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PHYTOCHEMICAL STANDARDIZATION OF POWDERED MIXTURE OF *FICUS RELIGIOSA* LEAF & BARK

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ABSTRACT

To evaluate various standardization parameters of powdered mixture of Ficus religosa leaf and bark. Method: Physico-chemical tests such as foreign organic matter, ash and extractive values, moisture content and fluorescent analysis as well as preliminary phytochemical analysis were used to evaluate the quality control parameters. Results: Physico-chemical constants such as foreign organic matter, ash and extractive values, moisture content and fluorescent analysis were established. Phytochemicals like alkaloid, steroids, flavanoids and tannins were found to be present in the extracts. Conclusion: Proven standardization parameters have a key role in identifying and authenticating the right plant material in the present research.

INTRODUCTION

It has now become common knowledge that herbal medicines are completely safe and have no side effects. 80% of the world's population is still relying on herbal medicines to treat illness, according to the WHO 2002 survey. In the current scenario, demand for herbal products is rising exponentially across the globe, and leading pharma companies are constantly

conducting extensive research into the possible medicinal value of plant materials. Therefore, it is essential to have quality control for the efficacy and safety of herbal products [1].

Ficus religiosa Linn. is an important species of medicinal tree which belongs to the Moraceae family. Because of its mythological and traditional heritage, it is widely known as the Peepal tree and is one of Asia's most revered trees. It is also known as the sacred fig tree or Bo tree, and in Indian cities and villages, it is the most planted tree species near religious or spiritual places. Since ancient times, a large number of medicinal plants have been used to treat different diseases [2,3].

Of all medicinal plants, *Ficus religiosa* (Peepal) holds an important role. There is therapeutic potential for almost every part of this tree and it is used in the preparation of herbal medicines. In curing a wide range of diseases, the therapeutic properties of this tree can be due to its richness in bioactive compounds, namely flavonoids, alkaloids, tannins, saponins, phenols, etc. It has become a common herbal tree owing to its antimicrobial, anti-diabetic, anticonvulsant, wound healing, anti-inflammatory and analgesic properties. Various parts of this plant are used in the modern pharmacological industry as an essential ingredient [2,4]. In order to complement different identification and standardization details, the current study used various physico-chemical and phytochemical parameters.

MATERIALS AND METHODS

Plant material

The fresh bark and leaves of *Ficus religiosa* plant were collected in the month of October 2019 from Kanpur, Uttar Pradesh and authenticated by Dr. Kamran Javed Naquvi (Associate Professor), Rama University, Kanpur. The leaves and bark of the plant were cleaned, dried under shade, mechanically reduced to moderate coarse powder and stored in amber colored airtight containers for further investigation.

Physico-chemical Analysis

The air dried moderately coarse powdered plant materials were used for quantitative determination of foreign organic matter, ash and extractive values and moisture content via reference methods as mentioned below [5-13]. The total ash value always bear variation because of possible presence of non-physiological substances like earthy matters. So counter this variation, other parameters such as acid insoluble and water soluble ash values were also determined. Extractive values with petroleum ether, chloroform, alcohol and water were also evaluated. Fluorescence analysis is a qualitative determinant of chemical nature of phytoconstituents present in the plant was evaluated by reference methods.

Foreign organic matter

The drug sample to be tested was measured and evenly distributed without overlap on a white tile. The foreign matter was manually isolated and examined with an unaided eye in daytime. In a Petri dish, the suspected particles were transferred. The weight of the foreign matter was taken after proper separation, and the percentage (percent) (w/w) was determined [5,13].

Moisture content

After accurately weighing, about 10 g of the drug (without preliminary drying) was placed in a tare evaporating dish and dried at 105 ° C. The drying and weighing continued at intervals of 1 h until there was no more than 0.25% difference between two successive weighings. When two consecutive weighings after drying for 30 min and cooling for 30 min in a desiccator showed no more than 0.01 g difference, a constant weight was supposed to have reached [5,10-13].

Ash values

Total ash

Around 2.0 grams of the crude substance was correctly measured in grams and then incinerated in a silica crucible at 450 degrees Celsius until free from carbon. The ash was cooled before being measured. This was replicated many times to avoid

variations from occurring. The percentage of total ash comparative to dosage for the air-dried drug was finally calculated [5,10-13].

Acid insoluble ash

25 ml of diluted hydrochloric acid was added to a crucible containing total ash. The insoluble substance was deposited on an ashless filter paper. It was washed with hot water until it became neutral and then ignited to a constant weight. The residue was left to cool for 30 minutes in a suitable desiccator, and was weighed immediately. This experiment was done repeatedly to obtain constant weight. Finally, the percentage of acid-insoluble ash was calculated with respect to the air-dried drug [5,10-13].

Water soluble ash

25 ml of water was added to the crucible containing the total ash and boiled for 5 min. On an ashless filter paper, the insoluble matter was collected. It was rinsed with hot water and then ignited at a temperature not exceeding 450 ° C for 15 minutes. To obtain a constant weight, the procedure was repeated. The difference was calculated between the weight of the ash and the weight of insoluble matter. Finally, the percentage of water-soluble ash was determined with reference to the air-dried drug [5,10-13].

Extractive value

The amount of active constituents extracted with solvents from a given qualtity of medicinal plant material is calculated by this process. For this, approximately 500 gm of dried coarse powdered material of bark and leaves of *Ficus religiosa* was successively extracted using the Soxhlet apparatus with solvents of increasing polarity order such as petroleum ether, chloroform, methanol and hydro-alcoholic (50%) for 24 hours each. The powdered material was air-dried below 50 ° C each time before extraction with the next solvent and then subjected to further extraction. The condensed extract was reduced to a semi-solid mass by drying at 40±5 ° C in a water bath and packed into separate air-tight containers. Finally, with regard to the air-dried drug, the percentage of extractive value for each solvent was determined. These extracts have been subjected to phytochemical screening to confirm the presence of various phytoconstituents [5,10-13].

Fluorescence analysis

On a grease-free clean microscopic slide, a small amount of dried and finely powdered crude drug was put and the same was treated separately with 1-2 drops of the freshly prepared reagent solutions, i.e. 0.1 N sodium hydroxide in water, Acetic Anhydride, Acetic Acid, 0.1 N Hydrochloric Acid and water. The added reagents were combined and waited for 1-2 minutes by gently tilting the slides. Then, inside the UV chamber, each slide was positioned and viewed in natural and ultraviolet lights. The colors observed by applying various reagents have been documented [5,10-13].

Phytochemical screening

The preliminary phytochemical studies for the identification of various organic plant constituents such as carbohydrates, proteins, alkaloids, glycosides, saponins, flavonos, tannins and steroids have been performed on petroleum ether, chloroform and hrdro-alcoholic (50%) extracts [5,10-13].

RESULTS

Physico-chemical evaluation

The parameters for physic-chemical evaluation such as foreign organic content, total ash, acid insoluble ash and water soluble ash and moisture content are listed in table 1. The foreign organic content was recorded to be nil. The total ash value was 11.0%. While acid insoluble and water soluble ash values were 5% and 6%, respectively. The moisture content was found to be 1.64%. The successive extractive values of powdered mixture of leaf and bark of the plant for various solvents such as petroleum ether, chloroform, methanol and hydro-alcohol (50%) were found to be 7.2%, 10.4%, 4.8% and 11.2%, respectively (Table 2).

The organoleptic characteristics of the obtained extracts were also mentioned in the table 2. The highest extractive value was found to be for hydroalcoholic extract and lowest was for methanolic extract. The results for fluorescence analysis were mentioned in table 3.

Table 1: Foreign organic content, ash values and moisture content for powdered mixture of *F. religiosa* leaf & bark

Foreign organic content	Total ash	Acid insoluble	Water soluble ash	Moisture content
Nil	11.0% w/w	5.0% w/w	6.0% w/w	1.64% w/w

Table 2: Successive extractive values of powdered mixture of *F. religiosa* leaf & bark

Solvent	Color of extract	Odor	Consistency	Sense of touch	% yield
Petroleum ether	Brownish dark green	Characteristic	Semisolid	Sticky	7.2
Chloroform	Brownish dark green	Characteristic	Semisolid	Sticky	10.4
Methanol	Reddish Brown	Characteristic	Semisolid	Sticky	4.8
Hydro-alcohol (50%)	Reddish Brown	Characteristic	Semisolid	Sticky	11.2

Table 3: Fluorescence analysis of powdered mixture of *F. religiosa* leaf & bark

Sample	Observation under Day/Visible light	Observation under UV
Powder	Pale green	Green
Powder+ 0.1N sodium Hydroxide	Green	Dark Green
Powder+ Acetic anhydride	Pale green	Green
Powder+ Acetic acid	Green	Brownish Green
Powder+0.1N Hydrochloric acid	Pale grey	Dark Green
Powder+Water	Yellowish green	Green

Phytochemical analysis

The preliminary phytochemical analysis of petroleum ether, chloroform, methanol and hydroalcoholic extracts showed the presence of various phytochemicals as listed in table 4. The tests for phytosterols were recorded positive in all four extracts. Carbohydrates, proteins, glycosides, and alkaloids were found to be present in methanolic and hydroalcoholic extracts. The flavanols were present only in methanolic extracts. The phytochemical tests for phenolic compounds and tannins were positive for all except petroleum ether extract.

Table 4: Phytochemical test of powdered mixture of *F. religiosa* leaf & bark

Phytochemical test	Result			
	Petroleum ether	Chloroform	Menthol	Hydro-alcohol (50%)
Test for Carbohydrates				
Molish's test	-	-	+	+
Benedict's test	-	-	+	+
Fehling's test	-	-	+	+
Test for Glycoside				
Legal's Test (test for cardenoloids)	-	-	+	+
Keller killiani's Test (for deoxysugars)	-	-	+	+
Brontrager's Test	-	-	+	+
Froth test	-	-	+	-
Test For Protein				
Biuret test	-	-	+	+
Test for Phytosterol				
Libermann-Burchard Test	+	+	+	+
Test for Phenolics & Tannins				
Ferric chloride test	-	+	+	+
Gelatin test	-	+	+	+
Iodine test	-	+	+	+
Nitric acid test	-	+	+	+
Test for Alkaloids				
Mayer's Reagent	-	-	+	+
Dragendroff's Reagent	-	-	+	+
Hager's Test	-	-	+	+
Wagner's Test	-	-	+	+
Test for Flavonoids				
Shinoda's Test	-	-	+	-
Lead acetate Test	-	-	+	-

Note: (+) positive test, (-) Negative test

DISCUSSION

The quality control of crude drugs and herbal formulations is of utmost importance in validating their suitability in modern medicine. The main problem faced by the herbal drug industry is a lack of quality control for herbal materials and the formulations they produce. The standardization is the basis of ensuring the quality of the drugs that are produced from the plants and also includes the field of study from the beginning of life of a plant up to its clinical application [10].

Ficus religiosa is a common plant recorded with medical and clinical significance and proven by strong scientific evidence. Different physico-chemical and phytochemical standardization parameters of the powdered mixture of leaves and bark of the plant have been examined in the current investigation. Foreign organic content depicts the unwanted parts of the same plant or the presence of parts of other

plants which have been found to be nil. At favorable temperature conditions, the moisture content in plant material can lead to the activation of enzymes and build an acceptable atmosphere for the growth of microorganisms that may be responsible for the deterioration of the drug [10-12]. Total ash reveals the existence of inorganic salts like carbonates, phosphates, and silicates of potassium, calcium, magnesium, sodium, etc. The residue obtained after boiling the ash with hydrochloric acid and igniting the insoluble component is acid insoluble ash. This provides a measure of sand and other silica matter. Water soluble extractive value suggests the presence of water soluble inorganic salts such as carbonates, phosphates, and chlorides of potassium, calcium, magnesium, sodium, etc [10-12]. Successive extractive value revealed the percentage yield in various solvents in order of their polarity when extracted successively by using Soxhlet method. Under UV radiation and ordinary visible light, the fluorescence properties of the crude powdered drug have been determined. They produced different color radiations when the powdered drug was treated with various chemical reagents and analyzed under UV and ordinary light. For the crude powder, the color transition was recognizable and reproducible, exposing the phytoconstituents' solvent properties. The phytochemical investigation confirms the presence of various organic phytoconstituents like alkaloids, glycosides, tannins, sterols and flavonoids in various extracts of the powdered mixture of leaves and bark *Ficus religiosa*.

CONCLUSION

In the current investigation, different quality control parameter including physico-chemical and phytochemical analysis have been performed on powdered mixture of leaves and bark of *Ficus religiosa*. In obtaining authentic drug samples of *F. religiosa* and establishing the pharmacopoeial standards for further research, these parameters might be useful for future use.

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