COMPARATIVE PHYTOCHEMICAL SCREENING OF AQUEOUS EXTRACT OF FOUR FICUS SPECIES

Awadhesh Kumar Pandey*[1], PathakMeenakshiS.N.[2], Prof.M.Sahu[3], Dr.Vijay Kumar[4]

[1]Assistant Professor, Department of Shalya Tantra, Patanjali Bharteeya Ayurvigyan Avum Anusandhan Sansthan, Haridwar, Uttrakhand.

[2]Assistant Professor, Department of Prasuti Tantra, Patanjali Bharteeya Ayurvigyan Avum Anusandhan Sansthan, Haridwar, Uttrakhand.

[3]Dean and Head, Department of Shalya Tantra, Faculty of Ayurveda, Institute of Medical Sciences, Banaras Hindu University Varanasi.

[4]Associate Professor, Department of Swasthavritta, Patanjali Bharteeya Ayurvigyan Avum Anusandhan, Sansthan, Haridwar, Uttarakhand.

*Correspondence for Author

Dr. Awadhesh Kumar Pandey

Assistant Professor, Department of Shalya Tantra, Patanjali Bharteeya Ayurvigyan Avum Anusandhan Sansthan, Haridwar, Uttrakhand.

ABSTRACT

India is a rich and varied flora of medicinal plants since the Vedic period. The barks of four *Ficus* species - Ficus benghalensis *L.* (Sanskrit-Vata), Ficus glomerata *Roxb.* (Sanskrit -gular) and Ficus religiosa *L.* (Sanskrit -Asvatthah) Ficus lacor Buch-Ham. (Sanskrit - plaksha), are important ingredients in many Ayurvedic and traditional formulations and considered to be very effective in various treatments, such as diabetes, skin diseases, healing of fractures, and in different type of wound. The present study deals with the scientific rationalization of four Ficus species with special reference to its pharmacognostical and phytochemical investigations. The barks of the four ficus species mentioned above are usually interchanged or adulterated with other species of *Ficus* because of the limited knowledge in identification and differentiation. Therefore, a detailed comparative pharmacognostic evaluation of the 4 species has been carried out with the aim to establish the diagnostic keys of these important drugs based on the macroscopic, microscopic, and HPTLC profiles. Detailed diagnostic and distinctive characteristics for the differentiation of the 4 *Ficus* species are discussed.

KEYWORDS: Bark, *Ficus racemosa, Ficus virens, Ficus religiosa, Ficus benghalensis*, Moraceae, Pharmacognosy.

INTRODUCTION

Wound was probably the primary ailment, which man had suffered and identified in early days. Similarly plants can be considered as the first living thing on the earth. So it was very natural that man tried to manage the wound by using various plants.[1]

In Ayurvedic texts, different types of remedies have been advocated in the management of wound. Normally healing of wound starts immediately after injury. Although there is a category of wound where either the wound healing is delayed or there is no healing at all. To counter all such types of wounds various authors have described a range of drugs.[2]

Acharya Charaka described 500 different drugs in fifty groups *i.e*., ten drugs in each group. This classification is based on pharmaco-dynamic actions (Karmanasura).[3]

Acharya Sushruta being parimaily a surgeon, classified drugs with their application in surgical diseases. He has enumerated a group of about 100 drugs for the management of wound.[4] Acharya Sushruta has classified drugs into 37 groups in accordance with their karma.[5] He has named these groups after the name of the main drug mentioned in those particular groups as Nayogradhadi varga etc. Several indigenous drugs have been mentioned specifically in Nayogradhadi varga its constituent as Vata, Udumbar, Ashwatha, Plaksh etc.[6]

Tree bark of all these four Ficus species are very complex in structure and has the potential of containing many primary and secondary metabolites. Products stored in the bark are useful for preparation of many drugs. The complex structure of the bark can be utilized for botanical identification to maintain the quality and purity of the drug.[7]

Barks of these four species, also equate with many other species like *F. microcarpa* L., *F. infectoria* Roxb., *F. arnottiana* Miq, *F. virens* Buch-Ham and *F. Talboti.* Hence, it is very difficult to identify the original from the adulterants /substitutes while procuring crude drug from the market.[8]

Based upon the macroscopic and microscopic features and HPTLC profiles of the barks of these 4 species, we can identify them with some specific characters.[9] The barks of 4 *Ficus* species contains tannin, wax, saponin gluanol acetate, β-sitosterol, leucocyanidin- $3-O-\beta-$

D - glucopyrancoside, leucopelargonidin– 3 – O – β – D - glucopyranoside, leucopelargonidin – 3 – O – α – L - rhamnopyranoside, lupeol, ceryl behenate, lupeol acetate, α-amyrin acetate, leucoanthocyanidin, and leucoanthocyanin.[10]

Morphological and anatomical diagnostic features of Stem barks of four *Ficus Species*

S. No.	Characters	*Ficus glomerata*	*Ficus lacor*	*Ficus religiosa*	*Ficus benghalensis*
1.	Thickness	8 mm	2-3 mm	5-8 mm	12-18 mm
2.	Physical features	Soft surface with minute papery flakes, smooth.	Hard and rough surface.	Hard and rough surface often covered with crustose lichen	Hard and rough surface
3.	Colour	Greyish-green colour with brown patches	Ash or greyish-brown	Greyish-white with green spots.	Grey colour with dark patches
4.	Fissure	Absent	Absent	Fissures shallow, vertical and irregularly oriented.	Fissures deep, irregular and vertically oriented.
5.	Lenticels	Absent	Lenticels irregular with black spots	Absent	Lenticels in longitudinal and transverse row, mostly circular and prominent.
6.	Periderm	Thin measuringabout72μm	Thin measuring about138μm and characteristic feature is the periderm tubes	Thick measuring about360μm and breaking in to irregular flakes.	Very thick measuring about288-576μm and distinct
7.	Phellem	Thin, membranous and easily peel off.	Thin, peel off as membranes of one cell thickness.	Thick and wavy, uneven in transaction. Older layer sex foliate in the form of thin tangential membranes.	Thick and homogeneous thin walled rectangular suberised cells.

**Phytochemical Screening of Stem Barks of Four *Ficus Species:***

Phytochemical analysis	*Ficus glomerata*	*Ficus lacor*	*Ficus religiosa*	*Ficus benghalensis*
Tannins	+	+	+	+
Saponin	+	+	+	+
Flavonoids	+	+	+	+
Steroids	+	+	+	+
Terpenoids	+	+	+	+
Cardiac glycosides	+	+	+	+
Alkaloids	-	-	-	-
Quinones	-	-	-	-

TLC and HPTLC of 4 *Ficus* Species
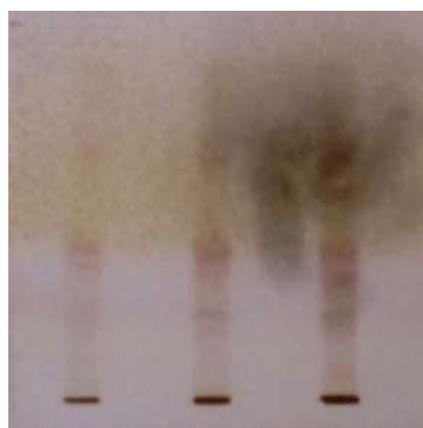
Preparation of Test solution

2gm of powdered drug was hydrolised with 50 ml of aqueous HCL by heating on a water bath at around 95^0 and cool. 50 ml of a mixture of Chloroform: Diethyl ether (1:2) added in this solution and intermittent shaking of mixture for 30 min was done.3 gm of sodium carboxy methyl cellulose added in the above mixture. This mixture was filtered and the mass was rinsed 4 times with 50 ml quantity of mixture of Chloroform: Diethyl ether (1:2) and then the filtrate was taken and exposed it to dry under reduced pressure and dissolved in 25 ml of methanol.

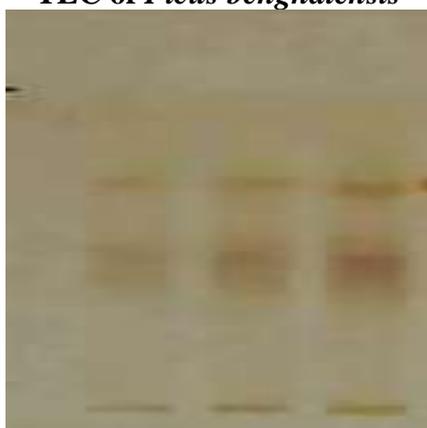Solvent system-Toluene:Ethyl acetate:Formic acid(5:5:0.5)

Standard Solution-5 mg of Caffic acid in 50 ml of methanol
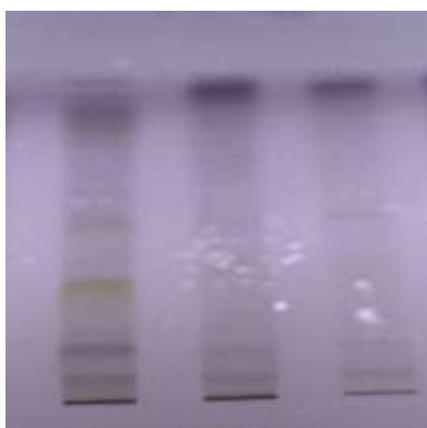
TLC of *Ficus benghalensis*   **TLC of *Ficus religiosa***

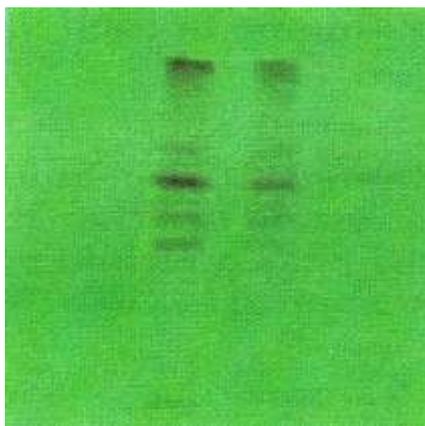**TLC of *Ficus glomerulata***   **TLC of *Ficus lacor***

Procedure

5 micro liter each of four ficus species test and standard solution applied on percolated silica gel of uniform thickness of 0.2 mm or Pre coated silica gel Sheet and dipped in a solvent system up to 8 cm.After dipping in the solution constituent are move upwards as seen in the sheet.

Visualization

Band of the Standard and test solution are at same level at Rf value 0.55 at 254nm.
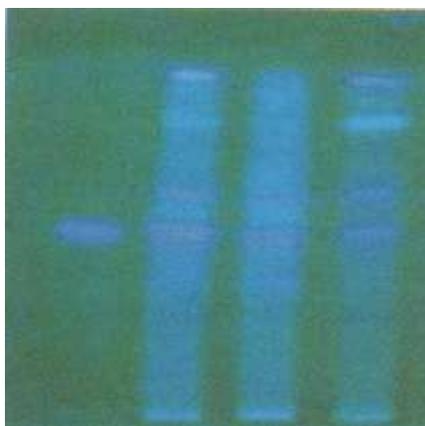
Color of the standard and test solution were fluorescent blue at 366 nm The plate was observed in UV 254 nm and UV 366nm, the Rf value and color of the band was noted in fluorescent light. Band in standard solution were exactly on the same level of the test solution. The colour of the band was fluorescent blue. Standard Rf value of the Caffic acid was 0.55 and colour of band in fluorescent light was blue.

Procedure for HPTLC of Four Ficus Species

Preparation of Test solution is as same as explained above for TLC**.**

Solvent system-Toluene: Ethyl Acetate: Formic acid (5:5:0.5).

Standard Solution

5 mg of Caffic acid was taken in 50 ml of methanol.10 micro lit of both the standard solution and test solution percolated on silica gel TLC plate. The plate was developed in solvent system scan in 327 nm and peak area was recorded then calibration curve was plotted and concentration of Caffic acid was calculated by measuring the peak area.

winCATS Planar Chromatography Manager
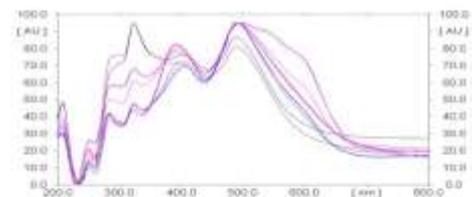
All tracks at all measured wavelengths
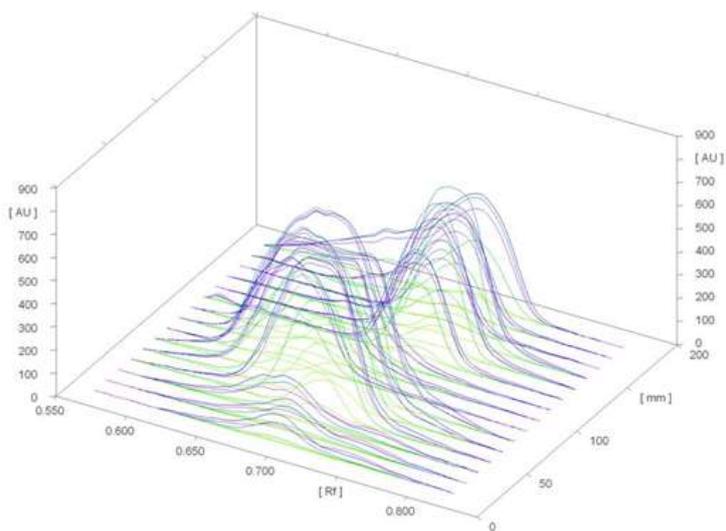
Track MWLTrackSc4 at all measured wavelengths

**winCATS Planar Chromatography Manager**

Detector properties

Y-position for 0 adjust	0.0 mm
Track # for 0 adjust	0
Analog Offset	10%
Sensitivity	Automatic (29)

AutoGenerated1 on all Tracks

T	Rf	Substance	Max. @
1	0.70 Rf	AutoGenerated1	323 nm
2	0.70 Rf	AutoGenerated1	323 nm
4	0.70 Rf	AutoGenerated1	489 nm
5	0.72 Rf	AutoGenerated1	490 nm
6	0.72 Rf	AutoGenerated1	490 nm
10	0.72 Rf	AutoGenerated1	493 nm
11	0.73 Rf	AutoGenerated1	496 nm
12	0.73 Rf	AutoGenerated1	497 nm
13	0.71 Rf	AutoGenerated1	491 nm
14	0.71 Rf	AutoGenerated1	491 nm
15	0.72 Rf	AutoGenerated1	490 nm

AutoGenerated2 on all Tracks
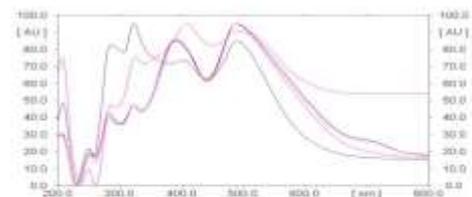
T	Rf	Substance	Max. @
3	0.69 Rf	AutoGenerated2	322 nm
5	0.68 Rf	AutoGenerated2	489 nm
6	0.68 Rf	AutoGenerated2	489 nm
9	0.67 Rf	AutoGenerated2	489 nm
12	0.65 Rf	AutoGenerated2	409 nm

CONCLUSION

Based upon the macroscopic and microscopic features and HPTLC profiles of the barks of these 4 species, we can identify them with some specific characters. The preliminary phytochemical screening shows that all the barks possess similar types of phytoconstituent groups. However, significant differences were observed in the physico-chemical analysis and successive soxhlet extractions with different solvents.[11]

Comparative HPTLC fingerprint also shows marked differences in their profiles. In UV 254 nm, except 2 common bands at Rf. 0.06 & 0.48, the other bands do not match. In UV 366 nm, all the barks show one similar common band at Rf. 0.24. *F. Religiosa* and *F. benghalensis* have 2 common bands at Rf. 0.62 & 0.76. *F. lacor* has 1 common band at Rf. 0.76and the band at Rf. 0.62 is absent. In visible light (after spray) all the barks shows 2 similar bands of violet colour at Rf. 0.28. *F. lacor, F.religiosa,* and *F. benghalensis* have 1 common band of pink colour at Rf. 0.22 and this band is absent in *F. Glomerata.* Though earlier researchers have studied and reported the pharmacognosy of the *Ficus* species individually, a relative and comparative study of the species providing key diagnostic tools has not been done earlier. We reported in the current study that on the basis of several cumulative characters, the bark of the 4 species of *Ficus* can be easily differentiated or distinguished from adulterants.

REFERENCES

1. Kaviraj Ambikadutta Shastri, Sushurt Samhita Chikitsa Sthan 1/47, Chaukhambha Sanskrit Sansthan, Varanasi, Edition-2010.

2. Kaviraj Ambikadutta Shastri, Sushurt Samhita Chikitsa Sthan 24/18, Chaukhambha Sanskrit Sansthan, Varanasi, Edition-2010.

3. Pandit Kashi Nath Pandey, Charak Samhita, Charak Sutra 30/23,Chaukhambha Bharati Prakashan, Varanasi, Reprint 2009.

4. Kaviraj Ambikadutta Shastri, Sushurt Samhita Chikitsa Sthan 36/21, Chaukhambha Sanskrit Sansthan, Varanasi, Edition-2010.

5. Kaviraj Ambikadutta Shastri, Sushurt Samhita Chikitsa Sthan 38/47, Chaukhambha Sanskrit Sansthan, Varanasi, Edition-2010.

6. Kaviraj Ambikadutta Shastri, Sushurt Samhita Chikitsa Sthan 40/21, Chaukhambha Sanskrit Sansthan, Varanasi, Edition-2010.

7. Brinda P, Saraswathy A & Jayaraman P. Micromorphological identification of the medicinal bark of *Ventilago madraspatana. J Med Arom Plant Sci,* 2000; 23: 619-622.

8. Nadkarni AK (1954). *Indian Materia Medica*, Popular Prakashan Pvt. Ltd., Mumbai.; Sing TB & K.C. Chunekar KC (1972). Glossary of vegetable drugs in Brhttrayu, Varanasi, India.

9. Koilpillai babu1, Sabesan gokul shankar, Sadananda rai Turk, Comparative pharmacognostic studies on the barks of four *Ficus* species J Bot (2010) 215-224 TÜBİTAK.

10. Sivarajan VV & Balachandran I (1994). *Ayurvedic Drugs and Their Sources*, Oxford & IBH Publishing Co. Pvt. Ltd., New Delhi.

11. Warrier PK (1994). *Indian Medicinal Plants*, Vol. 2, Orient Longman Ltd., Kottakkal, Kerala, India.