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Development of Quality Standards of *Ficus Carica* Linn. Leaves

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ABSTRACT

Traditional healing through herbs have been the experienced of many countries since ages, as they were generally whispered to be non toxic natural products. Contemporary medicine is more concern for the cure of diseases but remnants indifferent to health conservation. There is an urgent need to combine the best elements of traditional medicine and modern medicine to improve the health care system of human kind. For the reason that of the rapid progress of herbal drug an increasing need is felt to standardize the herbal products. It is needed to develop the scientific protocols such as SOP and pharmacopoeial standards of the herbal drug. *Ficus carica* Linn. (Moraceae) is commonly known as edible Due to the useful effect of leaves in skin diseases, Pharmacognostical standardization of *F. carica* leaves was carried out. The transverse section of leaves showed upper and lower epidermis with covering and glandular trichomes and midrib showed arc shaped vascular bundle. Successive extractive value was highest (23.606%) in case of aqueous extract. Mean ash values (%) were 23.04 (total), 6.48 (acid insoluble) 12.69 (water soluble). Loss on drying was 5.9107%. Resin content was found 1.33%. Phytochemical screening leaves powder showed the presence of carbohydrates, phenolic compounds, flavonoids, steroids, tannin, resin and acidic compounds.

Keywords: *Ficus carica*, Extractive values, Ash values, Pharmacognostical standardization.

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INTRODUCTION

Ficus carica Linn. (Moraceae) is a small or moderately sized deciduous tree, 15-30 ft high[1]. irregularly branched tree or large straggling bush. Branches are numerous, cylindrical, with a smooth redish or pale grey bark, marked, whilst young, with the scars of the petiole and fallen stipules, the youngest twigs downy[2]. The name is very similar in French (*figue*), German (*feige*), Italian and Portuguese (*figo*). It has been cultivated by humans for over 5000 years. In India it is cultivated in many parts of north India for its fruits. Since ancient times the fig have been used for human consumption, but it was only recently that their nutritive and pharmacological value has been investigated1. Medicinally leaves, roots, fruits and latex[3-5] are used. A decoction of the leaves is stomachic. The leaves are also added to boiling water and used as steam bath for painful or swollen piles. It also has an analgesic effect against insect sting and bites. Milky juicy of leaves is very acrid and has been used in some countries for raising blisters[3]. The juice of fig leaves has long been used to treat vitilgo due to presence of furanocoumarins principally psoralen and daidzein [6-8].The present work was undertaken to standardize the leaves of *Ficus carica*.

MATERIALS AND METHODS

Chemicals and reagents:

All the chemicals and reagents used were of analytical grade, purchased from Sigma chemical co. (St Louis, MQ, USA) and Merck (Darmstadt, Germany). The plant materials (leaves) were collected from Jamia Hamdard (Hamdard University), residential block A, Prahaladpur, New Delhi. The plant were authenticated by Dr. M. P. Sharma, Dept. of Botany, Faculty of science, Jamia Hamdard, New Delhi, India.

Morphological studies:

The morphological studies were carried out for shape, size, color, odor, taste and fracture of the *Ficus carica* leaves.

Microscopic studies and powder analysis:

The transverse section of leaf was prepared by standard method. Slides of powdered leaf material were also prepared and studied. Microphotography on different magnifications was carried out with motic microscopic unit.

Quantitative microscopy:

Leaf constants such as stomatal index, stomata number, vein islet, vein termination and palisade ratio of the drug were determined according to the method described[9].

Powdered microscopy:

The microscopic examination of powdered leaf material was performed to detect and established various identifying microscopic characters which will be help full in differentiation of the substitute of the drug supplied in the form of dried powder.

Physicochemical standardization:

The drug was standardized according to WHO guidelines and other pharmacopoeias procedures physicochemical standardization which includes total ash value, acid insoluble ash value and water soluble ash value, pH value, aflatoxins, heavy metals and pesticides residues in different extracts were analyzed as per the standard methods[10-11].

Determination of individual extractive values (cold extraction):

The accurately weighed amount (10 gm) of plant material was extracted with different solvents (Petroleum ether, chloroform and methanol) separately in a conical flask at a room temperature. The extracts were evaporated to dryness and their constant extractive values were recorded[10-11].

Determination of individual extractive values (hot extraction):

Weighed amount of drug (10 gm) was extracted with petroleum ether, chloroform and methanol separately in a Soxhlet apparatus[10-11].

Determination of successive extractive values:

The accurately weighed quantity (10 gm) of plant material was subjected to successive extraction in a Soxhlet apparatus with different solvents like petroleum ether, chloroform and methanol. The extracts were evaporated to dryness and their constant extractive values were recorded[10-11].

Determination of total ash values:

The drug (2 gm) was placed in a suitable tarred crucible of silica previously ignited and weighed. The formulation was spread in to an even layer and weighed accurately. The material was incinerated by gradually increasing the heat, not exceeding 450°C until free from carbon, cooled in a desiccators, weighed and percentage ash was calculated by taking in account the difference of empty weight of crucible and that of crucible with total ash[10-11].

Determination of acid insoluble ash values:

The obtained ash was boiled with 25 ml dilute HCl (6N) for 5 minutes. The insoluble matter collected on an ash less filter paper, washed with hot water and ignited in crucible at a temperature not exceeding 450^{o}C to a constant weight. The aired materials were then assayed for their acid-insoluble ash content[10-11].

Determination of water soluble ash values:

The ash obtained was dissolved in distilled water and the insoluble part was collected on an ash

less filter paper and ignited at 450^{o}C to constant weight. By subtracting the weight of insoluble part from that of the ash, the weight of soluble part of ash was obtained. The materials were then assayed for their water soluble ash content[10-11].

Loss on Drying (LOD):

The powder sample (10 gm) without preliminary drying was placed on a tarred evaporating dish and dried at 105ºC for 6 hours and weighed. The drying was continued until two successive reading matches each other or the difference between two successive weighing was not more than 0.25%. Constant weight was reached when two consecutive weighing after drying for 30 minutes in a desiccators, showed not more than 0.01 gm difference[10-11].

Determination of PH:

PH 1% Solution:

Dissolved an accurately weighed 1 gm of the drug in accurately measured 100 ml of distilled water, filtered and checked pH of the filtrate with a standardized glass electrode.

PH 10% solution:

Dissolved an accurately weighed 10 gm of the drug in accurately measured 100 ml of distilled water, filtered and checked pH of the filtrate with a standardized glass electrode.

Phytochemical screening:

The phytochemical evaluation of drug was carried out as per the method described. Previously weighed amount of powder drug (10 gm) was extracted in a Soxhlet apparatus with petroleum ether, chloroform, and methanol respectively. The extracts were evaporated to dryness under vacuum in rotary evaporator. These extract were used for the analysis of different phyto-constituents *viz*. alkaloids, carbohydrates, phenolics, flavonoids, proteins, amino acids, saponins, mucilage and resins etc.

Aflatoxins analysis:

Aflatoxins were analysed in the different plant extracts by HPLC method as described by Scott (AOAC method 980.20-ITEM-I) [12].The chromatographic separation was performed on a C18 column (3.9 · 300 mm i.d., Waters, Ireland) using a water–acetonitrile (4:1, v/v) mobile phase at a flow-rate of 21.0 ml/min. Detection of aflatoxin was carried out using365 and 418 nm as wavelengths for excitation and emission, respectively.

RESULTS AND DISCUSSION:

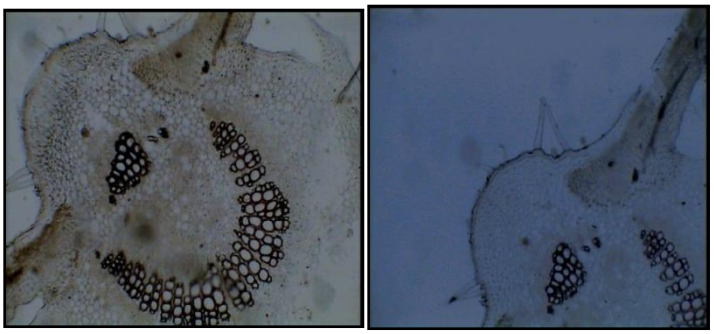
Macroscopical evaluation:

The leaves of *F. carica* were subjected to macroscopical evaluation and observations were

recorded. The proper examination of the leaves was carried out under sun light and artificial source similar to day light. The leaves of *F. carica* were long, palmate shape and dark green in colour.

Microscopical evaluation:

The T.S of *F. carica* leaves showed upper and lower epidermis showed single layered cell with covering and glandular trichomes and midrib showed arc shaped vascular bundle(Figure 1).



A **B**

Figure. 1: (A) Transverse section of normal leaf of *Ficus carica* showing magnified view of vascular bundle (5X20), (B) Transverse section of *F. carica* showing covering and glandular trichomes (10X10)

Extractive value:

Estimation of extractive values determines the amount of the active constituents in a given amount of drug when extracted with solvent. It is employed for that material for which no chemical and biological assay method exit. The extractions of any crude drug with a particular solvent yield a solution containing different phytoconstituents. The compositions of these phyto constituents depend upon the nature of the drug and solvent used. The use of a single solvent can be the means of providing preliminary information on the quality of particular drug. Extractive value also give the information regarding the quality of the drug (whether drug is exhausted or not). Methanol followed by petroleum ether proved to be highly effective for high cold extractive values. The maximum hot extractive values noticed in methanol extract. The maximum successive extractive values recorded in methanol (Figure 2).

Determination of ash values, loss on drying and pH:

The ash value of any organic material is composed of their non volatile inorganic components. Control incineration of crud drugs result in ash residue consisting of an inorganic material (metallic salt and silica). This value varies within fairly wide limits and is therefore, an important parameter for the purpose for evaluation of crude drugs. Unwanted parts of drug, some time

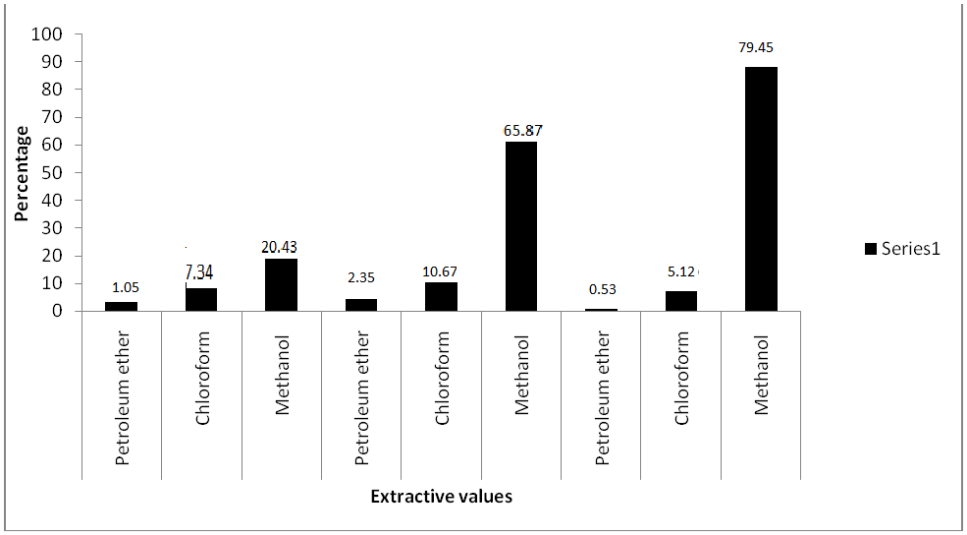


Figure.2. Percentage of individual extractive values

posses a character that will raise the ash value. A high value is indicative of contamination, substitution, adulterations or carelessness in preparing the crude drug for marketing. The total ash values, water soluble ash, acid insoluble ash, loss on drying (LOD), pH values and moisture content were determined. The results noticed were; loss on drying (5.91 ± 0.15%), total ash (23.04 ± 0.25), water soluble ash (12.69 ± 0.12), acid insoluble ash (6.48 ± 0.34), pH value 1% solution (4.3 ± 0.01), pH value 10%solution (7.2 ± 0.02) and moisture content (5.09 ± 1.07) respectively Table-1.

Table 1. Results of physicochemical evaluation of *F.carica*

Parameters	% w/w (Mean± SD)
Loss on drying	5.91 ± 0.15
Total ash	23.04 ± 0.25
Water soluble ash	12.69 ± 0.12
Acid insoluble ash	6.48 ± 0.34
Moisture content	5.09 ± 1.07
pH value 1% solution	4.3 ± 0.01
pH value 10%solution	7.2 ± 0.02

Phytochemical screening:

Phytochemical evaluation of the extracts may provide the information regarding various types of phytoconstituents present. Presence or absence of particular types of phytoconstituents in the sample of the interest may be helpful, partly in the development of metabolomics and analytical profile and in the differentiation of contravention plants. The extracts were subjected to preliminary chemical tests to detect the presence and absence of various phytoconstituents. Alkaloids, carbohydrates, phenolic compounds, flavonoid, glycoside, tannin, proteins and amino acids were present in different extracts Table-2.

Table 2. Phytochemical screening of different extract of *F.carica* ; (-: Absent, +: Present)

Extract Constituents	Hexane	Acetone	Chloroform	Benzene	Petroleum - ether	Methanol
Alkaloids	_	+	_	_	_	+
Carbohydrates	_	_	_	_	_	+
Phenolic compounds	_	_	+	_	+	+
Flavonoid	_	_	_	_	+	+
Glycoside	_	+	_	_	+	+
Tannin	_	_	+	_	_	+
Proteins and amino-acids	_	_	_	_	_	_
Saponins	_	_	_	+	_	+
Sterol	_	_	+	_	_	+
Resins	+	_	+	_	+	+
Lipids / Fats	+	_	+	_	+	+

Determination of Aflatoxins:

The chemical constituent in plants responsible for medicinal and nutritional properties and as well as toxicity. Men, animals and plants through air, water and food take up these materials from the environment. Medicinal plants which are the raw material for many of the popular nutrients supplements are sold all over the country. Effect of toxic material on human health and their interaction with essential trace may produce serious consequences. The amount of aflatoxins were determined in plant material. The results noticed were; absence of aflatoxin more ever neither B1and B2 nor G1 and G2 aflatoxin were detected in the plant sample. The results and observation are presented in Table 3. The aflatoxin B1, B2 and G1, G2are known to be mutagen, carcinogen and teratogen compounds. The intake of these toxins over a long period of time in very low concentrations may be highly dangerous. Thesecompounds can enter the food chain, mainly, by ingestion through the dietary channel of humans and animals.

Table 3: Aflatoxin residues of *F.carica;* MDL- maximum detection limit

S. No	Test parameter	Test method	Results	MDL
1	AflatoxinB1	AOAC 990.33	Not detected	1.0µg/kg
2	AflatoxinB2	AOAC 990.33	Not detected	1.0µg/kg
3	AflatoxinG1	AOAC 990.33	Not detected	1.0µg/kg
4	AflatoxinG2	AOAC 990.33	Not detected	1.0µg/kg

CONCLUSION

Hence the drug under study was subjected to physicochemical analysis, which is helpful in establishing the standard parameters. *Ficus carica* is an important medicinal plant in the Traditional System of Medicine. It is one of the most important components of various marketed preparations used in liver and skin diseases. The present study is an attempt in the direction of

standardization and preliminary phytochemical screening of *F. carica.* In the present investigation an attempt has been made to standardize leaves of *F. carica.* However, further work is warranted to isolate and quantify active constituents present in the leaves of *F. carica* by sophisticated techniques.

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