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# Development of quality control parameters for the standardization of stem bark of *Ficus benghalensis* Linn.

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ABSTRACT

**Objective:** *Ficus benghalensis* (*F. benghalensis*) the banyan, is a large and extensive growing tree of the Indian subcontinent. Although many of the experimental studies validated its traditional medicinal uses, but employed uncharacterized crude extracts. This is the major drawback of most of the studies conducted worldwide. With the uncharacterized crude extracts it is very difficult to reproduce the results and identification of the bioactive metabolite. Hence, there is a need of phytochemical standardization and bioactivity guided identification of bioactive metabolites. **Methods:** In present research work, the detailed comparative pharmacognostic study of *F. benghalensis* Linn. (Stem bark) was carried out to lay down the standards which could be useful in future experimental studies. The study includes macroscopy, physicochemical evaluation, preliminary phytochemical screening, chromatographic evaluation and fluorescence analysis etc. **Results:** Macroscopic study revealed the presence of brownish colour, stimulant odour, astringent taste and rough with fracture texture of *F. benghalensis* Linn. stem bark. Foreign matter, total ash, water soluble ash, acid insoluble ash, water soluble extractive value, alcohol soluble extractive value and loss on drying were found to be 1.58%, 11%, 5.13%, 2.46%, 13.00%, 8.2% and 10 % w/w respectively. Phytochemical investigation of various extracts of *F. benghalensis* Linn. stem bark indicated the presence of alkaloids, flavonoids, steroids, phenolic compounds and tannins. The powdered drug is also characterized on the bases of fluorescence, chromatographic analysis and by the treatment with different reagents. **Conclusion:** The results of the following study will definitely provide a convincing support to its future pharmaceutical application.

## 1. Introduction

*Ficus benghalensis* (*F. benghalensis*) Linn. known as Vata in Sanskrit, is one of the reputed Panchavalkala drugs of ayurveda. It is native to India where it grows from low altitudes to 2 000 ft (610 m), especially in dry regions. It grows in planes, in roadsides etc. It is native to a wide area of Asia from India through Myanmar (Burma), Thailand, Southeast Asia, Southern China and Malaysia. Useful parts includes Aerial root, Bark, Leaves, Buds, Fruits, Latex[1,2]. Different parts of the plants are used for various medicinal purposes. The leaf primordium (leaf bud) is known as Vata shrunga in Ayurvedic system of medicine. As per Ayurvedic Nighantus, Vata shrunga has the property of curing daha (burns), thrishna (thirst), moorcha (faintness), raktapitta (haemorrhage), kapha and pitta[3,4]. The group of four *Ficus*, all yielding latex according to ayurveda consist of Nyagrodha (*F. bengalensis*), udumbara (*Ficus glomerata*/*Ficus racemosa*), Plaksha (*Ficus lacor*/*Ficus retusa*) and ashvattha (*Ficus religiosa*) the bark and leaves of this group are used as astringent, haemostatic, anti-inflammatory, ant-septic: prescribed in diarrhea, dysentery, and in the treatment of skin diseases, ulcers, vaginal disorders, leucorrhoea, menorrhagia, deficient lactation[5-9].

Although the stem bark of ficus species are important but very less studies has been reported so far on pharmacognostic and phytochemical parameters. Hence this study was undertaken to develop comparative pharmacognostical and preliminary phytochemical standards for the stem bark of *F. benghalensis* Linn. This may be useful to researchers for authentication of commercial sample and also to explore the possibility of its use in medicines.

## 2. Material and methods

### 2.1. Processing of plant material

The plant material *F. benghalensis* Linn. (Figure 1, Figure 2) was collected from Poanta Sahib, Himachal Pradesh, India and identified with authentication number GUH 20727 by the Botanist Dr. R. M Painuli, Incharge GUH, Herbarium Department of botany, H. N. B. Garhwal University (Central University) (U.K.) India. The

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steam bark is separately dried in shade, and powdered in mixture grinder. The powdered plant material was preserved in air tight container for future use.

**Figure 1.** *F. benghalensis* Linn. tree[16].

**Figure 2.** *F. benghalensis* Linn. bark (WHO/PLIM)[16].

### *2.2. Plant extracts chemicals and reagents*

The powdered plant material was extracted successively with petroleum ether, chloroform, acetone, methanol and distilled water. All the extracts thus obtained and kept in desiccators for future use. All the other chemical and reagents used in this study are analytical grade and used without further purification.

### *2.3. Development of standard analytical parameters*[10]

Macroscopical evaluation, microscopic studies, physical parameters such as foreign matter, ash values, swelling index, fluorescence analysis, determination of pH, extractive value, moisture content, heavy metal analysis of the plant material were performed according to the standard official methods[11,12]. Successive extraction of the powdered plant material (stem bark) was carried out in Soxhlet extractor with the help of various solvents of different–different polarity. Preliminary phytochemical analysis of obtained extracts was carried out according to the standard methods. Thin layer chromatography analysis of petroleum ether, chloroform, acetone, ethanol and aqueous extracts were carried out in various solvents according to the standard protocols[13–15].

## 3. Result and discussion

### *3.1. Macroscopic characteristics*

Macroscopic study revealed the presence of brownish colour, stimulant odour, astringent taste and rough with fracture texture of *F. benghalensis* Linn. stem bark (Table 1).

**Table 1**
Macroscopical characteristics of *F. benghalensis* Linn. stem bark.

| Particulars | Bark |
|---|---|
| Condition | Dried |
| Colour | Outer surface – Brownish |
| | Inner surface – Reddish brown, Yellowish brown |
| Odour | Stimulant |
| Taste | Astringent |
| Texture | Rough with fracture |
| Fracture | Brittle with fibrous |
| Size | Length 4 to 6 cm |
| | Thickness 0.8 to 2.5 cm |

### *3.2. Foreign organic matter*

Foreign organic matter means the material consisting of material not coming from the original plant source or not covered by definition of the herbal drug. It also includes insects, moulds and other animal contamination, parts of the organ or organs from which the drug is derived. The results of foreign matter were recorded in the form of %w/w (Table 2)

**Table 2.**
Foreign organic matter of powdered stem bark of *F. benghalensis* Linn.

| Foreign matter |
|---|
| 1.58 |

### *3.3. Ash value*

Ash value is used to determine quality and purity of a crude drug. The ash of *F. benghalensis* Linn. (stem bark) contains inorganic radicals like phosphates, carbonates and silicates of sodium, potassium, magnesium, calcium etc. The results of ash values were given in Table 3.

**Table 3.**
Ash Value of powdered stem bark of *F. benghalensis* Linn.

| Total Ash (%) | Water soluble Ash (%) | Acid insoluble ash (%) |
|---|---|---|
| 11 | 5.13 | 2.46 |

### *3.4. Extractive values*

This method determines the amount of active constituents extracted with solvents from a given amount of medicinal plant material. It is employed for materials for which as yet no suitable chemical or biological assay exists. The air dried, accurately weighed powdered drug was treated with solvents: petroleum ether, chloroform, acetone, ethanol and distilled water. The values were recorded in Table 4.

**Table 4.**
Extractive values of powdered stem bark of *F. benghalensis* Linn.

| Water soluble extractive value (%) | Alcohol soluble extractive value (%) |
|---|---|
| 13.00 | 8.2 |

### *3.5. Determination of moisture (loss on drying)*

The most common method for the determination of moisture is to heat the drug till one gets constant weight at 100 ℃. For the substances which undergo change with consequent loss of weight

at a temperature of 100 ℃, other methods are used. Loss in weight after drying was found to be 10% w/w which was not so high as to facilitate bacterial growth.

### 3.6. Fluorescence analysis

The drug powder was taken and treated with various chemical reagents like sulphuric acid, hydrochloric acid, nitric acid, 5% iodine solution, 10% sodium hydroxide solution, picric acid and ammonium solution, methanol, ethanol, chloroform, petroleum ether, distilled water and the color obtained was visualized under ordinary light, short UV light (254 nm) and Long UV light (366 nm) in UV chamber. The results were recorded in Table 5.

**Table 5.**
Fluorescence analysis of powdered stem bark of *F. benghalensis* Linn.

| S. No | Treatment | Visible Light | UV 254 nm | UV 366 nm |
|---|---|---|---|---|
| 1. | As such | Dark brown | Brown | Blackish brown |
| 2. | Methanol | Brown | Black | Brownish black |
| 3. | 10% NaOH | Light green | Light brown | Black |
| 4. | Conc. HCl | Blackish brown | Pale brown | Black |
| 5. | Conc. HCl + $H_2O$ | Brown | Black | Black |
| 6. | Conc. Nitric Acid | Light brown | Brownish black | Black |
| 7. | Conc. Sulphuric acid | Brown | Brown | Black |
| 8. | Chloroform | Light brown | Brownish black | Black |
| 9. | Petroleum ether | Brown | Black | Black |
| 10. | Distilled water | Yellowish brown | Brown | Black |
| 11. | Conc. Sulphuric acid + $H_2O$ | Light brown | Dark brown | Brownish black |
| 12. | 5% Iodine | Dark green | Brown | Yellowish brown |
| 13. | Picric acid | Yellowish brown | Brownish black | Black |
| 14. | Ferric chloride solution | Green | Brownish black | Black |
| 15. | Ammonia solution | Brown | Brown | Black |

### 3.7. Treatment of powdered drug with different reagents

The powdered drug was taken and treated with various chemical reagents like hydrochloric acid, nitric acid, sulphuric acid, acetic acid, picric acid, sodium hydroxide and the change in color was observed. The results were recorded in Table 6.

**Table 6.**
Treatment of powdered stem bark of *F. bengalensis* Linn. with different chemicals.

| S. No. | Treatment with chemicals | Observation |
|---|---|---|
| 1. | Drug + Conc. HCl | Brown |
| 2. | Drug + Conc. $HNO_3$ | Radish brown |
| 3. | Drug + Conc. $H_2SO_4$ | Light brown |
| 4. | Drug + Acetic acid | Light brown |
| 5. | Drug + Picric acid | Reddish brown |
| 6. | Drug + 5% NaOH | Light brown |

### 3.8. Phytochemical screening

The various extracts of stem bark of *F. bengalensis* Linn. were subjected to qualitative chemical examination for the presence or absence of alkaloids, carbohydrates, flavanoids, proteins, saponins, tannins, phenolic compounds and glycosides. The results of preliminary phytochemical screening were recorded in Table 7.

### 3.9. Thin layer chromatography

TLC studies of the petroleum ether, chloroform, acetone, ethanol and aqueous extracts were carried out in various solvents at 30 ℃ using silica gel G as adsorbent[17]. The solvent systems, developer used and the RF values are given in Table 8.

### 3.10. Heavy metal analysis

In the heavy metal analysis the concentration of arsenic, cadmium & lead was found to be 0.006 7, 0.008 6 and 0.063 6 ppm respectively.

## 4. Conclusion

The extensive literature survey revealed *F. benghalensis* Linn. to be a sacred and important medicinal plant used for the treatment of skin diseases, ulcers, diarrhea, dysentery, vaginal disorders, leucorrhoea, menorrhagia, deficient lactation etc. Various parts including stem bark of *F. benghalensis* Linn. are also used as astringent, haemostatic, anti-inflammatory and ant-septics. Besides of these immense medicinal properties, there is lack of research work in order to identify the pharmacogonostical and physiochemical properties of *F. benghalensis* Linn. Our present research work is an attempt for providing a set of data which will be of immense use in carrying out further research and revalidation of its use in Ayurvedic system of medicine. All these parameters which were being reported in this research article could be useful in identification of distinctive features of the drug.

**Table 8.**
Chromatographic analysis of various extracts of *F. benghalensis* Linn. stem bark.

| S. No. | Solvent system | Developer | $R_f$ values |
|---|---|---|---|
| 1. | Petroleum ether | 5% concentrated sulphuric acid in methanol | 0.09, 0.20, 0.29, 0.44, 0.68, 0.84 |
| 2. | Chloroform | 5% concentrated sulphuric acid in methanol | 0.34, 0.65 |
| 3. | Acetone | 5% concentrated sulphuric acid in methanol | 0.12, 0.25 |
| 4. | Methanol | 5% concentrated sulphuric acid in methanol | 0.20, 0.36 |

## Conflict of interest statement

We declare that we have no conflict of interest.

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**Table 7**
Phytochemical investigation of various extracts of *F. benghalensis* Linn. stem bark.

| Plant constituent tests | Extracts | | | | |
|---|---|---|---|---|---|
| | Petroleum ether extract | Chloroform | Acetone | Methanol | Water |
| 1. Alkaloids | | | | | |
| Hager's reagent | −ve | +ve | −ve | −ve | −ve |
| Wagner's reagent | −ve | +ve | +ve | +ve | +ve |
| Mayer's reagent | −ve | −ve | −ve | −ve | −ve |
| Dragendorff's reagent | | | | | |
| 2. Phenolic compounds and tannins | | | | | |
| Ferric Chloride solution | −ve | −ve | −ve | −ve | −ve |
| Lead acetate test | +ve | +ve | +ve | +ve | −ve |
| Acetic Acid Solution | −ve | −ve | −ve | +ve | −ve |
| Dil. Nitric acid | −ve | −ve | −ve | −ve | +ve |
| Bromine Water | +ve | +ve | +ve | −ve | +ve |
| Dil. Iodine | +ve | +ve | +ve | −ve | +ve |
| Pot. Permanganate | −ve | +ve | +ve | −ve | −ve |
| Gelatin Solution | +ve | −ve | +ve | −ve | −ve |
| Pot. Dichromate | −ve | −ve | −ve | −ve | −ve |
| 3. Flavonoids | | | | | |
| Shinoda test | −ve | −ve | −ve | −ve | −ve |
| Lead acetate test | −ve | −ve | −ve | +ve | +ve |
| Alkaline test | −ve | −ve | −ve | +ve | +ve |
| 4. Proteins | | | | | |
| Biuret test | −ve | −ve | −ve | −ve | +ve |
| Million's test | −ve | −ve | −ve | −ve | −ve |
| Test proteins containing sulphur | −ve | −ve | −ve | +ve | +ve |
| Precipitation test | −ve | −ve | −ve | +ve | +ve |
| 5. Amino acids | | | | | |
| Ninhydrin test | −ve | −ve | −ve | −ve | −ve |
| 6. Fats and oils | | | | | |
| Solubility test | +ve | −ve | −ve | −ve | −ve |
| Filter paper test | +ve | −ve | −ve | −ve | −ve |
| 7. Steroids | | | | | |
| Salkowski reaction | +ve | −ve | −ve | −ve | −ve |

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