



STANDARDIZATION AND ANTIMICROBIAL ACTIVITY OF *FICUS RELIGIOSA* LINN. (FAMILY: MORACEAE)

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ABSTRACT

Ficus religiosa Linn. (Moraceae) has been traditionally claimed to be useful in asthmatic conditions, anti-diarrhoeal, antiviral and astringent. It also shows antivenom activity. It is used in the treatment of various diseases such as cancer, inflammation or infectious diseases. In the present study, it includes standardization parameters which are carried out on leaves where successive soxhlet extraction of dried powdered leaves was carried out using petroleum ether, chloroform, methanol and water. All these extracts were subjected for *in-vitro* antimicrobial activity against the strains *Escherichia coli* and *Staphylococcus aureus* by cup plate method.

Keywords: *Ficus religiosa*, Standardization, Antimicrobial activity.

INTRODUCTION

Ficus religiosa Linn. (Moraceae) is the most popular member of the genus *Ficus*, commonly named as Peepal. No other tree is claimed to have such long life. Various parts of the plant, like bark, fruit, leaves and seeds are widely used and have extensive medicinal uses. It is known to have astringent, antiprotozoal, anti-diarrhoeal and antiviral activity. It is used in the treatment of various diseases like cancer, inflammation and other skin and infectious diseases in popular indigenous systems of medicine like Ayurveda, Siddha, Unani and Homeopathy. The objective of this present study was to carry out the standardization and pharmacological activity i.e. *in-vitro* antimicrobial study on the leaves of *Ficus religiosa* to find out their usefulness to the human being.²

Plant Description

Ficus religiosa is a large dry season-deciduous or semi-evergreen tree up to 30 meters (98 ft) tall and with a trunk diameter of up to 3 meters (9.8 ft). The leaves are cordate in shape with a distinctive extended tip. They are 10–17 cm long and 8–12 cm broad, with a 6–10 cm petiole. The fruit is a small fig 1-1.5 cm diameter, green ripening to purple. It is a medium size tree and has a large crown with the wonderful wide spreading branches. It shed its leaves in the month of March and April. The fruits of the Peepal are hidden with the figs. The figs are ripening in the month of May. The figs which contain the flowers grow in pairs just below the leaves and look like the berries. Its bark is light gray and peels in patches. This plant is considered sacred by the followers of Hinduism, Jainism and Buddhism and hence the name 'Sacred Fig' was given to it. The bark of the plant is used as an astringent and a cooling agent. Roots are said to be good for gout and are chewed to prevent gum diseases. Fruits are used as a laxative, aphrodisiac and also used in cases of heart disease. The seeds are used as laxative, refrigerant and a cooling agent. Leaves are alone used to treat constipation.^{1,2}

MATERIALS AND METHODS

Collection of the Plant Sample

The fresh leaves of the whole plant of *Ficus religiosa* were collected from Pune, India. The time of collection was in the fruiting stage in early November. The leaves of the plant

were collected from the healthy host plants and any type of adulteration was strictly prohibited. The herbarium sheet was prepared,⁴ identified and authenticated by the experts of Botanical Survey of India, Koregaon Park, Pune, India (Reference No-BSI / WC / Tech. / 2012). Air-dried leaves were processed for size reduction by using cutter mill (Portable Mixer). Crushed material was passed through 40 mesh sieve (coarse powder) for uniform particle size, which gave efficient extraction and yield of extract/s.

Extraction of Plant Extract

The powdered *Ficus religiosa* Linn. was successively extracted with soxhlet extraction with solvents of increasing polarity beginning with petroleum ether, chloroform, methanol and water. The solvents were removed under reduced pressure on rotary evaporator until it became completely dry. The percentage yield for each extract was determined. All the crude extracts were subjected to antimicrobial assay.

Standardization

Evaluation of a drug means confirmation of its identity and determination of its quality and purity and detection of nature of adulteration. It deals with delivering a product with a specified minimum level of one or more phytoconstituent (s), where we make sure about the quality of the product; broadly covering the qualitative and quantitative part of analysis. The evaluation of a crude drug is necessary because of the biochemical variation in the drug, deterioration due to treatment and storage and substitution and adulteration, as a result of carelessness and ignorance. Over the years, the nature and degree of evaluation of crude drugs has undergone a systematic change. Initially, the crude drugs were identified by comparison only with the standard description available. Due to advancement in the chemical knowledge of crude drugs, at present, the quality of the medicinal plant material must be as high as that of other medicinal preparations for pharmaceutical purposes. Thus, plant materials and herbal remedies derived from them represent a substantial proportion of global drug market and internationally recognized guidelines for quality assessment.³

Physicochemical Parameters

Ash Value

In the determination of total ash values, the carbon must be removed at as low a temperature (450⁰C) as possible because alkali chlorides, which may be volatile at high temperatures, would otherwise be lost. If carbon is still present after heating at a moderate temperature, water soluble ash may be separated and the residue again ignited or the ash broken up, with the addition of alcohol and again ignited.^{5,6}

Method

2.5 g of the air-dried crude drug was weighed in a tared silica dish and maintained at a temperature not exceeding 450⁰C until free from carbon. After incineration the material was cooled and weighed. The percentage of ash value was calculated with reference to the air-dried drug.

Determination of Extractive values

The determination of water-soluble or alcohol-soluble extractive is used as a means of evaluating drugs, the constituents of which are not readily estimated by other means. But as suitable as said becomes available, the extractive tests are no longer required as pharmacopoeial standards.⁷

In-Vitro Antimicrobial Activity

An antimicrobial is a substance that kills or inhibits the growth of micro-organisms such as bacteria, fungi or protozoans. Antimicrobial drugs either kill microbes (microbiocidal) or prevent the growth of microbes (microbiostatic). Disinfectants are antimicrobial substances used on non-living objects or outside the body.⁸

Antimicrobial Activity Investigation Methods

Three types of method for investigation of antimicrobial activity^{9,10}

- Broth Dilution Technique
- Cup Plate Diffusion Technique
- Turbidimetric Assay or Technique

Cup Plate Diffusion methods are mostly used for investigation of antimicrobial activity of plant extracts. Cup plate method is easy to note the result and need small amount of extract.

Cup Plate Diffusion Method

All the glass wares and the petri plates were sterilized by dry heat in an oven at 160⁰C for one hour. Nutrient agar was

prepared in distilled water. The nutrient agar was poured in sterile petri plates aseptically and allowed to solidify at room temperature. All the petri plates were flooded with 0.1 ml of the standardized culture. The holes of 7 mm were bored aseptically using sterile cork borer. The agar plugs were taken out carefully so as not to disturb the surrounding medium. The holes were filled completely with desired extract and kept in incubator at 30⁰C for 48 hours. After this the Petri plates were observed for the antibacterial activity and zone of inhibition was measured.

Bacterial Culture

Gram positive: *Staphylococcus aureus*

Gram negative: *Escherichia coli*

Composition of nutrient agar media

Yeast/meat/beef extract	- 10 g
Peptone	- 10 g
Sodium chloride	- 5 g
Agar	- 20 g
Distilled water	- 1000 ml

Procedure

Plates are prepared with nutrient agar media medium of about 4 mm layer. Different dilutions of all the four extracts were prepared. The dried extracts were dissolved in 5 % dimethyl sulphoxide (DMSO) to the concentration 200 mg / ml and finally sterilized by filtration. The sterile discs (6 mm in diameter) were impregnated with 20 µl of the above extracts to achieve desired concentration of 4 mg / ml. The extracts were then inoculated for 24-48 hours and zones of inhibition were recorded.^{8,9} Sterile non-toxic cotton swab on a wooden applicator dipped in prepared inoculums and rotated soaked swab firmly against the upper inside wall of test tube. Streak the entire agar surface of the plate with a swab two to three times, turning plate at 60⁰ angle between each streaking. The inoculums allowed drying for 5-15 minutes with lid in place. Properly bore the plate with borer and disc is applied for standard drug. Cloramphenicol (30 mcg / disc) was used for standard antibiotics for activity being most resistant in both gram-positive and gram-negative species and inhibits bacterial protein synthesis by binding to the subunit of the ribosome. The antimicrobial activity was recorded by measuring the width of the clear inhibition zone around the disc using zone reader (Zone Size Interpretative Scale).

Table 1: Determination of Total Ash Value

Sr. No.	Reading	I	II	III
1.	Weight of crucible (g)	19.61	19.59	19.52
2.	Weight of crucible + air dried material (g)	20.57	20.53	20.56
3.	Weight of crucible + ash (g)	19.78	19.75	19.68
4.	Total ash (g)	00.17	00.16	0.16

The percentage total ash value was found to be 8.16% w/w

Table 2: Determination of Extractive Values

Extractive	Extractive Value (% w/w)
Water soluble extractives	12.00 %
Alcohol soluble extractives	10.16 %

Table 3: Determination of Extractive Values of four different extracts

Sr. No.	Extract	Colour of Extract	% (w/w) of extract obtained
1.	Petroleum ether	Green	1.5 %
2.	Chloroform	Green	1.4 %
3.	Methanol	Greenish brown	0.76 %
4.	Water	Brown	2.6 %

Table 4: Zone of Inhibition of extracts of *Ficus religiosa* by Cup plate diffusion method

Sr. No.	Solvent extracts	Conc of discs	Zone of Inhibition			
			<i>E. coli</i>		<i>S. aureus</i>	
			Std (mm)	Drug (mm)	Std (mm)	Drug (mm)
1.	Petroleum ether	4 mg / ml	-	-	20 mm	-
2.	Chloroform	4 mg / ml	30 mm	16 mm	27 mm	16 mm
3.	Methanol	4 mg / ml	27 mm	14 mm	27 mm	16 mm
4.	Water	4 mg / ml	27 mm	14 mm	27 mm	15 mm



E.coli



S. aureus

Figure 1: Zone of Inhibition Measurement of Blank DMSO



E.coli



S. aureus

Figure 2: Zone of Inhibition Measurement of Pet Ether Extract



E.coli



S. aureus

Figure 3: Zone of Inhibition Measurement of Chloroform Extract

*E. coli**S. aureus*

Figure 4: Zone of Inhibition Measurement of Methanol Extract

*E. coli**S. aureus*

Figure 5: Zone of Inhibition Measurement of Water Extract

RESULTS AND DISCUSSION

Medicinal plants are the local heritage with global importance as the world is endowed with a rich wealth of medicinal plants. Herbs have always been the principal form of medicine in India and presently they are becoming popular throughout the developed world, as people strive to stay healthy in the face of chronic stress and pollution and to treat illness with medicines that work in concert with the body's own defense. Little more needs to be said about the present day importance of medicinal plants, for it will be apparent from the foregoing, that the plant themselves either in the form of crude drugs or even more important, for the medicinally active materials isolated from them, have been and will always be an important aid to the physician in the treatment of disease. A wide range of medicinal plant parts is used which possess varied medicinal properties. The different parts used include root, stem, bark, flower, fruit, twigs exudates and modified plant organs. The present work was carried out on the leaves of *Ficus religiosa* family: Moraceae. The emphasis was given on the Standardization and *in-vitro* antimicrobial studies on the leaves of *Ficus religiosa* to find out their usefulness to the human being. This plant was collected from Pune, Maharashtra, India. Herbarium of the plant specimen was deposited at Botanical Survey of India, Pune, India. Standardization of leaf was done with the help of extractive values and total ash values. Antimicrobial activity was performed by two strains of microorganisms in which gram positive (*S. aureus*) and gram negative (*E. coli*) strains were studied. The chloroform extract showed good activity as compared to methanol and water extracts. The water extract showed a better activity than methanol extract. The petroleum ether extract does not show any activity. The extracts are more potent towards the gram positive bacteria (*Staphylococcus aureus*).

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