sample was applied on a precoated silica gel F254 on Aluminum plates to a band width of 8 mm using Linomat 5 TLC applicator. The plate was developed in Chloroform: Methanol (9.0:1.0) and the developed plates were visualized under UV 254nm, 366nm and after derivatisation in vanillin-sulphuric acid spray reagent the plates were scanned at 254nm, 366nm and 620nm post derivatisation. R_f, color of the spots and densitometric scan were recorded[7].

10. Powder microscopy [8]

A pinch of powder was warmed with drops of chloral hydrate on a microscopic slide and mounted in glycerine. Slides observed under microscope and diagnostic characters were observed and photographed using Zeiss AXIO

trinocular microscope attached with Zeiss Axio Cam camera under bright field light. Magnifications of the figures are indicated by the scale-bars

RESULTS OF ANALYTICAL STUDY:

Analytical study provides detail data of specific formulations. It helps in determination of the values of different parameters used for the analysis of a sample. It is a marking line to note the limits or range of the values.

Table 1: Organoleptic characteristics of *Apamarga* (whole plant)

Parameters	Observation
Color	Greenish brown
Odour	Characteristic
Taste	Mildly bitter
Appearance	Fine powder

Table 2: Physico-chemical parameters for *Apamarga (whole plant)*

Parameters	Result % w/w
Loss on drying	11.65
Total ash	9.75
Acid insoluble ash	2.29
Water soluble ash	2.49
pH	6.5

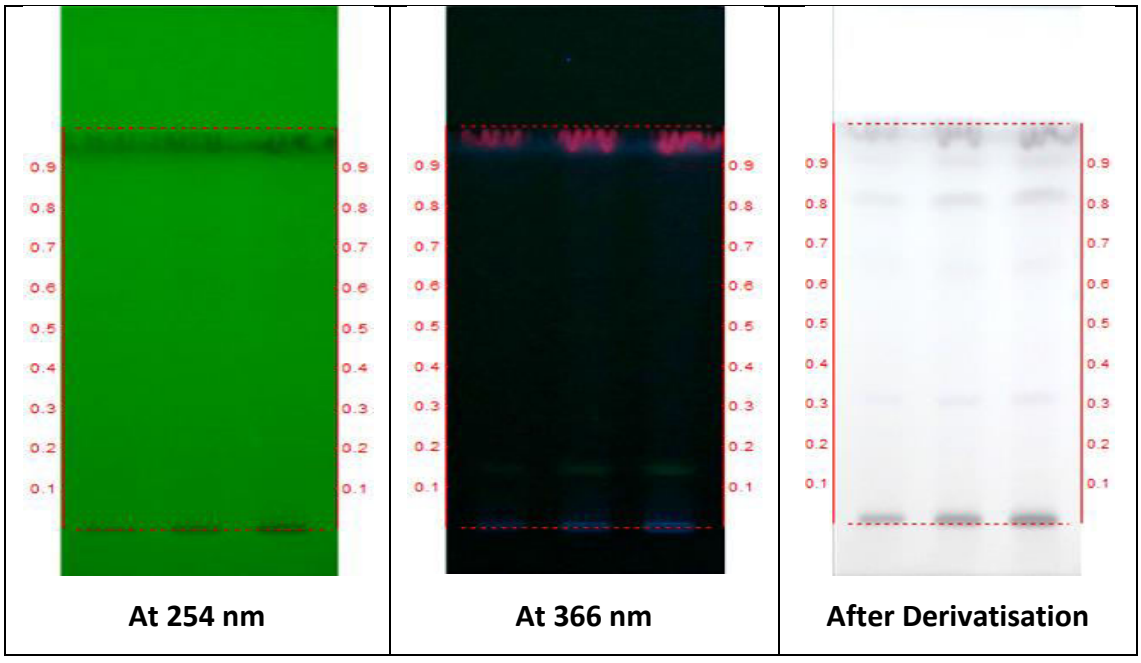


Figure 1. HPTLC photo documentation of Ethanol extract of *Apamarga* (Whole plant raw drug)

Track 1- 4µl; Track 2 - 8µl; Track 3- 12µl

Solvent system- Chloroform: Methanol (9.0: 1.0)

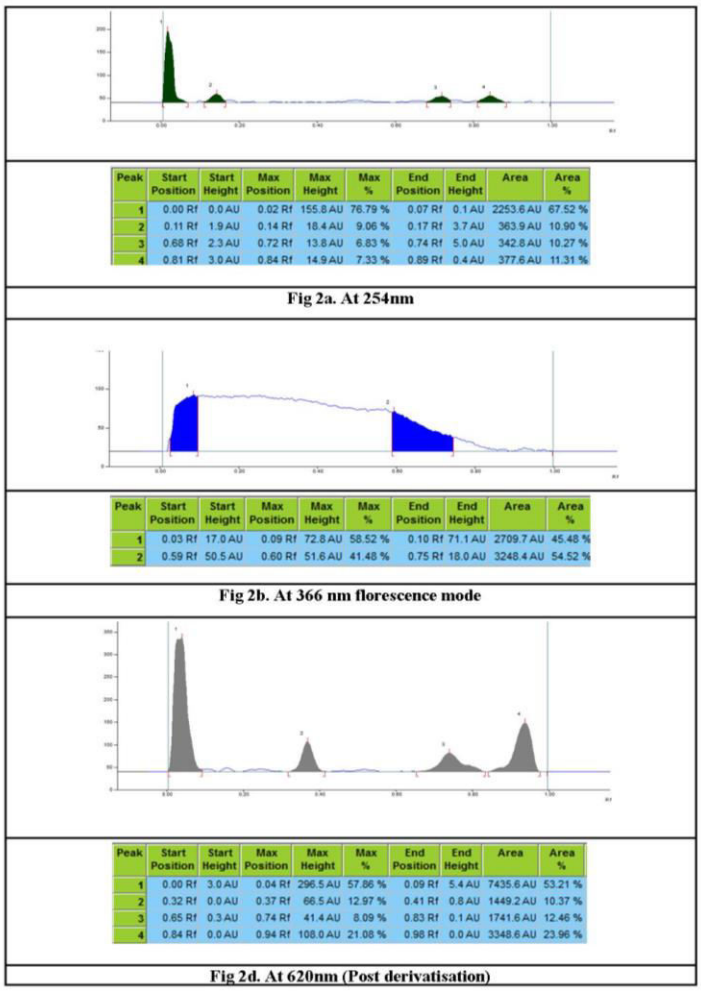
Table 3: R_f values of all the samples

At 254 nm	At 366 nm	After Derivatisation
-	0.08 (FL. blue)	-

-	0.09 (F. red)	-
0.12 (L. green)	-	-
-	0.15 (F. green)	-
-	-	0.31 (L. purple)
-	0.63 (F. red)	0.63 (L. purple)
-	-	0.76 (L. purple)
-	-	0.81 (D. purple)
-	0.90 (F. red)	0.90 (L. purple)

D – dark; L – light; F – fluorescent

Result: R_f value – 0.63 (purple) after Post Derivatization which is near to R_f value - 0.57 purple (oleanolic acid) – suggested the presence of oleanolic acid, is under the standards of - Quality standards of Indian medicinal plants (CCRAS) [9].



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	0.0 AU	0.02 Rf	155.8 AU	76.79 %	0.07 Rf	0.1 AU	2253.6 AU	67.52 %
2	0.11 Rf	1.9 AU	0.14 Rf	18.4 AU	9.06 %	0.17 Rf	3.7 AU	363.9 AU	10.90 %
3	0.68 Rf	2.3 AU	0.72 Rf	13.8 AU	6.83 %	0.74 Rf	5.0 AU	342.8 AU	10.27 %
4	0.81 Rf	3.0 AU	0.84 Rf	14.9 AU	7.33 %	0.89 Rf	0.4 AU	377.6 AU	11.31 %

Fig 2a. At 254nm

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.03 Rf	17.0 AU	0.09 Rf	72.8 AU	58.52 %	0.10 Rf	71.1 AU	2709.7 AU	45.48 %
2	0.59 Rf	50.5 AU	0.60 Rf	51.6 AU	41.48 %	0.75 Rf	18.0 AU	3248.4 AU	54.52 %

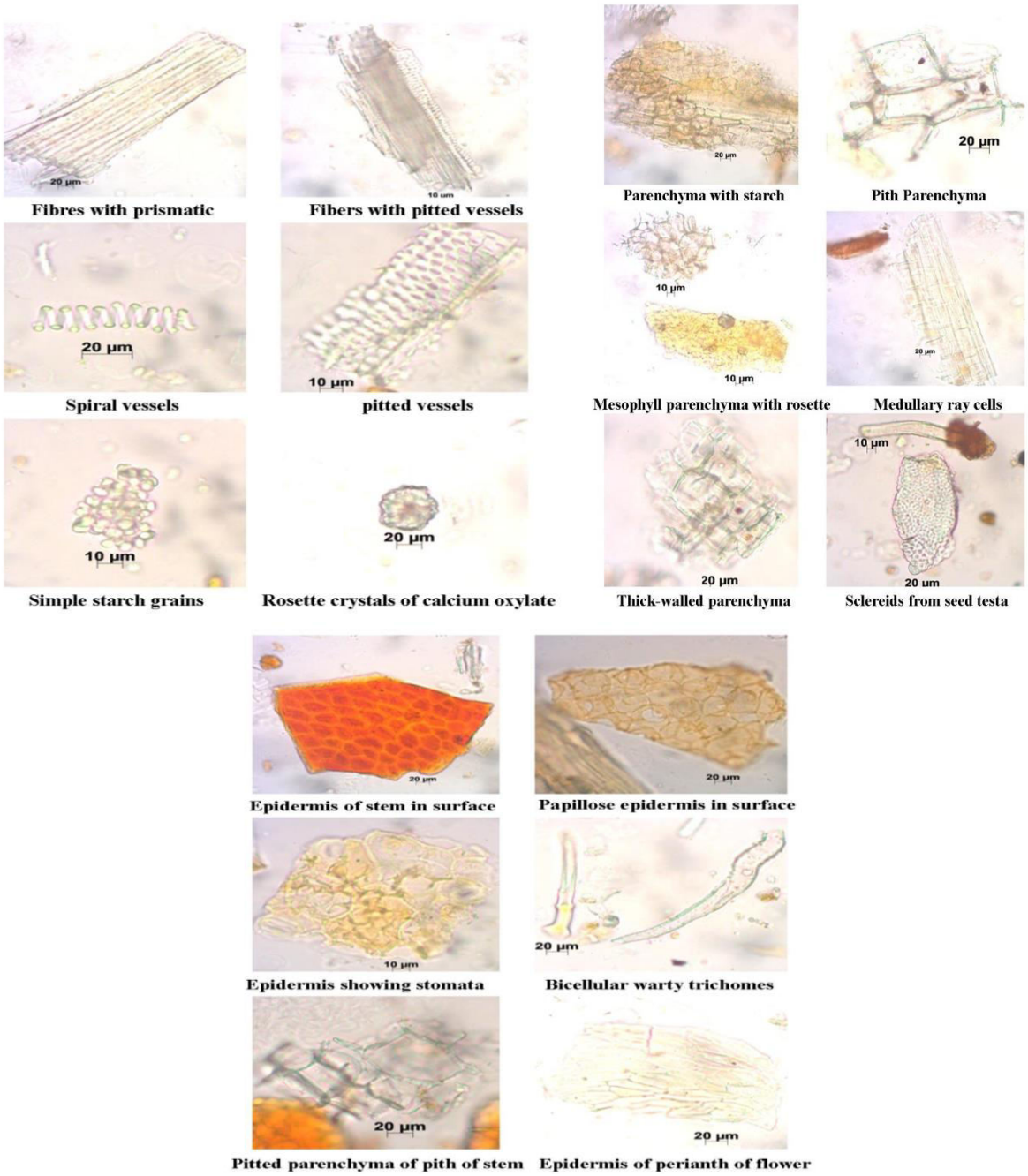
Fig 2b. At 366 nm florescence mode

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	3.0 AU	0.04 Rf	296.5 AU	57.86 %	0.09 Rf	5.4 AU	7435.6 AU	53.21 %
2	0.32 Rf	0.0 AU	0.37 Rf	66.5 AU	12.97 %	0.41 Rf	0.8 AU	1449.2 AU	10.37 %
3	0.65 Rf	0.3 AU	0.74 Rf	41.4 AU	8.09 %	0.83 Rf	0.1 AU	1741.6 AU	12.46 %
4	0.84 Rf	0.0 AU	0.94 Rf	108.0 AU	21.08 %	0.98 Rf	0.0 AU	3348.6 AU	23.96 %

Fig 2d. At 620nm (Post derivatisation)

Figure 2. Densitometric scan of ethanol extract of Apamarga (Whole plant raw drug)

Powder microscopy



DISCUSSION:

The word 'Apamarga'(*Achyranthus Aspera*) itself mean the plant which acts as a hindrance to passers. Apamarga can be authentified by various Physico- chemical analysis, the colour of the dried Apamarga powder shows greenish brown which may be due to colour of drug and also after drying in direct sunlight the moisture will evaporates turns to brownish colour, the pH shows mild acidic in nature this may be due to presence of certain chemical compounds like, Oleanolic acid glycosides, ecdysterone, ecdysone, betaine, tritriacontanone etc, Apamarga also

contain Ash value 9.75% which indicates the presence of ash content in it so it may be a reason Apamarga is used as one of main drug for the preparation of Apamarga kshara(Alkali preparation after burning whole plant)[10]. The other analysis like moisture content, acid insoluble ash, water soluble ash all are found under the standards of API (Ayurvedic pharmacopeia of India)[10]

The HPTLC photo documentation suggested the R_f value – 0.63 (purple) after post Derivatization – suggested the presence of oleanolic acid, also powder microscopy [11] , shows about the presence of Pith parenchyma, Parenchyma with starch, Sclereids etc, which indicates the genuenity of the drug. Hence it can be considered that the analytical parameters are important to know the genuenity of any drug the plant *Apamarga.*

CONCLUSION:

The authentic and genuine *Apamarga* can be inferred by above mentioned standards Organoleptic characters and analytical parameters like Moisture content(11.65%), Ash value(9.75), Acid insoluble ash(2.29%), water soluble ash(2.49%), pH(6.5), HPTLC(oleanolic acid.), and powder microscopy (presence of Pith parenchyma, Parenchyma with starch etc) which proved the drug is authentic and genuine .

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