

Comparative powder microscopy on the barks of four *Ficus* species

Singh D¹, Aeri V^{2*} and Ananthanarayana D B³

^{1,2}Department of Pharmacognosy and Phytochemistry, School of Pharmaceutical Education and Research, Jamia Hamdard, New Delhi 110062, India

³Chief Scientific officer, Ayurvedy Trust, Bengaluru, India

Received 06 September 2017; Revised 02 August 2018

The genus *Ficus* constitutes one of the largest genera of medicinal plants containing more than 800 species occurring in tropical and subtropical areas. The more famous species are *Ficus bengalensis* (Bargad), *Ficus religiosa* (Pipal), *Ficus racemosa* (Goolar) and *Ficus lacor* (Pakar). These plants are reported to possess antidiabetic, antidiarrhoeal, antiepilepsy, anti-inflammatory properties and are used in many Ayurvedic and traditional formulations. The barks of these species are usually interchanged or adulterated with other species of ficus due to limited knowledge of identification and differentiation. The slide preparation method was optimized to visualize common as well as distinguishing characters of the bark powder. The photomicrographs were taken with Motic microscope moticam 3.0 MP, AE 2000. The variation in the bark powder lies in size and shape of the stone cells and sclereids, their occurrence, type of wall and lumen. Most of the herbal drugs in the industry are supplied in powdered form. So, there are more chances of adulteration as it is very easy to spoil a drug in the powdered state. Industry emphasizes for powder microscopy as it is effortless. Hence, a detailed powder microscopy evaluation was carried out with an aim to establish diagnostic features to differentiate between these four bark powders.

Key words: Adulterants, Barks, *Ficus*, Herbal plants, Powder microscopy, Species.

IPC code; Int. cl. (2015.01)–A61K 36/00

Introduction

Ficus is a genus of about 850 species of woody trees, shrubs, vines, epiphytes and hemiepiphytes in the family Moraceae which is also referred to as Fig family¹. The *Ficus* species contain many active constituents namely stigmasterol, quercetin, bergaptene, campesterol, rutin, albumin, malic acid, alkaloids, glycosides, tannins, flavonoids, sterols, coumarins, saponins, phenolic compounds, amino acids, proteins, carbohydrates, lipids etc. The *Ficus* species have also been analysed for its biological activities like analgesic, anti-inflammatory, antidiabetic, antipyretic, antioxidant, antiulcer, anticancer, hepatoprotective². The barks of *Ficus religiosa*, *F. bengalensis*, *F. racemosa* and *F. lacor* has been mentioned in Ayurvedic Pharmacopoeia of India under the name *Asvattha*, *Nyagrodha*, *Udumbara* and *Plaksa* respectively³. The barks of these species form an important ingredient in many Ayurvedic formulations such as *Nalpamaraditailam*, *Chandanavasavam* and *Saribadyasavam*⁴. Today, there

are varieties of methods available to authenticate herbal drugs ranging from simple morphological examination to physical and chemical analysis and DNA molecular biology. Nevertheless, ordinary powder microscopy is still the most practical method for primary authentication and evaluating pharmaceutical quality⁵. In the present study, an attempt was made to study the comparative distinguishing pharmacognostic features of these four powdered barks on the basis of stone cells and sclereids, calcium oxalate crystals, cork and starch grains.

Materials and Methods

Collection of samples

For the present study fresh bark samples of four *Ficus* species were self-collected from National Institute of Science Communication and Information Resources (NISCAIR) Pusa campus under the supervision of Dr Sunita Garg, Scientist G. All the photomicrographs were taken with Canon 500D. (Fig. 1a, 2a, 3a and 4a).

Chemicals and reagents

All the chemicals used in the experiments were of analytical grade. Potassium chlorate was

*Correspondent author
Email: vidhuaeri@yahoo.com
Mobile no. 9873496942

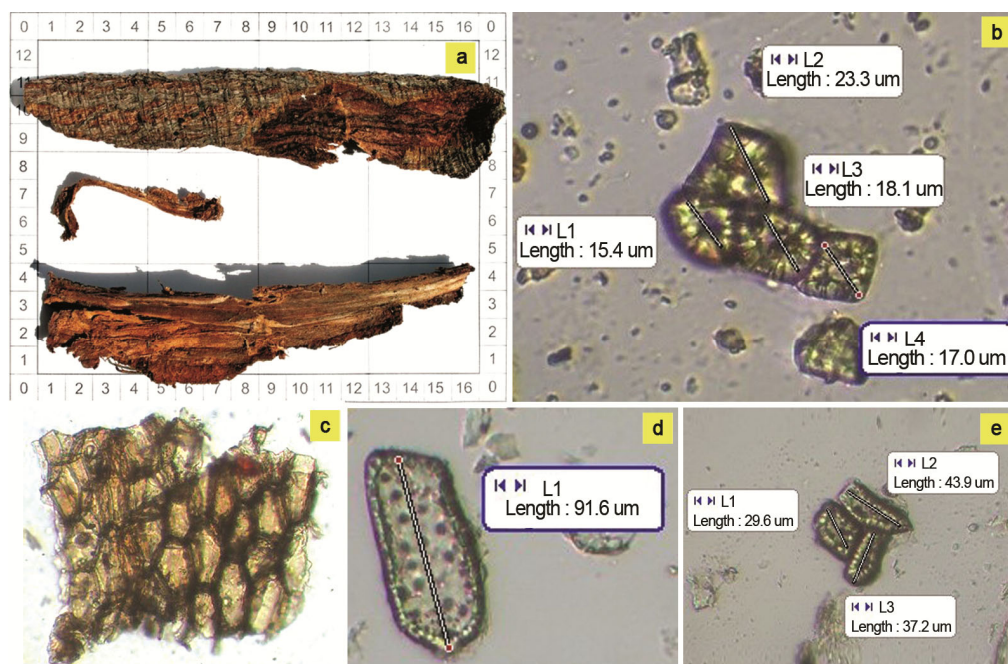


Fig. 1 — *Ficus lacor*, a- bark sample, b & e - Group of stone cells of various shape and sizes present in powdered bark sample, c - Cork cells present in powdered bark sample, and d - Isolated stone cells with pitted lumen present in powdered bark sample

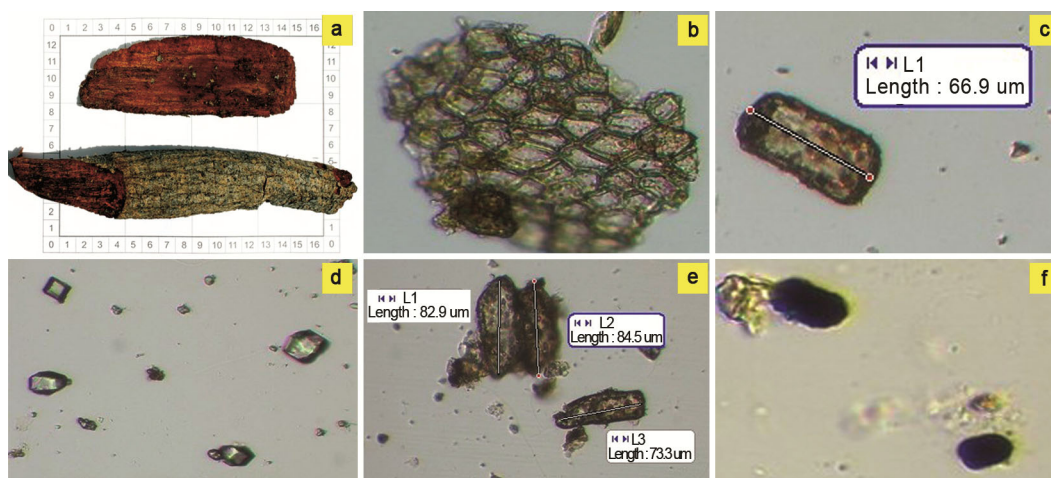


Fig. 2 — *Ficus racemosa*, a - bark sample, b - Cork cells present in powdered bark sample, c - Isolated stone cells present in powdered bark sample, d - Prismatic crystals present in powdered bark sample, e - Starch grains present in powdered bark sample, and f - Group of stone cells present in powdered bark sample

procured from Sigma Aldrich. Potassium chlorate and 50 % nitric acid forms a liquid known as Schulze's Maceration Fluid and is used to disintegrate hard woody substances such as sclereids, stone cells etc. This fluid is a powerful oxidizing agent and it rapidly oxidizes and removes lignin from vegetable tissues⁶.

Method of preparation of slides

The following two types of slides were prepared for visualization of microscopical features present

in a bark. The method of preparation of slide was optimized to determine the dilution of powdered bark in water to visualize common as well as distinguishing characters of the bark. Slide I: The slide was prepared by overnight soaking of the powdered material in water. 500 mg of material was taken in a test tube followed by addition of 10 mL of water (1: 20). Allowed it to soak overnight almost for 24 hours. After 24 hours, contents were poured in a Petri plate and slide was prepared by mounting the contents on a clean and

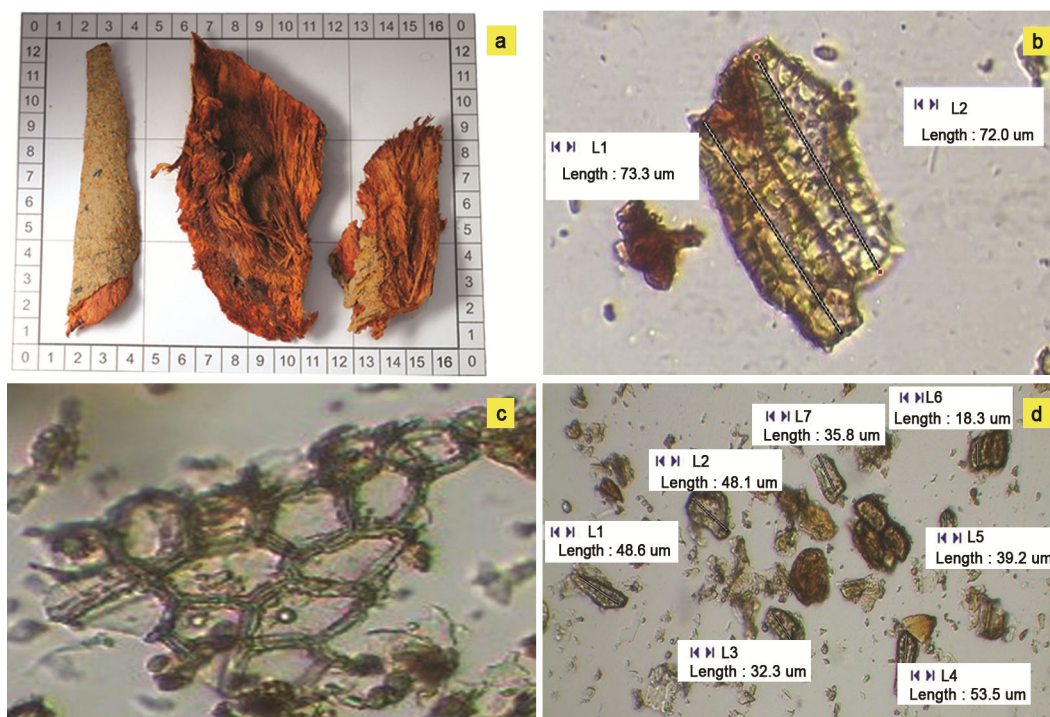


Fig. 3 — *Ficus religiosa*, a – bark sample, b - Group of sclereids present in powdered bark sample, c – Cork present in powdered bark sample, and d - Stone cells and sclereids of various shape and sizes present in powdered bark sample

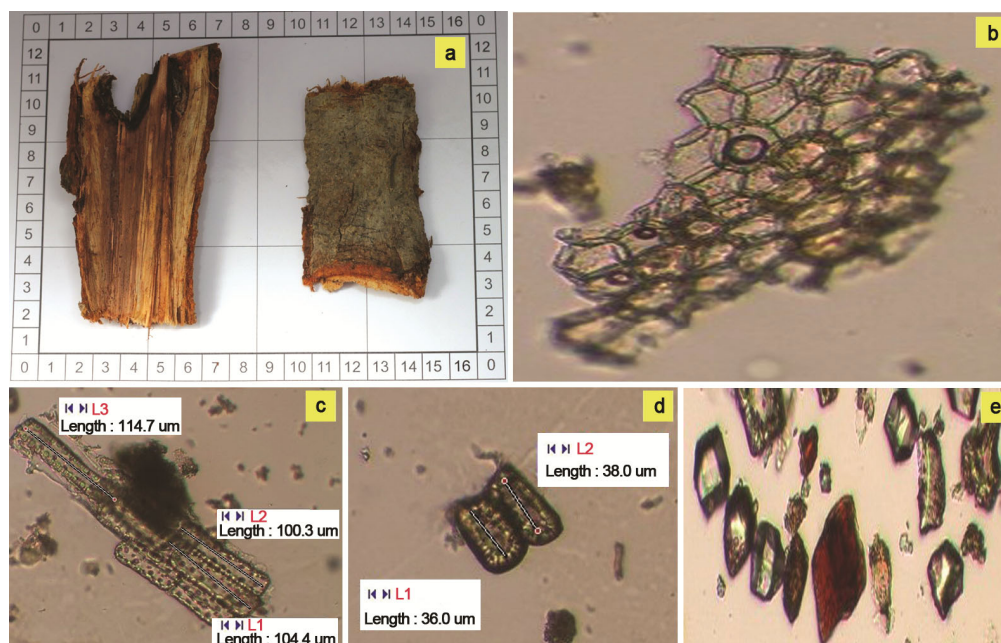


Fig. 4 — *Ficus bengalensis*, a –bark sample, b - Cork cells present in powdered bark sample, c - Group of very long sclereids present in powdered bark sample, d - Group of stone cells present in powdered bark sample, and e - Prismatic crystals present in powdered bark sample

dried slide with the help of a brush and observed under Motic microscope moticam 3.0 MP, AE 2000. Most of the features were visible by overnight soaking except stone cells and sclereids which require treatment by an oxidizing agent.

Slide II: The slide was prepared by potassium chlorate treatment which is used as an oxidizing agent used for disruption of stone cells and sclereids. However, calcium oxalate crystals and starch grains are destroyed using this method. 200 mg of

powdered material was boiled with 5 mL of 50 % nitric acid. To this, added a pinch ~ 100-150 mg of potassium chlorate and the contents were poured in a Petri plate after effervescence ceases. The contents were mounted on a clean and dried glass slide with the help of a brush and were observed under Motic microscope⁷.

Results

Organoleptic evaluation

The morphological description of each bark is tabulated in Table 1. Figure 1a-4a represents photographs of bark samples of *Ficus lacor*, *F. religiosa*,

F. racemosa and *F. bengalensis* respectively. Based upon the powder microscopic features, the bark powders of four *Ficus* species can be distinguished as shown in Table 2.

Powder microscopic characters

Ficus lacor

The extremely abundant stone cells which can be classified into three categories according to their size, shape, content etc. Smallest sized, isodiametric, pentagonal to hexagonal shaped stone cells with a very narrow lumen and indistinct pits, the majority being of this category. Bigger in size than the former,

Table 1 — Morphological characters of bark samples of *F. lacor*, *F. religiosa*, *F. racemosa* and *F. bengalensis*

S.No	Bark Sample	Shape	Outer bark	Inner bark	Fracture	Odour
1	<i>Ficus lacor</i>	Flat to curved pieces	Ash to whitish grey, numerous transversely arranged lenticels	Reddish brown, rough, fibrous and longitudinally striated	Fibrous	Characteristic
2	<i>Ficus religiosa</i>	Flat or slightly curved pieces	Uneven surface, light brown to ash coloured	Smooth and brownish	Fibrous	Indistinct
3	<i>Ficus racemosa</i>	Flat, curved or channelled	Rough, whitish papery flakes coming out of the outer surface	Pale brown, uneven, longitudinally striated	Fibrous	Nil
4	<i>Ficus bengalensis</i>	Flat or somewhat curved	Ashy white colour, transversely and longitudinally furrowed and cracked	Light brown in colour	Outer granular, inner hard, fibrous and pinkish in colour	Not characteristic

Table 2 — Specific comparative features of powdered bark samples of *Ficus lacor*, *Ficus racemosa*, *Ficus religiosa*, *Ficus bengalensis*

S. No.	Features	<i>Ficus lacor</i>	<i>Ficus racemosa</i>	<i>Ficus religiosa</i>	<i>Ficus bengalensis</i>
1	Common name	Pakar	Gular	Pipal	Bargad
2	Colour	Pale pinkish brown	Pinkish brown	Reddish brown	Dark greyish brown
3	Stone cells				
	Occurrence	Abundant	Isolated or in groups	Few	Isolated or in group
	Walls	Lignified, small sized and large	Lignified and beaded walls	Small sized	Lignified, very small sized
	Shape	Isodiametric, squarish to oblong, oval	Polygonal, spherical to squarish	Triangular to oval	Circular to oval
	Lumen	Narrow to wide pitted lumen	Wide pitted lumen	Narrow lumen	Central narrow lumen with radiating pits
	Size	15-92µm (Fig. 1b, d & e)	>60 µm (Fig. 2 c & f)	18-73 µm (Fig. 3d)	<50 µm (Fig. 4d)
4	Sclereids				
	Occurrence	NA	NA	Abundant, occasionally isolated, usually in groups	Few
	Walls	NA	NA	Striated walls with peg-like extensions	Beaded walls
	Shape	NA	NA	Rectangular to irregular	Rectangular to oval shape, very long
	Lumen	NA	NA	Narrow to wide lumen	Wide pitted lumen
	Size	NA	NA	<100 µm (Fig. 3b)	>100 µm (Fig. 4c)
5	Cork	Polygonal to hexagonal (Fig. 1c)	Polygonal to hexagonal (Fig. 2b)	Polygonal, thick-walled (Fig. 3c)	Polygonal to pentagonal to hexagonal (Fig. 4b)
6	Prismatic crystals	Present	Present (Fig. 2d)	Most abundant	Present (Fig. 4e)

squarish to oblong in shape with narrow, striated walls and distinct radiating pits almost reaching up to margin. These stone cells were much wider than earlier two, oblong, oval to somewhat irregular in shape with the wide pitted lumen and pitted striated walls. The abundant prismatic crystals of calcium oxalate scattered as such. The fragments of cork, cells were hexagonal to polygonal shape.

Ficus racemosa

Stone cells, isolated or in groups, polygonal, spherical too squarish, with a wide pitted lumen and beaded walls. The abundant fragments of cork cells, polygonal to hexagonal in surface view. Prismatic crystals of calcium oxalate and simple and compound starch grains (Figure 2E) scattered as such.

Ficus religiosa

The abundant sclereids of various sizes and shape, usually in groups, occasionally isolated, individual sclereids were rectangular to irregular in shape with pitted and striated walls and often bearing one or more peg-like extension. Stone cells, triangular to oval in outline, with highly thickened transversely running pitted and striated walls. The abundant prismatic crystals of calcium oxalate of various sizes and shape. The fragments of cork, polygonal thick walled and occasionally with pitted walls.

Ficus benghalensis

Stone cells, circular to oval, very small sized, isolated or in the group, the walls were highly thickened with central narrow lumen with radiating pits almost extending to the margins. Few rectangular to oval-shaped, thick and thin-walled sclereids with beaded walls and wide lumen were also present. The fragments of the oval to very long lactiferous canals filled with dark brownish granular content. Prismatic crystals of calcium oxalate scattered as such.

Discussion

A bark powder is identified microscopically by various features such as stone cells, calcium oxalate crystals, starch grains, medullary rays, fibres, sclereids, cork, isolated oil cells, tubular lactiferous canals, phloem parenchyma, masses, rhytidoma, parenchyma, secretory canals. Bark powder cannot be merely authenticated by observing the presence or absence of these features as these are common in all barks. The variation lies in the size and shape of the stone cells and sclereids, their occurrence, type of wall and lumen, type of calcium oxalate crystals. The

powder microscopy of bark was studied based on specific identifying features and their subfeatures. Barks of some of these four species are also equated with many other species of *Ficus*. Hence it is very difficult to identify the original from the adulterant and substitute while procuring crude drug from the market. Based upon the powder microscopic features of the barks of these four species, we can identify them with some specific characters. The diagnostic features of the four *Ficus* barks are tabulated in Table 1. *F. lacor* showed a wide range of stone cells in terms of size and shape whereas *F. bengalensis* showed smaller stone cells lesser than 50 μm in size. Sclereids were found to be absent in *F. lacor* and *F. racemosa* species while *F. bengalensis* showed longest sclereids greater than 100 μm in size. Prismatic crystals of calcium oxalate were found to be most abundant in *F. religiosa*.

Conclusion

Herbal medicines are mostly low cost medicine, should not be raised in price as a result of usage of unnecessary highly sophisticated methods for authentication. The complex chemical nature of herbs and their products require adequate quality control for herbal drug manufacturers. In addition, quality of botanical products and adulteration in the supply chain make it necessary to address the identity issues in a comprehensive manner. The natural products industry is growing in both complexity and compliance. The compliance requires more testing and the best way to work with a lab to minimize complications are crucial to understanding. Adulteration and substitution of herbal drugs can cause serious health problems to consumers. The first step for quality control and authentication of any herbal drug is to study its morphology followed by its anatomy or microscopy. Powdering of crude drugs in industries is a dust generating process. Ayurvedic herbal industries are also shifting towards GMP. Therefore industries are willing to purchase grounded herbs, which make it absolutely necessary to check its botanical identity at the first stage. Powder microscopy has been most commonly used for authentication of powdered herbal drugs because of its virtues of a small amount of sample needed, fast speed and low cost. Industry emphasizes for powder microscopy as most of the herbal plants supplied are in powdered form and it is very cumbersome to perform anatomy of every consignment. If highly

sophisticated technique like DNA fingerprinting is used for authentication of plants it will increase the cost of final product. Many reference books such as Chinese Materia Medica, European Pharmacopoeia, British Pharmacopoeia, United States Pharmacopoeia, Japanese Pharmacopoeia and Ayurvedic Pharmacopoeia of India records microscopic characters of herbal drugs. Though earlier researchers have studied and reported the pharmacognosy of *Ficus* species but the emphasis has not been laid on powder microscopy. In the current study, the bark of four species of *Ficus* has been differentiated on the basis of microscopical characters of powdered drugs.

Acknowledgement

The authors are grateful to Dr. Sunita Garg Scientist G, NISCAIR Pusa, New Delhi for her constant support in collection of plants with correct botanical identity.

References

- 1 Somashekhar M, Naira N and Mahesh A R, Botanical study of four *Ficus* species of family moraceae: A review, *Int J Univers Pharm Bio Sci*, 2013, **2**(6), 558.
- 2 The Wealth of India, Volume (F-G): In: A dictionary of Indian raw materials & industrial products Vol 4, New Delhi, Council of Scientific and Industrial Research, 1999, 24-26.
- 3 The Ayurvedic Pharmacopoeia of India, Government of India, Department of AYUSH, Ministry of Health and Welfare, Ghaziabad, 1999-2017, Part I, Vol. II, 144.
- 4 Sivarajan V V and Balchandran I, Ayurvedic drugs and their plant sources, (Oxford & IBH Publishing CO. Pvt Ltd, New Delhi), 1994.
- 5 Zhao Z, Application of microscopic techniques for authentication of herbal medicines, *Microsc Sci Technol Appl Educ*, Formatex, 2010, 803-812.
- 6 Chahuhan M G and Pillai A P G, Microscopic profile of powdered drugs used in Indian system of medicine, Vol. 1, Bark Drugs, Institute of Ayurvedic Medicinal Plant Sciences, Jamnagar, Gujarat, India.
- 7 Singh D, Aeri V and Ananthanarayana D B, Development of standard operating protocol for slide preparation of powdered bark samples with varying grinding techniques, *Pharmacog J*, 2018, **10**(2), 265-71.