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Development of Pharmacognostic standards of aerial parts *Alysicarpus vaginalis*

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Abstract: *Alysicarpus vaginalis* is a perennial climber belonging to family Fabaceae. It is a plant of significant medicinal importance in the indigenous system of medicine. All the parts of plant are reported for various ethno botanical and therapeutic uses. Vegetative aerial parts, leaves, stem and roots were collected form acroscopical, anatomical, physicochemical, and phytochemical studies. Microscopically leaf of *A.vaginalis* showed presence of stomata, unicellular trichomes. Stem showed wheel shaped appearance on a transverse cut surface, a particular characteristic feature of fabaceae family. Stem and aerial root exhibit dense sclerenchyma and characteristics wedge shaped medullary rays. Quantitative Phytochemical screening shows presence of higher amount of steroid, phenolic, tannins and flavonoids compounds.

Keywords : Pharmacognostic, Phytochemical, Qualitative Microscopy, Quantitative Microscopy**.**

Introduction

Alysicarpus vaginalis(*A. vaginalis*) is a flowering plant of Fabaceae family. It is widely found in the India and other parts of Asia along with the other continents, such as Australia and the Americas. It is commonly identified asalyce clover, buffalo clover, buffalo-bur, one-leaf clover, and white moneywort. This species is an annual or perennial herb; different varieties may be either annual or perennial, and some behave as perennials in wet conditions but grow as annuals in dry regions. [1, 2]

Phytochemistry of *A.vaginalis* consist to different classes such as alkaloids, diterpenoidlactone, steroid, phenolics and aliphatic compounds. It is a traditional useful as a Ayurvedic medicine in India employed for its anti-inflammatory, immunomodulatory, antipyretic activity, antioxidant, antidiabetic,antiallergic and various other medicinal properties.[3] In the present study the vegetative parts leaf, stem and roots of this plant were investigated for macro and microscopical characters with qualitative and quantitative estimation along withphysicochemical analysis and phytochemical study.

Materials and methods

Plant material

The *A. vaginalis* fresh aerial parts like leaves, stem, and roots were collected in the monsoon August-September month, from Aurangabad District, Maharashtra. The plant identification and authentication has been done at Botany Department, BAMU, Aurangabad, Maharashtra (India) and plant specimen was submitted at the

herbarium section of departmental museum of University Botany Department (Accession no. 0607) for future reference.The crude material was dried in shade for five days and then powdered with the help Warring blender.

Qualitative Microscopic evaluation

Using free hand sectioning, very thin sections of the transverse section of the leaf across the mid rib, the stem and the root were obtained. The sections were rinsed with water in the petridishes. The few drops of ethanol were added to dehydrate the cells. The sections were later stained with phloroglucinol and 25% hydrochloric acid before mounting in glycerine on the glass slide and covered with cover slips. The prepared slides were examined under light microscope.

Quantitative Microscopic Evaluation

Quantitative characters of the leaf epidermis were assessed[4]. Slides were prepared for each epidermal surface. Stomata number, stomata index, palisade ratio, and type of stoma were recorded according to Gray, 1964; Evans, 2002.[5,6]

Stomata index was calculated using the formula:

Stomata index = Stomata number x100/ Number of epidermal cells + stomata number

Phytochemical and Physicochemical Screening

Phytochemical and Physicochemical Screening Following the methods of Evans, 2002 and Sofowora, 1993, [6,7] various phytochemical tests were carried out to determine the presence or absence of various phytochemicals and results reported accordingly.

Estimation of various parameters such as ash value and extractive valuewas carried out according to Ajazuddin and Shailendra, 2010.[8]

Quantitative estimation:

Estimation of Total Flavonoids Content

Aluminium chloride colorimetric method was used for determination of total flavonoids content. [9, 10] Plant extract mixed separately with 1.5 mL of methanol, 0.1 mL of 10% aluminium chloride and 0.1 mL of 1M potassium acetate. Above solution was kept at room temperature for 30 min and the absorbance of mixture was measured at 415 nm. The calibration curve was prepared by serial dilution of quercetin in the concentration range 20 to 100 µg/ mL.

Total steroids Estimation

Total steroids of extract had been estimated by the method of David and Plummer, 1987.[11] Standard calibration curve of Diosgenin was prepared form the stock solution of standard Diosgenin (1 mg/mL). To the sample solution, 2 mL of Liberman-Burchard reagent was added and kept it for 10 to 15 min in cold water. The absorbance of solution was measured at 620 nm. The amount of total steroids calculated as a diosgenin equivalent from calibration curve.

Estimation of Total Phenolic Content

The assay was used to determine the total phenolics concentration by using Folin-Ciocalteu's phenol according to Edeoga, Okwu & Mbaebie 2005.[10] Total soluble phenolic compounds in the extracts were expressed as gallic acid equivalents. The 1 mg of extract was dissolved in 2 mL of distilled water. To the above solution 0.3 mL of saturated solution of sodium carbonate (Na2CO3) and 0.1 mL of 1N Folin–Ciocalteu's phenol reagent was added. The mixture was placed in dark for 1h at room temperature and the absorbance was measured at 725 nm against the blank. The total phenolic content was expressed as mg of gallic acid equivalents.

GAE = [(C x V) / M] x 100

C = the conc. of Gallic acid established from calibration curve mg/mL
V = Volume of extract (mL)
M = the weight of dried plant extract (mg)

Results& Discussion

Macroscopic characteristic

Leaf: The leaves are not divided into leaflets. The blades are variable in shape and up to about 6.5 cm long.

Stem: The stems take an erect form or run along the ground,more often erect when growing in dense stands.They reach one meter in length and usually have branches.Racemesare of up to 12 flowers occur at the stem tips and grow from the leaf axils. The flower corolla is half a centimeter long and can be shades of red, purple, blue, or yellow.

Fruit: The fruit is a lightly hairy, cylindrical but compressed legume pod up to 2.5 cm long.

Seed: The dark red seeds are no more than 1.5 mm long.

Microscopy of leaf

The cross section passing through the midrib region showed slight convex at the upper side,broad hump at lower side, single median and well developed collateral vascular bundle. The cross section of lamina shows dorsiventral structure with its mesophyll was differentiated into the palisade and spongy tissue. Mesophyll is divided into palisade and spongy parenchyma. The tubuller palisade cell constitutes about 2-3 layers below the upper epidermis, radially arranged at right angle to the upper epidermis a little more than half of width of mesophyll. Palisade ratio range lies from 3-6.Anomocytic stomata vary from 200-600 mm,measuring 35-47 um length and 15-30 um. Venation is reticulating a number of principal veins. Veins are multicostate and prominent on dorsal side.Vein –islet and vein-termination number are 2-4 mm^{-2} and 6-7 mm.The petiole is slender and fairly long ranging from 3-9cm. The base of the petiole is pulvinate and slightly twisted at base. Transverse section (Fig. 1) of petiole is more or less circular in outline. It shows single layer epidermis and wide zone of cortex,single layered endodermis, wavy arrangement of 3-5layer of fibrous pericycles,and 8-10 vascular bundles arranged in ring and broad zone of central parenchymain the wings.

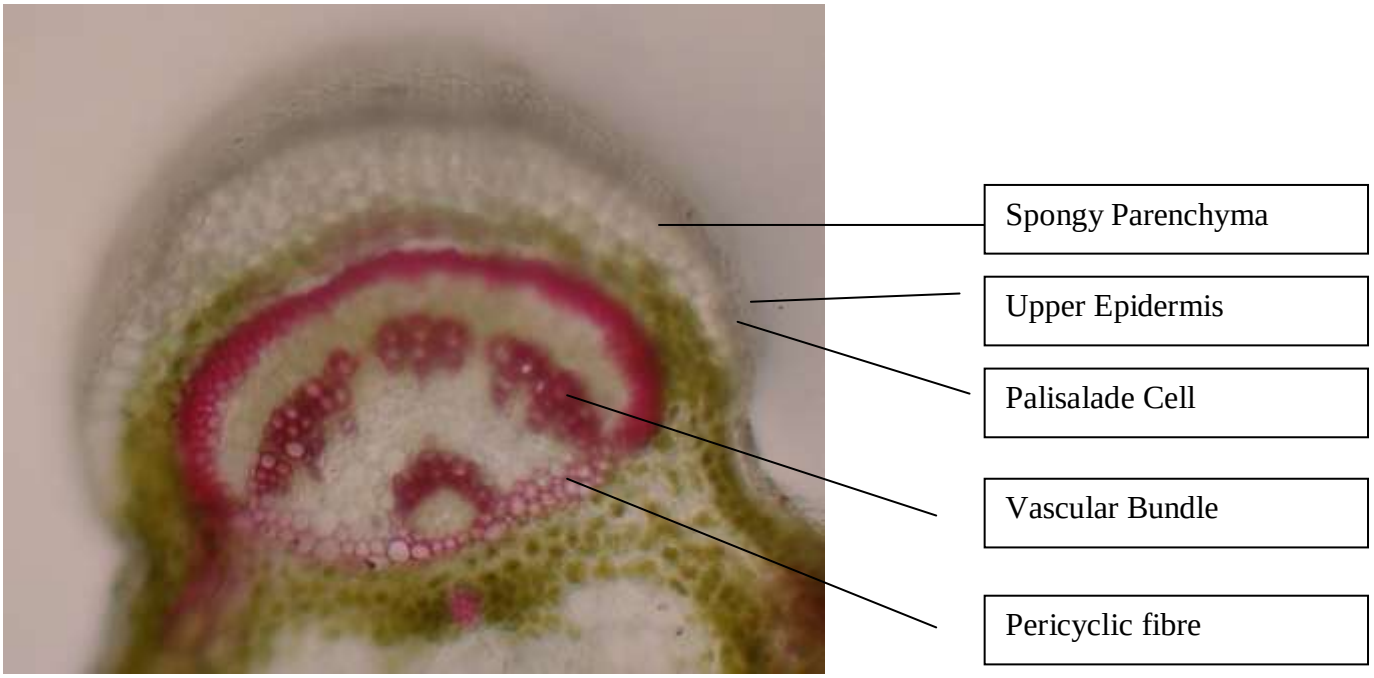

Fig 1: Transverse Section of Leaf of *A. vaginalis*

Microscopy of Stem-

Microscopy of stem shows 2-3 layers of cork followed by 4-5 layered phellogen. Outer most layer of cork is differentiating into outer zone of thick walled brownish and compressed cells, inner zone of thin walled colorless, tangentially arranged 3-7 rows of cells. Cork is broken at some places due to opening of lenticels. Cortex is wide parenchymatous zone contain large columnar type cells filled with mucilage. Cortical cells of outer rows smaller than the inner one, just within the opening of lenticels, groups of sclereids consisting of 2-8 cells found in this region, outer zone of cortex consists of 2-4 rows of irregularly arranged, tangentially elongated chlorenchymatous cells. Several mucilage canals are found scattered in the cortex along with tannin containing cells. Transversely cut surface of stem show secondary anomalous growth. Xylem is stellate in structure. Xylem is united at the center, thereby completely obliterating the pith and giving xylem a stellate appearance with the phloem at the ends of radii. Stem characterized by the presence of bicollateral vascular bundles with wedge shaped strips of xylem externally surrounded by semicircular strips of phloem surrounded by pericylcicfibres. It is dicot stem, containing 2 to 3 layer of epidermis, present in the outermost region with well-defined cuticle extending over it. Hypodermis is present which is made up of collenchymatous tissue forming a zone of 5 to 6 layers of tangentially elongated cells. Endodermis present is followed byschlerenchymatouspericylcicfibres which are lignified, forming 3 to 4 layered bundle cap. Vascular bundles are arranged in a ring forming a continuous cylinder due to activity of inter-fascicular cambium(Fig. 2). Phloem lies externally, consisting of sieve tubes, companion cells, phloem parenchyma and fibres. Phloem is followed by lignified elements of xylem.

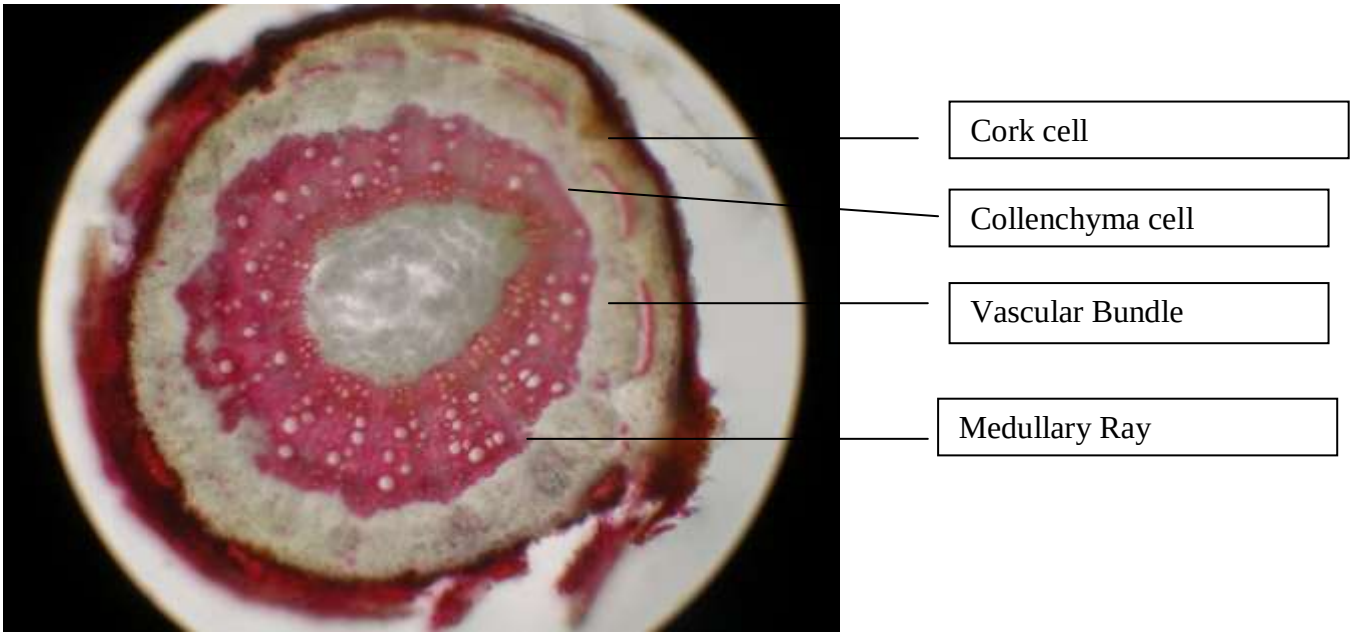

Fig 2: Transverse Section of Stem of *A. vaginalis*

Quantitative and Powder Microscopy

Quantitative microscopy is an important analytical tool in the plant drug analysis, as it gives the reliable data for the identification of drug out of which some of the parameter are independent on the age of drug. Quantitativemicroscopy includes identification and estimation stomata, stomatal no., stomatal index, palisalideration and vein islet and termination no. The results are shown in Table 1.

Table 1: **Quantitative Microscopy of Leaf of *A. vaginalis***

Particulars	**Upper surface**	**Lower surface**
Stomatal number	220- 250	423- 550
Stomatal Index	17-21.2	13.1- 20.4
Vein-Islet Number	18- 26	
Vein Termination Number	43.75- 62.5	
Palisade Ratio	5.5- 10	4- 7.4

*Determination Values per Square mm 1

Stem and root powder is slightly creamish-brown to dark brown with characteristic odour and bitter taste. Under the microscope it shows vessels with reticulate secondary wall thickening tracheids and tracheidal (Fig. 3). Fibers with bordered pits and horizontal perforations; starch grains oval, ovoid elliptical, oval to rounded, or elliptical in shape, mostly simple but sometimes as compound grains of 2 - 5 components, with faintlymarked concentric striation and central hilum appearing like a point.

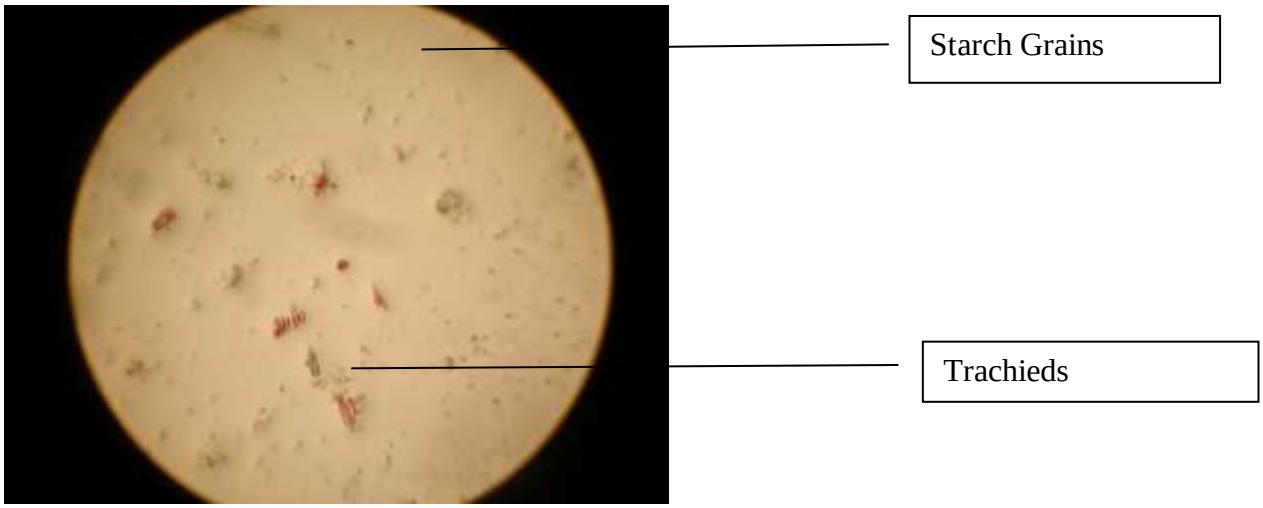

Fig 3: Powder Microscopy of *A. vaginalis*

Physicochemical and phytochemical analysis

In the evaluation of crude drug assessment of ash value is an important tool in the process of standardization of herbal drugs, which mark the impurity level of drug sample. The ash value of plant is shown in Table 2. The histochemical analysis using various reagents showed the presence and absence of primary and secondary metabolites. In the previous study[3] the phytochemical screening of chemical constituents of the plant in different solvents studied such as in petroleum ether, successive chloroform extract, successive methanol extract, and aqueous extracts showed that the alkaloids, flavanoids, phenols, tannins, steroids, and proteinsare present in all extracts but the quantity may vary. Phytochemical previous study [3] show that the maximum presence of compound in aqueous and methanol extracts. Petroleum ether and chloroform were found to be the least effective solvents in extracting phytochemicals, which could be due to lesser amount of non-polar compound in the plant parts. The extractive value in different polarities of solvent is shown in Table 3.

Table 2: Determination of Ash content of *A. vaginalis*

Particulars	**Ash content (% w/w)**
Water soluble	0.93-3.08
Acid insoluble	0.57-0.96
Sulphated ash	0.02-0.04

Table 3: Extractive values of *A. vaginalis*

Particulars	Extractive value (% w/w)
n- Hexane	1.01-5.01
Chloroform	2.01-2.83
Alcohol	5.43-8.6
Water	10.4-14.3

Table4: Estimation of Secondary metabolites in A. vaginalis

Particulars	Secondary Metabolites (% w/w)
Total Steroid content	1.25-3.29
Total Tannin content	2.41-7.10
Total Phenolic content	2.07-5.73
Total Flavonoid content	1.01-3.01

Quantitative analysis of phytochemicals values revealed that the plant posses the higher amount plant secondary metabolites which may be responsible for the biological activity as claim in literature. The amount of various secondary metabolites is shown in Table 4.

Conclusion

The plant A. vaginalis is widely found in the India and uses as important herbal medicine as per the literature, but till date it is not explored in terms of Pharmacognostic standards, In the presentstudy an attempt has been made for morpho-anatomicalas well as physicochemical evaluation of this plant for contribution in the pharmacognostic quality control and knowledge of Fabaceae family.

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