



FLEMINGIA STROBILIFERA: REVIEW ON PHYTOCHEMISTRY AND PHARMACOLOGICAL ASPECTS

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ABSTRACT

The genus *Flemingia* (family Leguminosae) comprises over forty species in the world. In India, this genus is represented by fifteen species, including *Flemingia strobilifera*. *Flemingia strobilifera* (R.Br.), an important medicinal plant, is commonly known as Kusrunt and is found in Sind, Rajputana, Bengal, South India and Andamans. Several plants of this genus have been used in folk medicine to treat fever, diarrhoea, indigestion and as vermifuge. The plants are reported to possess antimicrobial, antifungal, anthelmintic, anticancer, anti-rheumatic, anti-inflammatory, antioxidant and anti-histamine activities. Previous chemical studies showed that flavonoids, flavonoid glycosides, chalcones, epoxychromenes and pterocarpanes were the main constituents found in this genus. This review article includes the detailed exploration of the morphology, phytochemistry, and pharmacological aspects of *Flemingia strobilifera* in an attempt to provide a direction for further research.

Key words: *Flemingia strobilifera*, Flavonoids, Pterocarpanes, Anthelmintic, Anti-inflammatory.

INTRODUCTION

The genus *Flemingia* Roxb. ex comprises erect or prostrate shrubs and herbs native to the tropics and subtropics (Thuan, 1979 & Verdcourt, 1979). About fifteen species of *Flemingia* occur in India. *Flemingia strobilifera* (R.Br.) (*F. strobilifera*) is one of them which is an important medicinal plant, commonly known as Kusrunt in Hindi; Kanphuti, bundar in Marathi; Nallabaddu, Kannad in Telugu; Kumalu, kumbilteri in Malayalam; Makhioti in Assam; Simbusak in Bihar and belongs to the Leguminosae family. The root of this plant is used in epilepsy, hysteria, also to induce sleep and to relieve pain. The leaves are reported to be used as vermifuge for children. Figure 1 shows the root of *F. strobilifera*.

It is distributed almost throughout India, Andaman and Nicobar Islands, ascending to an altitude of 4,500 ft. Himalayas from Simla and Kumaon, ascending to 8000 ft. to Assam, Khasla, Chittagong, Siam, Malacca

and Ceylon. Dehradun and Siwalik range, Bundelkhand, from Sind, Rajasthan and Bengal to South India. It is an erect shrub, 5-10 ft high, with slender terete branches velvety towards their tips. Leaves 3- 4 inch long, oblong or ovate-lanceolate, acute, rounded at the base; lateral nerves 8-10 pairs; petioles half inch; stipules lanceolate, scarious. Racemes terminal, 3-6 inch long, the slender zigzag rachis rusty-pubescent; bracts 1 inch long, shortly stalked, deeply cordate, usually cuspidate at the apex. Calyx-teeth lanceolate, pilose, exceeding the tube. Corolla yellowish or greenish-white. Pod about half inch, oblong, turgid, finely downy, 5 2-seeded (Duthie, 1994). Two varieties are distinguished in this species: (i) var. *bracteata* syn. *F. bracteata* (ii) var. *fructiculosa* syn. *F. fructiculosa* (Hooker, 1978).

Macroscopic Study

The root of *Flemingia strobilifera* was cylindrical or slightly tortuous. Root was externally earthy brownish in color and internally yellowish brown, its surface was fissured, rootlets and lenticels were present. It has no specific odour and taste. The thin young root of about 1.6 mm diameter was studied.

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Microscopic Studies

The transverse sections (TS) of root were obtained by usual techniques. Thin sections were collected and placed on a grease free microscopic slide along with a drop of glycerine water (1:1). The sections were covered with clean cover slip and observed under the compound microscope at 100 & 400 magnifications. A camera Lucida was attached with the microscope and the sections were suitably traced out. Transverse section of root of *Flemingia strobilifera* showed the upper layer of cork cells, ten or more layers of tabular cells, outer layer contain reddish brown amorphous matter and inner layer show thick walled colourless cells. Phelloderm is arranged one to three layers of radially arranged parenchymatous cells. Secondary phloem in which presents phloem fibers which is thickened wall, lignified in the outer parts and thin walled cells phloem parenchyma. Medullary rays, distinct, bi to multiseriate parenchymatous cells, narrow in the xylem region and wider in the phloem region. Secondary xylem contains xylem vessels, with thick and lignified and lignified xylem fibers were presents. Xylem parenchyma contains starch grains which were oval, round in shape and prismatic type's calcium oxalate crystals were present. Parenchymatous cells with intercellular spaces. Pith was absent in root.

Powder Microscopy

Powder of *Flemingia strobilifera* root was cleaned with chloral hydrate solution mounted with glycerin and observed under microscope. Microscopic studies of the powdered root of *Flemingia strobilifera* showed the presence of xylem fibers, xylem vessels, starch grains, cork cells and calcium oxalate crystals which were abundant in different parts of the root. The crystals were predominantly prismatic type.

Preparation of Stain Solution

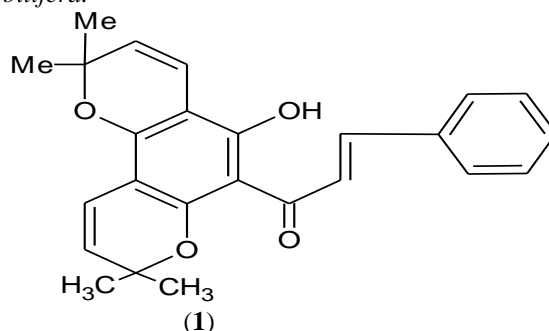
The stain solutions are solutions that are appropriate for each kind of cell to distinguish the tissue or cells. The stain solutions for medicinal plant powder are specific to each constituent such as distilled water, tests parenchyma cells, starch, crystals and basic cell components.

2% Iodine solution dyes starch grains in blue or violet and 1-2% Phloroglucinol solution in alcohol + 20% hydrochloric acid, dyes lignin fibers and selereids in pink or red colour. The internal characteristics of powder of *Flemingia strobilifera* root exhibited various types of elements such as calcium oxalate crystals were abundant in different parts of the root. The crystals were predominantly prismatic type and xylem fibers were lignified long narrow, thin-wall cell. Xylem vessels large with numerous bordered pitted Surfaces are lignified. These were cylindrical cells with wide simple perforated

plates at the ends. The perforations were slightly oblique or horizontal, lateral wall pits were abundant. Starch grains were both simple and compound in high quantities, also inside and outside of parenchyma cells. Most are simple, oval or round and Cork cells were abundant fragments of orange-brown composed of thin walled polygonal cells. Figure 2 shows the transverse section of *Flemingia strobilifera* root and Figure 3 shows the powder microscopy of *Flemingia strobilifera* root (Kumar et al., 2011).

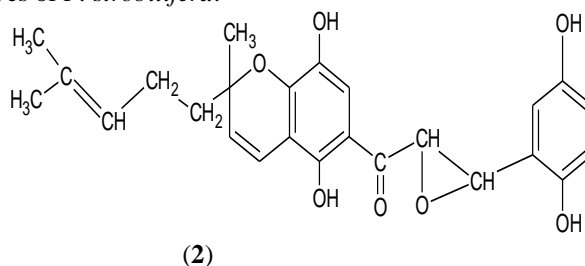
Phytoconstituents

Nigam and Saxena., 1975 reported the presence of aurone glycoside, Leptosin (1) from the leaves of *F. strobilifera*.

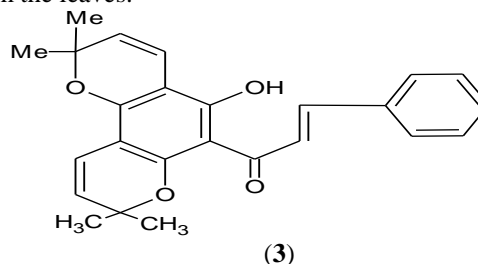


Tandon et al., 1974 has reported the presence of glycine, leucine, aspartic acid and proline as free amino acids and glycine, norvaline, leucine, serine, proline, alanine, valine and methionine as form of protein by chemical examination of the leaves of *F. strobilifera*.

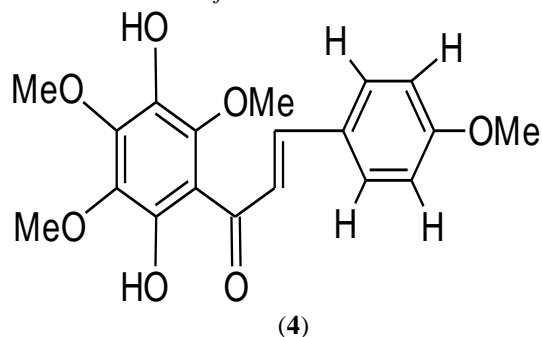
Saxena et al., 1976 and Saxena., 1995 reported the isolation of glycosidic principles: phloridzin, naringin and epoxy chromenes, flemingin X, Y and Z (2) from the leaves of *F. strobilifera*.



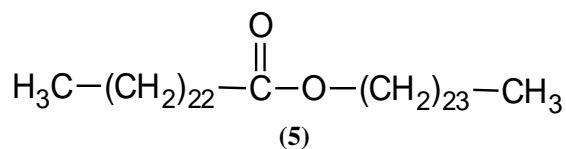
Sarathi et al., 1984 reported the isolation and characterization of a novel chalcone Flemiculisin (3) from the leaves.



Merlini *et al.*, 1968 and Bhatt.,1975 reported the isolation of chalcones, 3', 6'-dihydroxy-2', 4', 5', 4'-tetramethoxy chalcone (4), β -sitosterol, *n*-triacontane from the root *F. strobilifera*.



Lignoceryl lignocerate (5) was isolated by Bhatt., 1975 from the aerial parts of *F. strobilifera*.



Bhatt, 1976 isolated quercetin glycosides; quercitrin, rutin and quercimeritrin from the aerial parts of *F. strobilifera*. A new isoflavone isolated from the roots of *Flemingia strobilifera* (L) R. Br. was identified as 5,7,4'-trihydroxy 8,2',5'-tri(3-methylbut-2-enyl)isoflavone along with the known phytoconstituents: 5,7,2',4'-tetrahydroxyisoflavone, 5,7,4'-trihydroxyisoflavone and β -sitosterol (Madan S *et al.*, 2009). A new flavanone isolated from the roots of *Flemingia strobilifera* known as Flemingiaflavanone (8, 3'-diprenyl-5, 7, 4'-trihydroxy flavanone), Genistin (5, 4'-dihydroxy isoflavone 7-O-glucoside) and β - sitosterol-D glucoside (Madan S *et al.*, 2008).

A comparative study of the roots of two species of *Flemingia* is also reported. The species, *Flemingia strobilifera* and *Flemingia macrophylla*, have been reported to possess 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* (Ghalot K *et al.*, 2012).

BIOACTIVITIES

Anthelmintic activity

Das *et al.*, 2006 reported a vermifugal / vermucidal effect in the fowl tapeworm *Raillietina*

echinobothrida by treating the alcoholic crude root-peel extract of *Flemingia vestita* (*F. vestita*) and its major isoflavone, genistein (Das *et al.*, 2006). The treatment of parasite *Fasciolopsis buski* with 20 mg/ml of crude peel extract of *F. vestita*, 0.5 mg/ml of genistein showed a marked decrease in the levels of free amino acid; arginine, ornithine, tyrosine, leucine, isoleucine, valine, alanine, glycine, proline, serine, threonine, taurine and increase in the levels of glutamic acid, glutamine, phosphoserine, citrulline and GABA. The ammonia level increased by 40.7%, 66.4% and 18.16% in treatment with *F. vestita*, genistein. The changes in the levels of the amino acids and nitrogen components post treatment suggest that the amino acid metabolism in the parasite may have been altered under the influence of the test materials (Kar *et al.*, 2004).

The root-tuber peel of *F. vestita* and its active component genistein, were tested in respect of glucose metabolism in the cestode, *Raillietina echinobothrida*. In the treated worms, there was a significant decrease in the glycogen concentration accompanied with the decrease of glucose by 14-32%, whereas the malate concentration increased by 49-134% as compared to controls.

Das *et al.*, 2004 studied the role of phytochemicals from *F. vestita*, in particular genistein, which influence the key enzymes of gluconeogenesis-pyruvate carboxylase (PC), phosphoenolpyruvate carboxykinase (PEPCK) and fructose 1, 6-bisphosphatase (FBPase)-in *R. echinobothrida*, which is perhaps a function of high energy demand of the parasite under anthelmintic stress (Das *et al.*, 2009).

Tandon *et al.*, 2003 reported the effect of the crude root-peel extract (5 mg/ml) and pure genistein (0.2 mg/ml) from *F. vestita* which were tested in respect of glycogen metabolism in the fowl tapeworm, *Raillietina echinobothrida*. The glycogen concentration was found to decrease by 15-44%, accompanied by an increase of activity of the active form of glycogen phosphorylase (G Pase a) by 29-39% and decrease of activity of the active form of glycogen synthase (G Sase a) by 36-59% in treated parasites as compared to untreated controls, but without affecting the total activity (a + b) of both the enzymes.

Pal and Tandon, 1998 has studied the mode of action of genistein and its effect on the activity of tegumental enzymes of the parasite *Raillietina echinobothrida* using acid phosphatase (AcPase), alkaline phosphatase (AlkPase), adenosine triphosphatase (ATPase) and 5'-nucleotidase (5'-Nu). The crude extract of *F. vestita* (50 mg/ml) and genistein (0.5 mg/ml) suppressed the activity of AcPase, AlkPase, ATPase and 5'-Nu by 97, 95, 88 and 57% respectively. Tandon *et al.*, 1997 reported the *in-vitro* activity of root-tuber-peel extract of *F. vestita*, which was tested against helminth parasites. Live parasites (nematode: *Ascaris suum* from

pigs, *A. lumbricoides* from humans, *Ascaridia galli* and *Heterakis gallinarum* from domestic fowl; cestode: *Raillietina echinobothrida* from domestic fowl; trematode: *Paramphistomum* sp. from cattle) were collected in 0.9% physiological buffered saline (PBS) and maintained at $37 \pm 1^\circ\text{C}$. The treated parasites showed structural alteration in their tegumental architecture. This study suggests the vermifugal activity of the plant extract against trematodes and cestodes.

Kar *et al.*, 2002 studied the effect of the crude peel extract of *F. vestita* and genistein, for nitric oxide (NO) and the enzyme nitric oxide synthase (NOS) in *Fasciolopsis buski*, the large intestinal fluke of swine and human host. In biochemical analysis, the NOS activity showed a significant increase in the parasites treated with the test materials and reference drug, compared to the untreated controls. The increase in NOS activity in the treated parasites can be attributed to an inducing effect of the plant-derived components.

Roy and Tandon, 1996 reported the effect of ethanol root-tuber extract of *F. vestita* on a leguminous plant on *Artyfechinostomum sufrartyfex* and *Fasciolopsis buski* by a scanning electron microscopy. *A. sufrartyfex* became paralyzed within 1.1-1.4, 0.8-1.0, and 0.3-0.5 h, respectively. Stereo scanning observations were noticed on the tegumental surface of treated {(20 mg extract/ml phosphate-buffered saline (PBS))}, *A. sufrartyfex* revealed the sloughing off of most of the spines or their deformation as well as wrinkles and rupture of the general tegument. Severe tegumental alterations and deformities were also displayed by *F. buski* when exposed to 20 mg extract/ml PBS. *Flemingia macrophylla* have been shown to produce anti-parasitic effect due to the presence of condensed tannins and also shown to have effects on larvae of *Haemonchus in vitro* (Nguyen *et al.*, 2005).

Antioxidative activity

Pan *et al.*, 2005 has compared the antioxidative effect of the extracts of *Glycine radix* which showed higher activities in free radical-scavenging activity determined with DPPH, reduction in hemoglobin-catalyzed lipid auto-oxidation and inhibition of the lipoxigenase (LOX) and cyclooxygenase (COX)-catalyzed arachidonate oxidation when compared to the activities of extract of *Flemingia*.

Antimicrobial activity

Grosvenor *et al.*, 1995; Mahato and Chaudhary, 2005 studied the antimicrobial activity of leaf, fruit and stem extracts of *F. strobilifera* which were active against *Staphylococcus aureus*. Flemiflavanone-D from *Flemingia stricta* has been found to be active *in-vitro* against *Staphylococcus aureus* and *Mycobacterium smegmatis*. The compound has shown significant activity against Gram-positive *Staphylococcus aureus* (6.15

$\mu\text{g/ml}$) and acid-fast *Mycobacterium smegmatis* (6.25 $\mu\text{g/ml}$) (Mitscher *et al.*, 1985). *F. strobilifera* is also used for kidney problems (Lans., 2006). Saha *et al.*, 2011 reported the antimicrobial and antioxidant activities of *Flemingia strobilifera*.

Cytotoxicity activity

The 70% ethanol extract of the root of *Moghania philippinensis* (*M. philippinensis*) was reported to have cytotoxic activity against P-388 lymphocytic leukemia cells in culture (Chen *et al.*, 1991). The prenylated flavonoids isolated from the root of *M. philippinensis* was also found to have appreciable estrogenic activity when tested for its effect on the proliferation of MCF-7 human breast cancer cells (Ahn *et al.*, 2003).

Analgesic activity

Anil kumar *et al.*, 2011 studied the potent analgesic activity of *Flemingia strobilifera* at the dose levels of 300, 500 and 1000 mg/kg. The *Flemingia strobilifera* showed significant analgesic activity at low dose of 300 mg/kg even in the first hour of the test. The analgesic activity shown by *Flemingia strobilifera* at 300 mg/kg was almost comparable to that produced by acetylsalicylic acid, while at the dose levels of 500 mg/kg and 1000 mg/kg. *Flemingia strobilifera* showed better analgesic effect than the reference drug and at the dose level of 1000 mg/kg the duration and intensity of analgesia was also greater than acetylsalicylic acid. The activity was also evaluated by Tail flick method (Chen *et al.*, 1991).

Anti-inflammatory activity

Anil kumar *et al.*, 2011 also studied the anti-inflammatory activity of *Flemingia strobilifera*. The anti-inflammatory activity was evaluated by Carrageenan-induced paws edema method. The albino rats of either sex were divided into six groups of six animals each. Group I received 0.2 ml of 2% w/v carboxy methyl cellulose suspension orally for 7 days as a control group, Group II received 400 mg/kg body weight of ethanolic extract of *Flemingia strobilifera* (EFS-I) orally for 7 days, Group III received 600 mg/kg body weight of ethanolic extract of *Flemingia strobilifera* (EFS-II) orally for 7 days, Group IV received 400 mg/kg body weight of aqueous extract of *Flemingia strobilifera* (AFS-I) orally for 7 days, Group V received 600 mg/kg body weight of aqueous extract of *Flemingia strobilifera* (AFS-II) orally for 7 days and Group VI received 10 mg/kg of body weight of Indomethacin intraperitoneally for 7 days as a standard drug. Acute inflammation was induced in all groups by injecting 0.1 ml of 1% w/v Carrageenan into the sub-plantar region of the right hind paw of rats. On 7th day, paw volume was measured 1 hr prior to Carrageenan injection using plethysmometer and at 0 and 3 h after the

Carrageenan injection. Mean increase in the paw volume was measured and percent inhibition was calculated.

Hepatoprotective and antioxidant activity

The present study was carried out to evaluate the hepatoprotective and antioxidant effect of the Chloroform extract of *Flemingia strobilifera* R.Br. leaf (CEFS) in Wistar albino rats. Antioxidant was studied using 1, 1-Diphenyl-2-Picrylhydrazyl (DPPH) assay. Protective action of CEFS leaf extract was evaluated using animal model of hepatotoxicity induced by ethanol-Carbon tetrachloride (CCl₄). Liver marker enzymes were assayed in serum and antioxidant status was assessed in liver tissue. Histopathology was also studied. CEFS leaf did not demonstrated *in vitro* scavenging of DPPH radicals. Levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) and total bilirubin were increased and the levels of total protein were decreased in ethanol-CCl₄ treated rats. CEFS leaf at both the doses did not decreased the elevated levels of all these biochemical parameters and did not restored the normalcy of total protein significantly. Lipid peroxidation (LPO) was increased significant in liver tissue in the ethanol-CCl₄ treated rats while the activities of reduced glutathione (GSH), catalase (CAT) and superoxide dismutase (SOD) were decreased. CEFS leaf at both the doses did not decreased the elevated levels of lipid peroxide and did not restored the normalcy of GSH, CAT and SOD. Histopathology's also showing similar results. From this study it can be concluded that the CEFS leaf did not showed significant hepatoprotective and antioxidant action (Mohammad, 2009).

Anti-ulcerogenic properties

Chloroform extract of *Flemingia strobilifera* root was found to be safe up to 300 mg/kg body weight when administrated orally in female wistar rats. Water immersion stress produced characteristic lesions in the glandular portion of the rat stomach. Pre-treatment with chloroform extract of *Flemingia strobilifera* root reduced the characteristic lesions in a dose dependent manner (P<0.001) when compared with the control. Pre-treatment with chloroform extract of *Flemingia strobilifera* root at a dose of 15 and 30 mg/kg body wt. increased the gastric mucosal glutathione level, total protein content significantly (P<0.001) as compared to control group. Whereas there is significant (P<0.05, P<0.001) reduction in gastric mucosal Malonaldehyde levels when compared to control. Free radical scavenging activity of chloroform extract of *Flemingia strobilifera* root was observed in the concentration range tested, the IC₅₀ value was calculated. Antimicrobial activity of the chloroform extract of *Flemingia strobilifera* root exhibited activity against both gram positive and negative bacteria at concentration of 10

mg/ml. The root extract of *Flemingia strobilifera* possess antiulcerogenic properties could justify folklore uses of the plant in peptic ulcer diseases (Anil Kumar *et al.*, 2009).

Antidiabetic activity

Hsieh *et al.*, 2010 reported the antidiabetic activity of aqueous extract of *Flemingia strobilifera*.

Neuroprotective activity

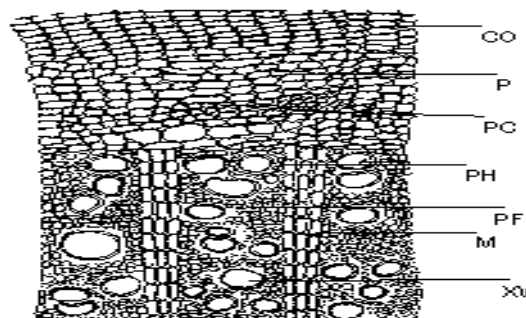
Shiao *et al.*, 2005 have shown the neuroprotective activity of flavonoids from *Flemingia macrophylla* against A β -induced neurotoxicity with EC₅₀ values of 31.43 \pm 3.16, 5.01 \pm 1.28, 11.25 \pm 1.51, 4.47 \pm 0.65, 12.09 \pm 2.55. The methanolic extract of *F. strobilifera* root and leaf possesses good antioxidant activity, which might be helpful in preventing the progress of various oxidative stresses (Chen *et al.*, 1991). *Flemingia* flavanone isolated from *F. strobilifera* showed significant antimicrobial activity against Gram-positive (*S. aureus*, *S. epidermidis*, MRSA), Gram-negative bacteria (*P. aeruginosa*, *E. coli*) and fungi (*C. albicans*) (Grosvenor *et al.*, 1995).

Anil kumar KV reported the anti-ulcer effect of chloroform extract of *F. strobilifera* root. Anti-ulcer effect was evaluated by water immersion induced ulcer in rats. Pre-treatment with chloroform extract of *Flemingia strobilifera* root at a dose of 15 and 30 mg/kg body wt. increased the gastric mucosal glutathione level, total protein content.

Therapeutic uses

The root of this plant is used in epilepsy, hysteria, to induce sleep and to relieve pain. The leaves are reported to be used as vermifuge for children. (Nigam SS and Saxena, 1975) & (Bhatt,1975). Decoction of *F. strobilifera* leaves are used as a health tonic by rubbing it on the body.

In Malay Peninsula and the Philippines, it is used as general purpose tonic and a postpartum protective medicine (Nguyen *et al.*, 2005). The root juice of *F. strobilifera* is used for fever, diarrhea, indigestion (Hsieh *et al.*, 2010). The root of the plant *F. macrophylla* is used by hill tribes as an external application to ulcers and swellings (Shiao *et al.*, 2005). The dried pods of the plant are used for preparing the dye "Wars" (Manandhar, 1995). *M. macrophylla* has been used as an anti-rheumatic, anti-inflammatory and anti-histamine in Taiwan and Southern China (Anonymous, 1956). Decoction of the tuber of *F. vestita* is used for vermifugal activity. (Krishnamurty & Prasad,1980). The powdered seed pods of *F. rhodocarpa* are used as cosmetic and dye (Kan, 1981).

Fig 1. Root of *F. strobilifera*Fig 2. T.S. of *F. strobilifera* root

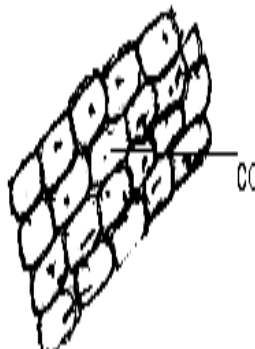
CO- Cork Cells, P- Phelloderm, C- Parenchymatous Cells, PH- Secondary Phloem, M- Medullary Rays, XV- Xylem Vessels

Fig 3. Powder microscopy of *Flemingia strobilifera* root

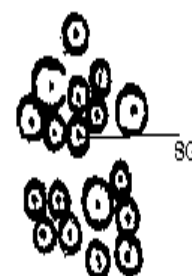
FS: Xylem fibers



BPV: Bordered pitted vessels



CO: Cork cells



SG: Starch grains

CONCLUSION

About 105 species of the genus *Flemingia* have been reported in various floras. An exhaustive survey of literature revealed that sporadic information is available only on 15 species. Among these 15 species, most of ethnopharmacological reports are available on 5 species of *Flemingia*. Further, only 6 species of *Flemingia* have been partially investigated for their phytoconstituents. A close scrutiny of literature on *Flemingia* reveals that 5 species have been investigated pharmacologically. Among these, *F. chappar* and *F. strobilifera* have been exhaustively explored for their antimicrobial and antioxidant activity. Despite a long tradition of use of *Flemingia* species for treatment of various ailments, no pharmacological work has ever been carried out to prove its traditional claims for epilepsy. Additionally, the plant has been included in number of herbal formulations, which are in clinical use for the treatment of various ailments. Keeping in view the traditional, alternative and complimentary medicinal uses, sporadic phytochemical

and pharmacological reports, low toxicity, *Flemingia* species seems to hold great potential for in depth investigation for various biological activities, especially its effect in the epilepsy and central nervous system. The authors are involved in bioactivity-directed-fractionation of this plant with a view to isolate bioactive fraction / constituent(s). The extracts of *Flemingia strobilifera* may contribute to the development of potent phytomedicine as antibacterials, antioxidants, anti-inflammatory and anticancer drugs.

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