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Pharmacognostic evaluation and physico-chemical analysis of *Ficus benjamina* Linn.

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ABSTRACT: Background: *Ficus benjamina* Linn. (Family: *Moraceae*) commonly known as Weeping Fig is native to Island of South Pacific, American Samoa, French Polynesia, Marshal Islands, Majuro, Tonga and Florida. *F. benjamina* has been used for treating various ailments. Traditionally, indigenous communities have been used fruit extract of this plant for the treatment of skin disorders, inflammation, piles, leprosy, malaria, cancer, ulcer and as insect repellant. **Aim:** An extensive literature survey on *F. benjamina* showed that pharmacognostic studies were not done on this plant till date. Therefore, the present study involves pharmacognostic standardization studies like macroscopic and microscopic examinations and physico-chemical analysis like ash values, extractive values and loss on drying. **Methods:** Macroscopic and microscopic characters of *F. benjamina* fruits and leaves were studied using the simple and trinocular microscope respectively, whereas procedures for physico-chemical analysis were followed WHO guidelines. **Results:** Powder microscopy of fruit showed the presence of various cell characters that are lignified fibers, lignified vessels and testa, whereas powder microscopy of the leaf showed the presence of various cell characters such are covering trichome, glandular trichome and calcium oxalate crystal. The transverse section of *F. benjamina* leaves shows the presence of various characters such are upper epidermis, vascular bundles (xylem and phloem), upper collenchyma, lower collenchyma and lower epidermis. **Conclusion:** The results of the present study help to authenticate of the plant and would be helpful for establishing the pharmacopoeial standards for *F. benjamina*.

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INTRODUCTION:

The popularity of the herbal drugs is increasing worldwide particularly in the developed countries but one of the obstacles in its acceptability is lack of standard quality control profile. World health organization (WHO) emphasizes pharmacognostic and physico-chemical evaluation of crude drug materials for developing standardized quality control profile of herbal medicine [1]. *Ficus benjamina* Linn, commonly called as

Weeping Fig, is distributed throughout the forests of eastern Himalayas of India, Fego, Travancore and China [2]. *F. benjamina* has been used by folk people due to its medicinal potential. The plant is used in wound healing, bruises, rheumatic headache [3], gonorrhea, syphilis, dysentery, erysipelas, inflammation, fever and as general tonic [4]. Traditionally, indigenous communities have been using fruit extract for the treatment of inflammation, skin disorders, piles, vomiting, cancer, malaria, leprosy [5] and as insect repellant [6]. Bark of the plant is used for the treatment of rheumatism [7]. Leaves of *F. benjamina* are used in the treatment of bruises and wound [8]. An exhausted review of literature revealed that fruits and leaves of this plant hold a great potential for pharmacognostic standardization. Hence, the present study was aimed to establish pharmacognostic parameters and physico-chemical analysis of *F. benjamina* fruits and leaves.

MATERIALS AND METHODS:

Chemicals:

Petroleum ether (60-80) AR, Chloroform 99.8 % AR, Ethyl acetate 99.5 % AR and Ethanol 95 % AR were purchased from Loba, Chemie, Mumbai, India. Whereas Wagner's reagent, Dragendorff's reagent, Hager's reagent, Mayer's reagent, Million's reagent were purchased from HiMedia, Mumbai, India.

Plant material:

Plant materials were collected from Forest Research Institute, Dehradun, Uttarakhand, India. The plant material was identified and authenticated by Kumar Ambrish (Scientist-D), Department of Botany, Botanical Survey of India, Northern Region Centre, 192, Kaulagarh Road, Dehradun, Uttarakhand, India, vide reference no. 118762. The voucher specimen was maintained in Botanical Survey of India laboratory, Dehradun, India, for the further reference.

Macroscopic study:

In macroscopic studies were done on the basis of visual characterization and identification of color, odor, taste, fracture, texture, size, and shape [9]. In the current study, fresh fruits and leaves were studied for color, odor, taste, shape and surface characteristics.

Microscopic study:

***Transverse section (TS) of leaf*:**

Thin sections of the plant parts were made by cutting along the midrib and mounted on the glass slide and the sections were stained with different reagents as per the standards and observed under trinocular microscope (Olympus, India) [10].

***Powder drug microscopy*:**

Fine powdered drug was placed uniformly on the glass slides and then tapped again for ensuring the uniform distribution of powder on the surface of glass slides. Powdered drug was then stained with the help of different reagents as per the standards, and observed under trinocular microscope [9,10].

Physico-chemical parameters (Determination of ash values):

***Determination of total ash*:**

About 2 g of moisture free powdered drug was used and was put on a pre-ignited silica crucible. The crucible along with its contents i.e. powdered drug were accurately weighed and then ignited by placing the crucible in the muffle furnace at 500 to 600 ^{0}C in a gradual way and kept for approximately 6 h. After the ignition process the ignited content was kept for 30 min in a desiccator and weighed again without any postponement. Then total ash was calculated in mg per gram of plant material [11,12].

***Determination of acid-insoluble ash*:**

The empty pre-ignited crucible was accurately weighed. Placed about two gram of powdered drug into the silica crucible and put it in muffle furnace at 500 to 600 ^{o}C for 6 h. The temperature of the muffle furnace was slowly and gradually increased. The ash obtained after ignition was weighed accurately and total ash was calculated in mg with reference to the air-dried sample of powdered drug. The ash obtained was boiled for 5 min with 25 ml hydrochloric acid in the crucible. Insoluble matter obtained was collected in an ash-less filter paper and washed with the help of hot water. This was done until the filtrate became neutral. Then the filtrate was transferred in the original crucible and was ignited again until a constant weight was obtained. Placed the crucible for 30 minutes in the desiccator and after that calculated the acid-insoluble ash in mg/g of plant material [11,12].

***Determination of water-soluble ash*:**

Powdered drug (2 g) was put into a previously weighed crucible and placed it in muffle furnace at 500 to 600 ^{o}C for 6 h. The ash obtained was kept in the desiccator along with the crucible and cooled for about 30 min. Total ash value was calculated in mg per gram with reference to the air-dried sample of the powdered drug. The ash was boiled with 25 ml of water for 5 min.

Collected and ignited the insoluble matter of the ash and further ignited it at 450 ^{0}C. The obtained residue was weighed and calculated the water-soluble ash in mg per gram with reference to the air-dried sample of the powdered drug [11,12].

Physico-chemical parameters (Extractive values):

***Determination of alcohol soluble extractive (ASE) value (Hot extraction method)*:**

Powdered drug weighing 4 g was transferred into the glass-stoppered conical flask. To the flask, 100 ml of 95 % ethanol was added and weighed it again. The flask was shaken and kept it stagnant for 1 h after that boiled it with a reflux condenser and attached to it for 1 h. it was filtered with the help of filter paper and placed 25 ml of the filtrate to a tared china dish. After evaporating it to dryness, the dried content was weighed and alcohol soluble extractive value was calculated in mg/g of air dried powdered drug [12].

***Determination of ASE value (Cold extraction method)*:**

Powdered drug weighing 4 g was transferred into the glass-stoppered conical flask. The drug was macerated with 100 ml of 95 % ethanol for 6 h with frequent shaking. It was filtered after 18 h and 25 ml of filtrate was placed in a tared china dish. After evaporating it to dryness the dried content was weighed and alcohol soluble extractive value was calculated in mg/g of air-dried powdered drug [12].

***Determination of water-soluble extractive value* (*Hot extraction method)*:**

Powdered drug weighing 4 g was transferred into the glass-stoppered conical flask. About 100 ml of water was added to flask and weighed it again. Shake the flask and kept it stagnant for 1 h after that it was boiled with a reflux condenser for 1 h. It was filtered and placed 25 ml of the filtrate to a tared china dish. After evaporating it to dryness the dried content was weighed and alcohol soluble extractive value is calculated in mg/g of air-dried powdered drug [12].

***Determination of water-soluble extractive value (Cold extraction method)*:**

About 4 g powdered drug was put into the glass-stoppered conical flask. Macerated the drug with 100 ml of water for 6 h with frequent shaking. After 18 h, it was filtered and 25 ml was placed in tared china dish. After evaporating it to dryness the dried content was weighed and alcohol soluble extractive value was calculated in mg/g of air-dried powdered drug [12].

***Determination of foreign organic matter*:**

Drug (250 g) was weighed and the sample was spreaded in a thin layer on a white tile without overlapping. The sample inspected with naked eye i.e. visual inspection or by magnifying lens (6 or 10X) [13]. Sifted the sample through sieve no 250. Weighed the sorted foreign matter and calculated the percentage of foreign organic matter.

***Determination of moisture content*:**

Accurately weighed 2 g powdered drug and kept it in a porcelain dish. It was dried in hot air oven at 105 ^{o}C. This was done until a constant value is obtained. The content was cooled in a desiccator. The initial weight was subtracted from the final constant weight. The loss in weight was recorded as moisture loss [11,14].

***Fluorescence analysis*:**

The powdered drug and the extract obtained was placed on grease free glass slides and were treated with different solvents and chemicals. Furthermore, glass slides were placed in the UV chamber and observed under visible light, long UV light (365 nm), short UV light (254 nm) for the investigation of the fluorescence produced by the powered drug [14].

RESULTS:

Macroscopic characters of *F. benjamina* fruits and leaves:

Fruits of *F. benjamina* found green (un-ripened) and orange to reddish orange (ripened) in color (Fig 1). They were fleshy and sweet in taste. The fruits were ovate in shape with a diameter of 1.8 to 2.0 cm and with a length of 2.0 to 2.3 cm (Table 1).

Table 1. Macroscopic characters of *Ficus benjamina* fruits.

Characters	Observations
Color	Green (Unripened), Yellow, Orange, Reddish orange
Odor	None
Taste	Sweet
Surface	Smooth
Texture	Fleshy
Diameter	1.8 to 2 cm
Length	2 to 2.3 cm
Fruit type	Fleshy
Shape	Ovate, Round

The leaves of *F.s benjamina* found green in color and elliptic in shape. Leaf arrangement was alternate and venation was pinnate. The results of macroscopic characters of leaves are shown in Fig 2 and Table 2.

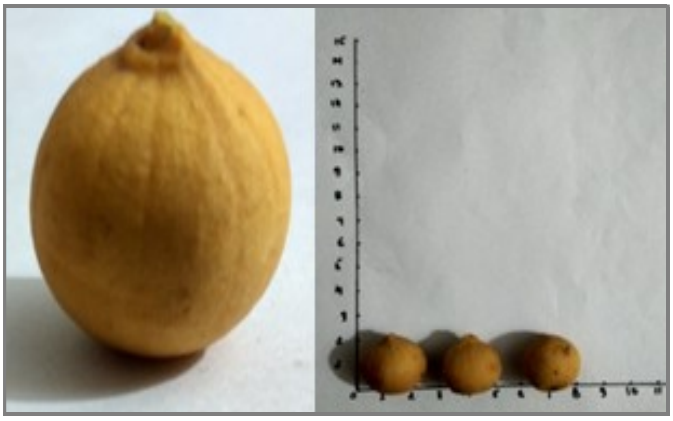

Fig 1. Fruits of *Ficus benjamina.*

Fig 2. Leaves of *Ficus benjamina.*

Table 2. Macroscopic characters of *Ficus benjamina* leaves.

Characters	Observations
Color	Green
Odor	None
Diameter	5 to 5.2 cm
Length	10 to 11 cm
Shape	Elliptic, oval
Arrangement	Alternate
Leaf margin	Entire, undulate
Venation	Pinnate
Persistence	Evergreen
Leaf blade length	9 to 9.3 cm

Microscopic characters of *F. benjamina* fruits and leaves:

Powder microscopy of fruits showed the presence of cell characters that are lignified fibers, lignified vessels and testa (Fig 3), whereas the powder microscopy of leaf showed covering trichome, glandular trichome and calcium oxalate crystals (Fig 4). The transverse section (TS) of *F. benjamina* leaf showed the presence of upper epidermis, vascular bundles (xylem and phloem), upper collenchyma, lower collenchyma and lower epidermis as presented in Fig 5.

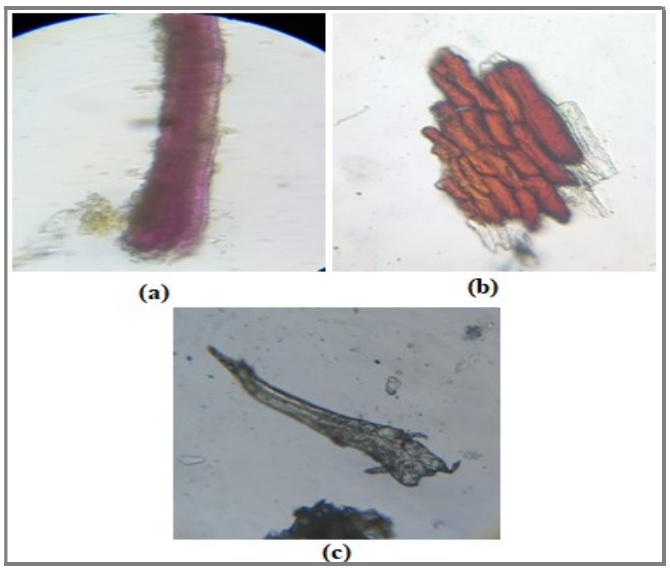

Fig 3. Powder microscopy of *Ficus benjamina* fruits.
(a) Lignified vessel [40 X], (b) Lignified vessel [40 X] and (c) Testa [40 X].

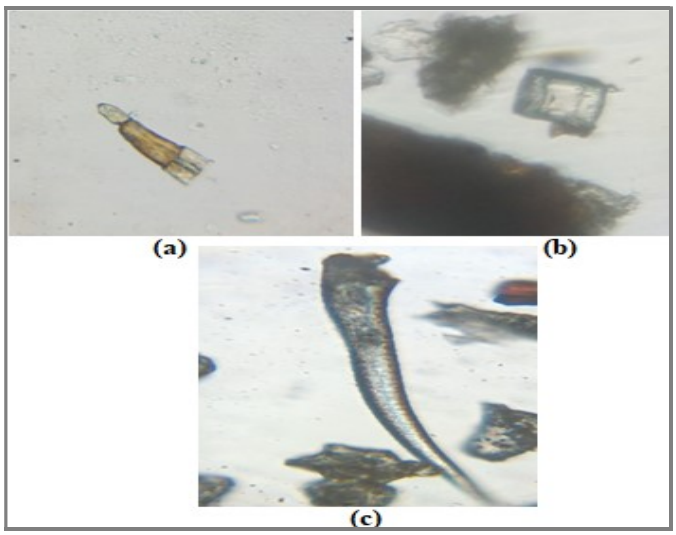

Fig 4. Powder microscopy of *Ficus benjamina* leaves.
(a) Glandular trichome [40 X], (b) Calcium oxalate crystal [40 X] and (c) Covering trichome [40 X].

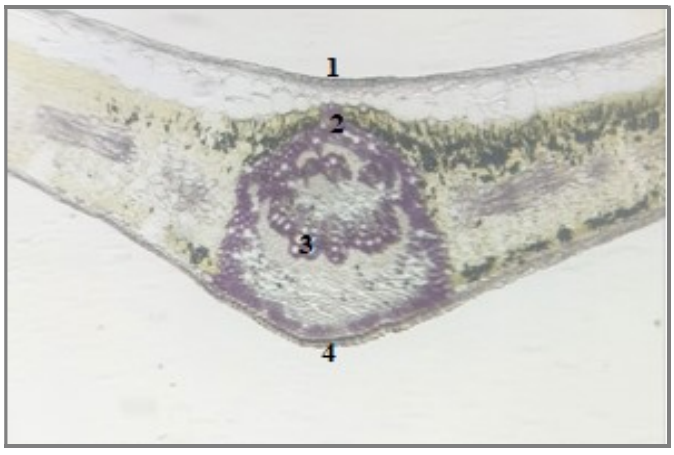

Fig 5. TS of *Ficus benjamina* leaf.
1 – Upper Epidermis, 2 – Upper Collenchyma, 3 – Xylem and 4 – Lower Epidermis.

Physico-chemical parameters of *F. benjamina* fruits:
Ash value like total ash, water soluble and acid insoluble ash value was determined as per WHO recommended procedures. The ash value gives an idea of inorganic composition and other impurities in crude drug. The total ash, acid-insoluble and water-soluble ash values were found to be 7.5, 0.85 and 4.2 % respectively as shown in Table 3.

Table 3. Ash values of *Ficus benjamina* fruits.

Sl. No.	Ash value	Yield (%w/w)
1	Total ash value	7.5
2	Water soluble ash value	4.2
3	Acid-insoluble ash value	0.85

Extractive values were determined by cold maceration and hot maceration methods. Results of extractive values are shown in Table 4. In this investigation extractive values such as petroleum ether, chloroform, ethyl acetate, alcohol soluble and water soluble by cold maceration method were calculated as 1.12, 1.26%, 1.47, 1.65 and 2.787 % respectively and by hot maceration method were calculated as 1.79, 2.09, 2.57, 2.88 and 3.74 % respectively.

Table 4. Extractive values of *Ficus benjamina* fruits.

Extractive value	CMY (%w/w)	HMY (%w/w)
Petroleum ether	1.12	1.79
Chloroform	1.26	2.09
Ethyl acetate	1.47	2.57
Ethanol	1.65	2.88
Aqueous	2.787	3.74

CMY-Cold maceration yield, HMY- Hot maceration yield.

Foreign organic matter is used to determine the amount of adulterants present in crude drugs. The foreign organic matter was found to be 0.9 % w/w. Moisture content is used to determine the amount of moisture present in a crude drug. The moisture content was found to be 6.2 % w/w. Swelling index is used to determine the quantity of gums, hemicelluloses and mucilage. The swelling index was found nil. Fluorescence analysis of fruits of *F. benjamina* was determined and results of fluorescence analysis are shown in Table 5.

DISCUSSIONS:

Quality control deals with the study of purity, safety, potency and efficacy. Thereby, standardization and quality control of herbal raw materials are always required. Quality standard of any herbal drug is related to its uniformity in quality which is numerical quantities by which the quality of commodities may be assessed. The information upon which standards may be based is obtained by a study of the genuine drug, the method used for adulteration and means adopted for the detection of adulterants. There are various several aspects are to be considered as pharmacognostical standards. The popularity of the herbal drugs is increasing worldwide particularly in the developed countries but one of the obstacles in its acceptability is lack of standard quality control profile [15]. WHO emphasizes macroscopic, microscopic and physico-chemical evaluation of crude drug materials for developing standardized quality control profile of herbal medicine [1]. The present study emphasized upon pharmacognostic evaluation and physico-chemical analysis of *F. benjamina.* Macroscopic studies help to determine the identity and degree of purity of herbal materials. Microscopic examinations are helpful for the identification of anatomical characters of a plant. The pharmacognostic investigations of some physical parameters are helpful in setting standards for a crude drug as these parameters are mostly constant for a plant. Various physico-chemical parameters were evaluated as mentioned in the WHO guidelines. These parameters are important for the detection of drug adulteration or improper handling of raw materials. The extractive value gives an idea about the nature of the chemical constituents present in a crude drug. Fluorescence analysis shows the emission of light by a substance against absorbed light. This Technique of observing plant material under fluorescence light has been used as a Pharmacognostic tool to distinguish closely related plant species.

CONCLUSION:

Since, there have been no reports available on the Pharmacognostic standardization of *Ficus benjamina*, thus, the present investigations had been taken up with a view to lay down standards that could be helpful for identification of purity, quality, authentication and also compendium of quality control of crude drugs.

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Table 5. Fluorescence analysis of powder of *Ficus benjamina* fruits.

Sl. No.	Powdered drug + Reagents	Visible light	Short UV light (254nm)	Long UV light (366 nm)
1	Powder as such	Yellowish brown	Dark brown	Light brown
2	Conc. HCl	Dark brown	Blackish brown	Dark brown
3	Conc. HNO_3	Dark brown	Blackish brown	Dark brown
4	Conc. H_2SO_4	Dark brown	Blackish brown	Blackish brown
5	Glacial acetic acid	Brown	Dark green	Green
6	Ammonia	Dark yellow	Green	Dark green
8	10 % HCl	Pale brown	Dark green	Light green
9	10 % H_2SO_4	Dark brown	Blackish brown	Dark brown
10	10 % HNO_3	Pale brown	Dark brown	Light brown
11	5 % $FeCl_3$	Dark brown	Dark brown	Light brown
12	1 M NaOH	Light brown	Blackish green	Green
13	Petroleum Ether	Yellowish brown	Dark green	Light green
14	Chloroform	Yellowish brown	Dark green	Light green
15	Ethyl acetate	Yellowish brown	Green	Light green
16	Ethanol	Yellowish brown	Green	Light green

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