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PHARMACOGNOSTIC STUDIES ON STEM BARK OF *FICUS ASPERRIMA*

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ABSTRACT

Ficus asperrima Roxb. commonly known as Sandpaper tree, belongs to Family: Moraceae (Mulberry family). Different parts of the *F. asperrima* are used for house-hold, industrial purposes and medicinal purposes. Many researches across the globe have reported that the leaf extract of *F. asperrima* possesses anti-hypertensive, anti-arthritic, anti-oxidant, anti-diabetic, anti-pyretic, anti-convulsant, ulcer protective activity. The present investigation includes macroscopy, microscopy, preliminary phytochemical screening, physicochemical evaluation and HPLC fingerprinting of the stem bark of the plant.

Keywords: *Ficus asperrima*, pharmacognosy

INTRODUCTION

Medicinal plants are moving to main stream use with a greater of people seeking herbal remedies. Herbal formulations generally use fresh or dried parts of the plants. Correct knowledge of such crude drugs is an important aspect in preparation, safety and efficacy of the herbal product. Thus, pharmacognosy is a reliable tool that provides the information for correct identification (Gokhale, S.B., 1979; Jeyaprakash *et al.*, 2011; Kadam *et al.*, 2011; Kaur *et al.*, 2011).

Ficus asperrima is a deciduous tree, bark is pale-greenish in colour; exudates watery sticky sap, leaves are simple, alternately arranged, ovate-elliptic leaves have a very rough surface, young leaves are often lobed; inflorescence is syconia; flowers are unisexual; figs arise in leaf axils, figs are spherical or obovoid, 1-1.5cm in diameter, rough, yellow, orange or red. Trees are distributed in evergreen to semi-evergreen forests up to 1200m in India, East Africa, Arabia and Sri Lanka, in Western Ghats-

South, Central and Maharashtra Sahyadris (Ayinde *et al.*, 2007; www.biotik.org).

Ficus asperrima belongs to family Moraceae, and commonly called sand paper tree, widely spread in all eco-regions of Nigeria. The plant has been ethnobotanically reported to have diverse medicinal uses. The leaf extract is reported to be effective as hypotensives (Buniyamin *et al.*, 2007), haemostatic, ophthalmia, coughs and haemorrhoids (Odunbaku *et al.*, 2008). The root bark is reported to be useful in the treatment of high blood pressure (Lawal *et al.*, 2009). The leaf is used to scratch skin parts affected by ringworm while the grounded leaves are applied topically to treat boils (Okoli *et al.*, 2007). Polyherbal formulation containing *F. asperrima* is used in treatment of sleep disorders (Mshana *et al.*, 2000). Also, sap of *F. asperrima* is used for haemostatic ophthalmia while leaves are used as hemostatic externally (Cousins *et al.*, 2002). One handful of leaves are macerated in a glass of water and given to treat diarrhoea with one litre of water for four days. People of Nigeria also use these leaves for treating ulcers. Chewing and swallowing a few leaves for four to eight weeks is believed to produce complete cure from peptic ulcers (Akah *et al.*, 1998).

Synonyms

Ficus exasperata, *Ficus punctifera*, *Ficus serrate*, *Ficus silicea*.

Vernacular names

English: Sandpaper tree, Sanskrit: Karapatra, Marathi: Karvat, Malayalam: Theerakam

MATERIALS & METHODS

Procurement of plant materials:

The stem bark of *Ficus asperrima* was collected from the Borivali National Park, Mumbai. The identity of plant (Voucher no.: LWG Acc. no. 97373) was taxonomically authenticated by National Botanical Research Institute in Lucknow (India) and voucher specimens were deposited in Department of Zoology, R. Jhunjhunwala College (Mumbai) as authentic specimen for future reference.

Macroscopic evaluation:

Macroscopic characters like colour, odour, taste, fracture and size were studied.

Microscopic evaluation:

Microscopic evaluation was carried out by taking transverse sections of fresh bark using razor blade; few sections were stained with safranin. Microscopic characteristics of the stained and unstained sections were observed under microscope.

Physicochemical evaluation:

Physicochemical properties of stem bark of *Ficus asperrima* were evaluated as per Indian Pharmacopoeia 2010. Parameters

such as foreign organic matter, ash values, extractive value (water, alcohol and hydroalcoholic {1:1} extractive value) and loss on drying were carried out.

Preliminary phytochemical evaluation:

Aqueous, hydroalcoholic and alcoholic extracts for phytochemical analysis were prepared from the powdered bark of *F.asperima*. The aqueous and hydroalcoholic (water:alcohol = 1:1) extract were prepared by double maceration technique, where 500g of powdered bark was macerated with 5L of solvent for 7 days, was then filtered; the residue was again macerated for 7 days, filtered again; both the filtrates were mixed, then evaporated using rotary vacuum evaporator and stored in air-tight container in refrigerator till further use. Alcoholic extract was prepared using Soxhlet apparatus. 500 g of powdered crude drug was packed in Soxhlet and was extracted with ethanol. Ethanol was evaporated using rotary vacuum evaporator. Extract was stored in refrigerator till further use. For the tests, each extracts were dissolved in methanol and filtered. The filtrates were then tested as per Gokhale *et al.*, 2011.

HPTLC fingerprinting:

HPTLC fingerprinting of the plants were developed using DESEGA (Germany) HPTLC system and the densitometric scanning was performed at visible, 254 &

366 nm using TLC scanner DESEGA CD60 and ProQuant software.

RESULTS

Macroscopic study of the stem bark:

Mature dried stem bark showed following features,

- Size: 0.4 – 0.9 cm in thickness
- Shape: slightly curved
- Outer surface: rough due to presence of lenticels, blackish-brown in colour
- Inner surface: dark brown in colour
- Fracture: fibrous
- Odour: characteristic
- Taste: mild aromatic.

Microscopic features of the stem bark:

The transverse section of mature stem bark of *Ficus asperima* shows the following features:

- Bark is differentiated into outer thick periderm and inner secondary phloem.
- The phellem cells are thick, tangential, wavy and many filled with tannin.
- Phelloderm is broad and distinct. The cells are interrupted by patches of stone cells. Stone cells are highly lignified, round-oval-square in shape having narrow-wide lumen. Starch grains and prismatic calcium crystals are present in this region.

- Secondary phloem is traversed by phloem rays. Cells are filled with tannin, starch grains, calcium oxalate crystals.



Fig 1: Stem bark of *Ficus asperima*; A: Outer surface, B: Inner surface

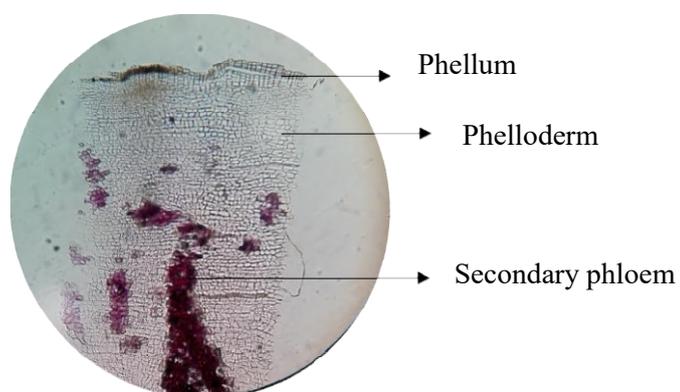


Fig 2: Transverse section showing phellum, phelloderm and secondary phloem region (100x)

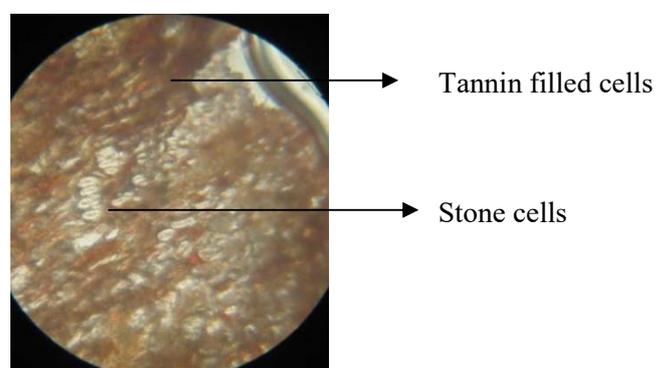


Fig 3: Transverse section of *Ficus asperima* showing phelloderm region with tannin filled cells and stone cell patches (450x)

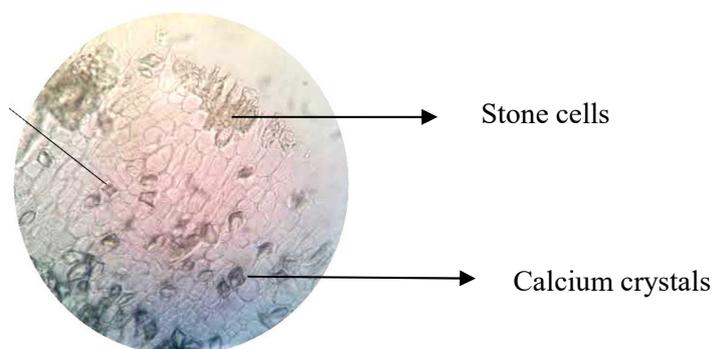


Fig 4: Transverse section of *Ficus asperima* showing prismatic calcium crystals and stone cells (450x).

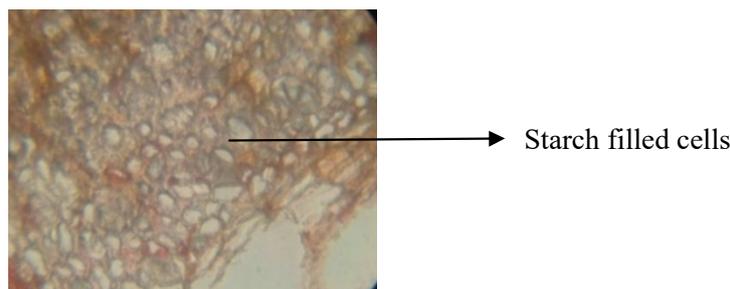


Fig 5: Transverse section of *Ficus asperima* showing secondary phloem region with starch filled cells (450x)

Physicochemical evaluation:

The results of the physicochemical evaluation of the powdered bark of *Ficus asperima* have been summarized in the **Table 1** below.

Phytochemical evaluation:

The **Table 2** below reveals the results of the qualitative analysis of phytochemical constituents of the stem bark of *Ficus asperima*.

HPTLC fingerprinting:

a) Aqueous extract of *Ficus asperima* bark- The developed HPTLC plates of the aqueous extract of *F.asperima* can be seen in Figure 6, while the Figure 7 depicts the densitogram of the same. In Figure 6, a single prominent band though jagged can

be noticed. The resolution factor of it was 0.45.

b) Hydroalcoholic extract of *Ficus asperima* bark - The figure 8 shows the developed HPTLC of the hydroalcoholic extract of *Ficus asperima*. It shows the bands with the Rf value of 0.29, 0.37, 0.56, 0.60, 0.66 and 0.75. The figure 9 reveals the densitograms of the same.

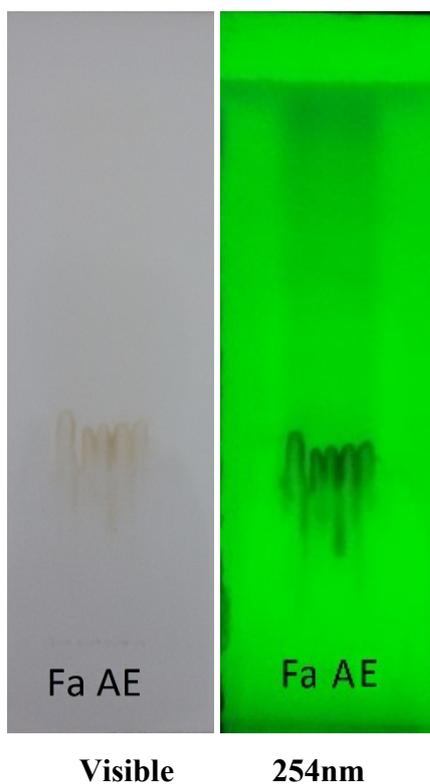
c) Alcoholic extract of *Ficus asperima* bark - The result of HPTLC of the alcoholic extract of the bark of *F.asperima* can be seen in figure 10 visualized under visible, 254nm and 266nm. Figure 11 depicts the densitogram of the same. As evident in there alcoholic extract of *Ficus asperima* showed five peaks in densitogram at Rf value of 0.27, 0.54, 0.59, 0.67 and 0.74.

Table 1: Physicochemical evaluation of *Ficus asperrima*

Parameter	Results
1.Foreign organic matter	Foreign matter content of raw material was found to be less than 0.05 gm/ 100 gm dried sample.
2. Ash values	
a. Total ash	18.22 ± 1.07 (%w/w)
b. Acid insoluble ash	4.88 ± 0.91 (%w/w)
3. Solvent extractive values	
a. Water-soluble extractive value	14.32 ± 0.26 (%w/w)
b. Alcohol soluble extractive	4.64 ± 0.32 (%w/w)
c. Hydroalcoholic extractive	8.12 ± 0.24 (%w/w)
4. Loss on drying	16.18 ± 0.66 (%w/w)

Table 2: Phytochemical evaluation of the extracts of *Ficus asperrima*

Sr. No.	Phytochemicals	Aqueous extract	Hydroalcoholic extract	Alcoholic extract
1	Carbohydrates	+	+	+
2	Saponins	+	+	+
3	Glycosides	+	+	+
4	Flavonoids	+	+	+
5	Alkaloids	-	-	-
6	Steroids	+	+	+
7	Phenolic Compounds	-	-	-
8	Tannins	+	+	+

Fig 6: HPTLC of aqueous extract of *Ficus asperrima* bark

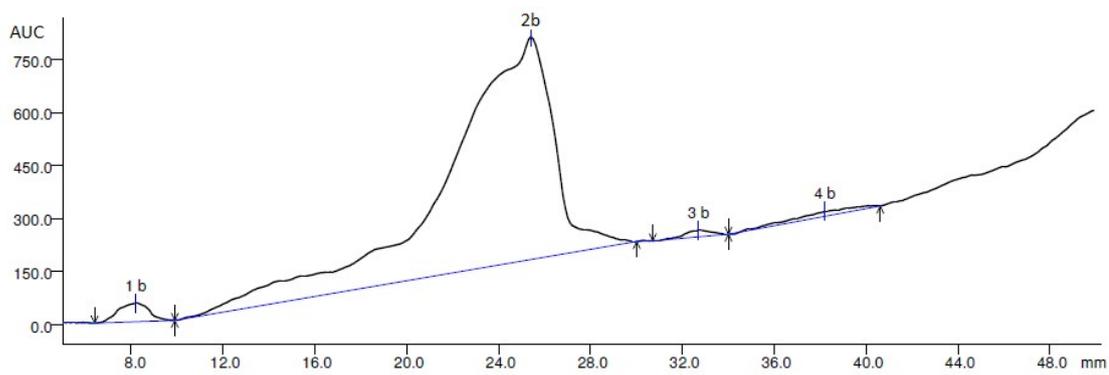


Fig 7: HPTLC densitogram of aqueous extract of *Ficus asperrima* bark



Fig 8: HPTLC of hydroalcoholic extract of *Ficus asperrima* bark

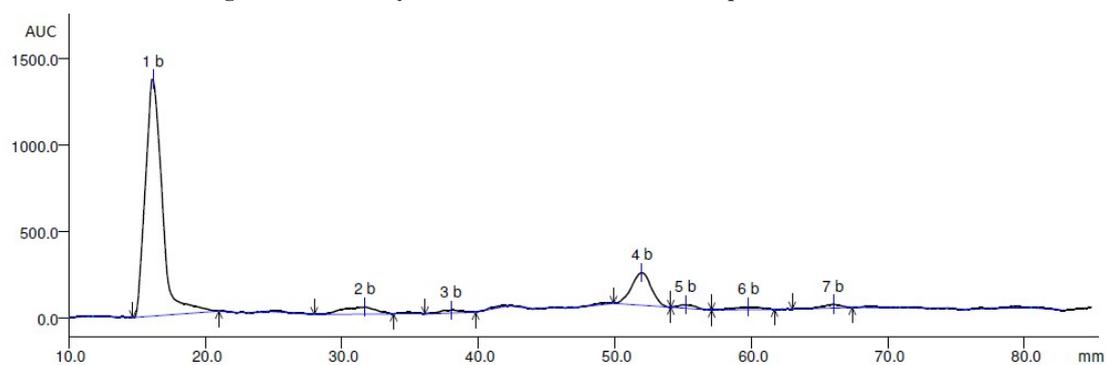


Fig 9: HPTLC densitogram of hydroalcoholic extract of *Ficus asperrima* bark

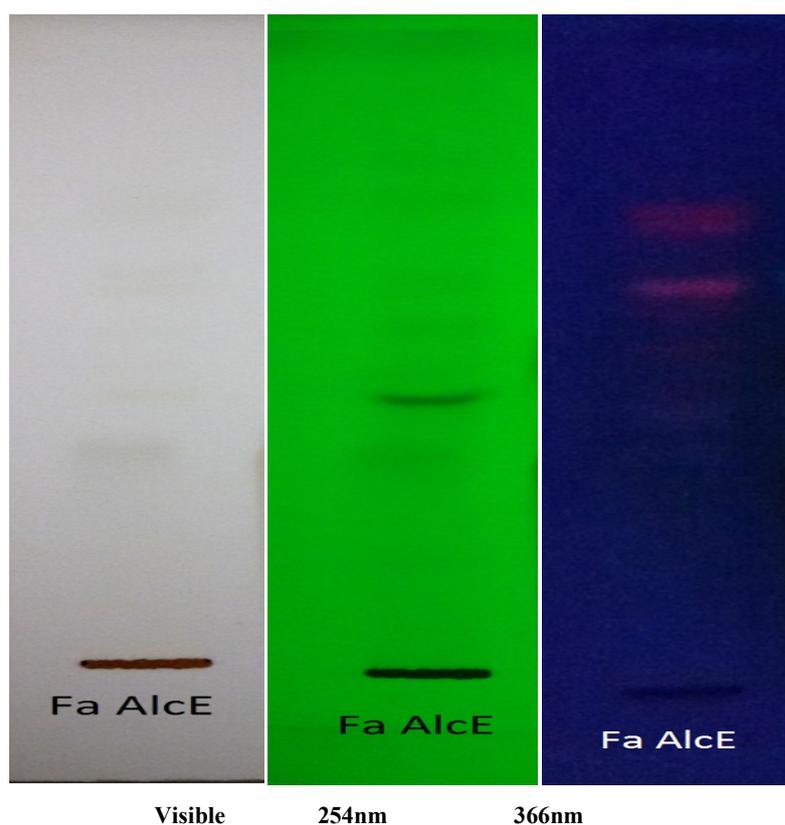


Fig 10: HPTLC of alcoholic extract of *Ficus asperima* bark

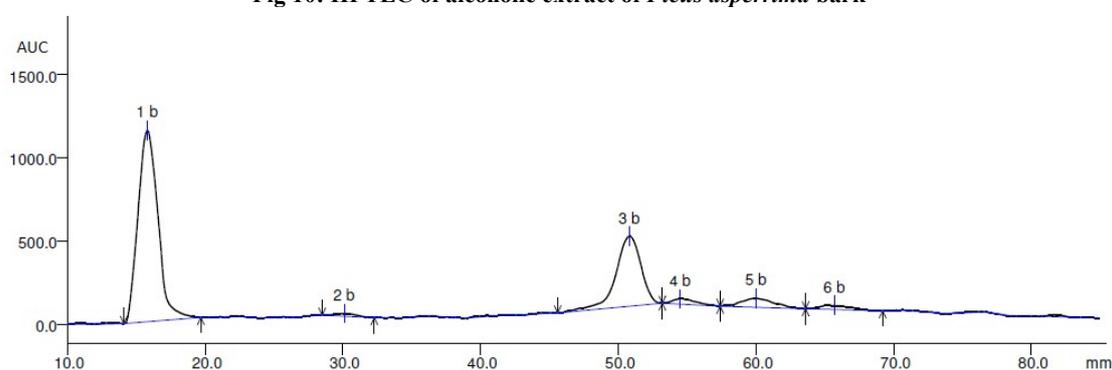


Fig 11: HPTLC densitogram of alcoholic extract of *Ficus asperima* bark

DISCUSSION

Only a few previous works were found on *Ficus asperima* stem bark. Macroscopic and microscopic analysis of the plant is concurrent with the previous works (E.A Adebayo *et al.*, 2009; Adeniyi I.W *et al.*, 2010; B.A Ayinde and F.C Amaechina, 2011; B.N Tripathy *et al.*, 2011; L. G Radhika *et al.*, 2011).

The total ash value and acid insoluble ash value of *Ficus asperima* reported by Ayinde *et al.* (2011) have been $28.49 \pm 0.87\%$ and $9.93 \pm 0.2\%$ respectively. The percentage of loss on drying reported by them has been $6.1 \pm 0.15\%$, while the water and alcohol soluble extractive value were $1.87 \pm 0.04\%$ and $0.59 \pm 0.03\%$ respectively. In the current study of *Ficus asperima*, the total ash value and acid insoluble ash value

have been found to be $18.22 \pm 1.07\%$ and $4.88 \pm 0.91\%$ respectively. The hydroalcoholic extractive value of *F. asperima* in the current study has been $8.12 \pm 0.24\%$. Also, the water and alcohol soluble extractive value have been $14.32 \pm 0.26\%$ and $4.64 \pm 0.32\%$ respectively. Preliminary phytochemical screening of the extracts gives an idea about the active constituents present in the plants. Phytochemical analysis of stem bark of *F. asperima* concurrent with the previous works (B.A Ayinde and F.C Amaechina, 2011; E.A Adebayo *et al.*, 2009; Adeniyi I.W *et al.*, 2010; A. Bisht *et al.*, 2011; B.N Tripathy *et al.*, 2011; L. G Radhika *et al.*, 2011). In current study, carbohydrates, glycosides, flavonoids, saponins, steroids and tannins are found to be present in *Ficus asperima*; while the test for alkaloid and phenolic compounds have been negative. Chromatographic fingerprint helps in authentication and correct identification of herbal medicines. Thus, it is very important to obtain reliable chromatographic fingerprints that represent pharmacologically active and chemically characteristic component of the herbal drug (Liang *et al.*, 2004). The extracts of the plant have also been standardized using HPTLC technique. No previous HPTLC analysis of *F. asperima* has been reported. However, HPTLC analysis of the alcoholic

extract of stem bark *Ficus religiosa* has been done by Verma *et al.*, 2014. It has revealed bands with Rf values of 0.14, 0.28, 0.41, 0.56, 0.65 and 0.71. Also, Tripathi and Sikarwar's (2014) work on *Ficus virens* has revealed bands with Rf values of 0.36, 0.42, 0.62, 0.64, 0.11, 0.18, 0.36, 0.50, 0.54, 0.62, 0.64, 0.68, 0.74 and 0.80. In the current study, the densitogram of the alcoholic extract of *F. asperima* has shown peaks with Rf value of 0.27, 0.54, 0.59, 0.67, 0.74. While the aqueous extract has shown a prominent peak with Rf value of 0.45 and the hydroalcoholic extract has shown peaks with Rf values of 0.29, 0.37, 0.56, 0.60, 0.66, 0.75.

CONCLUSION

Morphologically, stem bark of *Ficus asperima* is blackish-brown in colour and dark brown internally, having characteristic odour and mild aromatic taste; it shows fibrous type fracture. Few diagnostic characters of the bark anatomy include thick, tangentially elongated, tannin filled cells of phellum; patches of stone cells which are round-oval-square in shape with narrow-wide lumen and presence of starch grains and prismatic calcium crystals in the phelloderm region; secondary phloem cells filled with tannins, starch grains, calcium oxalate crystals.

The physiochemical standards like foreign organic matter, ash values, loss on drying

and solvent extractive values are considered to be reliable for the crude drugs, are also determined in the current study. Solvents used for extraction were water, alcohol and water + alcohol (50:50) which were an addition to the pharmacopiel standard.

If the chemical profile of the plant is known, one can relate it, to its therapeutic activities. In the present study, qualitative pythochemical analysis of different extracts viz. aqueous, alcoholic and hydroalcoholic of the plant. Carbohydrates, glycosides, flavonoids, saponins, steroids and tannins are found to be present in these extracts.

The fingerprinting for the extracts of stem bark of *F.asperima* in alcohol, mixture of alcohol & water and water differ from one another sufficiently to believe that different chemicals get extracted in different solvent system. This data can be used and enable further *in-vivo* studies using all three types of extracts to establish the efficacy of the drug.

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