Mahajon et al., J Homeop Ayurv Med 2014, 3:4
DOI: 10.4172/2167-1206.1000171

Homeopathy & Ayurvedic Medicine

Research Article Open Access

Preliminary Analysis of Botanical and Phytochemical Features of *Kamalu* - Root of *Flemingia strobilifera* (L.) W.T. Aiton

Bidhan Mahajon[1]*, Remadevi R[1], Sunil Kumar KN[2] and Ravishankar B[3]

[1]*Department of Dravyaguna Vijnanam, Vaidyaratnam P. S. Varier Ayurveda College, Kottakkal, Kerala, 676501, India*
[2]*Pharmacognosy, SDM Centre for Research in Ayurveda and Allied Sciences, Laxminarayana Nagar, Kuthpady, Udupi, 574 118, India*
[3]*Department of Pharmacology and Toxicology, SDM Centre for Research in Ayurveda and Allied Sciences, Udupi, 574 118, India*

Abstract

Flemingia strobilifera (Fabaceae) is an important medicinal plant, commonly known as *Kamalu* in Malayalam and *Kusrunt* in Hindi. It is distributed in the region of the tropical area of India. Root of the plant is used for various ailments such as insomnia, epilepsy, ulcer, inflammation and microbial infection. However, Pharmacognostical standardization of this important medicinal plant has not been reported. Present study aims to characterize botanical and phytochemical features of the root, which could be used for the standard testing protocols in the future. Botanical and phytochemical features of root of this plant were analyzed. These features would be of great help in authentication and standardization of the raw drug. The phytochemical tests revealed the presence of carbohydrates, flavonoids, phenol, tannin, terpenoids, saponin, coumarins, quinone and steroids in the root of this plant. The HPLTC fingerprint obtained would be useful in chemical standardization of the raw drug of *Kamalu.*

Keywords: *Flemingia strobilifera*; HPTLC; Macro-microscopy; Phytochemical analysis; Physico-chemical study; Standardization

Introduction

Flemingia strobilifera (L.) W.T. Aiton commonly known as *Kamalu* in Malayalam belongs to the family Fabaceae. The plant is abundantly distributed in Assam, Sind, Rajputana, Bengal, South India and Andamans [1]. The roots of this plant have been indigenously used in epilepsy, insomnia and hysteria and the leaves were as also used for vermifuge. Arabians use it in cosmetics, as anthelmintic and as a remedy for coughs and cold [1,2]. Methanolic extract of *F. strobilifera* was found to be a source of natural antioxidant to prevent progress of various oxidative stresses [3]. Analgesic activity of *F. strobilifera* had been studied, at the dose level of 100 mg/kg, the duration and intensity of analgesia in the extracts of this plant was also greater than that of acetylsalicylic acid [4]. A comparative study of the roots of two species of *Flemingia viz. F. strobilifera* and *F. macrophylla* have been reported [5]. Crude ethanol extract and ethyl acetate fraction of roots of *Flemingia strobilifera* showed to possess a central nervous system depressant action and act as a potential anticonvulsant [6]. Chloroform extract of leaf of *Flemingia strobilifera* did not show significant hepatoprotective and antioxidant activity [7]. Phytochemicals like flavanone [8], chalcones [9], glycoside [10], aurones [11] and chromenes [12] can be isolated from the plant. In spite of above important research works, a detailed pharmacognostic study to exam the authenticity and quality of root of *Kamalu* has not been reported so far. In present study detailed macroscopic and microscopic features of root as well as root powder was studied for authentication of the drug *Kamalu*. Physico-chemical tests such as weight loss on drying, total ash, acid insoluble ash, water soluble extractive, alcohol soluble extractive of root were also studied. Preliminary phytochemical tests were conducted as per standard procedure. HPTLC photo documentation, densitometric scan and R_f values of the root sample has been documented for authentication of the extract by standard procedures.

Materials and Methods

The fresh mature roots of *Flemingia strobilifera* were collected from Jagiroad District of Assam in December 2013. Morphological features were compared with those of the regional floras [1] at Department of Dravyaguna Vijnanam, V.P.S.V Ayurveda College, Kottakkal and further authentication was done by the department of Pharmacognosy at SDM Centre for Research in Ayurveda and Allied Sciences, Udupi. A voucher specimen (No. 385/14020702) has been deposited for further reference.

Dried root pieces were used for characterization of macroscopic and microscopical features. The shade-dried coarsely powdered material was used for physico-chemical analysis, extraction and chromatographic fingerprinting of phytochemicals using High Performance Thin Layer Chromatography (HPTLC). Thin hand sections were done using blades, stained as the standard methodology and then examined under microscope [13,14]. Photomicrographs of the microscopical sections were captured in Zeiss Axio Lab. A1 microscope (fitted with Ziess AxioCamERc5s) and images were analyzed using the Zeiss Axio Vision software. Percentage of total ash, acid-insoluble ash, water soluble ash, alcohol and water soluble extractive and loss on drying was performed according to the standard protocol [14]. Preliminary phytochemical investigation was done to detect the presence of alkaloid, triterpenoid, steroid, tannin, glycoside, flavonoid and coumarin in chloroform and alcohol extracts [15,16].

For HPTLC fingerprinting, one gram of powdered samples were soaked in 10 ml ethanol and kept for cold percolation for 24 h and filtered. 4, 8 and 12 µl of the above samples of were applied on a pre-coated silica gel 60 F_{254} on aluminum plates of 0.2 mm thickness to a band width of 7 mm using Linomat 5 TLC applicator. The plate was developed in Toluene: Ethyl acetate (8.1). The developed plates were visualized in UV 254, 366 nm and then derivatzed with vanillin sulphuric acid reagent and scanned under UV 254 and 366 nm. R_f, colour of the spots and densitometric scan were recorded [17].

***Corresponding author:** Bidhan Mahajon, PG Scholar, Department of Dravyaguna Vijnanam, Vaidyaratnam P. S. Varier Ayurveda College, Kottakkal, Kerala, 676501, India, Tel: +91-8593038842; E-mail: bidhanmahajon@gmail.com

Received October 25, 2014; **Accepted** December 22, 2014; **Published** December 29, 2014

Citation: Mahajon B, Remadevi R, Sunil Kumar KN, Ravishankar B (2014) Preliminary Analysis of Botanical and Phytochemical Features of *Kamalu* - Root of *Flemingia strobilifera* (L.) W.T. Aiton. J Homeop Ayurv Med 3: 171. doi:10.4172/2167-1206.1000171

Results and Discussions

Macroscopically the roots were cylindrical to slightly tortuous in shape, ash brown externally and yellowish brown internally, about 0.7 to 1.8 cm in diameter; surface rough with fissures and longitudinal striations, rootlets and lenticels distributing on the surface; fracture fibrous, a bitter and pungent taste without odour (Figure 1).

The transverse section observation showed that the outer layer of root was composed of ten or more tabular cork cells, which were filled with reddish brown amorphous matter, and the inner layer was composed of thick walled cells without any contents. Cortex is formed by two to three layers of radially arranged parenchymatous cells. Thick walled lignified phloem fibers were observed in the outer phloem and thin walled parenchyma cells in the inner phloem. Outer xylem contains vessels, lignified fibers, and parenchyma with round to oval starch grains prismatic crystals of calcium oxalate. Medullary rays were composed of distinct, two to multiseriate parenchymatous cells, narrow in the xylem region and wider in the phloem region. Pith was absent, but few parenchymatous cells with intercellular spaces occurred at the centre (Figure 2).

Microscopic studies of the powdered root showed the presence of fragments of vessels, fibers, cork cells; starch grains and abundant prismatic calcium oxalate crystals. Xylem fibers are long, narrow heavily lignified and thin-walled. Cylindrical vessels were composed of numerous bordered pits forming perforations, in which slightly oblique or horizontal lateral walls were observed. Starch grains are simple, oval to round, and abundantly distributing inside and outside of parenchyma cells. The cork cells were was thin-walled and polygonal, and composed of the orange-brown matter in the cross-section and the surface (Figure 3).

The total ash and the acid insoluble ash, direct indicator of inorganic substances, were 5.78 and 1.70% w/w respectively in the sample tested. Loss on drying was 5.193% w/w. The water soluble and alcohol soluble extractives, which gives percentage solubility of active metabolites in the solvents, were 3.61 and 5.67% w/w respectively (Table 1).

Preliminary phytochemical analysis showed the presence of carbohydrate, coumarins, flavanoids, phenol, quinine, saponins, tannin, and terpenoids in the root of *F. strobilifera* (Table 2). The roots were in rich of different categories of secondary metabolites. This might be responsible for the medicinal functions derived from the plant.

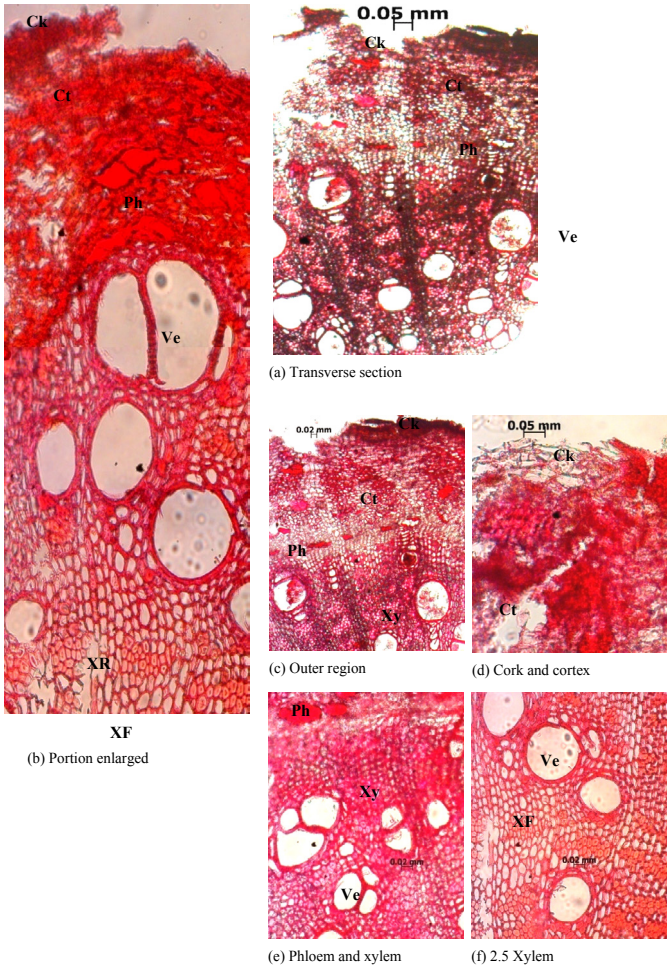
TLC finger print profile of ethanol extract of root under UV light

(a) Flowering plant

(b) Roots

(c) Dried root pieces

Figure 1: Overview of *Flemingia strobilifera*.



(a) Transverse section

(b) Portion enlarged

(c) Outer region

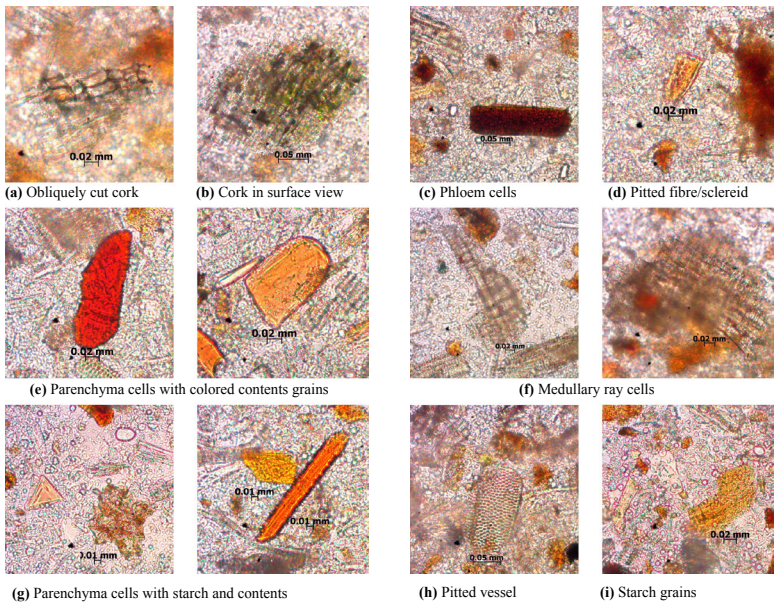
(d) Cork and cortex

(e) Phloem and xylem

(f) 2.5 Xylem

Ck: cork; Ct: cortex; Ph: phloem; VB: vascular bundle; Ve: vessel; XF: xylem fibre; XR: xylem ray; Xy: xylem

Figure 2: Microscopic features of root of *Flemingia strobilifera*.



(a) Obliquely cut cork **(b)** Cork in surface view **(c)** Phloem cells **(d)** Pitted fibre/sclereid

(e) Parenchyma cells with colored contents grains **(f)** Medullary ray cells

(g) Parenchyma cells with starch and contents **(h)** Pitted vessel **(i)** Starch grains

Figure 3: Microscopic features of root powder of *Flemingia strobilifera*.

Parameters	Data (*n*=3, %, w/w)
Loss On Drying	5.19
Total ash	5.78
Acid Insoluble Ash	1.70
Alcohol Soluble Extractive	3.61
Water Soluble Extractive	5.67

Table 1: Physico-chemical parameters of root of *Flemingia strobilifera*.

Tests	Colour if positive	*Flemingia strobilifera*
Alkaloids		
Dragendrof's test	Orange precipitate	Light brown Solution
Wagners test	Red precipitate	Light brown Solution
Mayers test	Dull white precipitate	Light yellow colour
Hagers test	Yellow precipitate	Light yellow colour
Steroids		
Liebermann- Buchard test	Bluish green	Brownish pink colour
Salkowski test	Bluish red to cherry red	Reddish brown colour
Carbohydrate		
Molish test	Violet ring	Violet colour at junction
Fehlings test	Brick red precipitate	Red precipitate
Benedicts test	Red precipitate	Red precipitate
Tannin		
With $FeCl_3$	Dark blue or green or brown	Dark Blue solution
Flavanoids		
Shinoda's test	Red to pink	Pink colour
Saponins		
With $NaHCO_3$	Stable froth	Froth formed
Triterpenoids		
Tin and thionyl chloride test	Pink	Pinkish red colour
Coumarins		
With 2N NaOH	Yellow	Orange Red colour ppt
Phenols		
With alcoholic ferric chloride	Blue to blue black, brown	Blue black colour
Carboxylic acid		
With water and $NaHCO_3$	Brisk effervescence	No brisk effervescence
Resin		
With aqueous acetone	Turbidity	No turbidity
Quinine		
5% NaOH	Pink/purple/red	Orange red colour

2.1 Colour formation

Alkaloid	–
Carbohydrate	+
Carboxylic acid	–
Coumarins	+
Flavanoids	+
Phenol	+
Quinine	+
Resins	–
Steroid	–
Saponins	+
Tannin	+
Terpenoid	+

2.2 Inference

Table 2: Results of preliminary phytochemical tests in *Flemingia strobilifera*.

at 254 nm showed 8 spots with R_f values of 0.1, 0.18, 0.28, 0.32, 0.42, 0.50 and 0.56. When the plates were viewed at 366 nm, the TLC plate showed 13 spots. The spots with R_f values 0.1, 0.32, 0.42, 0.50 and 0.56 were observed under both 254 nm and 366 nm. After post derivatization of the plate with vanillin sulphuric acid, the plate showed 5 spots with R_f values 0.05, 0.14, 0.32, 0.39 and 0.56. Spots with R_f of 0.32 and 0.56 were common in all the three visualization methods indicating that the chemical structure of the compound could be the same (Table 3 and Figure 4).

Densitometric scan at UV 254 nm in HPTLC of ethanol extract revealed 11 peaks suggesting 11 different compounds in the ethanol extract, the R_f were 0.04 (13.53%), 0.07 (2.31%), 0.13 (20.77%), 0.20 (15.10%), 0.38 (17.30%), 0.46 (9.54%), 0.55 (8.60%), 0.65 (2.27%), 0.81 (2.27%), 0.90 (4.30%), and 0.97 (4.01%), respectively (Figure 5). At UV 366 nm, the plate showed 6 peaks. 0.02 (0.69%), 0.03 (18.64%), 0.24 (21.86%), 0.41 (16.18%), 0.52 (10.60%), and 0.57 (32.03%), respectively (Figure 6).

The TLC and HPTLC fingerprint would serve as an important parameter to standardize the ethanol extract of *Kamalu*.

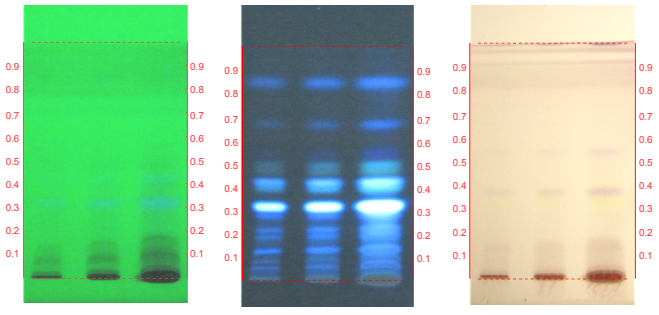
Conclusion

The Pharmacognostical standards of the root of *Kamalu* were analyzed in this study. The macroscopic and microscopic features of the plant were characterized, which could be used for the future identification of the plant. Physico-chemical pharmacopoeial standards for this plant were also studied. Phytochemical entities such as carbohydrate, coumarins, flavonoids, phenol, quinine, saponin, and tannin were detected in the root of this plant. The HPTLC fingerprint

At 254 nm	At 366 nm	Post derivatisation
-	0.05 (FL Blue)	0.05 (L Pink)
0.1 (D Green)	0.1 (FL Blue)	-
-	0.14 (FL Blue)	0.14 (L Purple)
0.18 (D Green)	-	-
-	0.2 (FL Blue)	-
-	0.23 (FL Blue)	-
0.28 (L Green)	-	-
0.32 (L Blue)	0.32 (F Blue)	0.32 (L Yellow)
-	0.39 (F Blue)	0.39 (L Purple)
0.42 (L Green)	0.42 (F Blue)	-
-	0.47 (FL Green)	-
0.50 (L Green)	0.50 (F Blue)	-
0.56 (L Green)	0.56 (FD Blue)	0.56 (L Purple)
-	0.68 (FD Blue)	-
-	0.85 (FD Blue)	-

D-Dark; L-Light; F-Fluorescent

Table 3: R_f values of all the samples.



Track 1- Alcoholic extract of *Flemingia strobilifera* 4 µl; Track 2-8 µl; Track 3-12 µl; Solvent system- 8:1 (Toluene: Ethyl Acetate)

Figure 4: TLC data of the alcoholic extract of Flemingia strobilifera.

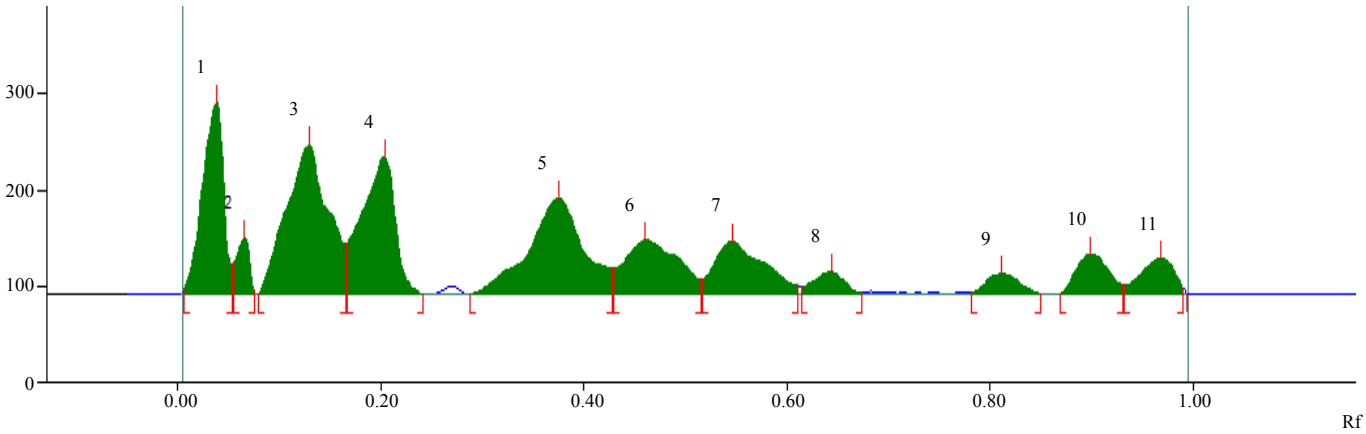
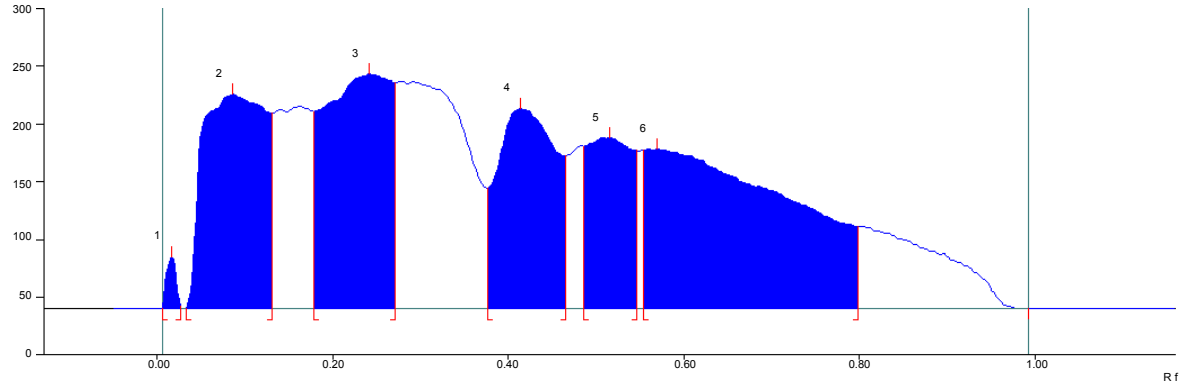


Figure 5: HPTLC densitometry of *Flemingia strobilifera* at 254 nm.



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	0.0 AU	0.02 Rf	44.5 AU	4.99 %	0.03 Rf	1.6 AU	352.5 AU	0.69 %
2	0.03 Rf	0.2 AU	0.09 Rf	185.4 AU	20.78 %	0.13 Rf	69.0 AU	9579.5 AU	18.64 %
3	0.18 Rf	170.6 AU	0.24 Rf	203.1 AU	22.76 %	0.27 Rf	95.1 AU	11239.5 AU	21.86 %
4	0.38 Rf	104.1 AU	0.41 Rf	173.0 AU	19.39 %	0.47 Rf	32.3 AU	8317.2 AU	16.18 %
5	0.49 Rf	140.5 AU	0.52 Rf	147.8 AU	16.57 %	0.55 Rf	36.8 AU	5451.2 AU	10.60 %
6	0.55 Rf	136.7 AU	0.57 Rf	138.3 AU	15.50 %	0.80 Rf	70.9 AU	16465.3 AU	32.03 %

Alcoholic extract (12 µl)

Figure 6: HPTLC densitometry of *Flemingia strobilifera* at 366 nm.

would be used for analysis of the *F. strobilifera* extract. The set of data obtained in the present investigation may be served as standards for the identification of this Indian Medicinal plant.

Acknowledgement

The authors are grateful to Mr. Puneeth and Mrs. Suchithra Kini, Trainee Research Officers- Pharmaceutical Chemistry, SDM Centre for Research in Ayurveda and Allied Sciences, Udupi for their helps.

References

1. Kirtikar KR, Basu BD (1935) Indian Medicinal Plants. Vol 1. Allahabad, India: Lalit Mohan Basu Publishers, p. 813.
2. Duthie JS (1994) Flora of the Upper Gangetic Plain. Vol I. Dehradun: Bishen Singh Mahendrapal Singh Publisher, p. 215.
3. Madan S, Singh GN, Kumar Y, Kohli K (2010) Phytochemical analysis and free-radical scavenging activity of *Flemingia strobilifera* (Linn.) R. Br. Research Journal of Pharmaceutical, Biological and Chemical Sciences 1: 183-190.
4. Anil K, Kavita G, Jyotsna D, Pankaj S (2011) Analgesic activity of methanolic extract of *Flemingia strobilifera* (R.Br). IJRPC 1: 825-827.
5. Kavita G, Lal VK, Jha S (2012) Comparative morpho-anatomical and Preliminary Phytochemical studies of *Flemingia strobilifera* (L.) R.Br. and *Flemingia macrophylla* (Willd.) Merr (Fabaceae). International Journal of Pharm Tech Research 4: 495.
6. Gahlot K, Lal VK, Jha S (2013) Anti convulsant potential of ethanol extracts

and their solvent partitioned fractions from *Flemingia strobelifera*. Phcog Res, 5: 265.

7. Anil Kumar KV, Veere Gowda K (2011) Evaluation of hepatoprotective and antioxidant activity of *Flemingia strobilifera* R. Br. Against experimentally induced liver injury in rats. Int J Pharm Sci 3: 115-119.
8. Madan S, Singh GN, Kumar Y, Kohli K, Singh RM (2008) New Flavanone from *Flemingia strobilifera* (Linn.) R. Br. and its Antimicrobial Activity. Trop J of Pharmaceu Res 7: 921-927.
9. Bhatt S (1975) Chalcones and some other constituents of *Flemingia strobilifera*. Ind J Chem 13: 1105-1108.
10. Saxena VK, Nigam SS, Singh RB (1976) Glycosidic principles from the leaves of *Flemingia strobilifera*. Planta Med 29: 94-97.
11. Nigam SS, Saxena VK (1975) Isolation and study of the Aurone glycoside Leptosin from the leaves of *Flemingia strobilifera*. Planta Med 27: 98-100.
12. Saxena VK (1995) Epoxy chromenes: Therapeutic Agents from *Flemingia strobilifera*. Asian J Chem 7: 307-310.
13. Johansen DA (1940) Plant Micro techniques, New York: Mc. Grow Hill, p. 182-203.
14. World Health Organization (1998) Quality Control Methods for Medicinal Plant Materials. Geneva p. 16-20: 25-28.
15. Brain KR, Turner T (1975) The Practical Evaluation of Phyto pharmaceuticals, (1stedn.) Bristol: Wright-Scientechnica, p. 10-12.
16. Harborne JB (1998) Method of extraction and isolation, In: Phytochemical methods, (2ndedn.) London: Chapman & Hall, p. 60-66.
17. Sethi PD (1996) High Performance Thin Layer Chromatography. 1st ed. New Delhi: CBS Publishers and Distributors, p. 1-56.