



Preliminary phytochemical screening and HPTLC fingerprinting of fruits of three *Ficus* species

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ABSTRACT

India being a rich and varied flora of medicinal plants since the Vedic period. The present study deals with the scientific validation of three *Ficus* species - *Ficus benghalensis* (Indian banyan), *Ficus racemosa* (gular) and *Ficus religiosa* (bo tree or sacred fig) with special reference to its pharmacognostical and phytochemical investigations. This is commonly known as gular, Indian banyan, sacred fig and is known to have medicinal properties, also even used as home remedies in the rural and the remotest parts of the India. The fruit of *Ficus benghalensis* used as astringent, haemostatic, anti-septic, anti-inflammatory, antioxidant and anticancer agent and also in the treatment of diarrhoea, dysentery and in the treatment of skin diseases, ulcers, vaginal disorders, leucorrhoea, menorrhagia and deficient lactation, the fruit of *Ficus racemosa* is medicinally more used for the treatment of diabetes, diarrhoea, dyspepsia, menorrhagia, haemoptysis, intrinsic haemorrhage, excessive thirst, visceral obstructions,. The seeds and fruits of *Ficus religiosa* are digestive, laxative and refrigerant. The dried fruit asthma, diseases of vagina. vomiting, anorexia and edema; therefore an attempt has been made to carried out the detailed quality control and assurance of the drug followed by HPTLC profiles, physiochemical analysis, preliminary phytochemical studies and fluorescence analysis of drug to get authentic therapeutic value.

Key words: *Ficus* species, fruit, phytochemical screening, HPTLC fingerprinting.

INTRODUCTION

Fruit of three *Ficus* species (family-Moraceae) viz. *F. religiosa* L. (the Bo tree which sheltered the Buddha as he divined the "Truths"), *F. racemosa* L. (syn. *glomerata*, the giant cluster tree). *Ficus benghalensis* L. commonly known as 'Pipala', 'Gular' and 'Chinese banyan or the banyan tree or Bargad' respectively has been most widely used in traditional medicinal system of all over world including India [1-3]. *Ficus* species are native to India, Nepal, Sri Lanka, Southwest China and Indochina also found throughout the plains of India upto 170 m altitude in the Himalayan region [4].

F. religiosa is a well known ethnomedicinal tree used in Ayurveda. Its use in the Indian traditional folk medicine also well documented. The use of different parts of *F. religiosa* in traditional system of medicine [5]. The dried fruit and seed used tuberculosis, fever, paralysis, hemorrhoids, laxative, digestive, refrigerant, laxative, respiratory disorders and scabies [6-9]. Powder dried fruits are given for 14 days is believed to promote fertility (conception) in women. Ripe fruit are used in stomach ache and constipation. In diabetes fruit are used [10]. The dried fruit, pulverized and taken in water for a fortnight removes asthma. The ripe fruit is cold in potency and good for burning

sensation. It act as cardiac tonic and is useful to cure the diseases of Vagina. It also cures vomiting, anorexia and edema and the fruit extract of plant have anti tumour and antibacterial activity [11].

Ficus racemosa Linn. Syn. *Ficus glomerata* Roxb. (Moraceae) commonly known as 'Jagya-dumur' (Bengali), 'Gular' (Hindi) and 'Udumbara' (Sanskrit) is a well known moderate sized to large spreading tree with ovate, ovate-lanceolate leaves [12-13]. Most parts of this plant are being used traditionally in Bangladesh for the treatment of various diseases. In an ancient Literature of Ayurveda [14-16]. It has been mentioned for therapy of various disorders like: diabetes, amlapitta (acidity), asthma, dysentery, menorrhagia, glandular enlargement, sore throat etc. It has chemomodulatory effect and is believed to be a good remedy for visceral obstructions and extract of the fruit is used in leprosy, diarrhoea, dyspepsia excessive thirst, visceral obstructions, haemoptysis, leucoderma and intrinsic haemorrhage [17-22].

Ficus benghalensis is a major source of drugs in medicine and other ancient systems in the world. Herbalism is a traditional medicine or folk medicine practice based on the use of plants and plant extracts [23]. *Ficus benghalensis* is commonly known as a Banyan tree. This tree is considered to be sacred tree in India. The plant is a large evergreen tree distributed all over India from sub Himalayan region and in the deciduous forest of Deccan and south India. It is grown in gardens and road sides for shades. The fruits are used as astringent, haemostatic, anti-septic, anti-inflammatory, antioxidant and anticancer agent and also in the treatment of diarrhoea, dysentery and in the treatment of skin diseases, ulcers, vaginal disorders, leucorrhoea, menorrhagia and deficient lactation. In the traditional system of medicine, the plant is used for various health problems and diseases [24-25].

Although the fruit of these species are important but very less studies has been reported so far on pharmacognostic and phytochemical parameters [26]. Hence this study was undertaken to develop comparative quality standards and HPTLC fingerprinting of fruit of these *Ficus* species and their evaluation. This may be useful to pharmaceutical industries for authentication of commercial sample and also to explore the possibility of using other species as complementary to each other.

EXPERIMENTAL SECTION

Plant materials

Ficus benghalensis (Indian banyan), *Ficus racemosa*, and *Ficus religiosa* (bo tree or sacred fig) fruit are collected from Arogyadham campus garden, Chitrakoot, Satna, Madhya Pradesh. They were washed with tap water, rinsed with distilled water and shade dried until the fracture is uniform and smooth. Then the dried fruit material was powdered. Then the final uniform powder was used for the extraction of active constituents of the fruit.

Pharmacognostic Evaluations

Physico-chemical analysis

Air dried fruit material was used for the quantitative determination of loss on drying, total ash, acid insoluble ash, alcohol and water soluble extractive values according to standard procedure of Indian Pharmacopoeia and WHO/QCMMPPM [27-28].

Fluorescence analysis

Fluorescence analysis of the fruit powder was treated with different chemicals and seen under the normal light and UV radiations at 254 and 365 nm wavelengths as per the standard procedure. The colour development under the day light was also observed for the presence of various phytochemical compounds [29].

High Performance Thin Layer Chromatography (HPTLC)

For HPTLC, 5 g of coarsely fruit powdered in 250 ml stoppered conical flask & extracted with 100 ml ethanol for 24 hours by maceration technique with occasional shaking. The extract was extracted and volume was raised up to 100 ml in a volumetric flask. 25 ml of the extract was taken from the above stock solution and concentrated on a water bath to similarly, ethanol extracts were prepared for two samples of *F. religiosa* L., *F. racemosa* L. and *F. benghalensis* L. as reference. TLC of extracts of all the samples and the reference ingredients was carried out on Silica Gel 60 F₂₅₄ precoated plates (0.2 mm thickness; from Merck India Limited). Camag Linomat 5 applicator was used for band application and Desaga Video documentation Unit 3 was used for documentation of fingerprinting. The mobile phase used was Toluene: Ethyl acetate (7:3). The plate was developed over a distance of 10 cm in a saturated development chamber (Twin trough chamber (10×10 cm with SS lid) and visualized under visible light,

254nm and 366nm. After spraying with 5% methanolic-sulphuric acid followed by heating at 110°C for 5-10 min [30-32].

RESULTS AND DISCUSSION

Physiochemical analysis

Air dried fruit material was used for quantitative determination of phytochemical values. Loss on drying, total ash, acid insoluble ash, water soluble and alcohol soluble extractive were determined for five times as per WHO recommendations. Water soluble extractive value was found in *Ficus racemosa* L. to be very high when compared to other *Ficus* species extractable matter in the drug (Table 1).

Table 1: Physico-chemical analysis of three *Ficus* species fruit powder

Sl.No.	Parameters	Samples		
		<i>Ficus benghalensis</i> L.	<i>Ficus racemosa</i> L.	<i>Ficus religiosa</i> L.
1.	Loss on drying at 105°C (%)	5.66	10.57	8.99
2.	Total Ash (%)	7.44	8.25	5.88
3.	Acid-insoluble ash (%)	1.58	0.83	0.60
4.	Alcohol-soluble extractive (%)	17.90	17.25	28.80
5.	Water-soluble extractive (%)	17.35	47.25	33.10

Fluorescence analysis

The fluorescence analysis is a tool for the determination of constituents in the plant that gives a definite idea of the chemical nature. Thus the fluorescence analysis of the powder was carried out and data is presented in the (Table 2).

Table 2: Fluorescence analysis of three *Ficus* species fruit powder

Sl.No.	Treatment	Samples								
		<i>Ficus benghalensis</i> Linn.			<i>Ficus racemosa</i> Linn.			<i>Ficus religiosa</i> Linn.		
		Day light	UV light at 254 nm	UV light at 366nm	Day light	UV light at 254 nm	UV light at 366nm	Day light	UV light at 254 nm	UV light at 366nm
1.	Powder as such	Yellow green	Dark brown	Dark brown	Brown	Dark brown	Dark brown	Light brown	Dark brown	Dark brown
2.	Powder+ Distilled water	Yellow green	Dark brown	Lemon yellow	Grass green	Brown	Grass green	Dark brown	Brown	Yellow green
3.	Powder + KOH	Grass green	Brown	Grass green	Brown	Dark brown	Grass green	Light yellow	Black	Dark brown
4.	Powder + HCl	Light brown	Dark brown	Dark brown	Yellow green	brown	Dark brown	Light black	Dark brown	Dark brown
5.	Powder + H ₂ SO ₄	Black	Black	Black	Black	Black	Grass green	Black	Black	Light Blue
6.	Powder + NaOH	Yellow green	Black	Brown	Brown	Dark brown	Brown	Brown	Brown	Dark brown
7.	Powder + HNO ₃	Yellow green	Grass green	Black	Yellow	Grass green	Black	Yellow	Yellow green	Grass green
8.	Powder + FeCl ₃	Grass green	Dark brown	Dark brown	Brown	Dark brown	Dark brown	Light green	Dark brown	Dark brown
9.	Powder + Ammonia solution	Grass green	Brown	Yellow green	Yellow green	Grass green	Yellow green	Grass green	Light brown	Yellow green
10.	Powder + Acetic acid	Grass green	Dark brown	Dark brown	Grass green	Brown	Yellow green	Light green	Yellow green	Grass green
11.	Powder + Ethanol	Yellow green	Brown	Grass green	Grass green	Light brown	Grass green	Grass green	Yellow green	Grass green
12.	Powder + Methanol	Yellow green	Brown	Grass green	Grass green	Brown	Grass green	Grass green	Yellow green	Grass green

High Performance Thin Layer Chromatography (HPTLC)

The TLC plate were examine under ultra violet light at 366 nm; at visible for both before and after derivetization with 5% methanolic- sulphuric acid reagent (Figure 1). The R_f values and colours of the bands obtained were recorded (Tables 3-5).

Figure 1

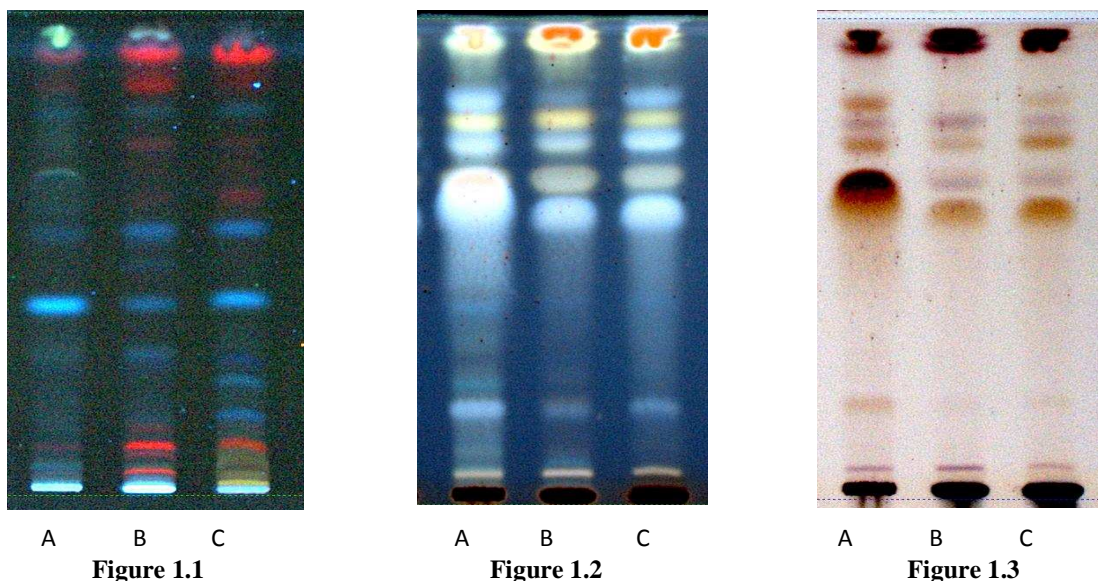


Figure 1.1-TLC profile of three *Ficus* species fruit observed under 366 nm,
 A -*Ficus benghalensis* Linn., B- *Ficus racemosa* Linn., C- *Ficus religiosa* Linn.

Figure 1.2-TLC profile of three *Ficus* species fruit after derivatization with 5% methanolic sulphuric acid reagent observed under 366 nm

Figure 1.3-TLC profile of three *Ficus* species fruit after derivatization with 5% methanolic sulphuric acid reagent observed under visible light

Table 3: R_f values in TLC finger prints of fruit of three *Ficus* species at 366nm (before derivatization)

R_f value	Samples		
	<i>Ficus benghalensis</i> Linn.	<i>Ficus racemosa</i> Linn.	<i>Ficus religiosa</i> Linn.
R_f 1 (red)	NA	0.05	NA
R_f 2 (red)	NA	0.11	0.11
R_f 3 (blue)	NA	NA	0.16
R_f 4 (blue)	NA	NA	0.22
R_f 5 (blue)	NA	0.29	NA
R_f 6 (fluorescent)	0.39	0.39	0.39
R_f 7 (blue)	0.55	0.55	0.55
R_f 8 (red)	NA	0.85	NA
R_f 9 (red)	NA	0.91	0.91
R_f 10 (white)	0.95	NA	NA

NA- Not appeared

Table 4: R_f values in TLC finger prints of fruit of three *Ficus* species at 366 nm (after derivatization)

R_f value	Samples		
	<i>Ficus benghalensis</i> Linn.	<i>Ficus racemosa</i> Linn.	<i>Ficus religiosa</i> Linn.
R_f 1 (white)	0.06	0.06	0.06
R_f 2 (grey)	0.19	0.19	0.19
R_f 3 (white)	NA	0.66	0.66
R_f 4 (light brown)	0.67(white)	0.67	0.67
R_f 5 (white)	0.74	0.74	0.74
R_f 6 (yellow)	0.78	0.78	0.78
R_f 7 (fluorescent)	0.83	0.83	0.83
R_f 8 (brick red)	0.95(white)	0.95	0.95

NA- Not appeared

Table 5: R_f values in TLC finger prints of fruit of three *Ficus* species at visible light (after derivatization)

R _f value	Samples		
	<i>Ficus benghalensis</i> Linn.	<i>Ficus racemosa</i> Linn.	<i>Ficus religiosa</i> Linn.
R _f 1 (brick red)	NA	0.66	0.66
R _f 2 (red)	0.67	NA	NA
R _f 3 (brick red)	0.74	NA	0.74
R _f 4 (pink)	0.78	0.78	0.78
R _f 5 (brown)	0.83	NA	NA
R _f 6 (red)	0.95	0.95	0.95

NA- Not appeared

CONCLUSION

There are over 400 different tribal and other ethnic groups in India which constitute about 7.5 % of India's population. Tribal, rural and primitive societies have discovered solution for treatment of disease to almost all their needs and problems from the natural resources around them. Hence, in recent years, ethnomedicinal studies received much attention as this brings to light the numerous little known and unknown medicinal virtues especially of plant origin which needs evaluation on modern scientific lines such as phytochemical analysis and HPTLC fingerprinting. The present work was taken up with a view to lay down standards which will contribute significantly to quality control of these medicinally useful *Ficus* species. It also provides suitable criteria to differentiate the fruit of three *Ficus* species.

Acknowledgement

Authors are grateful to Dr. Bharat Pathak, General Secretary, Deendayal Research Institute, Chitrakoot, Satna (M.P.) for providing the infrastructure and support to conclude this type of research work successfully.

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