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Pharmacognostical Standardization of roots of *Flemingia chappar* Benth.

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Abstract

Flemingia chappar Benth (Fabaceae) roots have been used traditionally in the treatment of epilepsy, hysteria, to induce sleep and relieve pain. Despite a long tradition of use in the treatment of various ailments, no work has ever been carried out for standardizing this potential plant. The authors suggest that the major stumbling block in systematic exploration of the plant is non-availability of authentic plant material. In the present investigation, various pharmacognostic standards for the plant have been generated so that authentic *F. chappar* could be explored for its traditional claims. The macroscopic studies revealed that the roots are yellowish brown, cylindrical and slightly tapering, fracture splintery, taste and odour indistinct. Microscopically root of *F. chappar* showed the presence of cork, cortex, scattered groups of pericyclic fibres and radial vascular bundles alternating with medullary rays. Powdered roots of the plant showed lignified bordered pitted tracheidal vessels, fibers, calcium oxalate crystals and starch grains. Moisture content, total ash, acid insoluble ash, water soluble ash alcohol soluble extractive, and water soluble extractive were 6.30,4.05,1.65,1.24,8.37 and 4.43% respsctively Thin-layer chromatography of the petroleum ether extract showed five spots using hexane: dichloromethane (1:1 vol/vol), while the chloroform extract showed two spots using toluene: ethyl acetate: glacial acetic acid (35:4:1 by volume) and methanolic extract showed three spots using toluene: ethyl acetate: pyridine (17:2:1) phytochemically, the plant was found to contain fats and oil steroids, flavonoids, tannins and carbohydrates. All these diagnostic feature may be useful for proper identification of the crude drug.

Key words, *Flemingia chappar,* Fabaceae, standardization, physicochemical parameters, Quantitative microscopy, TLC finger printing, Flavonoids

INTRODUCTION

Flemingia (Fabaceae) is genus of shrubs or under shrubs a native of the tropical and subtropical regions of the world. About fifteen species occur in India. These are *Flemingia strobilifera, F. chappar, F.vestita, F. macrophylla* and *F.grahamiana.* The roots of *F.strobilifera* are used to treat epilepsy. The dried pods of *F. macrophylla* and *F.grahamiana* yield warrus by gentle shaking and rubbing to denude them of their outer hairy covering. The tubers of *F.vestita* have been used as vermifuge and anthelmintic. *Flemingia chappar* (Syn: *Mogahnia chappar*) is commonly known as Salpan and Galfulli, belongs to family Fabaceae. *F. chappar* is a shrub 0.9-1.2m height, the branches terets appressed tawny pubescent. In India it is distributed in Bengal, Bihar, S.India, Uttarakhand and Gujrat. Leaves are l-foliolate, cordate, orbicular on a 3.8cm long petiole shortly and rather abruptly acuminate 5-7.5cm long and as broad or sometimes broader, 3 to almost 5 nerved at the base, indistinctly velvety above, minutely tawny, pubescent beneath. Flower small, yellowish, very shortly pedicelled. Pods are 8-12mm long, clothed with bright red glands. Flowering and fruiting time is Nov-March. [1, 2]. Roots have been used to treat epilepsy and hysteria, also used to induce sleep and relieve pain [3]. The plant has also been used as vermifuge, anticancer, antifungal and in the treatment of filarial [4]. A literature survey revealed that roots of *F.chappar* contain a chromenochalcone flemichapparin A, a pterocarpan flemichapparin B and a coumestan flemichapparin C [5]. The roots of *F.chappar* also contain an anthocyanin two sugars galactose and rhamnose [6], β-sitosterol, 7-hydroxyflavanon, Flemingin A, B and C, homoflemingin and desoxy- homoflemingin .From the whole plant of *F.chappar* two cryst. Chalcones 2', 4'-dihydroxychalcone and 2', 4'-dihydroxy-5'-methoxychalcone have been isolated [7].

In the light of traditional uses and sporadic recent pharmacologic reports, *F. chappar* seems to be a potential candidate for thorough investigation .Thus the present investigation has been undertaken with an objective to establish Pharmacognostical standards for *F. chappar* roots so that authentic plant material could be explored properly for its traditional claims .

MATERIALS AND METHODS

Plant material

The plant material was collected from the local areas of Kaladungi near National Corbett Park (Nainital) in the month of Jan 2008. The plant

was identified and authenticated by Dr. H.D. Pandey a senior botanist there. A voucher specimen has been preserved in the Department of Pharmacognosy, College of Pharmacy, IFTM, Moradabad for further reference. The roots were separated, washed and dried in shade, coarsely powdered passed through sieve no 40 and stored in a closed container for further use. All reagents used were of analytical grade

Macroscopic and microscopic studies

The macroscopical characters (size, shape colour, odour, texture, fracture) of the roots were studied following standard methods [8, 9], 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. [10] and the representative diagrams were taken with the help of inverted microscope for photodocumentation (Leitz, Japan)

Determination of physico chemical parameters

The ash values and moisture content with various reagents were determined as per the Indian Pharmacopoeia [11]. Extractive values with various solvents like alcohol and water was performed as per standard procedure [12]. The fluorescence characters of the powder with various acids were observed under visible light and UV light following reported methods [13, 14].

Thin layer chromatography

Thin layer chromatography of pet ether, chloroform and alcoholic extracts was performed using standard method of Stahl [15] and the R_f values were determined.

Preliminary phytochemical screening

Preliminary Phytochemical screening of the extracts were carried out for different groups of phytoconstotuents following standard procedures described by Harborne [16] and Khandelwal [17].

RESULTS

Macroscopic characteristics (Figure 1)

The root is externally yellowish brown, cylindrical and slightly tapering, longitudinally wrinkled, with transverse fissures and lateral portions with several roots and root scars. Taste and odour is indistinct. Fracture of the drug is splintery.

Fig.1: Roots of *F.chappar*

Microscopic characteristics

The transverse section of the root shows narrow cork consisting of 2-4 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. 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 2A, B). The powder microscopy reveals the presence of lignified bordered pitted xylem vessels, fibres, prism shaped Calcium oxalate crystal and starch grain (Fig 3a, b, c, d).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.

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

Parameter	***Mean length(µm)***	***Mean width(µm)***
Pericyclic fibres	640.7	9.52
Vessels	285.4	24.36
Calcium oxalate crystals	12.5	8.8
Starch grains	*Mean diameter* 7.45 *µm*	
Average length of fibres per g of root powder	310.6m	
Average no. of starch grains per mg of root powder	67614	

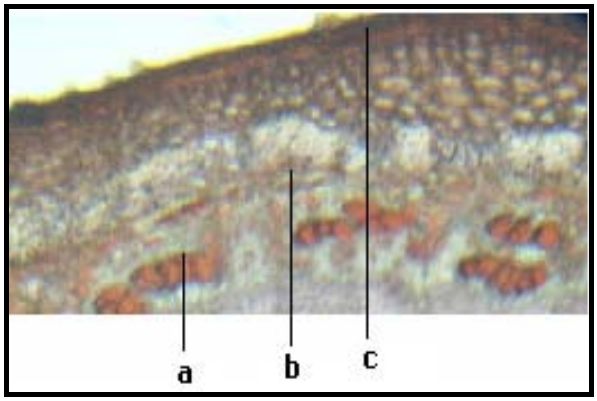

Fig.2A

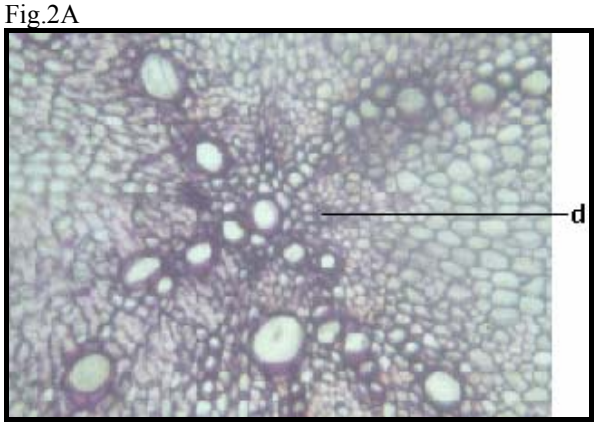

Fig.2B

Fig.2: Transverse section of root of *F.chappar* **a**: pericyclic fibres **b**: phelloderm **c**: cork cells **d**: vascular bundle region including xylem.phloem and medullary rays

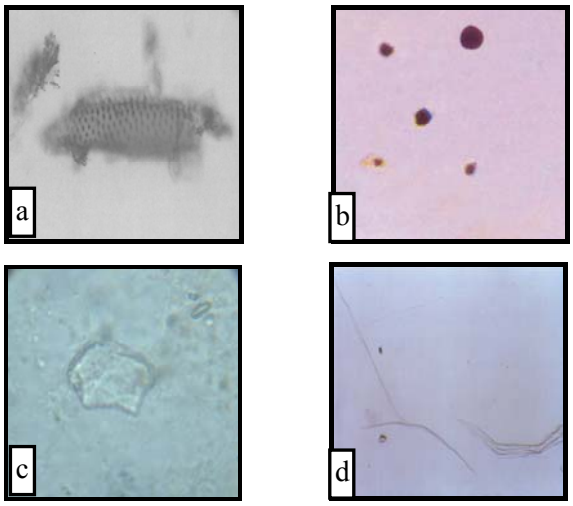

Fig.3 Powder characteristics of *F.chappar* root. **a**: Bordered pitted xylem vessels **b**: starch grains **c** : fibres **d** : calcium oxalate crystal.

Physicochemical parameters

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. The behavior of root powder upon treatment with different chemical reagents under visible light and UV light was also observed and reported in Table-3.

Table 2: Mean value of various physicochemical parameters of *F.chappar*

Parameter	***Mean ^{n} (n=3)***
Moisture content	6.30
Total ash	4.05
Acid insoluble ash	1.65
Water soluble ash	1.24
Ethanol soluble extractive	8.37
Water soluble extractive	4.43

Table 3: Fluorescence analysis of *F.chappar* root powder

Treatment	***Normal Light***	***Under U.V light***	
		254 nm	***365 nm***
Dry Powder(P)	Yellowish brown	Brown	Blackish brown
P + 5% NaOH	Brownish	Greenish Brown	Blackish
P + 5% KOH	Brownish	Greenish Brown	Blackish
P+Ferric Chloride	Deep Brownish	Greenish	Blackish
P + Conc. H_2SO_4	Deep Brown	Brownish Green	Dark Brown
P + Dil. Ammonia	Brownish yellow	Green	Brown
P + Conc. HCl	Brownish	Greenish	Blackish
P + Conc. HNO_3	Yellowish brown	Greenish Brown	Blackish Brown
P+ Iodine Solution	Blackish Brown	Greenish	Blackish
P + Dil. HCl	Brownish yellow	Light Green	Dark Brown
P + Dil. H_2SO_4	Brownish yellow	Light Green	Black
P+ Sodium Carbonate	Brown	Greenish	Blackish brown
P + Alcoholic KOH	Brownish Black	Brownish Green	Dark Brown
P + NH_4OH	Deep Brownish	Deep greenish	Brownish black
P + Sod. nitropruside	Brownish Black	Greenish	Blackish
P+ Bromine water	Brownish black	Brownish yellow	Brownish yellow
P + $KMnO_4$	Blackish	Green	Blackish
P + Silver Nitrate	Blackish	Greenish	Blackish
P + pyridine	Brownish Black	Greenish	Blackish

Table 4: Preliminary Phytochemical screening of different extracts of *F.chappar* root

Class of phytoconstiuents	*Petroleum ether extract*	*Chloroform extract*	*Alcoholic extract*	*Aqueous extract*
Alkaloids	-	-	-	-
Anthraquionone glycosides	-	-	-	-
Cyanogenic glycosides	-	-	-	-
Cardiac glycosides	-	-	-	-
Steroides/triterpinoides	+	-	-	-
Fat and oils	+	-	-	-
Saponins	-	-	-	-
Flavanoides	-	+	+	-
Coumarins	-	-	-	-
Tannins	-	-	+	+
Carbohydrates	-	-	+	+
protiens	-	-	-	-

+ present: - absent

Preliminary phytochemical screening

Preliminary phytochemical analysis revealed the presence of Fatty acids, Steroids, Flavonoids, Tannins and Carbohydrate (Table 4).

TLC finger print profile

Thin layer chromatography of the petroleum ether extract, chloroform extract and methanol extract were done using Hexane: Dichloromethane (1:1), toluene:ethyl acetate:glacial acetic acid (35:4:1)and toluene:ethyl acetate : pyridine (17:2:1) as mobile phase respectively and the R_f value were recorded (Table 5).

Table 5: Thin layer chromatography of petroleum ether, chloroform and methanol Extract of *F.chappar* root

Extract	*Mobile phase*	*No. of spots and their Rf value*
Petroleum ether	Hexane: Dichloromethane (1:1)	Five spots Rf values: 0.19, 0.42, 0.53,0.73 and 0.80
Chloroform	Toluene: Ethyl acetate: Glacial acetic acid (3:1:0.5)	Two spots Rf value- 0.66, 0.74
Methanol	Toluene: Ethyl acetate: Pyridine (17:2:1)	Three spots Rf value- 0.29, 0.69,0.80,

DISCUSSION

To check the potential of crude drug with reference to its Phytochemistry and pharmacology, the proper botanical identification is of paramount important [18]. Hence, in the present study the detailed pharmacognostical characters of the root of *Flemingia chappar* have been evaluated. Microscopically root of *F. chappar* showed the presence of cork, cortex, scattered groups of pericyclic fibres and radial vascular bundles alternating with medullary rays. Powdered roots of the plant showed lignified bordered pitted tracheidal vessels, fibers, calcium oxalate crystals and starch grains. Total ash of the underground parts of *F. chappar* was approximately 2.5 and three times more than acid-insoluble and water-soluble ash, respectively. The ethanol-soluble extractive value of the plant was slightly higher than its water-soluble extractive value. In addition TLC profile and preliminary phytochemical screening in this study would help for botanical identification of the crude drug.

CONCLUSION

Various Pharmacognostic standards including qualitative and quantitative microscopic characters, Fluorescence, various physicochemical parameters , TLC fingerprint profile and phytochemical screening of various extracts were generated to substantiate standardization data on *F.chappar*.This study would be useful for preparation of a monograph and selecting the authentic plant material for exploring its phytochemical and pharmacological potential.

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