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Comparative morpho-anatomical and Preliminary Phytochemical studies of *Flemingia strobilifera* (L.) *R.Br* and *Flemingia macrophylla* (Willd.) Merr (Fabaceae)

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Abstract: A comparative study of the roots of two species of *Flemingia* is reported. Both the species, *Flemingia strobilifera* and *Flemingia macrophylla*, have been reported to possesses antibacterial, antifungal, antioxidant and most concerning antiepileptic properties. In view of its medicinal importance and taxonomic confusion, the individual morphological and histological characteristics of these two species have been described through certain parameters. In anatomical studies, transverse section and macerated tissue has been examined. In preliminary phytochemical evaluation Ash value, Extractive value, moisture content and phytochemical screening was performed for comparative study of *Flemingia strobilifera* and *Flemingia macrophylla*. These findings will provide referential information for identification of the species *F. strobilifera* and *F.macrophylla*.
Key words: Morphology, anatomy, *Flemingia strobilifera*, *Flemingia macrophylla,* Fabaceae.

INTRODUCTION

Both the species *Flemingia strobilifera* and *Flemingia macrophylla* belongs to family Fabaceae and subfamily Paplionaceae. *F. strobilifera* (Linn) *R.Br*, (Syn. *Moghania strobilifera*) an important medicinal plant, is commonly known as Kusrunt and is found in Sind, Rajputana, Bengal, South India and Andaman's [1, 2]. Literature reveals that the various parts such as its bracts, leaves, flowers and roots of the plant *Flemingia strobilifera* found to be useful in folkloric medicine for its different pharmacological activities such as leaves and flower for tuberculosis, roots for epilepsy, hysterea and swellings, roots juice for diarrhea and dysentery [3]. In Burma, the roots of *F. strobilifera* are used to treat epilepsy. Previous chemical studies showed that flavonoids, flavonoid glycosides, chalcones, epoxychromenes and pterocarpans were the main constituents found in *F. strobilifera* [4, 5].

Flemingia macrophylla is commonly known as Barasalpan, belongs to family Fabaceae. It is distributed throughout the hotter parts of India, Malay peninsula, Java. It is a source for the dye 'wars' and is one of the host plant for the lac insect. *F. macrophylla* is an erect shrub 4-6ft in height, the branches angular sulcate appressedly pubscent. Leaves are trifoliolate

pubescent. Flower racemes, purple.Pods are oblong turgid, finely pubescent. Seeds two, shiny Black. Flowering and fruiting time is Jan-march [6,7]. Roots are used by santals as an external application to ulcers and swellings, mainly of the neck. The plant has also been used as antibacterial and hypoglycemic. Various plant parts are reported to be used in smallpox spleen complaints,cholera dysentery and blindness Phytochemical reports on *F. macrophylla* indicate that the plant contain flavonoids flavanones, flemiflavanone A, B, C, D flemichin A, B, C, D narigenin genistin genistein 5,7,2' 4'-tetrahydroxyisoflavone a sitosterol. homoflemingin flemiwallichin C, myricitrin robinin and flemistricains D,E and F , FleminginA,B and C,D chalcone, lupeol,sitosterol, procyanidin and α- amyrin [8,9].

Literature regarding anatomical and comparative phytochemical details is not available to distinguish *Flemingia strobilifera* and *Flemingia macrophylla*, hence it was decided to establish the morphoanatomical characters and comparative phytochemical studies of these two plants.

EXPERIMENTAL

Material and methods

The roots of *Flemingia strobilifera* and *Flemingia macrophylla* were collected from the Regional Research Institute tarikhet, Ranikhet in the month of Jan 2008. The plants were identified and authenticated by Dr. G.C.Joshi Research officer there. A voucher specimen (No.COP/IFTM/FS-1 and COP/IFTM/FM-1) has been preserved in the Department of Pharmacognosy, College of Pharmacy, IFTM, Moradabad for further reference.

Macroscopic and microscopic studies

The macroscopical characters (size, shape colour, odour, texture, fracture) of the roots were studied following standard methods [10, 11], transverse section of root and powder was Identified with routine reagents to study the lignified cells, fiber, calcium oxalate crystal etc.Permanent slide of TS of root was prepared to observe the presence and arrangement of cellular structures as per the procedure of Johansen. [12] and the representative diagrams were taken with the help of inverted microscope for photodocumentation (Leitz, Japan).Micrometric determinations viz., length and width of vessels ,pericyclic fibres and calcium oxalate crystals were also made using eye piece and stage micrometers(Erma, Japan). For micrometric determinations roots were cleared using Schulz's macerating fluid (Aqueous nitric acid solution (50%v/v) +potassium chlorate).

Determination of physico chemical parameters

The ash values and moisture content with various reagents were determined as per the Indian Pharmacopoeia [13]. Extractive values with various solvents like alcohol and water was performed as per standard procedure [14].

Preliminary phytochemical screening

Preliminary Phytochemical screening of the extracts were carried out for different groups of phytoconstituents following standard procedures described by Harborne [15] and Khandelwal [16].

RESULTS

Morphological details

The root of *Flemingia strobilifera* is 0.7-1.8 cm in diameter, cylindrical with rough surface. Root is brown in color externally and internally it is yellowish brown, its surface is fissured, rootlets and longitudinal striations are present. Fracture is fibrous in nature. No characteristic odour and taste **(**Fig.1).

The root of *Flemingia macrophylla* were 0.8 cm to 2.2 cm in diameter, Sub - cylindrical in shape and longitudinally wrinkled with transverse fissures. Root is dark brown in colour, odourless, taste is pungent. Fractures is long, irregular and fibrous. Several long rootlets and root scars are present (Fig.2).

Fig.1: Root of *F.strobilifera*

Fig.2: Root of *F. macrophylla*

Anatomical characters

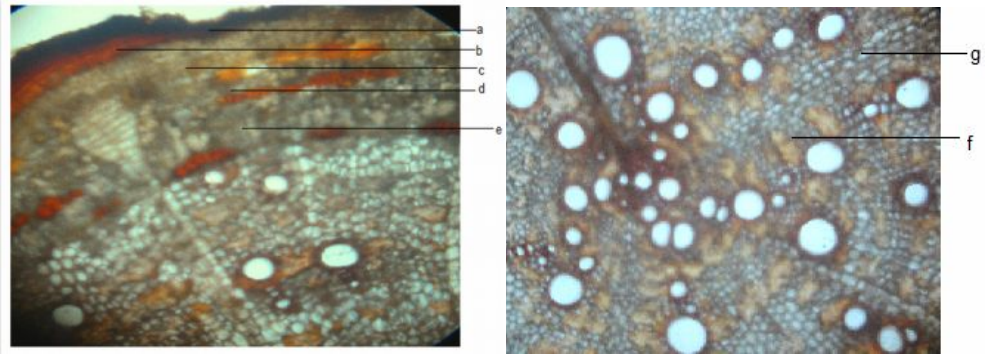
***Flemingia strobilifera*:** Transverse section of root of *Flemingia strobilifera* showed the 2-4 layer of cork cells, radially arranged, thin walled, polygonal, tabular cells. Outer layer contain reddish brown amorphous matter. Phelloderm is arranged one to three layers of radially arranged parenchymatous cells. Secondary phloem consisting of fibres slightly lignified alternating with sieve tissue. Medullary rays, distinct, bi to multiseriate parenchymatous cells, narrow in the xylem region and wider in the phloem region.

Xylem present as protoxylem and metaxylem and consist of xylem fibres, lignified xylem vessels and xylem parenchyma were present. Pith was absent in root (Fig.3).

Microscopic studies of the powdered root of *Flemingia strobilifera* showed the presence of bordered pitted xylem vessels, lignified phloem fibres, starch grains, cork cells and calcium oxalate crystals (Fig 5a, b, c, d).

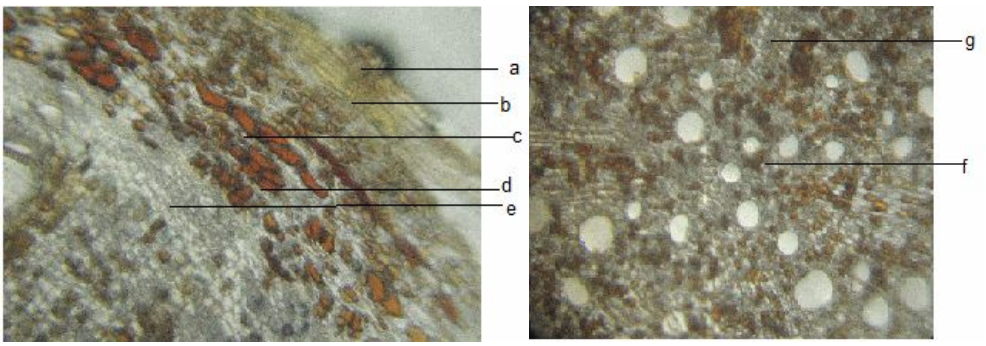
***Flemingia macrophylla* :** The transverse section of the root shows narrow cork consisting of 4-6 layers of yellowish brown cork cells followed by wide phelloderm made up of several layers of thin walled paraenchymatous cells. Secondary phloem consisting of sieve- tubes, companion cells, phloem parenchyma and fibres being traversed by uni-biseriate medullary rays which are narrower towards the pith region and broad towards the cortical region. The xylem is a solid core consisting of vessels, tracheids, fibres parenchyma and uni-biseriate medullary rays, as the cells being thick walled and lignified (Fig.4).

The powder microscopy shows the presence of cork cells, and reticulate xylem vessel, lignified bordered pitted xylem vessel, phloem fibre, starch grains and prismatic calcium oxalate crystals (Fig 6a, b, c, d).



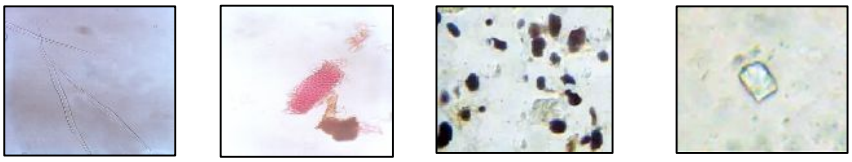
A **B**

Fig 3: T.S. of root of *F.strobilifera* :Representative photomicrographs (×100) of transverse section of root (A) showing cork (a), cork cambium (b), secondary cortex (c), pericycle (d), secondary phloem (e), B showing xylem (f) and medullary rays (g)



A **B**

Fig.4 :T.S. of root of *F.macrophylla* Representative photomicrographs (×100) of transverse section of root (A) showing cork (a), cork cambium (b), secondary cortex (c), pericycle (d), secondary phloem (e), B showing xylem (f) and medullary rays (g) .



a b c d

Fig. 5: Powder characteristics of *F.strobilifera* root (a) Fibres (b) Bordered pitted xylem vessels (c) Calcium oxalate crystal (d) Starch grains

a b c d e

Fig. 6: Powder characteristics of *F.macrophylla* root (a) Bordered pitted xylem vessels (b) Fibres (c) Calcium oxalate crystal (d) Reticulate xylem vessel (e) Starch grains

Physicochemical parameters

Physicochemical parameters like moisture content, ash value and extractive value were performed and their percentage (w/w) was also calculated. The average length and width of vessels and pericyclic fibres were determined. In quantitative microscopy average no. of starch grain per mg of powder and average length of fibres per g of powder were also determined and the data are represented in Table 1.

Various physicochemical constants like moisture content, total ash, acid insoluble ash, water soluble ash, water soluble extractive and ethanol soluble extractive value were determined and depicted in Table 2.

Preliminary phytochemical screening

Preliminary phytochemical analysis revealed the presence of Fatty acids, Steroids, Flavonoids, Tannins and Carbohydrates (Table3).

Table 1: Mean values of length and width of vessels, fibres, calcium oxalate crystals and Starch grains of *F.strobilifera and F.macrophylla* roots.

Parameter	Mean^{n}Length(µm)	Mean^{n}Width(µm)
	F.strobilifera/F.macrophylla	
Vessels	202.5/261.3	20.46/20.12
Pericyclic fibres	468.21/525.4	12.36/11.64
Calcium oxalate crystals	11.22/10.5	8.5/7.8
Starch grains	**Mean diameter(µm)**	
	6.4/8.5	
Average no. of starch grains Per mg of root powder	149812/37840	
Average length of fibres per mg of root powder	159.9/177.8m	

n=50

Table 2: **Mean value of various physicochemical parameters of *F. strobilifera and F.macrophylla* roots.**

Parameter	Mean [n]	
	F. strobilifera	*F.macrophylla*
Moisture content	5.85v/w	5.05v/w
Total Ash	3.07 w/w	2.17 w/w
Acid insoluble Ash	0.72 w/w	0.64 w/w
Water soluble Ash	0.52 w/w	0.42w/w
Ethanol soluble extractive value	8.12 w/w	9.23 w/w
Water soluble Extractive value	10.18 w/w	8.57 w/w

n=3

Table 3: Results of Phytochemical screening of various extracts of *F. strobilifera and F.macrophylla* roots.

Class of Phytoconstiuents	Petroleum ether extract		Chloroform extract		Alcoholic extract		Aqueous extract	
	F.str	*F.mac*	*F.str*	*F.mac*	*F.str*	*F.mac*	*F.str*	*F.mac*
Alkaloids	-	-	-	-	-	-	-	-
Anthraquionone glycosides	-	-	-	-	-	-	-	-
Cyanogenic glycosides	-	-	-	-	-	-	-	-
Cardiac glycosides	-	-	-	-	-	-	-	-
Steroides/triterpinoides	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-
Fat and oils	+/-	+/-	-	-	-	-	-	-
Saponins	-	-	-	-	-	-	-	-
Flavanoides	-	-	+	++	+	+	-	-
Coumarins	-	-	-	-	-	-	-	-
Tannins	-	-	-	-	+	+	+	+
Carbohydrates	-	-	-	-	+	+	+	+
Proteins	-	-	-	-	-	-	-	-

DISCUSSION

As per World Health Organisation (WHO) norms an examination to determine the sensory, macroscopic and microscopic characteristics is the first step towards establishing the identity and the degree of purity of medicinal plant materials and should be carried out before any further tests are undertaken [17]. Organoleptic evaluations can be done to establish the identity and purity and thereby ensure the quality of a particular sample. A number of different bases are used for morphological studies and a natural variation in these characteristics plays an important role for preliminary evaluation of crude drugs. The basis of analysis by evaluation of microscopic characters is that there are always sufficient differences in the same type or different types of plants as far as the cell characteristics are concerned.

F.strobilifera and *F. macrophylla*, both belonging to the same family Paplionaceae, share several common features, in both the species roots are longitudinally wrinkled and fracture is fibrous. These two species can be easily confused and to distinguish one from the other poses little difficulty. Therefore, some diagnostic features have been evolved to identify and to differentiate the two species. There are many microscopic common features between these two species. However, cortex and cork cell layer is relatively small in *F.strobilifera*. Medullary rays are multiseriate in *F.strobilifera*. In powder microscopy only bordered pitted xylem vessels are present in *F.strobilifera* but bordered pitted as well as reticulate xylem vessels are present in *F. macrophylla.*In quantitative microscopy there is difference in dimensions of fibres, vessels, calcium oxalate crystals and starch grains. Various physicochemical parameters viz. moisture content, ash values, extractive values and phytochemical screening of various extracts were generated to substantiate standardization data on both the species.

CONCLUSION

Since roots of these two species have close resemblance in external profile and there is no detail

anatomical work on records, there are always possibilities of adulteration of the roots of these two plants. The present work is taken up with a view to lay down the macroscopic, microscopic standards and physicochemical parameters, which could be used in deciding the genuineness of the above described drugs. Afore-mentioned pharmacognostical standards generated by the authors for proper identification of *F.strobilifera and F. macrophylla* would be useful for preparation of monograph and selecting the authentic plant material for exploring its therapeutic potentials.

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