Standardization of bark of *Ficus religiosa* Linn by developing different quality control parameters

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ABSTRACT

Objective: To develop novel techniques of standardization, this can overlay the method of repid determination of phytoconstituents of *Ficus religiosa* (F. religiosa) Linn. (Moraceae). From the review of literature it was revealed that no reports were available on standardization parameters of bark of *F. religiosa* Linn. Method: Phytochemical analysis, TLC analysis, Foreign matter, Ash values, swelling index, foaming index, Extractive value, Moisture content, Microbiological Analysis, Crude fibre content, Amino acid content, Aflatoxin, toxic elements analysis and pesticide analysis were performed for quality control of drug. Results: thus it was thought worthwhile to explore this plant on the basis of its standardization parameters. Alkaloid, steroids, flavanoids and tannins were found to be present in *F. religiosa* extracts. Ash value, insoluble ash value, soluble ash value, crude fibre content and crude fat were found to be 13.1%, 0.41%, 7.45%, 9.9% & 1.7% respectively . The study will provide referential information for correct identification of crude drug. Conclusion: the data of *F. religiosa* extracts thus produced by different phytochemical analysis is useful for further studies. It will be useful for pharmacological screening in future studies. Also, the study will be helpful for qualitative and quantitative analysis of phytoconstituents for isolation of newer molecule from *F. religiosa* Linn.

Keywords: *F. religiosa*, Phytoconstituent, Phytochemical,Standardization parameters.

Introduction

Ficus religiosa (F. religiosa) Linn commonly known as 'Peepal tree' is a large widely branched tree with leathery, heart shaped, long tipped leaves on long slender petioles and purple fruits growing in pairs [1] The tree is regarded as a sacred tree to both Hindus as well as Buddhists. It has got mythological, religious and medicinal importance in Indian culture since ancient times [2,3]. The tree grows throughout India and widely cultivated in south-east Asia especially in vicinity of temples. In Ayurveda, *F. religiosa* belongs to a class of drugs called rasayana. Rasayana are rejuvenators, antioxidants and relieve stress in the body [4,5]

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Taxonomic classification

Domain: Eukaryota
Kingdom: Plantae
Subkingdom: Viridaeplantae
Phylum: Tracheophyta
Subphylum: Euphyllophytina
Infraphylum: Radiatopses
Class: Magnoliopsida
Subclass: Dilleniidae
Superorder: Urticanae
Order: Urticales
Family: Moraceae
Tribe: Ficeae
Genus: Ficus
Specific epithet: *Religiosa Linnaeus*
Botanical name : *Ficus religiosa*

VERNACULAR NAMES

In India it is known by several vernacular names, the most commonly used ones being Asvatthah (Sanskrit),

Sacred fig(Bengali), Peepal (Hindi), Arayal (Malayalam), Ravi (Telgu) and Arasu (Tamil).

Description

F. religiosa is an evergreen or deciduous tree, 20 m tall and 1.5-2 m wide, irregularly-shaped, with wide-spreading branches and without aerial roots from the branches. The trunk is regularly shaped, often with low buttresses. Bark is grey with brownish specks, smooth, exfoliating in irregular rounded flakes.Leaves alternate, spirally arranged and broadly ovate, glossy, coriaceous (leathery), dark green leaves, 10-18 by 7.5-10 cm, with unusual tail-like tips, pink when young, stipulate, base-cordate. Petioles is slender and 7.5-10 cm long. Galls on leaves. Flowers axillary sessile, unisexual. Figs in pairs, rounded, flat-topped green, to 1.5 cm across, axillary, sessile, smooth, ripening to purple with red dots, basal bracts 3 and broad.[6]

Biophysical limits

Altitude: up to 1520 m
Mean annual temperature: 16-35ºC
Mean annual rainfall: 500-5000 mm
Soil type: It grows on a wide variety of soils but prefer deep, alluvial sandy loam with good drainage. It is also found on shallow soils including rock crevices. It is native to Chad, India, Nepal, Thailand and also exotic in Israel, US. [7]

Cultivation

F. religiosa is widely planted in the tropics [8]. The tree is very long lived and one tree near Bombay is reported to be over 3,000 years old [9]. *F. religiosa* are mostly planted near Buddhist temples. It is also cultivated as an ornamental, for medicinal uses, such as toothaches, and in the making of shellac. The fruit (syconium or fig) and reproduction systems of species in the genus *Ficus* are unique. Each species of *Ficus* has an associated species of agaonid wasp (Hymenoptera: Chalcoidea: Agaonidae). *Ficus* species can only be pollinated by their associated agaonid wasps and in turn, the wasps can only lay eggs within their associated *Ficus* fruit. The pollinator wasp for *F.religiosa* is *Blastophaga quadraticeps*. *Ficus religiosa* is grown by specialty tree plant nurseries for use in tropical and subtropical climates. Peepal trees are native to India and thrive in hot, humid weather. They prefer full sunlight and can grow in most soil types, though loam is the best. When planting, use soil with a pH of 7 or below. While it is possible for the plant to grow indoors in a pot, it grows best outside. Young peepal needs proper nourishment. It requires full sunlight and proper watering. It is found scattered in forests, where it propagates as an epiphyte on other trees especially widely found in uplands and plane area. [9].

Material and method

Plant material

The plant material *F. religiosa* Linn. (Bark) was collected from Shiv mandir Batala distt Gurdaspur (Pb.). The plant material were identified and authenticated by Dr. Adarsh Pal Vig head of the department of department of botanical & Environmental Sciences of Guru Nanak Dev University, Amritsar-143005 under ref No. 203 Bot. & Env. Sc.

Preparation of extract

The plant material bark was air dried then grounded to moderately fine to coarse powder and extracted with different solvents in their increasing polarity order (Petroleum ether, Chloroform, Ethanol methanol and water) by soxhlet and cold percolation method. Rotary evaporator was used to evaporate the solvent and dry the extract under reduced pressure. The powder was subjected to successive hot continous extraction with various solvent. The extract was air dried each time before extracting with next solvent system. Various concentrated extracts were stored in air tight container for further studies. [10]

Nutriotional and mineral assay

The bark of *F. religiosa* was analyzed for moisture content, ash value, fat, fiber, as per method reported in AOAC. Total nitrogen value was obtained by following method given by microkjeldhal and for crude protein the value was multiplied by 6.25. Total carbohydrate were obtained by subtracting the value moisture, crude protein, crude fat, crude fiber and ash from 100%.The total energy value equal to addition of fat, protein and sugar calorie, each gram of fat give 9 Kcal, protein and sugar give 4 Kcal energy. The minerals analysed were potassium using atomic absorption spectrophotometer, calcium and phosphorus by flame photometer. Ascorbic acid was estimated.[11-14]

Successive value

500 g dried extract powder was subjected to hot successive continous extraction with soxhlet apparatus using different solvent in their increasing polarity order : petroleum ether, chloroform , ethanol, methanol and

finally with distilled water. The extracts thus produced were filtered and solvents were evaporated by vacuum distillation. The extracts were dried in dessicator and remaining residues were weighed. Chemical categories retained are subjective to the solvent nature and types.[15]

Detection of chemical compound through TLC

TLC, thin layer chromatography is a chromatography mobile: stationary phase technique employed to separate mixtures. TLC is performed on a sheet of glass, plastic, aluminium foil or readymade TLC sheets which are coated with a very thin layer of silica gel, aluminium oxide, or cellulose used as adsorbing material as a stationary phase. It works on capillary action principle, after the sample has been applied on the plate, a solvent or solvent mixture (mobile phase) is drawn up the plate by capillary action.TLC plates are prepared by spreading silica gel G with the help of spreader on glass plate using distilled water as solvent. The plates thus produced are activated in oven at 110 °C for 30 minutes. All five extracts were applied separately and run in different solvent system of varying polarity. These plates are developed in UV chamber, Iodine Chamber or spraying different reagents for different spots of constituents. [16]

Phytochemical analysis

The extracts produced by different solvent system were subjected to preliminary phytochemical analysis. This analysis was done by using standard methods to detect presence or absence of the primary and secondary phytocontituent. [17]

Analysis of toxic elements

Analysis of toxic elements and essential minerals was carried out by Atomic absorption spectroscopy (Perkin Elmer-400, carrier gas-Argon, Flow rate-2mL/3min) by following method.

500 mg of extract was powder was taken and mixed with 5mL of conc. Nitric acid in round bottomed flask and was refluxed for 30 minutes in a hot plate at 60°C - 80°C. After cooling, 5mL of conc. Nitric acid was added and warmed on water bath. Later 2mL of 30% H_2O_2 solution was added to the above mixture and warmed till clear solution was obtained. It was then cooled, filtered through whatman-42 filter paper diluted with deionised water and made upto 100 mL in volumetric flask.

Analysis of pesticide residue

Analysis of pesticide residue was carried out by Gas Chromatography-Mass spectra (GC-MS) (Intrument – Agilent, Detector-Mass selective detector, Column specification-DB5MS, carrier gas – Helium, flow rate – 1mL/min, column length – 30 m, internal diameter – 0.25mm, column thickness – 0.25µm)

Sample preparation

Accurately weighed 25 g of coarsely powdered air-dried material is taken in aconical flask. 65 mL of acetonitrile and 35mL of deionised water was added to it. After shaking it was allowed to stand for 2 hours with constant shaking. Filtered though whatman-41 filter paper and filtrate was collected in separating funnel. Add 3X65 mL of petroleum ether (boiling point of petroleum ether 60-80 °C).

Sample was shaked vigorously and solvent layer was collected in round bottom flask. It was evaporated to dryness. Then 1mL of acetonitrile was added to the residue . then the sample was injected in GC-MS(Gas Chromatography-Mass Spectra).

Determination of aflatoxins

Aflatoxins were determined by Kobra cell technique using Agilent HPLC instrument as per the method ASTA. (Lachrome Merck HPLC D7000 series, detector – flurescent detector, column specification – ODS – 3 V, column thickness & length - 5µ & 150 m, internal diameter – 4.6mm, flow rate – mL/min).

Procedure

50 g of powered plant material was transferred to a glass stoppered conical flask. 100 ml of methanol and water (4:1) was added and vigorously shaken for 15 min in dark condition by wrapping the flask in aluminium foil and filtered. The filterate (30ml) was passed through ALFA preparative column. The flow rate was 1ml/min. the column was washed with 20 ml distilled water and then eluted with 2 ml of methanol followed by 1 ml of water and the elute is collected. 100µl of the sample was injected into HPLC. The mobile phase employed was water: acetonitrile: methanol (3:1:1) along with 0.109 g potassium bromide and 0.35 mL of 4 M nitric acid. The aflatoxin present in the sample was converted to the bromide derivative in the kobra cell. The fluorescence detector with 362 nm excitation and 455nm emissions detected the bromide derivatives. 10µL of standard aflatoxins containing 9.6 ppb of B1 and G1 each, 2.88 ppb of B2 and 2.81 ppb of G2 was injected for quantitative estimation. The eluting order was of B1, B2, G1, and G2. The area of the graph obtained in the both sample

and standard was calculated to give the total residual Aflatoxin present. [18]

$$\text{Aflatoxin} = \frac{\text{Sample area}}{\text{Standard area}} \times \text{Standard concentration} \times \text{Sample dilution}$$

Results

Plants have been used for the treatment of different ailments in traditional medicine. In modern era plants have proved to be the important source of bioactive constituents both bioactive and adjuvant for the development of new chemotherapeutic agents.

The process begins with phytochemical screening, amino acid screening, nutritional profile, extractive value, TLC, Aflatoxin, toxic element analysis. The results of phytochemical screening, amino acid screening, nutritional profile, extractive value, TLC analysis, Aflatoxin, toxic element analysis and analysis of pesticide residue are given in tables 1,2,3,4,5,6,7 and 8.

Discussion

Phytochemical have been used for the treatment and prevention Phytochemicals have been used for the treatment and prevention of various health ailments from time immemorial. a large percentage of the drugs prescribed worldwide are derived from plants and 121 such active compounds are in use. Who's essential medicine list contain large number of drug from plant origin. Physicochemical standards were generally used for deciding the identity, purity and strength of the drug source. These parameters were also used to detect the adulterants if any present in the plant materials[19,20].

Physical parameters such as Moisture content, Ash value, insoluble ash, soluble ash, crude fibre, crude fat, total nitrogen, total protein, carbohydrate, Organic matter, Preliminary phytochemical analysis, Thin layer chromatography analysis and heavy metal detection can be used as reliable aid for detecting adulteration. These are simple, but reliable standards will be useful to a layperson in using the drug as a home remedy. Effective formulations have to be developed using indigenous medicinal plants, with proper pharmacological experiments and clinical trials. The manufacture of plant products should be governed by standards of safety and efficacy. In future, these characters are also used to check the genuine nature of the crude drug, Thus it plays an important role in preventing the possible steps of adulteration.

The bark, of *F. religiosa* Linn. contains phytoconstituents like alkaloids, steroids, fats & fixed oil, flavonoids & tannins. So finally we concluded that these physicochemical data and phytochemical analysis of different extracts of *F. religiosa* Linn. is useful for further studies of Pharmacological parameters. More detailed study must be done for further isolation leading to the pure compounds.

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Table 1:Phytochemical investigation of various extrats of *F. religiosa* bark

Sr.No.	Test	FRPE	FRCE	FRME	FREE	FRAE
1.	Alkaloids					
I.	Mayer's reagent	-	-	-	-	-
II.	Hager's reagent	-	+	-	-	-
III.	Wagner's reagent	-	+	-	-	-
2.	Flavonoids					
I.	Shinoda test	+	-	-	-	-
II.	Lead acetate test	-	+	-	-	+
III.	Alkaline test	+	+	-	-	-
3.	Steroids					
I.	Salwoski test	+	-	-	-	-
II.	Leibermann test	+	+	-	-	-
4.	Tannins & Phenolic					

	compounds					
I.	Lead acetate	-	-	+	+	-
II.	Test with iron salt	-	-	+	-	-
III.	Dilute HNO_3	+	-	+	-	-
IV.	Bromine water	-	-	-	+	-
V.	Potassium dichromate	-	+	-	+	-
5.	Amino acids					
I.	Ninhydrin test	-	-	-	-	-
6.	Proteins					
	Biuret test	-	-	-	+	+
	Precipitation test	-	-	-	-	-
7.	Fat & Oil					
I.	Solubility Test	+	-	-	-	-
II.	Filter paper test	+	-	-	-	-

+ present ; + Present;FRPE: *F.religiosa* Pet. Ether Extract; FRCE: *F.religiosa* Chloroform extract FRME: *F.religiosa* Methanolic extract; FREE: *F.religiosa* Ethanolic Extract; FRAE: *F.religiosa* Aqueous extract

Table 2:Qualitative estimation of F.religiosa bark amino acid screening

Sr. No.	Amino acid test	F.religiosa
1.	L–Hydroxy proline	-
2.	DL Serine	+
3.	DL–Alanine	+
4.	Dl-Tryptophan	+
5.	DL-Iso-leucine	+
6.	Dl Valine	+
7.	DL-Nor-Leucine	+
8.	L-Cystein hydroxyl	-
9.	L-Ornithin	-
10.	DL-2-Amnobutyric acid	-
11.	DL-Aspartic acid	+
12.	Glycine	+
13.	3-C-3-4Dihydroxy phenyl	-
14.	L-Glutamic acid	-
15.	L-Tyrosine	+
16.	DL-Threonine	+
17.	L-Proline	+
18.	L-Arginine	+

19.	L-Leucine	+
20.	L-Lycine monochloride	-
21.	DL-Methionine	+
22.	L-Histidine	-
23.	L-Cystein hydroxychloride	+
24.	DL-β-Phenyl alanine	-

+ Present; - Absent

Table 3:Nutritional value of *F.religiosa* Linn. bark

Sr. No.	Nutrients	Value
1.	Moisture (%)	62.4
2.	Ash (%)	13.1
3.	Insoluble ash (%)	0.41
4.	Soluble ash (%)	7.45
5.	Crude fibre (%)	9.9
6.	Crude fat (%)	1.7
7.	Total nitrogen (%)	0.95
8.	Total protein (%)	2.5
9.	Carbohydrate (%)	15.4
10.	Organic matter (%)	83.65
11.	Na (mg/100 g)	1.5
12.	Ca (mg/100 g)	16.1
13.	K(mg/100 g)	27.9
14.	Mg (mg/100 g)	11.9
15.	P (mg/100 g)	3.3
16.	Fe (mg/100 g)	623

Table 4:Extractive values of *F.religiosa* Linn. bark

Sr. No.	Method of extraction	Values of three Replicates (%w/w)	Mean (%w/w) ±SEM
1	COLD MACERATION:		
	Water soluble	(15.23,15.74,15.33)	15.43±0.156
	Alcohol soluble	(7.21,7.28,7.42)	7.303±0.061
2.	HOT EXTRACTION		
	Pet. Ether soluble	(3.20,3.22,3.42)	3.28±0.072
	Chloroform soluble	(4.85,4.82,4.57)	4.647±0.102
	Methanol soluble	(15.29,15.24,15.22)	15.25±0.028
	Ethanol Soluble	(13.31,13,38,13.33)	13.34±0.020
	Water soluble	(19.95,19.91,19.92)	19.93±0.12

Table 5:Obseravtion of Thin Layer Chromatography (TLC) of bark extract of *F.religiosa* Linn.

Sr. No.	**Extract**	**Mobile Phase**	**No. of spots**	**R_f values**
1.	Pet. Ether Extract	(C:M:W) 60:30:10	2	.62,.54
2.	Chlorform Extract	(C:M:W) 75:20:5	1	.64
3.	Methanol Extract	(C:M:W) 65:25:10	3	.44,.72,.32

4.	Ethanol Extract	(C:M:W) 90:5:5	4	.57,.38,.46 .63
5.	Water Extract	(C:M:W) 60:30:10	3	.48,.39,.71

Table 6 :Analysis of aflatoxins of bark of *F.religiosa*

Sr. No.	Aflatoxins	**F. religiosa**
1.	Aflatoxins B1	BDL(DL:0.5 ppb)
2.	AflatoxinsB2	BDL(DL:0.5 ppb)
3.	AflatoxinsG1	BDL(DL:1.0 ppb)
4.	AflatoxinsG2	BDL(DL:0.5 ppb)

BDL: Below detectable Limit, DL: Detectable Limit

Table 7:Toxic element analysis of bark of *F. religiosa.*

Sr.No.	Heavy Metals	F. religiosa (ppm)	Permissible Limits
1.	Cadmium	0.0012	10.0 ppm(WHO)
2.	Lead	0.0042	0.3 ppm (WHO)
3.	Mercury	0.001	10.0 ppm (FDA)
4.	Arsenic	0.0005	1.0 ppm (FDA)

Table 8:Analysis of pesticide residues of bark of *F. religiosa*

Sr. No.	Pesticides	*F.religiosa*
1.	a-HCH	ND
2.	b-HCH	ND
3.	g-HCH	ND
4.	o p´- DDT	ND
5.	d - HCH	ND
6.	pp´- DDT	ND
7.	Op – DDE	ND
8.	a - Endosulfan	ND
9.	b – Endosulfan	ND
10.	op – DDD	ND
11.	pp - DDD	ND

Detectable limit – 0.01 ppm, ND: Not detectable

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Conflict of Interest: None