Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2016; 8(5); 800-806

ISSN: 0975-4873

Research Article

Development of Quality Control Parameters for Standardization of Leaves of *Ficus* Species

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Available Online: 1st May, 2016

ABSTRACT
Ashvatha (*Ficus religiosa Linn.*), Nyagrodha (*Ficus benghalensis Linn.*), Udumbara (*Ficus glomerata Roxb.*) are few of the reputed panchavlkal drugs of ayurveda found abundantly throughout India. Various parts of these plants are used as antidiabetic, antidiarrheal, antistress, antioxidant, antiinflammatory, antiallergic, antiulcer, antimicrobial, hypolipidemic, immunomodulatory and analgesic. In present study, a detailed comparative pharmacognostic study of leaves of Ficus religiosa Linn., Ficus benghalensis Linn and Ficus glomerata Roxb. was carried out to develop quality control parameters which could be useful as a reference for experimental study of these plants in future. The study includes macroscopy, physicochemical evaluation, preliminary phytochemical screening, fluorescence analysis, UV Spectroscopic and thin layer chromatographic evaluation.

Keywords: *Ficus religiosa*, *Ficus benghalensis*, *Ficus glomerata*, Macroscopic character, Physico-chemical study, fluorescence analysis, UV Spectroscopic and chromatographic evaluation

INTRODUCTION

The herbal medicines is one of the oldest form of health care known to the mankind. It can be safely used if parameters such as authentication, quality control standards and its efficacy and safety are maintained. In the view of the tremendous increase in use of herbal medicines, it is necessary to have standard parameters to ensure its quality. *Ficus religiosa*, *Ficus benghalensis*, *Ficus glomerata* are large and extensively growing trees found in Indian subcontinent known not only for its religious but also for its medicinal value. The leaves, bark and fruits of these plants are used as antiseptic, astringent, haemostatic, antiinflammatory, anticancer, antioxidant and also in the treatment of skin diseases, diarrhoea, dysentery, vaginal disorders, ulcers, leucorrhoea, deficient lactation, menorrhagia[1]. Although, the leaves of these plants are important but very few studies reported so far on comparative quality control parameters of these theree plants from ficus species. Hence this study is undertaken to develop comparative quality control parameters of leaves of *Ficus benghalensis* (FB), *Ficus religiosa* (FR) *and Ficus glomerata* (FG).

MATERIALS AND METHODS

Collection of plant material

The leaves of *Ficus religiosa, Ficus benghalensis and Ficus glomerata* were collected from Sindhudurg district of Maharashtra, India and identified with authentication no. 14-235, 14-236 and 15-023 respectively by botanist Dr. A. S. Upadhye of Agharkar Research Institute, Pune, India. The leaves were dried in shade and powdered using mixture grinder. The powder leaf material was preserved in air tight container for future use.

Plant extracts, chemicals and reagents

The powdered plant material of leaf was successively extracted with petroleum ether, benzene, chloroform, ethanol and distilled water. All the extracts obtained are preserved in desiccator for future use. All the chemicals

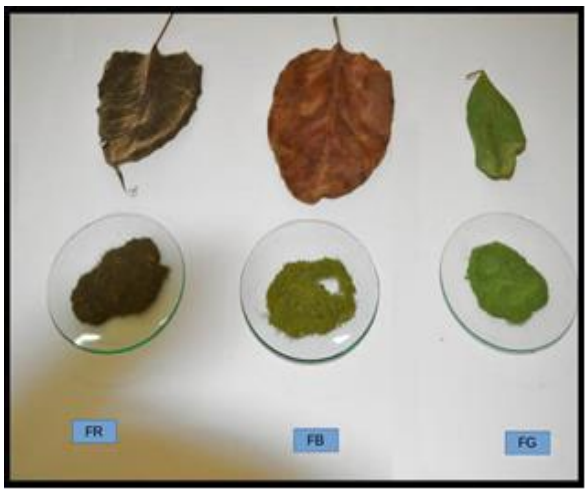

Figure 1: Leaf and powder drug of FR, FB and FG

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Table 1: Comparative macroscopic study of FB, FR and FG leaf

Leaf parts and Characteristics	FB	FR	FG
Duration	Evergreen	Evergreen	Evergreen
Leaf arrangement	Alternate	Alternate	Alternate
Petiole Color Size	Brownish yellow (dried) 3-5 cm	Brownish yellow (dried) 7-12 cm	Dark brown (dried) 3-8 cm
Lamina	Dark green (Fresh)	Dark green (Fresh) Dark brown (dried)	Olive green (Fresh)
Color	Brownish yellow (dried)		Light green (dried)
Shape	Ovate-cordate	Cordate with extended drip tip	Ovate- Lanceolate
Size	Length -15-25 cm Width - 7-18 cm	Length -10-15 cm Width -8-12 cm	Length - 8-17cm Width - 3.5-7 cm
Nature or composition	Simple	Simple	Simple
Incision	Absent	Absent	Absent
Venation	Pinnate-Reticulate	Pinnate-Reticulate	Reticulate
Margin	Entire	Entire and slightly undulate (wavy)	Entire
Apex	Mucronate	Caudate- Acuminate	Acuminate - Obtuse
Base	Cordate-rounded	Cordate	Cuneate
Surface			
Adaxia	*Pubescent*	Glabrous, Shiny	Pubescent, glabrous
Abaxial	*Coriaceous*	Coriaceous	Glabrous
Texture	Thick and leathery, medium course texture	Thin, papery, smooth	Thin,Papery, Smooth, Glaucouse
Taste	Not significant	Not significant	Slightly acrid
Odour	Odorless	Odorless	Odorless

Table 2: Comparative study of physical parameters of powdered leaf of of FB, FR and FG

Sr. No	Parameters	FB	FR	FG
1	Total ash (%w/w)	13.5	14	14.5
2	Water soluble Ash (%w/w/)	2	6	2
3	Acid insoluble ash (%w/w/)	5	7	8
4	Water soluble extractive value (%w/w/)	21.2	19.6	18
5	Alcohol soluble extractive value (%w/w/)	9.2	2.8	8
6	Loss On drying (%w/w/)	5.55	3.05	4.48

Table 3: Comparative study of percentage yield for different extracts of FB, FR and FG

Sr. No	Solvent	Percentage yield		
		FB	FR	FG
1	Pet. ether	3.325	2.676	2.125
2	Benzene	1.075	1.595	0.675
3	Chloroform	0.7	0.925	0.85
4	Ethanol	1.975	3.55	2.371
5	Water	-	12.775	7.447

and reagents used in this study are of analytical grade.

Development of standard analytical parameters:

Macroscopic evaluation and physical parameters such as ash value, extractive value, loss on drying and percentage yield were performed according to the standard official method[2]. Preliminary phytochemical analysis of extracts was carried out as per the standard method[3]. UV spectroscopic analysis of crude extracts was carried out using Jasco UV Spectrophotometer in the range of 200 to 500 nm. Thin layer chromatography analysis is carried out using silicagel GF-254 as adsorbent, and benzene: chloroform (ratio of 7:3) is used as a mobile phase[4]. Plate was prepared by pouring silica gel on glass plate and activated by heating at 110 0 c for 30 min. The sample was spotted on the plate and the plate was kept in the solvent system until it reaches to 3/4th of the plate. The spots are detected under long uv at 365 nm and short uv at 254 nm and Rf value were calculated.

RESULTS AND DISCUSSION

Quality control of plants materials used in traditional system of medicine is important for the commercialization of new therapeutic agents. Adulterated and substituted plants materials may produce various

Table 4: Comparative fluorescence analysis of powdered leaf sample of FB, FR and FG

Treatment	FB		FR		FG	
	UV 254 nm	UV 366 nm	UV 254 nm	UV 366 nm	UV 254 nm	UV 366 nm
As such	Brown	Black	Brown	Black	Brown	Black
Methanol	Greenish brown	Reddish brown	Light green	Reddish brown	Greenish brown	Reddish brown
10 % NAOH	Light green	Black	Light green	Black	Greenish black	Black
Conc. HCL	Light green	Black	Light green	Black	Blackish green	Black
Conc. HCL + H2O	Greenish black	Blackish green	Light green	Blackish brown	Light green	Black
Conc. Nitric acid	Green	Black	Green	Black	Greenish white	Black
Conc. Nitric acid + H2O	Blackish brown	Bluish black	Light green	Black	Light green	Black
Conc Sulphuric Acid	Blackish green	Bluish black	Blackish green	Black	Black	Black
Chloroform	Light brown	Black	Light green	Light orange	Light green	Reddish brown
Pet Ether	Light green	Reddish brown	Transparent	Brownish black	Light green	Black

Table 5: Comparative study of treatment of powdered leaf with different chemicals

S. No	Treatment with chemicals	FB	FR	FG
1	Drug + Conc. HCL	Dark brown	Light brown	Brown
2	Drug + Conc. HNO3	Reddish brown	Orange	Brisk red
3	Drug + Conc. H2SO4	Black	Reddish black	Black
4	Drug + Glacial acetic acid	Light brown	Light green	Greenish brown
5	Drug + Picric acid	Brown	Light orange	Yellowish brown
6	Drug + 5 % NaOH	Light brown	Light brown	Brown

Table 6: Comparative phytochemical analysis of petroleum ether (PE), benzene (BE), chloroform (CH), ethanol(ET) and aqueous (AQ) extracts of the powdered leaf of FB, FR, FG

Sr. No.	Extracts	Alkaloids	Amino acids	Anthraquinone	Flavonoids	Glycoside	Polyphenols	Saponins	Tanins	Triterpenoids	Steroids
1	FBPE	+	-	-	-	+	-	+	-	+	+
2	FRPE	+	-	-	+	-	-	-	+	+	-
3	FGPE	+	-	-	+	+	+	+	-	+	+
4	FBBE	-	+	-	+	-	+	+	-	+	+
5	FRBE	+	-	-	+	-	+	+	+	-	+
6	FGBE	+	-	-	+	+	+	-	-	+	-
7	FBCH	-	-	-	+	-	+	-	-	+	+
8	FRCH	+	-	-	+	+	+	+	+	-	+
9	FGCH	+	-	-	+	+	+	+	-	-	-
10	FBET	+	-	-	+	+	+	+	+	+	+
11	FRET	+	-	-	+	+	+	+	+	-	-
12	FGET	-	-	-	+	+	+	-	+	-	+
13	FBAQ	+	+	-	+	+	+	+	+	+	-
14	FRAQ	+	-	-	+	+	+	-	+	+	-
15	FGQ	+	-	-	+	-	+	+	-	-	-

health related issues when used by the patients. Therefore, comparative study of macroscopic features and quality control parameters of three plants of ficus species mentioned in the present work may be helpful for identification and standardization of these plants material.

Macroscopic evaluation:

Data of different aspects of macroscopic evaluation is given in Table 1. Although the three varieties belong to

Table 7: Comparative UV spectroscopic analysis of extracts of leaf of FB, FR and FG

Sr. No.	Solvent	λ max of 0.1 mg/ml extract in respective solvents		
		FB	FR	FG
1	Pet. ether	369.7	268.6	269.6
2	Benzene	414.5	415	414.6
3	Chloroform	235.5	414.8	272.5
4	Ethanol	207.8	267.8	267.7
5	Water	215	218.5	213.9

Table 8: Comparative TLC analysis of petroleum ether (PE), benzene (BE), chloroform (CH), ethanol (ET) and aqueous (AQ) extracts of the powdered leaf of FB, FR, FG

Sr. No.	Extracts	*UV 254 nm*		*UV 365 nm*	
		Total No of Spots	*Color & Rf Value for Spot No 1,2, n..*	*Total No of Spots*	*Color & Rf Value for Spot No 1,2, n..*
1.	FBPE	2	1. Greenish yellow, 0.0363 2. Greenish yellow, 0.090	2	1. Orange, 0.090 2. Light blue,0.127
2.	FBBE	-	-	1	1. Light Orange, 0.0769
3.	FBCH	1	1. Greenish yellow, 0.0363	2	1. Green, 0.0363 2. Orange, 0.0727
4.	FBET	-	-	2	1. Orange, 0.145 2. Light blue, 0.181
5.	FBWA	-	-	-	-
6.	FRPE	2	1. Yellow, 0.0727 2. Yellow, 0.109	3	1.Orange, 0.0727 2.Blue, 0.163 3.Blue, 0.545
7.	FRBE	1	1. Greenish yellow, 0.0909	2	1. Orange, 0.0909 2. Blue, 0.545
8.	FRCH	1	1. Greenish yellow, 0.0909	2	1. Orange, 0.0909 2. Light Blue, 0.5272
9.	FRET	1	1. Light green, 0.145	2	1. Light orange, 0.0545 2. Dark orange,0.145
10.	FRWA	-	-	-	-
11.	FGPE	2	1. Greenish yellow, 0.036 2. Greenish yellow, 0.109	3	1. Orange, 0.0727 2. Yellow, 0.1636 3. Orange, 0.2727
12.	FGBE	2	1. Light yellow, 0.0363 2. Greenish yellow, 0.109	2	1. Light orange, 0.0363 2. Dark orange, 0.109
13.	FGCH	1	1. Greenish yellow, 0.0909	2	1. Orange, 0.0909 2. Light blue, 0.527
14.	FGET	1	1. Light green, 0.196	1	1. Orange, 0.2
15.	FGWA	-	-	-	-

same species, they vary with each other in many respects; FB and FR leaves are large in size, dark green whereas FG leaves are smaller and dark olive green in color with following macroscopic features:

Shape: FB leaves are ovate and cordate and FR leaves are cordate with extended drip teep while FG leaves are ovate lanceolate in shape (Photo slide 1- A, B, C).

Venation: Both FB and FR showed pinnate- reticulate while FG showed reticulate type of venation.

Margine: Both FB and FG showed entire margine while FR showed entire and slightly wavey called as undulate margine.

Apex: All three leaves showed different type of apex. In FB leaf apex is mucronate and FR it is Caudate-acuminate while in FG it is acuminate- abtuse.

Leaf base: In FB base is caurdate- rounded and FR it is cordate while FG leaf shows cuneate base.

Surface: In both FB and FR abaxial surface is coriaceous while in FG it is glabrous whereas upper adaxial surface is pubescent in FB, glabrous and shiney in FR and pubescent and glabrous in FG.

Texture: In FB leaf it has thick leathery medium course texture while FR and FG leaf showed thin, papery and smooth texture where as FG have additional glaucouse texture.

Determination of physico-chemical parameters:

Ash value

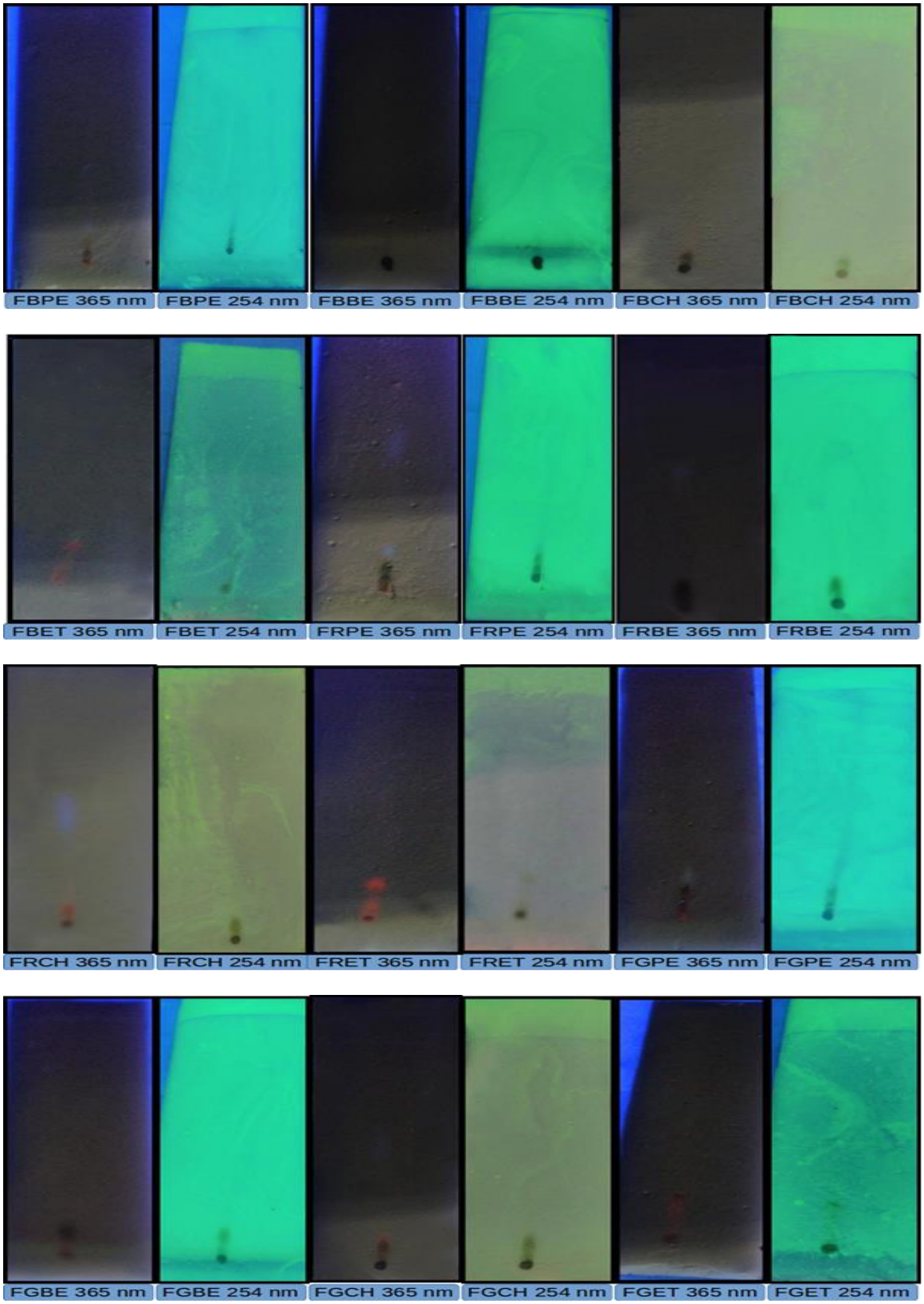

Figure 2: Thin layer chromatography of petroleum ether (PE), benzene (BE), chloroform (CH), ethanol (ET) and aqueous (AQ) extracts of the powdered leaf of FB, FR, FG at 365 and 254 nm

Ash values reflect quality, purity and authenticity of crud drug and also it is an important quantitative standard[5].

The comparative results of total ash, water soluble ash, acid insoluble ash of the leaf of three plants expressed as average % w/w for three samples is given in table 2. Total ash of leaves of all three plants were found to be more

than water soluble ash and acid insoluble ash. Water soluble ash of both FB and FG was found to be very less than total ash, acid insoluble ash of the leaf.

Extractive value

Extractive value is an indicator of total soluble components in particular solvents. The comparative result of water soluble and alcohol soluble extractive value expressed as % w/w for three samples is given in table 2. Water soluble extractive value of leaves of all three plants were found to be more than alcohol soluble extractive value which indicate presence of more amount of water soluble components in the plants.

Loss on drying

Loss on drying for leaves of FB, FR and FG were found to be 5.5 % w/w, 3.05 % w/w and 4.48 % w/w which indicate presence of less moisture content in the crud drug (Table 2). Less moisture content could prevent herbal drugs from the growth of bacteria, fungi or yeast during the storage[6].

Percentage yield

Percentage yield is the measure of the efficiency of solvents to extracts specific components from the powder drug material[7]. Comprehensive comparative percentage yield of powder leaf sample of three plants of ficus species is given in table 3. Maximum yield is observed in water extract of FR and FG followed by ethanol extract of FR. However, benzene extract of FG followed by chloroform extract of FB showed minimum percentage yield.

Fluorescence analysis of leaf powder

Fluorescence analysis of powdered leaf sample has been carried out in visible light, UV light at 254 nm and 365 nm. The powdered leaf sample of all three plants were treated with various organic solvents and solutions and observed under Visible and UV light (Table 4). The crude drugs produces fluorescence because in presence of different solvents phytoconstituents in the drugs are converted into fluorescent derivatives[8]. Since fluorescence produced by the individual powdered drug is the characteristic of phytoconstituents present in it, fluorescent analysis could be one of the important parameter for evaluation of quality of herbal drugs.

Behavior of leaf powdered materials towards chemical reagents:

The behavior of the powdered leaf sample of all three plants treated with conc. Hydrochloric acid, conc. Nitric acid, conc. Sulphuric acid, glacial acetic acid, picric acid, 5% Sodium hydroxide were observed (Table 5).

Preliminary phytochemical analysis

Preliminary phytochemical screening is a valuable in detection of bioactive principles present in medicinal plants[9]. Various extracts of these plants showed presence of alkaloids, amino acids, glycosides, polyphenols, saponins, flavonoids, tannin, triterpenoids and steroids. Comparative phytochemical analysis of petroleum ether, benzene, chloroform, ethanol and aqueous crude plant extracts of the powdered leaf of FB, FR and FG is given in Table 6.

UV spectroscopic analysis

Lambda max of different extracts of leaf of all three plants in the range of 200 to 500 nm were determined by using jasco UV Spectrophotometer. Extract of leaves of all three plants showed different lambda max which is given in table7. Determination of Lambda max is indicative of wavelength at which compound shows maximum absorption. Since all extracts of three plants showed different lambda max values which reflect that the extracts are different in their composition of phytoconstituents present in it.

Thin layer chromatography

Thin layer chromatography is a very good tool to monitor the progress of a reaction, identify compounds present in a given test drug and for ensuring the purity of a substance[10]. Thin layer chromatography of different extracts of leaves of all three plants was carried out using silica gel GF-254 as adsorbent and benzene: chloroform in the ratio of 7:3 as a mobile phase. Different extracts showed blue and orange colored fluorescence at 365 nm with different Rf value which indicate presence of flavonoids in the extracts (Table 8).

CONCLUSION

Ficus benghalensis Linn., Ficus religiosa Linn. and *Ficus glomerata Roxb. a*re few of the reputed panchavlkal drugs of ayurveda. Various parts of these plants are used as antidiabetic, antidiarrheal, antistress, antioxidant, antiinflammatory, antiallergic, antiulcer, antimicrobial, hypolipidemic, immunomodulatory and analgesic etc. In spite of these immense medicinal value, there is lack of research work on comparative quality control parameter of these three plants. Our present research work on the leaves of *Ficus benghalensis Linn., Ficus religiosa Linn. and Ficus glomerata Roxb.* is an attempt for providing a set of data that can be used as a reference to differentiate these closely related plants in future.

ACKNOWLEDGEMENT

The author wish to thanks Dr. Abdul Razak Honnutagi, Director, AIKTC and Dr. Rajani Shettigar, Dean, SoP, AIKTC for providing facilities to carry out this work and Dr. Shariq Syed, Associate Professor, SoP, AIKTC for his valuable edits.

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