TÜBİTAK

Research Article

Turk J Bot
34 (2010) 215-224

doi:10.3906/bot-0907-115

Comparative pharmacognostic studies on the barks of four *Ficus* species

Koilpillai BABU[1,*], Sabesan GOKUL SHANKAR[2], Sadananda RAI[1]

[1]R & D Center, Cholayil Private Limited, 31-A/24, SIDCO Industrial Estate (North Phase), Ambattur, Chennai - 600 098, Tamilnadu, INDIA

[2]Microbiology Unit, Faculty of Medicine, AIMST University, Kedah, MALAYSIA

Received: 20.07.2009
Accepted: 04.04.2010

Abstract: The barks of 4 *Ficus* species, namely *F. racemosa, F. virens, F. religiosa* and *F. benghalensis*, are important ingredients in many Ayurvedic and traditional formulations. The barks are considered to be very effective in various treatments, such as diabetes, skin diseases, ulcers, and nervous disorders. During market research, we observed that various species of *Ficus* barks were sold in Indian market under traditional names, such as Plaksah, Udumbarah, Asvatthah, and Vatah. The barks of the species mention 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.

Key words: *Ficus racemosa, Ficus virens, Ficus religiosa, Ficus benghalensis*, Moraceae, Bark, Pharmacognosy

Introduction

Plants are utilized extensively as raw drugs for many formulations in traditional systems of medicine. To check the genuineness of the raw drugs and to detect adulteration of these materials, an authentic pharmacognostic study is needed for each raw drug. Usually the drugs are collected by traditional practitioners who have inherited Ayurvedic or other herbal practices. Their identification is mostly based on morphological features or other traditionally known characteristics. In such cases, there is a chance of selecting incorrect raw drugs/adulterants. Therefore, an extensive anatomical and phytochemical screening is needed for each raw drug used in the formulation to avoid any ambiguity and such a study will serve also as a reference for further studies (Vaibhav & Kamlesh, 2007). Anatomical studies are helpful in describing a particular drug with a special emphasize on quantitative microscopy, such as sclereids, starch grains, crystals, stomata, and trichomes, and qualitative microscopy, such as xylem, phloem, and other tissues (Brinda et al., 2000).

* E-mail: babu_plantsci@yahoo.co.in; babuk@cholayil.com

The genus *Ficus* belonging to the family Moraceae constitutes an important group of trees with immense medicinal value. Among the many species, the most important are the 4 trees with milky latex, namely *Ficus racemosa* L. (Sanskrit - Udumbarah), *Ficus virens* Aiton (Sanskrit - Plaksah), *Ficus religiosa* L. (Sanskrit - Asvatthah), and *Ficus benghalensis* L. (Sanskrit - Nyagrodhah, Vatah), that constitute the group "*Nalpamaram*" in Ayurveda. The barks of these species form an important ingredient in many Ayurvedic formulations, such as *Nalpamaradi tailam, Chandanasavam,* and *Saribadyasavam* (Sivarajan & Balachandran, 1994). They are used separately or in combination in different formulations. The barks are used for various purposes: as an astringent medicine, for cooling in action, as haemostatic, as laxative, in improving complexion, in cleaning vagina, and it is useful in *pitta* and *kapha*. They are used in diabetes, diarrhoea, leucorrhoea, menorrhoea, nervous disorder, and vaginal diseases. It is also widely used in the treatment of skin diseases, ulcer and soreness in the mouth (Nadkarni, 1954; Aiyer & Kolammal, 1957; Mooss, 1976; Kurup et al., 1979; Warrier, 1994; Joshi & Upadhye, 2008; Gayathri & Kannabiran, 2008).

In the present study, an attempt was made to study the comparative pharmacognostic features of the barks of the above 4 species used in *Nalpamaram*, an important Ayurvedic drug.

Materials and methods

Morphological and anatomical studies

For the present study fresh stem barks of 4 *Ficus* species were collected from Cholayil Medicinal Plants Conservation Park (CMPCP), Velagapuram, Thiruvallur district, near Chennai after authentication, and voucher specimens were preserved. Fresh barks were cut into small pieces and immediately fixed in Formalin:acetic acid:70% alcohol (5 mL + 5 mL + 90 mL) for 24 h and dehydrated, paraffin infiltrated and embedded in wax using customary techniques (Johansen, 1940; Sass, 1940). Serial transections and tangential longitudinal sections were obtained at 10-12 mm thickness with rotary microtome and the sections were stained with safranin and fast green. All the photomicrographs were taken with a Nikon E400 microscope.

Phytochemical analysis

For the chemical analysis all the barks were shade dried separately for 10 days and powdered using a pulveriser. Powdered samples were subjected to physico-chemical analysis, such as the percentage of water and alcohol soluble extractive, total ash, acid-insoluble ash (Ayurvedic Pharmacopoeia of India, 2001) and preliminary phytochemical screening was carried out using standard procedures (Evans, 1989; Sofowara, 1993; Harborne, 1998).

HPTLC analysis

Two grams of powdered material was refluxed with 10 mL of methanol in a water-bath at 60 °C for 30 min, consecutively 3 times, and then concentrated and dried. The final extract was re-dissolved in methanol and used for the HPTLC analysis. Pre-coated Silica Gel F_{254} (Merck) plate was used for stationary phase and Chloroform:Ethylacetate:Formic acid (2.5:2:1.5) used as mobile phase. After development, the plate was sprayed with 1% vanillin sulphuric acid and heated at 105 °C in hot air oven for 5 to 10 min to develop the colour of the bands.

Results

Ficus racemosa L.

Macroscopic features

The bark has a thickness of about 6 to 15 mm and is grayish-green with a fairly smooth and soft surface, with minute separating papery flakes of white tissue emerging out from outer surface, no fissures and homogeneously leathery texture. Inner surface light brown (Figure 1a).

Microscopic features

The transection of bark measuring about 8 mm thickness consists of an outer periderm measuring 72 mm thick. The rest of the bark includes a secondary phloem. The periderm is superficial in origin. It consists of regular tangentially arranged thin layers of cells (Figure 1b). The older layers of phellem exfoliate in the form of thin membrane due to separations of tangential walls between successive layers of cells. Phelloderm is evident and consists of a few layers of cubical parenchymatous cells (Figure 1b).

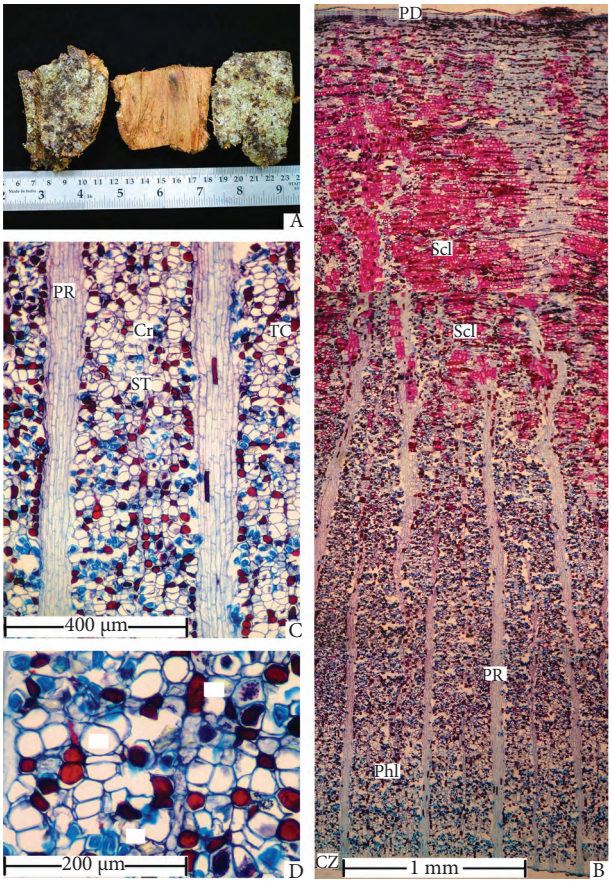

Figure 1. *Ficus racemosa*: A. External features of bark, B. Cross section of bark, C and D. Portions enlarged.
Abbreviations (Figures 1-4): AP – Axial parenchyma; Cr – Crystal; CZ – Cambial zone; Fi – Fissure; LV – Latex vessel PD – Periderm; Phl – Phloem; PP – Phloem protein; PR – Phloem rays; Scl – Sclereids; ST – Sieve tube; TC – Tannin cell.

Secondary phloem represents the broad inner bark. It consists of a narrow zone or non-collapsed phloem, which is about 360 mm broad. The non-collapsed phloem has sieve tube members and axial parenchyma, which are randomly distributed. There are also small nests of unlignified phloem fibres possessing gelatinous inner walls. Most of the phloem parenchyma cells contain tannin. Large p-protein bodies are seen in almost all sieve tube members. The collapsed phloem zone is very wide. The phloem rays, which start as narrow canals in the non-collapsed zone, dilate extensively in the collapsed phloem zone. The phloem parenchyma cells and phloem ray cells become lignified forming sclereids. Prismatic calcium oxalate crystals are fairly abundant in the axial parenchyma and ray parenchyma cells. The phloem rays either uniseriate or multiseriate (Figures 1c and 1d).

Ficus virens Aiton

Macroscopic features

Bark is flat to curve, measuring 2 to 3 mm in thickness. External surface ash or grayish-brown in colour. Surface rough with numerous lenticels. Internal surface rough, fibrous, longitudinally striated, pale reddish, fracture, and fibrous (Figure 2a).

Microscopic features

Outer bark is represented by a narrow zone of simple periderm, which is superficial in origin. Periderm is 138 mm thick. It consists of a phellem zone, which is 5-10 layers of cells forming thin continuous membranes. The outermost layers of phellem peel off as membranes of 1-cell thickness. Phelloderm is also distinct, consisting of about 8-10 layers of cubical cells containing chloroplast or tannin. Along frequent places, the periderm zone invaginates towards the inner side forming a sac like pouch filled with tannin containing dead and crushed phellem cells. Sometimes the pouch is detached from the surface and gets buried in the inner part of the bark. In cross-sectional view this detached periderm appears as a circular structure and this type of structure is designated as "periderm tubes" (Figures 2b-2d).

Secondary phloem is clearly distinguished into inner broad non-collapsed phloem, which is 920 mm wide. This zone consists of randomly oriented sieve tube members, tannin containing axial parenchyma cells, and scattered groups of gelatinous fibres. The outer zone has collapsed sieve tube members, dilated phloem rays, and larger groups of gelatinous fibres. The dilated rays or the axial parenchyma of the phloem remain parenchymatous, non-differentiated in sclerenchymatous elements as in other cases. Phloem rays are both uniseriate and multiseriate. The rays appear homocellular or heterocellular. Laticifers are not abundant both in inner and outer phloem. Cubical p-protein bodies are seen almost in all sieve tube members somewhat abutting the sieve plates. Prismatic calcium oxalate crystals are fairly abundant in the axial parenchyma and ray parenchyma cells. They are mostly solitary in each cell. The frequency of crystals increases from centre towards the periphery.

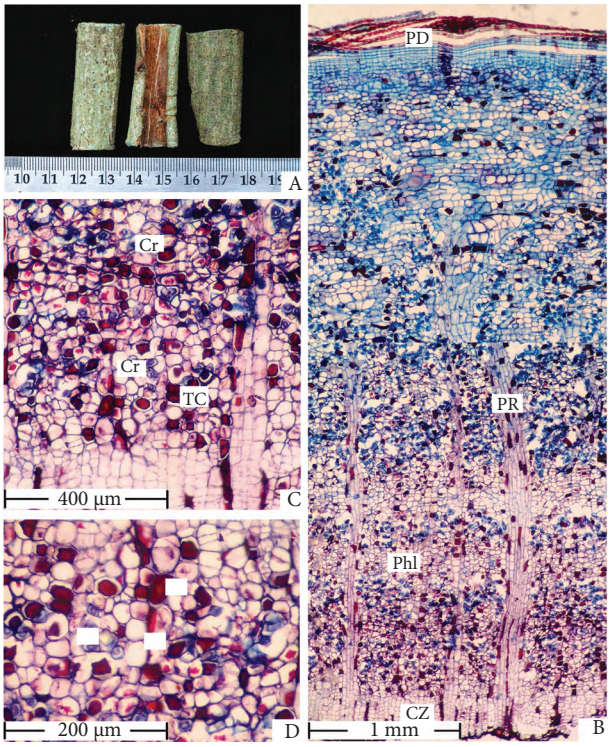

Figure 2. *Ficus virens*: A. External features of bark, B. Cross section of bark, C and D. Portions enlarged.

Ficus religiosa L.

Macroscopic features

The bark is flat or slightly curved, varying from 5 to 8 mm in thickness, outer surface is grey or ash with thin or membranous flakes and is often covered with crustose lichen brown or ash coloured, surface has shallow irregular vertical fissures and uneven due to exfoliation of cork, inner surface smooth, yellowish to orange brown and fibrous (Figure 3a).

Microscopic features

Bark differentiated into outer thick periderm and inner secondary phloem. Periderm is differentiated into phellem and phelloderm. Phellem zone is 360 mm thick and it is wavy and uneven in transection. Phellem cells are organized into thin tangential membranous layers and the older layers exfoliate in the form of thin membranes. The phelloderm zone is broad and distinct. Phelloderm cells are turned into lignified sclereids.

Secondary phloem differentiated into inner narrow non-collapsed zone and outer broad collapsed zone. Non-collapsed zone consists of radial files of sieve tube members, axial parenchyma, and gelatinous fibres. Outer collapsed phloem has dilated rays, crushed obliterated sieve tube members, thick walled and lignified fibres, and abundant tannin filled parenchyma cells. Laticifers are fairly abundant in the outer secondary phloem zone. Phloem rays are both uniseriate and multiseriate. Multiseriate rays are homocellular and uniseriate rays are either homocellular or heterocellular (Figures 3b-3f).

Ficus benghalensis L.

Macroscopic features

Mature bark is 12-18 mm thick, grey, closely adhered ashy white, light bluish-green or grey patches, slightly curve, thickness varies with the age of the tree. Surface is deeply fissured and rough due to the presence of longitudinal and transverse row of lenticels, mostly circular and prominent, fracture short in outer 2/3 of bark while inner portion shows a fibrous fracture (Figure 4a).

Microscopic features

Bark differentiated into outer bark or rhytidome and inner bark or secondary phloem. Outer bark measures 288-576 mm. Width and inner bark measures 2.9 –3.5 mm. Periderm is deeper in origin and consists of discontinuous irregular bands of sequential periderm, and originates from the deeper part of the secondary phloem. Periderm consists of phellem and phelloderm. Phellem cells are homogeneous thin walled rectangular and suberised. Phelloderm is wide and distinct. Phelloderm cells are turned into cubical sclereids arranged in radial files (Figure 4b).

Secondary phloem is differentiated into inner intact non-collapsed zone, lying next to cambial zone, and outer collapsed phloem zone. In the non-collapsed zone phloem elements occur in small clusters and consist of sieve tube members, companion cells, and axial parenchyma. Tannin is abundant in most of the axial parenchyma cells. The collapsed phloem zone consists of wide dilated rays and collapsed and obliterated sieve elements (Figures 4b and 4c).

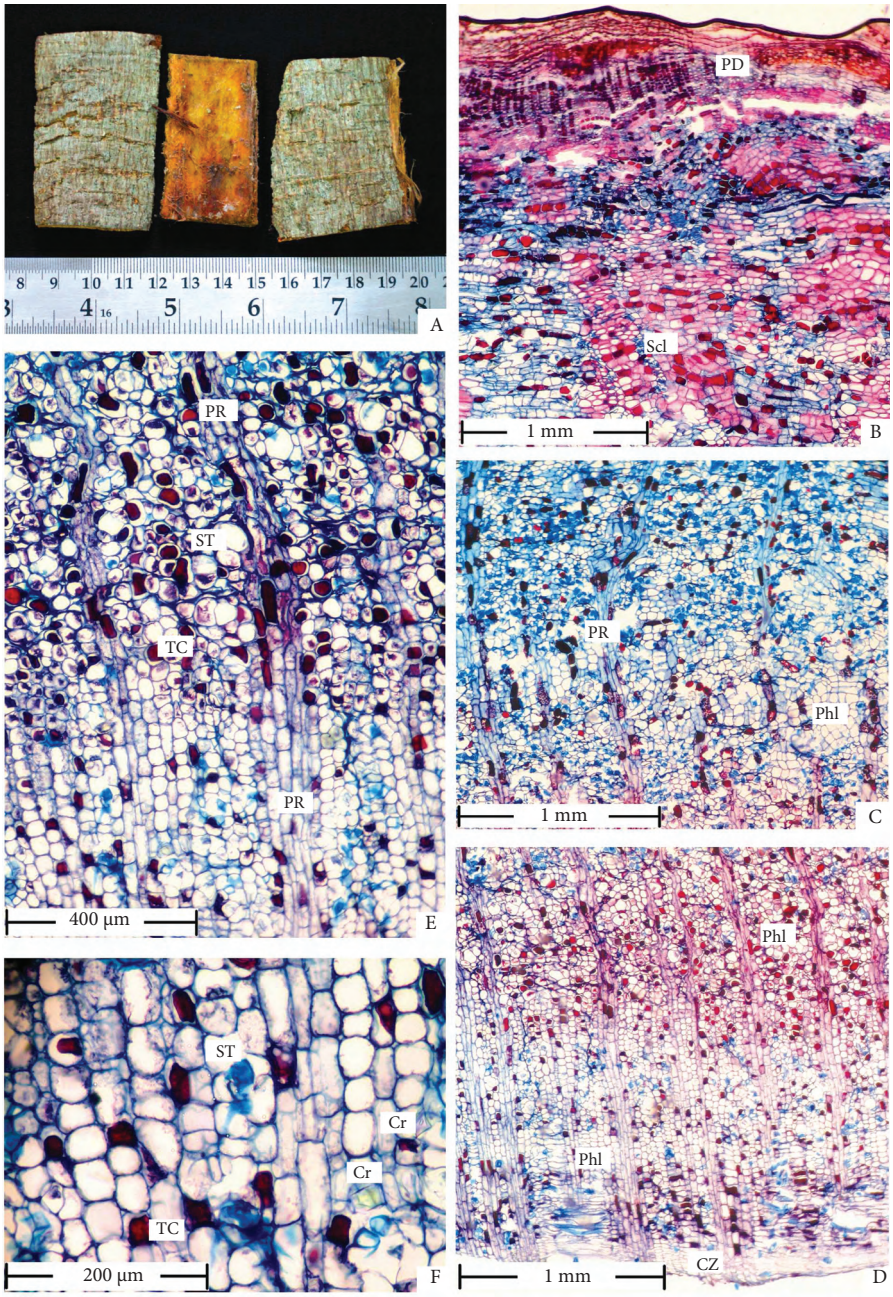

Figure 3. *Ficus religiosa*: A. External features of bark, B, C and D. Cross section of bark, E and F. Portions enlarged.

The ray cells are turned into thick walled lignified sclerenchyma cells in the peripheral part of the bark. The phloem rays are uniseriate or multiseriate. They are homocellular or heterocellular. The multiseriate rays are 72 mm in breadth and up to 900 mm in height. The sieve tube members have 288-360 mm height. Large vertically oblong p-protein bodies are invariably seen abutting the sieve plates. Laticifers are abundant in the inner bark. Each laticiferous canal is surrounded by distinct epithelial cells (Figures 4d-4f).

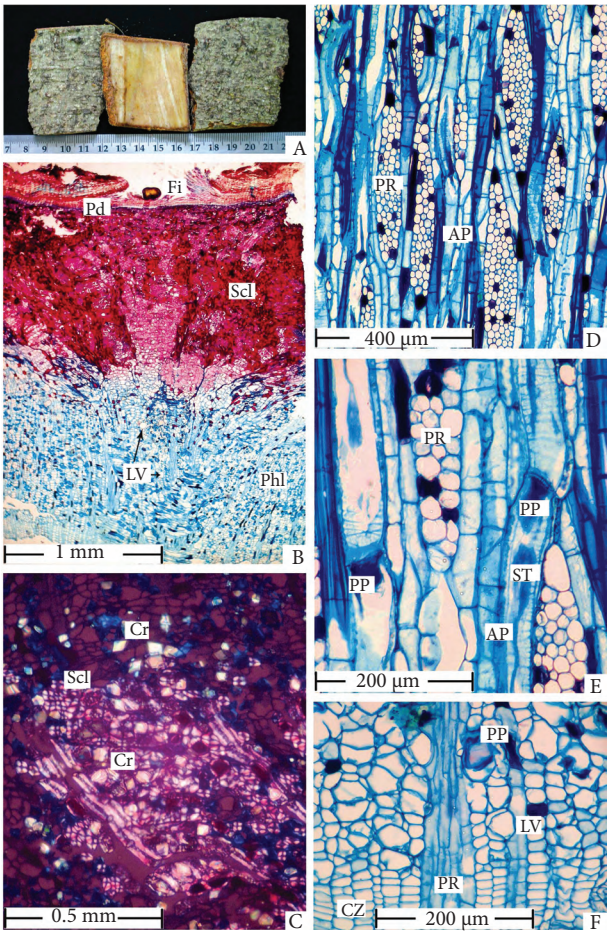

Figure 4. *Ficus benghalensis*: A. External features of bark, B. Cross section of bark, C. Portion under polarized light, D. Tangential longitudinal section of bark, E. Portion enlarged, F. Phloem region.

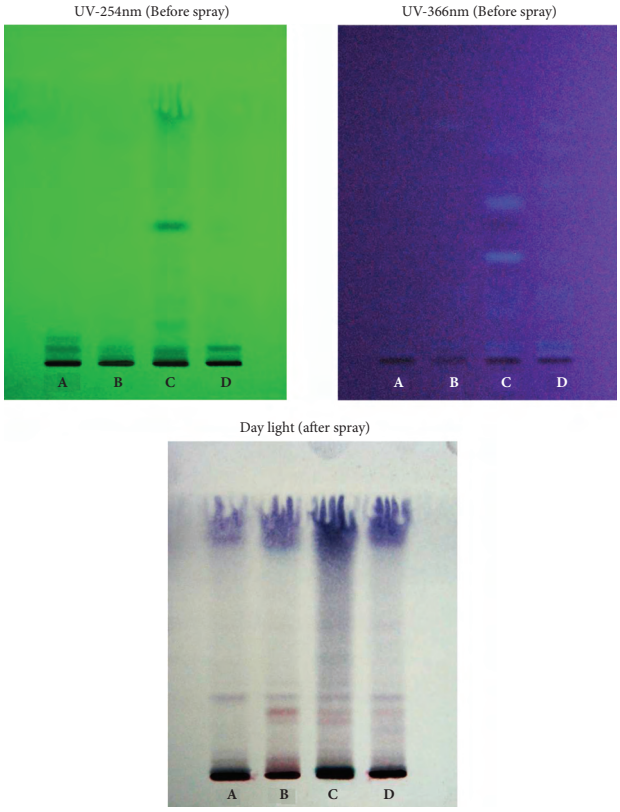

Figure 5. HPTLC profile of *Ficus* spp. barks: Track -A: *F. racemosa*, Track – B: *F. virens*, Track – C: *F. religiosa*, Track – D: *F. benghalensis*

Table 1. Physico-chemical analysis of 4 *Ficus* spp. barks.

Class of chemical compounds	***Ficus racemosa***	***Ficus virens***	***Ficus religiosa***	***Ficus benghalensis***
Alcohol soluble extractive	8.48 ± 0.30	2.26 ± 0.78	7.21 ± 0.92	4.43 ± 0.95
Water soluble extractive	11.27 ± 0.47	4.39 ± 0.83	15.76 ± 0.67	7.44 ± 0.86
Total ash	16.31 ± 1.45	11.97 ± 1.18	7.86 ± 0.8	5.45 ± 0.92
Acid insoluble ash	1.35 ± 0.21	2.59 ± 0.45	0.41 ± 0.03	1.21 ± 0. 23

Table 2. Preliminary phytochemical screening of 4 *Ficus* spp. barks.

Class of chemical compounds	***Ficus racemosa***	***Ficus virens***	***Ficus religiosa***	***Ficus benghalensis***
Tannins	+	+	+	+
Saponins	+	+	+	+
Flavonoids	+	+	+	+
Steroids	+	+	+	+
Terpenoids	+	+	+	+
Cardiac glycosides	+	+	+	+
Alkaloids	-	-	-	-
Quinones	-	-	-	-

+ Present; - Absent

Table 3. Rf. values of HPTLC analysis of 4 *Ficus* spp. barks.

F.racemosa	***F. virens***	***F. religiosa***	***F. benghalensis***
	Under 254 nm		
0.06	0.06	0.05	0.06
0.09	-	-	-
-	-	0.14	-
-	0.2	-	-
-	0.23	0.22	0.23
-	0.28	-	-
-	-	0.32	-
-	-	0.4	-
	0.48	-	0.48
-	0.64	0.65	-
0.68	-	-	-
-	-	-	0.71
0.75	-	-	-
0.81	0.8	-	-
-	-	-	0.84
0.89	0.9	-	0.9
	Under 366 nm		
0.05	0.06	0.05	0.06
0.14	-	-	-
-	0.17	0.18	-
0.23	-	0.22	-
0.24	0.25	0.24	0.24
-	-	0.27	-
-	0.38	0.37	-
-	-	0.43	-
-	0.48	—	-
-	-	0.56	-
-	-	0.62	0.63
-	0.76	0.76	0.76
-	-	-	-
-	-	-	-
0.83	0.83	0.84	0.84
			0.96
	Under 550 nm (After spray)		
-	-	-	0.15
-	-	0.19	-
-	0.23	0.22	0.22
0.28	0.28	0.28	0.27
-	-	0.32	-
-	-	0.42	-
-	-	0.53	0.53
0.84	0.85	0.84	0.86
-	-	-	0.88

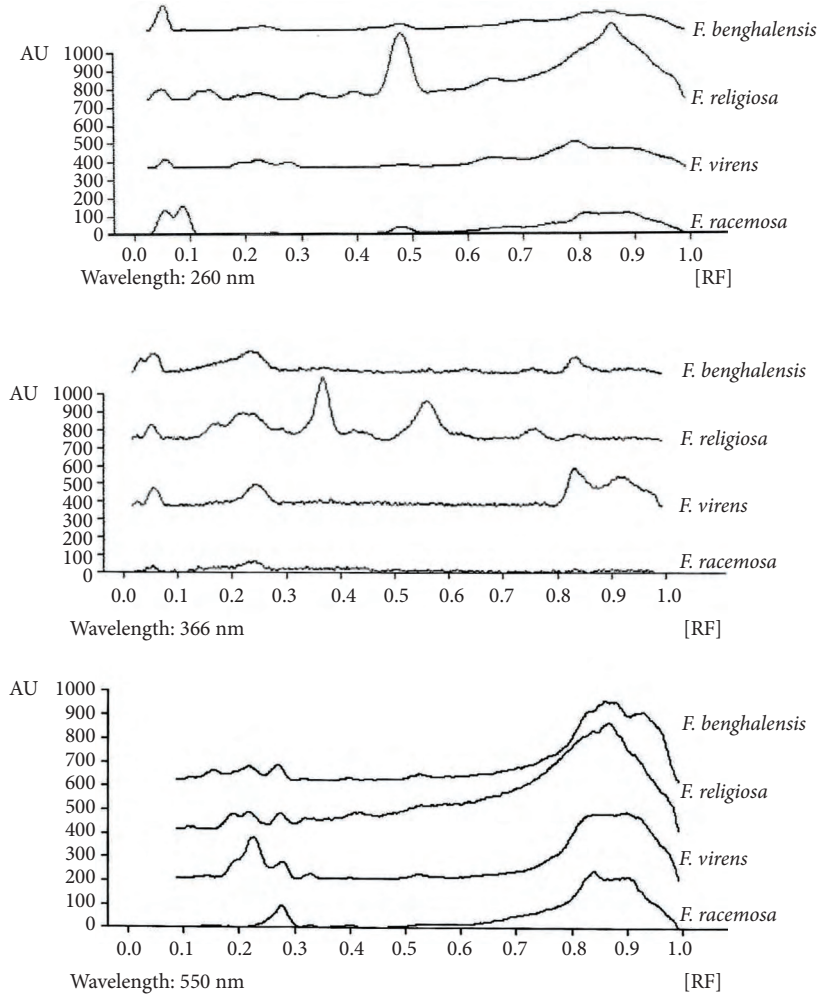

Figure 6. HPTLC densitometer scanning profile of *Ficus* spp. barks.

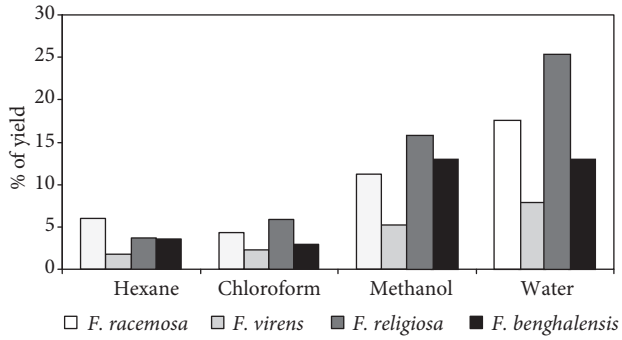

Figure 7. Successive soxhlet extractives of barks of *Ficus* spp. with various solvents.

Table 4. Morphological and anatomical diagnostic features of 4 *Ficus* spp. barks.

S.No	Characters	***Ficus racemosa***	***Ficus virens***	***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 spot.	Absent.	Lenticels in longitudinal and transverse row, mostly circular and prominent.
6	Periderm	Thin measuring about 72µm.	Thin measuring about 138µm and characteristic feature is the periderm tubes.	Thick measuring about 360µm and breaking into irregular flakes.	Very thick measuring about 288-576µm 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 layers exfoliate in the form of thin tangential membranes.	Thick and homogeneous thin walled rectangular suberised cells.

Phytochemical studies

The percentages of alcohol-soluble and water-soluble extractives, total ash, and acid-insoluble ash are tabulated in Table 1. Presence and absence of different phyto-constituents were detected (Table 2). HPTLC fingerprint profiles were developed and are presented in Table 3 and Figures 5 and 6. The percentages of successive soxhlet extractives were calculated and results are depicted in a histogram (Figure 7).

Discussion and conclusion

Tree bark is 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 (Brinda et al., 2000).

Nālpāmaram is an important group of Ayurvedic formulation that constitutes the barks of *Ksīrivrksās* (4 laticiferous tree species), namely *Ficus racemosa*, *F. virens*, *F. religiosa* and *F. benghalensis*, widely used in the treatment of skin diseases with *pitta* and *rakta* predominance and also used in various ailments (Sivarajan & Balachandran, 1994; Joy et al., 2001).

Barks of some of these 4 species, such as *Ficus virens*, are also equated with many other species like *F. microcarpa* L. f. *F. infectoria* Roxb., *F. arnottiana* Miq, *F. lacor* Buch-Ham and *F. talboti* King (Nadkarni, 1954; Singh & Chunekar, 1972; Kapoor & Mitra, 1979; Sharma, 1983). Hence, it is very difficult to identify the original from the adulterants/substitutes while procuring crude drug from the market.

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 diagnostic features of the 4 *Ficus* barks are tabulated in Table 4.

The barks of 4 *Ficus* species contains tannin, wax, saponin gluanol acetate, β-sitosterol, leucocyanidin-3 – O – β – 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 (Husain et al., 1992).

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.

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 1 similar common band at Rf. 0.24. *F. religiosa* and *F. benghalensis* have 2 common bands at Rf. 0.62 & 0.76. *F. virens* has 1 common band at Rf. 0.76 and the band at Rf. 0.62 is absent. In visible light (after spray) all the barks shows 2 similar common bands of violet colour at Rf. 0.28. *F. virens, F. religiosa,* and *F. benghalensis* have 1 common band of pink colour at Rf. 0.22 and this band is absent in *F. racemosa*. 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.

Acknowledgements

The authors are grateful to Dr. V.P. Sidhan (Chairman), Mr. V.S. Pradeep (Managing Director), and Mrs. Jayadevi Pradeep (Director) from Cholayil Private Limited for their constant support and encouragement. The authors are also thankful to Prof. P. Jayaraman (Director), Plant Anatomy Research Centre (PARC), Chennai, for the valuable guidance during the study.

References

Aiyer KN & Kolammal M (1957). *Pharmacognosy of Ayurvedic Drugs*, Trivandrum, India.

Ayurvedic Pharmacopoeia of India, (2001). Vol-3, Govt. of India, Ministry of health and family welfare, Dept. of ISM & H, New Delhi.

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

Evans WC (1989). *Trease and Evan's Pharmacognosy* (13th Ed), Baillière Tindall.

Gayathri M & Kannabiran K (2008). Antidiabetic and ameliorative potential of *Ficus bengalensis* bark extract in streptozotocin induced diabetic rats. *Indian J Clinic Biochem* 23(4): 394-400.

Harborne JB (1998). *Phytochemical Methods*, (3rd Ed), Chapman and Hall, London.

Husain A, Virmani OP, Popli SP, Misra LN, Gupta MM, Srivastava GN, Abraham Z & Singh AK (1992). *Dictionary of Indian Medicinal Plants*, CIMAP, Lucknow, India. 546p.

Johansen DA (1940). *Plant Microtechnique*, McGraw Hill Book Company, New York.

Joshi U & Upadhye M (2008). Evaluation of antioxidant activity of aqueous extract bark of *Ficus glomerata. Res J Pharm Tech* 1(4): 537-538.

Joy PP, Thomas J, Mathew S & Skaria BP (2001). Medicinal Plants In: Bose TK, Kabir J, Das P & Joy PP (ed.) *Tropical Horticulture* - Vol. 2. pp. 449-632. Calcutta: Naya Prokash.

Kapoor SL & Mitra R (1979). Herbal drugs in Indian Pharmaceutical industry, Lucknow, India.

Kurup PNV, Ramadas VNK & Joshi P (1979). *Handbook of Medicinal Plants*, New Delhi.

Mooss NS (1976). *Single Drug Remedies*, Kottayam, Kerala, India.

Nadkarni AK (1954). *Indian Materia Medica*, Popular Prakashan Pvt. Ltd., Mumbai.

Sass JE (1940). *Elements of Botanical Microtechnique*, McGraw-Hill Book Company, INC, New York.

Sharma PV (1983). Dravyaguna vijnana, Varanasi, India.

Sing TB & K.C. Chunekar KC (1972). Glossary of vegetable drugs in Brhttrayu, Varanasi, India.

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

Sofowara A (1993). *Medicinal Plants and Traditional Medicine in Africa*, Spectrum Books Ltd, Ibadan, Nigeria.

Vaibhav S & Kamlesh D (2007). Pharmacognosy: The Changing Scenario, *Pharmacog Rev* 1(1): 1-6.

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