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Original Research Article

Standardization of Different Plant Parts of *Adhatoda Vasica* Nees. for Validation of it's Quality Evaluation with Special Emphasis on Ayurveda

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Abstract

Ayurveda system nearly using 90 percent of the Crude drugs is obtained from the plant sources. The drugs of plant origin especially of herbaceous nature are frequently used as whole plant; otherwise their parts such as root, stem, leaf, flower, seed, fruit, rhizomes, bark of a stem or root, wood, and their exudates or gums etc. Adhatoda vasica Nees. is an important drug used mainly in the Ayurvedic industry in their formulations. The present study deals with the application of analytical methods for to find out the variations with in different parts of a single plant by adopting pharmacognostical phytochemical and analytical parameters in Adhatoda vasica plant .Identified fresh materials of Adhatoda vasica categorized in to 5 type of samples according to different ratio of stem and root portions of plant material were documented.

Keywords: Morphology, Pharmacognosy, Chemical parameters

INTRODUCTION

Adhatoda vasica is a well known plant in indigenous system of medicine.It shows various type of pharmacological actions like bronchodilatory[6], expectorant, antifungal,antiviral,hepatoprotective,antibacterial,anti inflammatory,anti ulcer,cardio vascular protective, and anti oxidant properties . A vasica belonging to the family Acanthaceae[2] is an dense shrub with many long opposite ascending branches, it is a glabrous shrub. Stem with brownish, yellow bark Leaves are elliptic lanceolate, petiolate and glabrous when mature, dark brown above and paler beneath ,main nerves are 10-12 pairs with reticulate venation .Flowers in short dense axillary pedunculate spikes.The plant is distributed in through out India, and often cultivated. The roots, leaves and flowers are active principles of the plant posses a number of pharmacological properties and are used in cough, chronic bronchitis, rheumatism, asthma and bronchial asthma. Root, Leaves, Flowers are the main parts of the plant is widely using for various traditional Ayurvedic preparations. All parts of the plant are bitter due to the presence of alkaloid. The leaves extract of Adhatoda vasica reported Antibacterial, Antimicrobial and Antioxidant activities [7].In India it is known as Malabar nut in English1,Arusa in Hindi,Vasa in Sanskrit and Atalodakam in Malayalam etc.It is an important and a powerful Ayurvedic medicinal plant mainly used for cough and asthma3.Main constituents of this plant are alkaloids (Quinazoline alkaloid,Vasicine and Vasicinol)and oils. The present study

deals with the application of analytical methods for finding out the variations with in different parts of a single plant by adopting Pharmacognostical and chemical analytical methods.

Materials and methods

Identified fresh materials of Adhatoda vasica five plants were collected from Vaidyaratnam Ayurveda Research Institute garden and to arrange the materials in to five category of samples, according to different proportions of root and stem. And the samples are washed under running tap water to remove soil particles.

Macroscopy of samples:

Sample 1- Root only (4 cm length),Sample 2 - Root with half portion stem (6 cm length),Sample 3 - Root with equal portion stem(9 cm length),Sample 4 - Root with 2parts of stem(More than 15 cm) ,Sample 5 - Whole plant without leaf (More than 30 cm) respectively.

All the samples dried under shade and powdered separately with the help of a mechanical pulverizer. These samples were used for carried out Microscopical & Thin layer chromatographic studies .For the preparations of Powder analysis with the help of microscopic slides : powdered samples are passing through 180 micron IS sieve separately is treated in this process of microscopical examination .Then mounted in a glass slide with 50% of glycerol S and viewed under microscope. Photographs are captured with the help of image capturing software and to taken the measurements of plant characters.Tradtional kashaya preparation method taken as a base to the extract preparation for TLC analysis and chemical analysis.

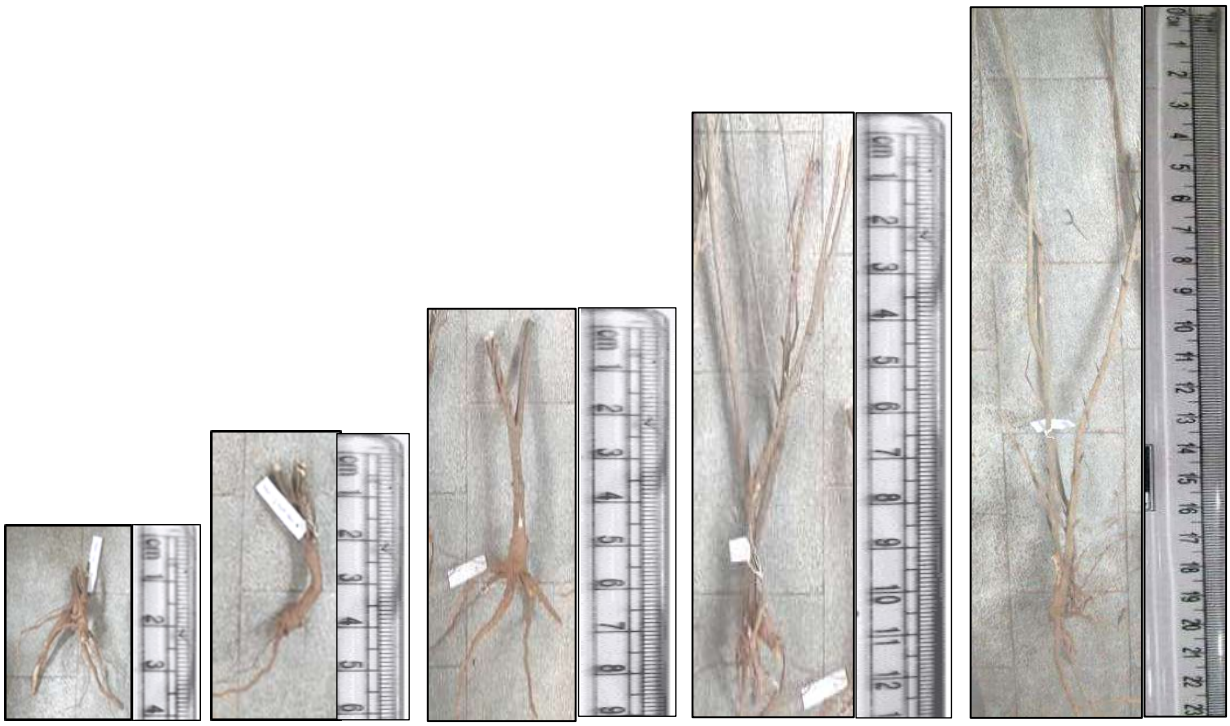
Experimental analysis of results

Macroscopy of plant

Macroscopy of test samples

Each sample is hard ,woody,and root having lateral branches.Surface is rough due to longitudinal cracks .Fresh samples shows stem with yellowish bark in fresh condition and it becomes greyish-brown to dark brown when dry. Internally creamish-white in colour. Taste- bitter. Odour-not specific.

1. Sample 1- Root only (4 cm length)
2. Sample 2 - Root with half portion stem (6 cm length)
3. Sample 3 - Root with equal portion stem(9 cm length)
4. Sample 4 - Root with 2parts of stem(More than 15 cm)
5. Sample 5 - Whole plant with out leaf (More than 30 cm)

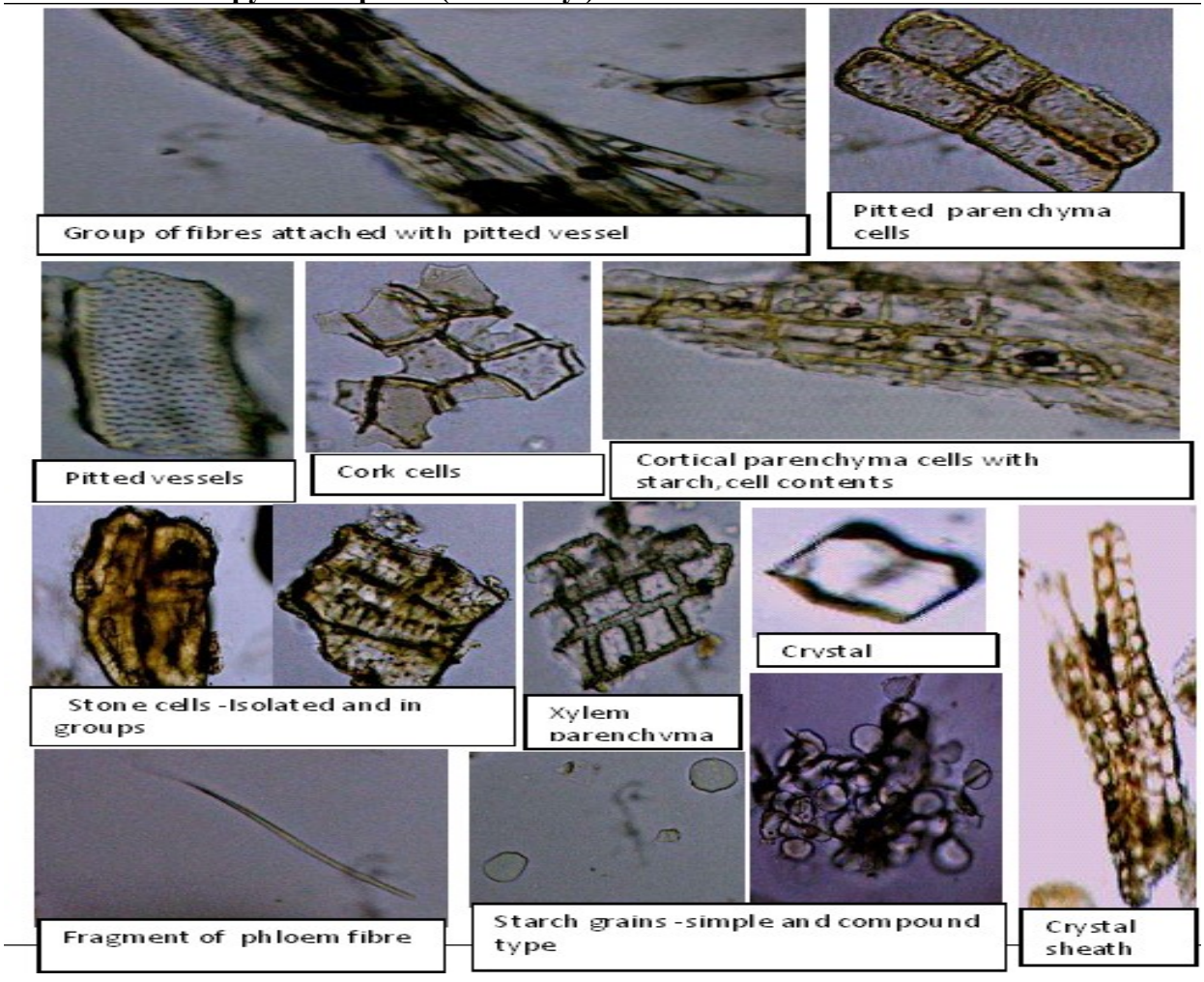


Sample 1 Sample 2 Sample 3 Sample 4 Sample 5

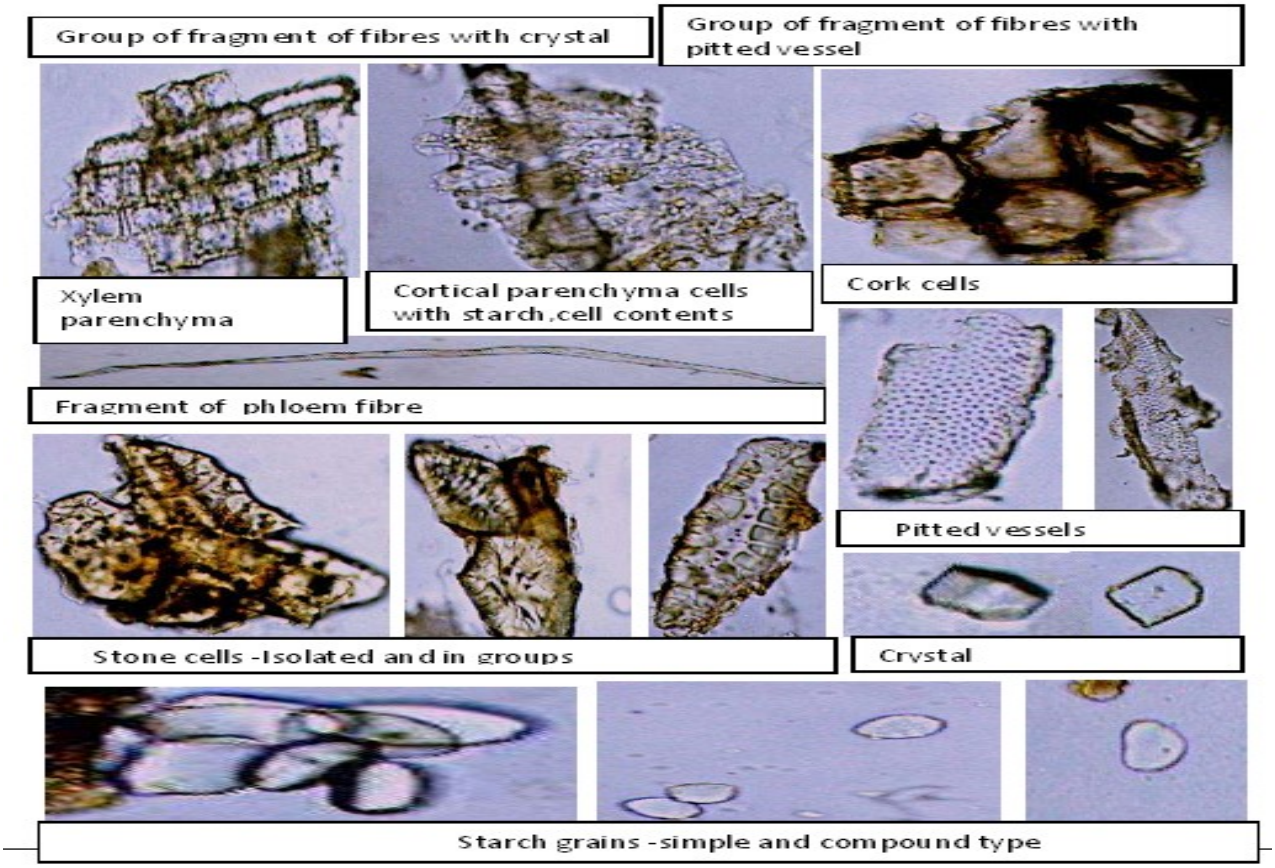
Powder microscopy

Powder microscopic analysis[4] is one of the method for to authenticate herbal drugs by identifying its powder characters.The characters are shown in Fig 3-7 and the main features are tabulated in Table1

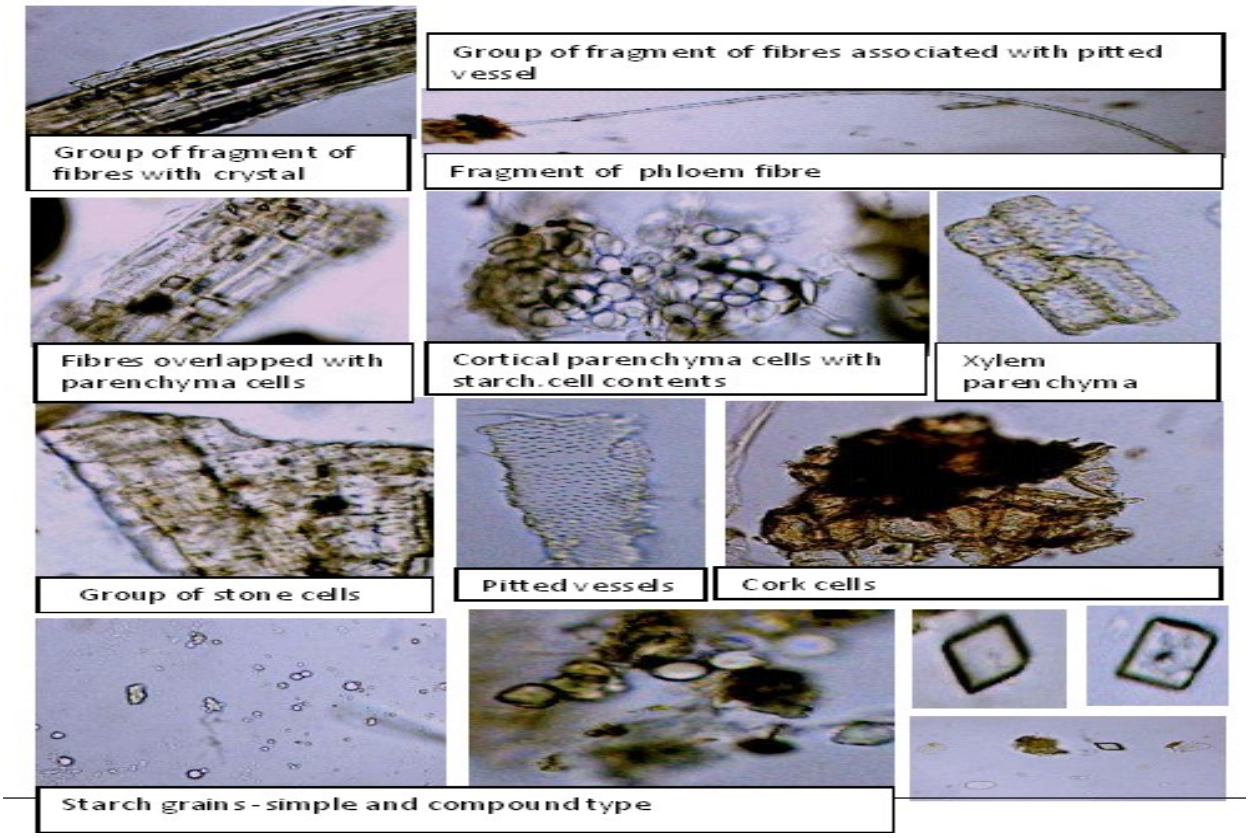
Powder microscopy of sample :1 (Root only)



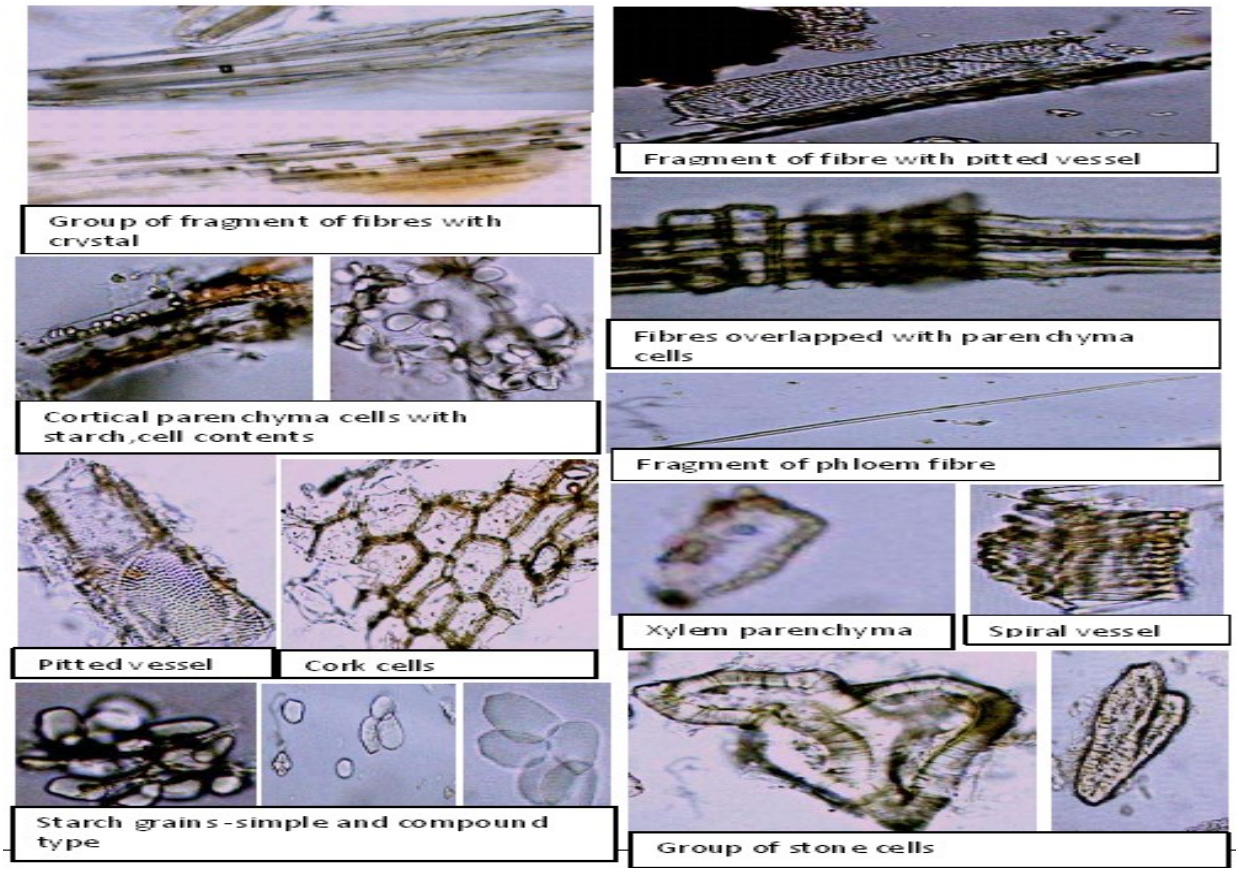
Powder microscopy of sample:2 (Root with half portion of stem)



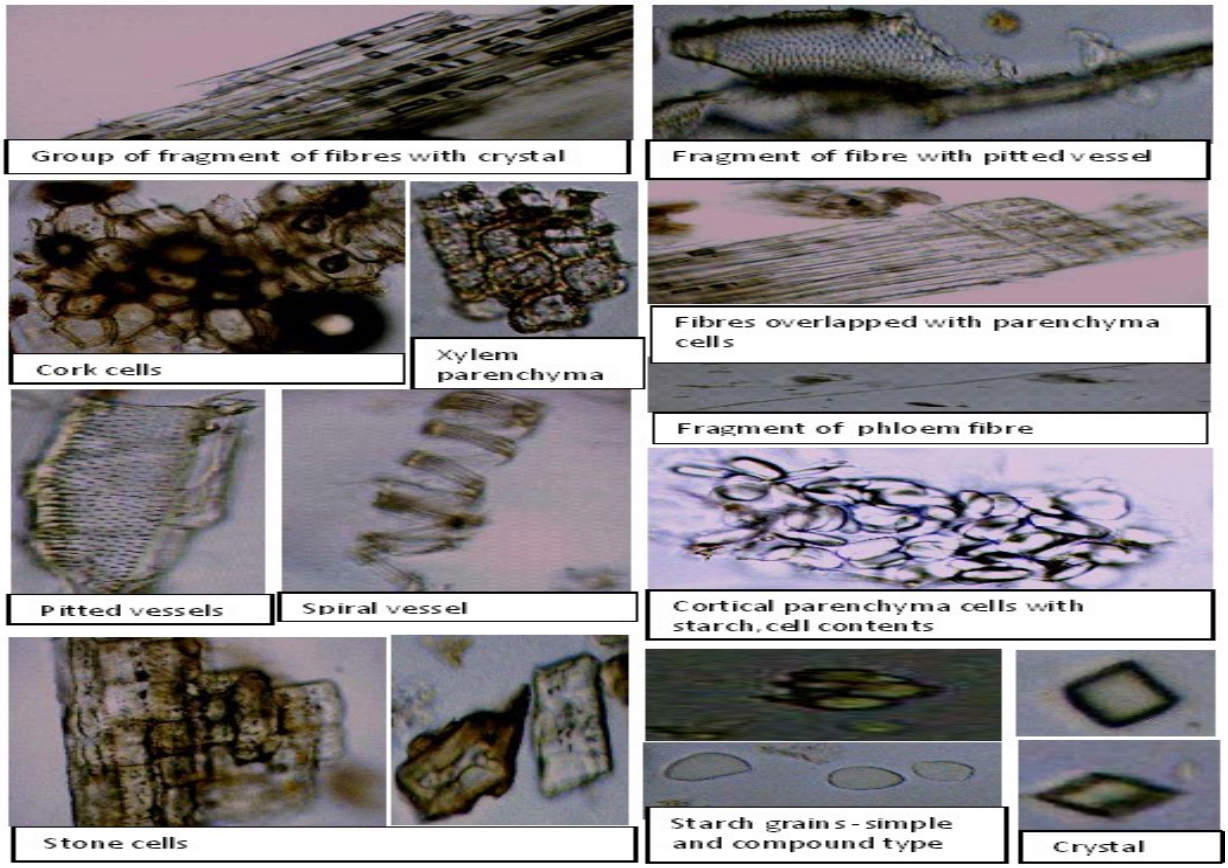
Powder microscopy of sample: 3(Root equal to stem)



Powder microscopy of sample :4 (Root with 2 parts of stem)



Powder microscopy of sample: 5 (Wholeplant without leaf)



Diagnostic characters:
Table 1

NO	POWDER MICROSCOPIC CHARACTERS	SAMPLE NAME				
		Sample 1 **ROOT ONLY**	Sample 2 **ROOT WITH 1/2 STEM**	Sample 3 **ROOT= STEM**	Sample 4 **ROOT WITH 2 PART STEM**	Sample 5 **WHOLEPLANT WITH OUT LEAF**
1	Group of entire /fragment of fibres with crystal	Present	Present	Present	Numerous	Numerous
2	Wide lumened isolated or group of xylem fibres attached with pitted vessels	Present . Fibres are in group	Present . Fibres are in group	Present . Fibres are in group	Present . Isolated type of fibre identified.	Present
3	Wide lumened group of xylem fibres overlapped with parenchyma cells	Present	Present	Present	Present	Present
4	Fragment of phloem fibre	Present	Present	Present	Present	Present
5	Fragments of pitted vessels	Present- Isolated or in groups	Numerous . Isolated or in groups	Numerous .Isolated or in groups	Numerous. Isolated or in groups	Numerous .Isolated or in groups
6	Cortical parenchymatous cells with starch	Present	Present	Present	Present	Present
7	Fragment of crystal Sheaths	Present	Not identified	Not identified	Not identified	Not identified
8	Pitted parenchyma	Present	Present	Present	Present	Present
9	Stone cells (isolated/group)	Mostly in groups	Mostly in groups	Only in groups	Numerous.only in groups	Numerous. Mostly in groups
10	Prismatic crystals	Present 41.03-81.76 µ in length and 54.84-117.02µ in breadth	Present 51.48-68.95 µ in length and 37.03-65.33 µ in breadth	Present 56.38-101.44 µ in length and 45.84-84.06 µ in breadth	Present .But isolated type of crystals are not identified	Present 38.66-65.33µ in length and42.54-103.85 µ in breadth
11	Starch grains -	Numerous- Big & small type 10.87-85.82 µ in diameter. Simple and compound type .Some grains divided in to 2-3 components, round to oval in shape	Big,small type 11.69-63.54 µ in diameter Simple and compound type.Some grains divided into 2 components, round to oval in shape	Big,small type 9.45-67.41 µ in diameter Simple and compound type .Some grains divided in to 2-3 components, round to oval in shape	Big,small type 25.16-81.81 µ in diameter Simple and compound type having 2-4 components, round to oval in shape	Big,small type 10.87-88.45 µ in diameter Simple and compound type having 2-3 components, round to oval in shape

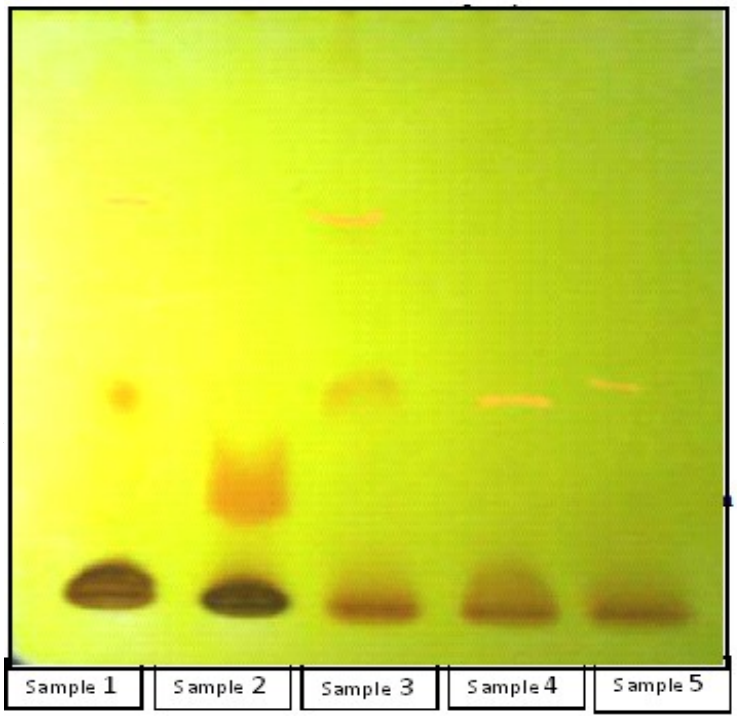
12	Surface view of cork cells	Present	Present	Present	Present	Present
13	Pitted parenchyma cells	Present -group type	Present -group type	Present - group type	Present- isolated type	Present - group type
14	Spiral vessel	Not identified	Not identified	Not Identified	Present	Present

Thin Layer Chromatography

Basic TLC technique involves the application of sample at one end of the silicate plate and allow to develop the plate in suitable solvent system (mobile phase) in a closed glass chamber to the specified height. Derivatization :For the purpose of better visualization of each bands derivatize the plate with suitable spraying reagents and allow to generate the bands in a controlled temperature condition . The $R_{f.}$ Values and the colours of the resolved bands are recorded for finger print profiles. Finger print profiling of Alkaloidal presence in each sample.

Extract preparation : Traditional kashaya preparation method was used for the extract preparation for TLC.1part of samples boiled with 16 times of water and reduce to $1/8^{th}$ final volume 10 ml of that suspension of each sample converted in to methanolic medium. Silica gel $60F_{254}$ (DC Kieselgel 60 $F_{254,}$TLC Silicagel 60 $F_{254,}$CCM Gel de silice 60 F_{254}) is act as stationary phase.Capilary tube is used for the application of samples into the silica plate. Solvent system: Ethyl acetate:Methanol:Ammonia (8:2:0:2).After the movement of mobile phase through the stationary phase derivatize the plate with Dragendroff's reagent.[8]And to calculate the Retention factor value. Present TLC analysis shows the primary detection of alkaloid content in all the sample.

TLC profile



Sample Name	Rf values	
Sample 1	0.25	0.47
Sample 2	0.18	0.31
Sample 3	0.24	0.47
Sample 4		0.32
Sample 5		0.35

Sample 1- Root only (4 cm length)
Sample 2 - Root with half portion stem length)
Sample 3 - Root with equal portion stem
Sample 4 - Root with 2parts of stem
Sample 5 - Whole plant with out leaf

Chemical analysis

For more scientific validation of results also we conducted chemical analytical methods to validate the quality and quantity of 5 samples .

Method of extract preparation for chemical analysis : Aqueous suspension used for further studies.Procedure for total Solids percentage referred from Ayurvedic Pharmacopeia of India,estimation of total

Phenol % by Folin-Ciocalteau method[5] and Total Alkaloid from Harborne method etc. Final results are tabulated in **Table: 2 and Graph :1.** From the analysis point of **Table**

Parameters	Total Solids	Total Phenol %	Total Alkaloid %
Sample 1	5.18 %	0.20 %	0.87 %
Sample 2	6.23 %	0.50 %	0.86 %
Sample 3	4.47 %	0.19 %	0.79 %
Sample 4	5.21 %	0.25 %	0.46 %
Sample 5	4.13 %	0.15 %	0.88 %

RESULTS AND DISCUSSION

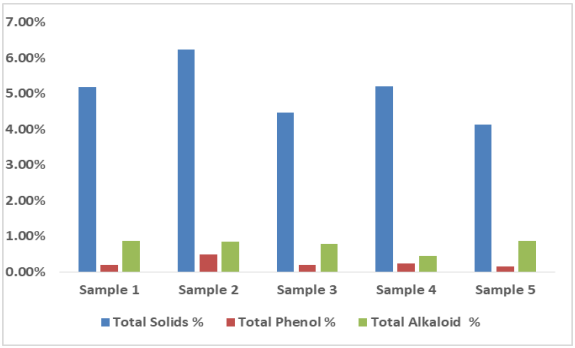
Especially Ayurvedic industry using different parts of *A.vasica* plant parts for various kinds of medicinal formulations. Aim of the present study is taken as a initiation to proves the variations with in different parts of a single plant by applying primary and modern analytical methods. Powder microscopic analysis showed no characteristic diagnostic features in comparative study, exceptional observation is the Spiral type of vessel is only present in sample 4 (Root with 2parts of stem). Traditional kashaya preparation forms of samples were applied to carried out the parameters like TLC, total solids % total phenol % and total alkaloid percentage etc.TLC finger printing of samples showed the detection of bands in all the samples when the plate was derivatize with Dragendroff's reagent. From the chemical analysis point of view Sample 2(root with half portion stem) shows maximum Total solids and Total Phenol content (Table 2).But the alkaloid content was high in sample 1(Root part only) sample 2((root with half portion stem)and sample 5(Whole plant with out leaf).

CONCLUSION

The literature survey revealed that different parts of *Adhatoda vasica* plant has been widely used for to prove it's pharmacological and phyto chemical studies. Now a days all the raw materials are facing a serious problem related with it's availability. It leads to adulterate the plant materials with different plant parts or unwanted parts of same plant. *Adhatoda vasica* plant is an important source of vasicine,Vascinone and some other alkaloids. In this present study five different forms of samples were prepared by *Adhatoda vasica* plant according to various proportions of stem and root taken as a study material to prove the more potential part of plant other than root. Chemical analytical methods proved that the whole plant form of plant contain almost equal amounts of alkaloids as present in root part or near by root portions(Root with half portions of stem).

view Sample 2(Root with half portion stem) shows maximum Total solids and Total Phenol content.

Graph



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