Pharmacognostical standardization of Ficus religiosa fruits

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ABSTRACT

Context: Ficus religiosa belonging to family Moraceae is a large glabrous tree, found throughout in India in the vicinity of temples. It is well known for curing a variety of ailments such as diarrhea, dysentery, vaginal and other urinogenital disorder, eczema, leprosy, rheumatism and used as anticonvulsant. Aim: The present study was undertaken to investigate the Pharmacognostical and Phytochemical parameters of fruits of Ficus religiosa. Settings and Design: Pharmacognostical investigations were carried out to study its macroscopical and microscopical characters. Various physiochemical parameters and histochemical color reactions were evaluated as per the IP method. Results: Macroscopical studies revealed that the fruit is purple colored, depress and globose shaped, 2-3 cm in diameter and sweet in taste. The results of microscopical studies showed the presence of epidermis, stone cells, pitted parenchymatous fibers, parenchymatous tissue, spiral vessels etc. The results of physiochemical parameters showed total ash- 6.74% w/w, water soluble ash- 5.40% w/w, acid insoluble ash-1.85%w/w, petroleum ether soluble extractive- 1.08% w/w, 90% methanol extractive-4.50% w/w, water soluble extractive-6.50% w/w. The qualitative evaluation of the extract indicated the presence of carbohydrates, steroids, free amino acids and phenolic compounds. Total phenolic content in the fruit was found to be 0.2% w/w. Keywords: Ficus religiosa fruits, Moraceae, Phytochemical evaluation, Total phenolic content

INTRODUCTION

After decades of serious obsession with the modern medicinal system, people have started looking at the ancient healing systems like Ayurveda, Siddha and Unani. This is because of the adverse effects associated with synthetic drugs. Herbal drugs play an important role in health care programs especially in developing countries. Ancient Indian literature incorporates a remarkably broad definition of medicinal plants and considers all plant parts to be potential sources of medicinal substances. However, a key obstacle, which hindered the acceptance of the alternative medicines, is the lack of documentation and stringent control. Therefore, there is a need for documentation and stringent quality control. With this backdrop it becomes extremely important to make an effort towards standardization of the herbal drugs. The process of standardization can be achieved by stepwise pharmacognostic studies. The pharmacognostical studies are one of the major criteria for identification of herbal drugs. Medicinal plants form a large group of economically important plants that provide the basic raw materials for indigenous pharmaceuticals. One approach to the discovery of new drugs is the study of the bioactive constituents of higher plants. The investigation of plants used as remedies in the traditional folk medicine can be an interesting tool to identify several biologically active molecules from the 250,000 higher plant bioactive constituents with antiinflammatory, analgesic, antipyretic and anti ulcerogenic activity. Ficus religiosa (F. religiosa) commonly known as peepal is a very big sacred tree and found throughout India in the vicinity of temples. F. religiosa leaf juice along with honey is used for treatment of asthma, cough, sexual disorders, diarrhoea, haematuria, earache and toothache, migraine, eye troubles, gastric problems and scabies. Fruits are used for the treatment of asthma and respiratory disorders. Fruit paste is taken to cure scabies. Stem bark is used in the treatment of gonorrhoea, bleeding, cuts, wounds, paralysis, diabetes, diarrhoea, bone fracture and used as antiseptic, astringent and antidote. Bark paste along with honey is used to treat...
cough and cold as well as accompanying mild fever. Aerial root juice is used for treatment of menstrual problems.[9] In the present study an attempt has been made to highlight this medicinal fruit through pharmacognostic and phytochemical studies. As per the available literature no pharmacognostical study has been carried out on the fruits; hence the present investigation was undertaken to evaluate various pharmacognostical standards like macroscopy and microscopy of fruits; ash values, extractive values, microscopical characteristics of powdered fruits and preliminary phytochemical analysis of *F. religiosa* fruits.

**MATERIAL AND METHODS**

**Collection of plant material**
The fruits of *F. religiosa* were collected from Chandigarh in the month of Nov-Dec, 2008 depending upon its easy availability. Fruits were authenticated by Dr. Promila Pathak, Dept. Of Botany, Punjab University, Chandigarh. The fruits were shade dried, coarsely powdered and stored in an airtight container.

**Chemicals and instruments**
Compound microscope, glass slides, cover slips, watch glass and other common glass ware were the basic apparatus and instruments used for the study. Microphotographs were taken using a motic images microscope. Solvents used for extraction includes viz. petroleum ether, chloroform, ethyl acetate, ethanol (95%), water and reagents viz. phloroglucinol, glycerine, HCl, chloral hydrate and sodium hydroxide were procured from Central Drug House (P) Ltd., New Delhi, India.

**Macroscopic and Microscopic analysis**
The macroscopy and microscopy of the fruit and powder were studied according to the method of Brain and Turner.[10] For the microscopical studies, cross sections were prepared and stained as per the procedure of Johansen. [11] The micropowder analysis was done according to the method of Brain and Turner[12] and Kokate.[13]

**Physico-chemical analysis**
Physico-chemical analysis i.e. percentage of ash values and extractive values were performed according to the official methods prescribed[14] and the WHO guidelines on the quality control methods for medicinal plant materials.[15]

**Preliminary phytochemical screening**
Preliminary phytochemical screening was carried out by using standard procedures described by Kokate[16] and Harborne.[17] The shade dried and powdered fruits of *F. religiosa*, were subjected to maceration with different solvents like petroleum ether (60–80°C), 90% methanol and finally macerated with water so as to get respective extracts. All extracts were filtered individually, evaporated to dryness. After drying, the respective extracts were weighed and percentage yields were determined separately and stored in freeze condition for further use. The qualitative chemical tests, for identifying the presence of various phytoconstituents, were carried out on various extracts of *F. religiosa* fruits. The extracts were screened for the presence of tannins, saponins, sterols/triterpenes, alkaloids, glycosides, flavonoids, polyphenolic compounds, protein/amino acids and carbohydrates on Silica gel G (Merck) plates (0.25 mm thickness). Development was carried out with various solvent systems viz: ethyl acetate: formic acid: methanol (6: 0.6: 0.4 v/v/v), ethyl acetate: methanol: water (10: 1.3: 1.0 v/v/v), ethyl acetate: formic acid: acetic acid: water (10: 1.1: 1.1: 2.7 v/v/v), chloroform: methanol: water (6.4: 5.0: 1.0 v/v/v), benzene: ethyl acetate (8.6: 1.4 v/v) and ethyl acetate: methanol: water: acetic acid (6.5: 1.5: 1.5: 1.0 v/v/v). After development in the different solvents, the plates were sprayed with Dragendorff’s reagent, AlCl₃, hydroxylamine–ferric chloride, ninhydrine and antimony trichloride reagents for the discovery of alkalds, flavonoids, proteins/amino acids and sterols/triterpenes respectively. Detection of glycosides, saponins, tannins, and carbohydrate are carried out using KOH, anisaldehyde–sulphuric acid, ferric chloride, and naphthoresorcinol reagents, respectively.[18]

**Estimation of total phenolic compounds**

**Principle:**
The total phenolic contents in the fruits of *F. religiosa* were determined by using Folin Ciocalteu’s method. Folin Ciocalteu reagent is a mixture of phosphomolybdate and phosphotungstate used for the colorimetric assay of phenolic and polyphenolic antioxidants. It works by measuring the amount of substance, being tested needed to inhibit the oxidation of the reagent. The sample extract dilution was oxidized with Folin Ciocalteu reagent and the reaction was neutralized with sodium carbonate. The absorbance of the resulting blue colour was measured at 765 nm after 30 min.

**Preparation of standard solution:**
Gallic acid was used to make the calibration curve. 10 mg of gallic acid was dissolved in 100 ml of 50% methanol (100 μg /ml) and then further diluted to 1, 2, 4, 6, 8 and 10 μg /ml. 1 ml aliquot of each dilution was taken in a test tube and diluted with 10 ml of distilled water. Then, 1.5 ml Folin Ciocalteu reagent was added and allowed to incubate at room temperature for 5 min. 4 ml of 20% (w/w) Na₂CO₃ were added, adjusted with distilled water up to the mark of 25 ml, agitated and left to stand for 30 min at room temperature. Absorbance of the standard was measured at 765 nm and distilled water was taken as a blank.

**Preparation of sample solution:**
1 g of sample (fruit powder) was added to 15 ml of methanol (50%) and extracted for three times by maceration of 2 hours.

Then filtered and make up the volume with methanol (50%) in volumetric flask upto 50 ml. 1 ml aliquot of the sample was taken in a test tube and diluted with 10 ml of distilled water. Then, 1.5 ml Folin Ciocalteu reagent was added and allowed to incubate at room temperature for 5 min. 4 ml of 20% (w/v) Na₂CO₃ were added, adjusted with distilled water up to the mark of 25 ml, agitated and left to stand for 30 min at room temperature. Absorbance of the sample was measured at 765 nm. Three parallel determinations were recorded. Quantification was done on the basis of a standard curve of gallic acid. Results were expressed as mg gallic acid equivalents (GAE) and percentage w/w. [19]

Calculation: Total phenolic contents (%) = GAE × V × D × 10⁻⁶ × 100/W

GAE - Gallic acid equivalent (μg/ml)
V - Total volume of sample (ml)
D - Dilution factor
W - Sample weight (g)

RESULTS AND DISCUSSION

Brief taxonomic description of the plant

The Sacred Figure (F. religiosa) or Bo-Tree (from the Sinhala bo) is a species of banyan figure native to Bangladesh, India, Nepal, Pakistan, Sri Lanka, southwest China and Indochina. In India, it occurs both wild and cultivated up to 5,000 ft (1,524 m). [20] F. religiosa is cultivated in various tropical areas of the world. In the United States, it is grown in southern California, Florida, and Hawaii. In Florida, seedlings were found in Homestead in 1975 and in Miami in 1988. [21] F. religiosa is a large, glabrous tree, with characteristic milky latex and the trunk often covered with epiphytes. It is a large dry season-deciduous or semi-evergreen tree with a pale stem up to 30 m tall and with a trunk diameter of up to 3 m, often appearing fluted on account of the numerous roots which have fused with the stem. The bark is light grey and peels off in patches. The leaves are leathery 4–8 inches long by 3–5 inches wide, somewhat egg-shaped or rounded, tailed at the tip and heart-shaped at the base, or sometimes rounded are large; alternate, with long petioles and a broadly ovate, subcoriaceous lamina cordate in shape. The tip of leaf is long, lanceolate and cuspidate. The margin sinuate and the base truncate. The young leaves are frequently pink, change to copper and finally to green. Flowers minute within the receptacle. Receptacles-sessile, dark purple when ripe, basal bracts, broadly ovate-elliptic obtuse. Male flowers-Sessile K₂₃₅ ovate, lanceolate. A₁, anther single. Female flower C₂₉₃ and gall flowers sessile or pedicillate. K₃₄, lanceolate, gall flowers without perianth, style short, stigma round. [22,23] The fruit is a small figure 1-1.5 cm diameter, green ripening purple.

Description of Fruit

Macromorphology:

F. religiosa fruits are syconus inflorescence containing drupe fruits, having depressed and globose shape with 2-3 cm in diameter. The colors of the fruit are green when unripe and purple when ripe. The outer surface of the fruit is smooth and the position of fruit is sessile in axillary pairs (Figure 1).

Fruits of this plant are odorless and ripe fruits are sweet in taste. The fruits (figures) are small, axillary, paired, sessile, obovoid or globose, purplish when ripe. The fruit, is developed from an entire inflorescence, the fleshy part being hollow receptacle, the entire inflorescence axis, to the interior of which very numerous small flowers are attached. The fruits of these flowers are drupes, the stones of which are minute seeds present in figures. These stones are about 1.5 to 2.0 mm long each contains an endospermic seed with a curved embryo. At one point of the surface may be seen the orifice of the receptacle surrounded by small bracts and at another part the short remains of the stalk is usually present. When young, the receptacle contains laticiferous vessels filled with milky latex; as it ripens the latex disappears, the fleshy walls fills with sugar and becomes edible.

Micromorphology:

Transverse section of F. religiosa fruit showed that the internal structure was divided into four compartments viz. Epidermis, Hypodermis, Pericyclic fibres and Ground tissue (Figure 2). Epidermis was single layered covered with thin cuticle followed by 4-5 layered hypodermis which consists of compactly arranged collenchymatous cell surrounded by 12-15 layered sclerenchymatous pericyclic fibres. The innermost layer was ground tissue which was made up of parenchymatous cells (Figure 3) in which stone cells (Figure 4) and spiral vessels (Figure 5) were scattered. Furthermore transverse section of single drupe fruit showed the presence of innermost curved embryo surrounded by

Figure 1: T.S. of whole inflorescence X 10X

Thick walled, polygonal sclerenchymatous cells (Also revealed in the L.S. Figure 6).

**Physico-chemical studies**

Ash values of a drug give an idea of the earthy matter or the inorganic composition and other impurities present along with the drug. The percentage of total ash, acid insoluble ash and water soluble ash were carried out (Table 1). The percentage extractive values were calculated and shown in (Table 2). Also the three extracts viz. petroleum ether, 90% methanolic and aqueous extracts were weighed and percentage yields were determined separately. The color, consistency and appearance of the extracts were reported in (Table 3).

**Preliminary phytochemical screening**

Preliminary phytochemical screening revealed the presence of steroids, carbohydrates, amino acids and phenolic compounds. Results showed the presence of carbohydrates in aqueous extract, steroids in pet ether (60-80°C), amino acids and phenolic compounds in both aqueous and 90% methanolic extracts.

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**Table 1: The percentage ash values of fruits of F. religiosa**

<table>
<thead>
<tr>
<th>Type</th>
<th>Yield % w/w</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total ash</td>
<td>6.74</td>
</tr>
<tr>
<td>Water soluble ash</td>
<td>5.40</td>
</tr>
<tr>
<td>Acid insoluble ash</td>
<td>1.85</td>
</tr>
</tbody>
</table>

**Table 2: The percentage extractive values of fruits of F. religiosa**

<table>
<thead>
<tr>
<th>Type</th>
<th>Yield % w/w</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol(hot)</td>
<td>4.50</td>
</tr>
<tr>
<td>Methanol(cold)</td>
<td>3.85</td>
</tr>
<tr>
<td>Aqueous(hot)</td>
<td>6.50</td>
</tr>
<tr>
<td>Aqueous(cold)</td>
<td>5.50</td>
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methanol extract (Table 4). The tested plant showed positive results for variable amounts of unsaturated sterols and polyphenols. In the methanolic extract amino acids and phenolic compounds are present in considerable amounts. Alkaloids, flavonoids, tannins, saponins, proteins and glycosides were not found in any of the tested extracts. The presence of these constituents was further confirmed by TLC studies on various extracts. TLC showed the presence of steroids mainly in petroleum ether extract, two prominent spots appears after derivatization with anisaldehyde-sulphuric acid reagent. Presence of polyphenols was confirmed in methanolic after spraying of KOH and anisaldehyde-sulphuric acid reagent; five spots appeared on the plate. Amino acids and polyphenols were also found in aqueous extract, while all other phytoconstituents were absent from all extracts.

**Total phenolic content of F. religiosa fruits**
The total phenolic content estimated in fruits of *F. religiosa* was 0.2% w/w of dry fruit powder. The calibration curve of standard gallic acid is shown in Figure. 7.

**CONCLUSION**
The Pharmacognostical study of *F. religiosa* fruit was done for the purpose of standardization. Standardization of natural products is a complex task due to their heterogenous
composition, which is in the form of whole plant, plant part/extracts obtained thereof. To ensure reproducible quality of herbal products, proper identification of starting material is essential. The parameters studied were macroscopy, microscopy, physico-chemical properties and phytochemical screening. The results of macroscopical study revealed that the fruit, known as syconus, is developed from an entire inflorescence, the fleshy part being hollow receptacle (the entire inflorescence axis) to the interior of which very numerous small flowers are attached. Fruit is green colored when unripe and purple upon ripening. Ripe fruit is sweet in taste and found odorless. The outer surface is smooth; shape is depressed and globose and 2-3 cm in diameter. Thus our findings confirm the characters of fruits of moraceae family.[24]

Microscopical studies revealed the presence of single layered epidermis covered with thin cuticle followed by 4-5 layers of hypodermis, sclerenchymatous pericyclic fibers 12-15 layers and stone cells and spiral vessels are scattered in ground tissue. Area of ground tissue is 3/4th of total fruit and each single drupe is embedded in ground tissue.

The fruit was also characterized for it physico-chemical properties. Water soluble and alcohol soluble extractive values increased gradually with ripening of fruit. The results of phytochemical screening of petroleum ether (60-80°C), methanolic and aqueous extracts have shown the presence of carbohydrates, proteins, amino acids, phenolic compounds and steroids by positive reaction with the respective test reagent.

The result of Folin Ciocalteu method revealed the presence of total phenolic compound content 0.2% w/w which was not reported earlier. These are the antioxidant compounds which act as free radical terminators.[25] This plant can also play a role in plant defensive mechanism by counteracting reactive oxygen species (ROS), thus minimizing molecular damage due to microorganisms, insects and herbivores.[26]

The fruits of F. religiosa were selected for the pharmacognostical standardization on the basis of literature review, as no such study has been reported earlier.

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