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Pharmacognostical and physiochemical standardization of *Ficus benghalensis* Linn. seed

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ABSTRACT

The objective of the study is to perform pharmacognostical and physiochemical standardization of the seeds of Ficus benghalensis Linn. Fresh seed samples were studied macroscopically and microscopically. WHO recommended parameters were estimated for standardization of seeds. Physiochemical investigation was also performed. The detail microscopy of F. benghalensis seed revealed in sectional view the seed is triangular in outline with thick sclerotic sclerotesta and a thin layer of parenchymatous sarcotesta. The parenchyma cells possess dense accumulation of tannin. The powder preparations exhibits calcium oxalate crystals are abundant in the lumen of the seed coat cells. The crystals are 15 µm long and 10 µm broad. Physiochemical properties of F. benghalensis seed revealed the presence of ash content (2.55% w/w), water soluble ash (78.34% w/w), acid soluble ash (98.05% w/w), moisture content (7.68% w/w), loss on drying (4.93% w/w) and foreign matter (0.78% w/w). The pharmacognostical and physiochemical standards developed in this study will provide referential information for identification and standardization of F. benghalensis seed crude drugs.

Key words: *Ficus benghalensis*, Macroscopic study, Microscopic study, Physiochemical studies

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INTRODUCTION

Nature always stands as a golden mark to exemplify the outstanding phenomena of symbiosis. In the western world, as the people are becoming aware of the side effects of synthetic drugs, there is an increasing interest in the natural remedies with a basic approach towards the nature. WHO estimates that about 80% of people in developing countries still realize on traditional medicine based largely on plants and animals for their primary healthcare [1]. Herbal medicines are promising choice over modern synthetic drugs [2]. Herbals are comparatively safe because of their low toxicities. Herbal medicines are currently in demand and their popularity is increasing day by day. In the healthcare sector, WHO recommends and encourages the use of traditional herbs/remedies because of easy availability and affordability.

However, a key obstacle, which has hindered the acceptance of the alternative medicines in the developed countries, is the lack of proper documentation, stringent quality control and standardization. These problems arise from the complex composition of drugs which are used in the form of whole plant, parts of the plant(s) and of plant extracts. Standardization of the presumed active compounds of drugs in general does not reflect reality. Only in a few cases, drug activity depends upon single component. Generally, it is the result of concerted activity of several active compounds as well as of inert accompanying substances treating the particular disease. There is a growing concern for documentation of research work carried out on traditional medicines needed for regulatory control [3].

With this backdrop, it becomes extremely important to make an effort towards standardization of the plant material used for therapeutic purposes. The process of standardization can be achieved by stepwise pharmacognostic studies and minimizing the inherent variation of natural product composition through quality assurance practices applied to the cultivation and manufacturing processes.

Pharmacognosy is a simple and reliable tool, by which complete information of the crude drug can be obtained [4 – 6]. Anatomy studies are helpful in describing a particular drug with a special emphasize on quantitative microscopy, such as sclereids, starch grains, crystals, stomata and trichomes, and qualitative microscopy, such as xylem, phloem, and other tissues [7].

Ficus benghalensis Linn (syn. *F. indica*) belongs to the family Moraceae. The plant is a large evergreen tree attaining 15-30 m or more and distributed all over India from the sub Himalayan region and in the deciduous forest of Deccan and south India. It is the national tree of India. Almost every part of the tree is being used to treat different human ailments. The extract of bark, leaves, fruits and roots has been used to treat astringent, haemostatic, inflammatory, and septic; prescribed in diarrhea, dysentery, and in the treatment of skin diseases, ulcers, vaginal disorders, diabetes, leucorrhoea, menorrhagia and deficient lactation [8]. The seeds are brownish in colour. The seeds of *F. benghalensis* are refrigerant, demulcent, diuretic, pectoral and tonic, and they are prescribed as a diet for peptic ulcer in the Ayurvedic system of medicine [9]. Protein, pentose, mucilage and tannins are reported in the seeds [10].

Keeping in view the above mentioned problems, an attempt has been made to standardize the ethnopharmacologically useful seeds of *Ficus benghalensis* Linn, commonly available and widely used in all over India, based on pharmacognostical and physiochemical characteristics.

EXPERIMENTAL SECTION

Chemicals

All the chemicals used were of analytical grade and obtained from E. Merck limited, India and Hi-Media laboratories, Mumbai, India.

Plant material

The fresh seeds of *F. benghalensis* were collected from a forest in the Thennampattu village of Thiruvannamalai district of Tamilnadu, India. The seeds were identified and authenticated by Dr. Jayaraman, Director, Plant Anatomy Research Center, Chennai. The collected seeds were washed, shade dried and pulverized with a mechanical pulverizer for size reduction. The size pulverized seed powder was passed through mesh 40-60 and used for determination of physiochemical parameters. The fresh seed samples were used for macroscopic and microscopic studies [11].

Macroscopic analysis

The macroscopy of the seeds were studied according to the method of Brain [12]. The macroscopic evaluations involve the study of morphological characters and organoleptic studies, like colour, odour, taste, texture, etc.

Microscopic analysis

In microscopy, the fresh seeds were cut into pieces of 2-5 mm without compression and immediately transferred into FAA solution (Farmalin-5ml+ Acetic acid-5ml + 70% Ethyl alcohol-90ml) for one day to kill and fix the tissues. After 24 hrs of fixing, the specimens were dehydrated with a graded series of Tertiary Butyl Alcohol (TBA). Infiltration of the specimens was carried by gradual addition of paraffin wax (melting point 58 - 60 $^{\circ}$C) until TBA solution attained super saturation. The specimens were cast into paraffin blocks [13]. The paraffin embedded specimens were sectioned with the help of Rotary Microtome. The thickness of the sections was 10-12 μm. Dewaxing of the sections was performed by a customary procedure [14]. The sections were stained with Toluidine blue [15]. Wherever necessary the sections were also stained with safranin and fast-green with KI (for starch).

Powder microscopic analysis

The powder was passed through a sieve 125 and 180, separately, to obtain fine and very fine powder respectively and then subjected to powder microscopic examination. The sample was treated with following reagents and studied for their components of diagnostic value (50% glycerin as a temporary mountant). A drop of 90% HCl and 2%

phloroglucinol for lignin; 5% alcoholic ferric chloride for phenolic compounds; 2% iodine solution for starch grains and 0.08% ruthenium red and 10% lead acetate for mucilage [15].

Photomicrographs

Microscopic descriptions of the sections were supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikonlabphoto2 microscopic Units. For normal observations bright field was used. For the study of crystals, starch grains and lignified cells, polarized light was employed. Since these structures have bi-refringent property, under polarized light they appear bright against a dark background. Magnifications of the figures were indicated by the scale-bars [16].

Physiochemical studies

The percentage of foreign matter, loss on drying, Moisture content, ash content, water soluble ash and acid soluble ash were determined according to the method described in WHO guidelines on quality control methods for medicinal plant materials [17].

Statistical analysis

All the experiments were done in triplicates. Statistical analysis was performed using Graph Pad Prism Software, Version 4.0.3 (Graph Pad Software, San Diego, CA, USA).

RESULTS

Macroscopic evaluation of seeds

The surface of the seeds is smooth and shiny, dark brown in color which is approximately 2-5 mm long and 3-4 mm wide. The whole seed of *Ficus benghalensis* is elliptical, conical and sometimes oval in shape. The powder of seeds appeared brown in color, coarse in texture, slightly aromatic and pleasant in odour with bitter taste. The sample includes both seeds of neutral florets and female florets (Fig. 1.1). The seed without long style and elliptical outline with the dark lateral mark are seeds from neutral flowers (Fig. 1.2). A seed with short conical stalk and long and flat style are seeds from female flowers (Fig. 1.3).

Fig. 1.1: A cluster of seeds

Fig. 1.2: Seeds from neutral flowers

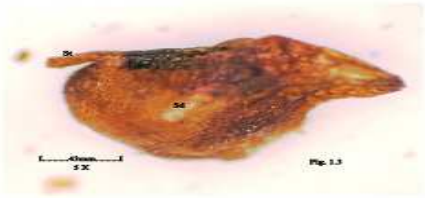

Fig. 1.3: Seed from female flowers

Figure 1 : Exomorphic features of the *F. benghalensis* seeds

(F- Seed from female flower, N- Seed from neutral flower, Sd- Seed, St- Stalk)

Microscopic evaluation of seeds

In sectional view the seed is triangular in outline with thick sclerotic sclerotesta and a thin layer of parenchymatous sarcotesta (Fig. 2.1 and Fig. 3.1). The parenchyma cells are angular, thick walled and highly compressed. The style

of the female floret is elliptical with outer parenchymatous zone and inner sclerenchyma cylinder. There is a central cavity which forms the stylar canal (Fig. 2.2).

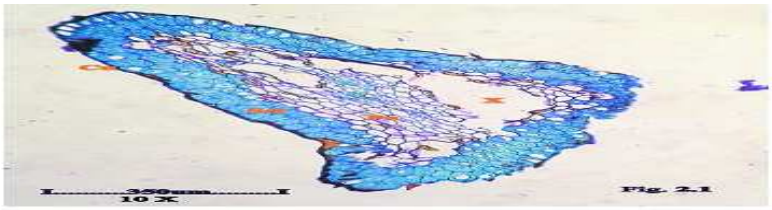

Fig. 2.1: L.S of the seed showing sclerotic outer layer and central parenchymatous zone possessing vascular bundles

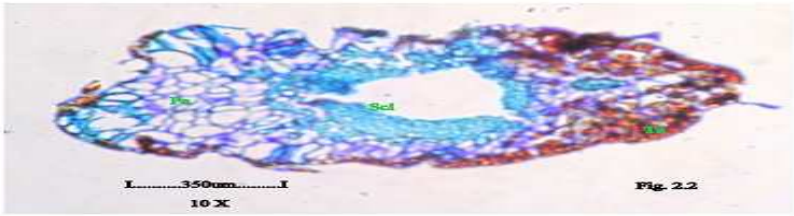

Fig. 2.2: C.S of the seed with central cavity surrounded by sclerotic zone and outer parenchyma zone

Figure 2: L.S and C.S of the *F. benghalensis* seed
(Cu- Cuticle, Pa-Parenchyma, Scl- Sclerotic zone, Ta-Tannin, X- Xylem)

The sclerotic layer has a thick darkly stained cuticle and 3 or 4 layers of circular brachy sclereids (Fig. 3.2). Inner to the sclerotic zone occur parenchymatous zone with well developed vascular strands (Fig. 2.1 and Fig. 3.2). The sclereids have thick secondary walls and narrow lumen. When viewed under polarized light the secondary walls of the sclereids exhibit bright light with radiating dark lines (Fig. 3.3). This feature indicates that the sclereids have lignified secondary walls.

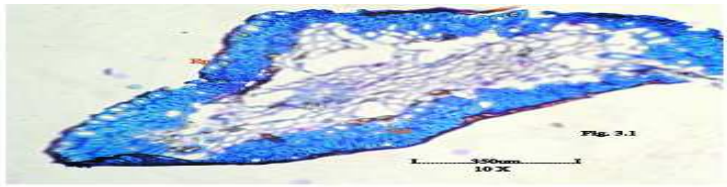

Fig. 3.1: One portion of the seed in sectional view enlarged

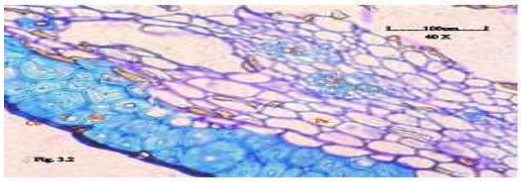

Fig. 3.2: Middle portion of the seed enlarged

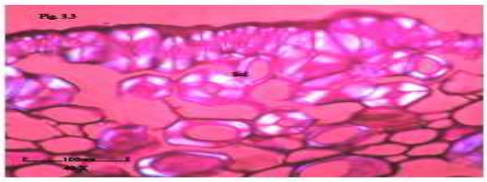

Fig. 3.3: Sclereids appearing bright viewed under polarized light microscope

Figure 3: *F. benghalensis* seed in different sectional view
(Cu- Cuticle, Ep- Epidermis, Pa-Parenchyma, Scl- Sclereids, X- Xylem)

The parenchyma cells possess dense accumulation of tannin. The cell contents exhibit light brown prominent masses of tannin (Fig. 4.1). The vascular strands possess a large cluster of polygonal, compact, thick walled lignified cells (Fig. 4.2). Phloem occurs at the ends of xylem strands.

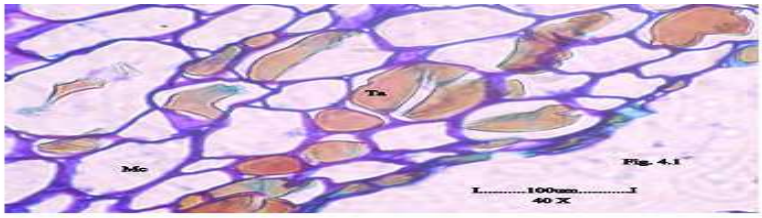

Fig. 4.1: Central parenchyma tissue of the seed possessing thick masses of tannin

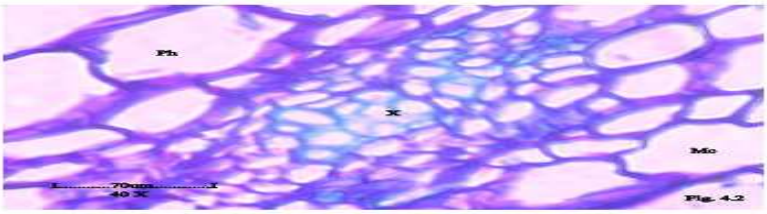

Fig. 4.2: Vascular strand found in the middle seed coat

Figure 4: *F. benghalensis* seed showing tannin and vascular strand
(Mc- Mesocarp, Ta-Tannin, X- Xylem)

Powder microscopic evaluation of seeds

The powder preparation of the seeds exhibits the following inclusions; Thick pieces of seed coat possessing sclereids are seen in different sizes. The sclereids are compact, elongated with thick secondary walls (Fig. 5.1). Brachy sclereids are more or less isodiametric with thick secondary walls and numerous canals like simple pits found in the secondary wall. The pits may be simple or branched. The lumen of the brachy sclereid is wide and sometime possesses some cell inclusions. The brachy sclereids are 50 µm in diameter (Fig. 5.2). Apart from brachy sclereids, there are also elongated, narrow, cylindrical sclereids. These sclereids have narrow lumen, thick lignified secondary walls. They are 140 µm long and 30 µm wide (Fig. 5.3). The elongated sclereids are seen mostly in groups.

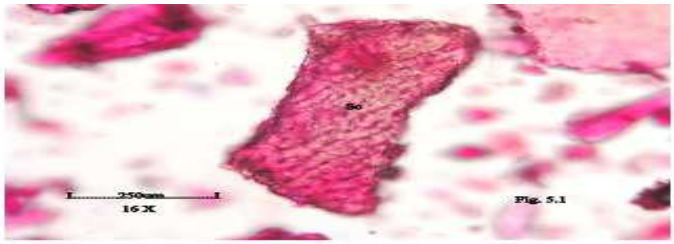

Fig. 5.1: A thick piece of seed coat comprising sclereids

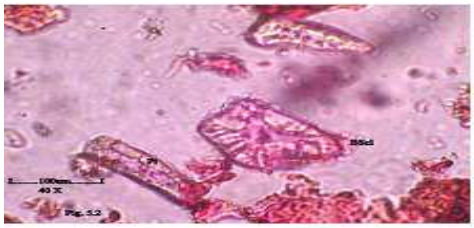

Fig. 5.2: Brachy sclereids, lumen and pits

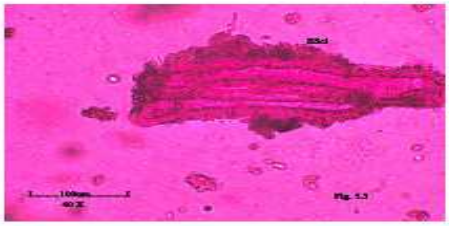

Fig. 5.3: Elongated sclereids

Figure 5: Powder microscopy of the seed
(B scl- Brachy sclereids, E scl- Elongated sclereids, Sc- Seed coat, Pi- Pits)

Calcium oxalate crystals are abundant in the lumen of the seed coat cells. The crystals are polyhedral and quite large. The crystals are located within the lumen of sclereids. The crystals appear bright when viewed under polarized light microscope (Fig. 6.1 and Fig. 6.2). The crystals are 15 µm long and 10 µm broad.

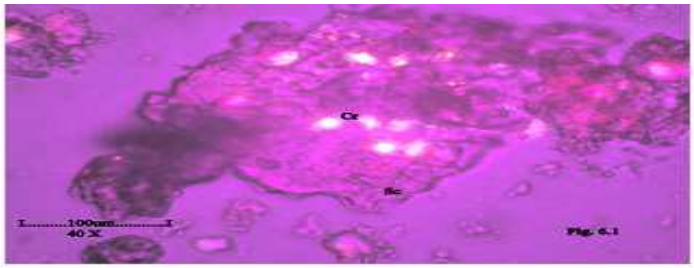

Fig. 6.1: Seed coat possessing calcium oxalate prismatic crystals

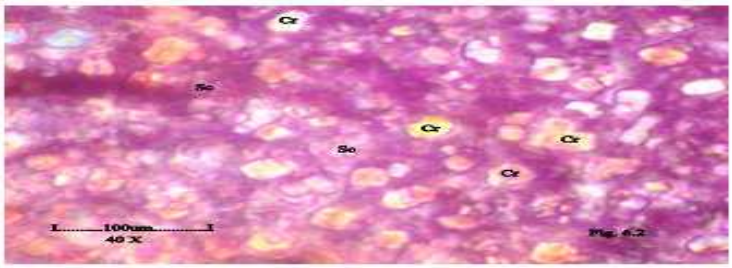

Fig. 6.2: Seed coat having dense accumulation of calcium oxalate crystals

Figure 6: Powder microscopy of the seed

(Sc- Seed coat, Cr- Crystals)

Physiochemical evaluation

Results of the physiochemical properties of *F. benghalensis* seed revealed (Figure 7) the presence of ash content (2.55% w/w), water soluble ash (78.34% w/w), acid soluble ash (98.05% w/w), moisture content (7.68% w/w), loss on drying (4.93% w/w) and foreign matter (0.78% w/w).

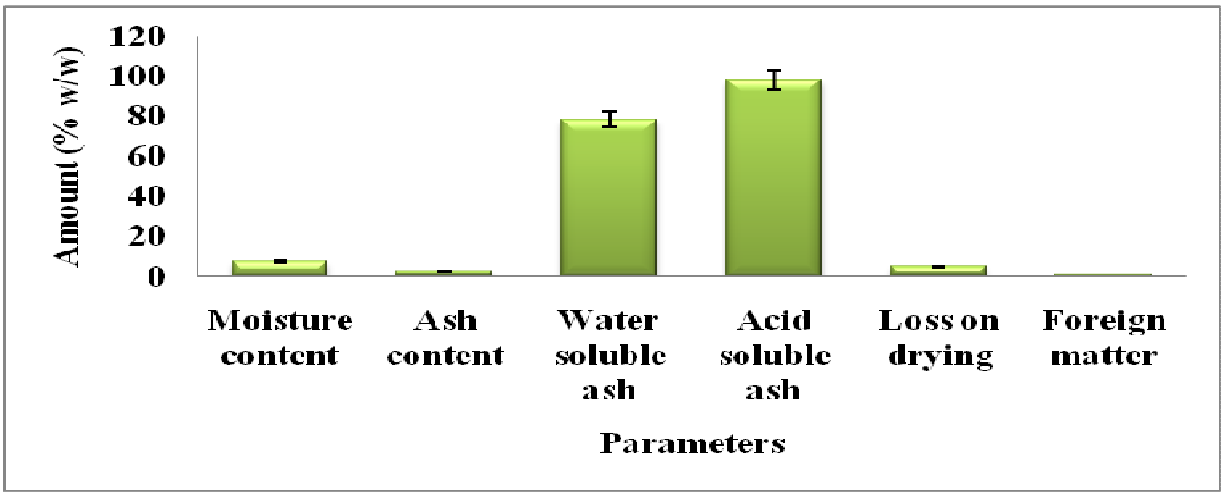

Figure 7: Physiochemical properties of the seeds of *F. benghalensis* (n = 3)

DISCUSSION

Despite the availability of hyphenated analytical techniques, identification and evaluation of plant drugs by pharmacognostical and physico-chemical parameter study is still more reliable, accurate and inexpensive. According to World Health Organization (WHO), the macroscopic and microscopic determination of the plant is the first step towards establishing its identity and purity and should be carried out before any tests are undertaken [18]. In the present work the macroscopic and microscopic study of *F. benghalensis* seeds were carried out. The results of macroscopic study might be useful for distinguishing it from its substitutes and adulterants. Microscopic evaluation allows more detailed examination of crude and enables to identify the organized structural features such as sclereids, stylar canal, cuticle, circular brachy sclereids, and tannin in parenchymatous cells, and calcium oxalate crystals as present in *F. benghalensis* seed.

The physico-chemical parameters are helpful in judging the purity and quality of the drug. The foreign matters were present in negligible amount in the seeds. This may be due to the first hand collection of plant material from the non polluted area [19]. Loss on drying for *F. benghalensis* seeds was nearly five percent. It signifies the considerable amount of moisture in the seeds. The percentage of the active chemical constituents in crude drugs is usually mentioned on air-dried basis. Hence, the moisture content of a drug should be determined and also should be controlled to make the solution of definite strength. The moisture content of a drug should be minimized in order to prevent decomposition of crude drug either due to chemical change or due to microbial contamination.

Ash values were used to detect the presence of any siliceous contamination and water soluble salts. These values are important quantitative standards as it is useful in determining authenticity and purity of drugs [20]. A lower content of total ashes in the results indicates a low level of carbonates, phosphates, silicates and silica in the seeds. The ash value of a crude drug is not always reliable, since there is the possibility of the presence of non-physiological substances. So, the authentication of acid soluble ash and water soluble ash was also performed which showed high content in the seeds.

The seeds under study can be utilized as a potential source of useful therapeutics and the outcome data will be beneficial for standardization of herbal preparations containing *F. benghalensis* seed.

CONCLUSION

In the present study various parameters such as macroscopy, microscopy, powder behavior and physiochemical investigation were carried out which could helpful in authentication of *Ficus benghalensis* Linn seed. The adulterants if any of the plant material can also easily identified by these studies. Further studies are in progress on these seeds in order to isolate, identify, characterize and elucidate the structure of bioactive compounds along with exploration of their pharmacological activity.

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