

## ORIGINAL ARTICLE

# Standardisation of *Ficus carica* L.: In homoeopathic perspective

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### ABSTRACT

**Background:** *Ficus carica* L. known as 'Common Fig' in English and 'Anjir' in Hindi, is a well known tree belonging to the family Moraceae. The unripe fruits are used as medicine in Homoeopathy.

**Objective:** The pharmacognostic and physico-chemical studies are carried out to facilitate use of correct species and standard raw materials.

**Material and Methods:** Pharmacognostic studies on unripe fruits of authentic samples of *Ficus carica* L. have been carried out; Physico-chemical parameters of raw drug viz., extractive value, ash values, formulation, besides weight per mL, total solids, alcohol content along with High Performance Thin Layer Chromatography (HPTLC) and Ultra violet-visible studies have been worked out for mother tincture.

**Results:** The unripe fruit is a syconium, globose, light green and externally tomentose. Stomata are anomocytic or actinocytic. Trichomes are unicellular conical. The 1-layered epidermis in transection (T.S.) is marked by hair bases with conspicuously large encircling cells. Inner cortex is aerenchymatous and possesses laticiferous tubes. Vascular bundles are present in the cortex towards inside. In T.S. inner fruit wall possess female flowers. Physico-chemical properties and HPTLC values of this plant have been standardised.

**Conclusion:** The powder microscopic features and organoleptic characters along with anatomical and physico-chemical studies are diagnostic to establish the standards for the drug.

**Keywords:** *Ficus carica* L., Figs, High performance thin layer chromatography, Syconium, Laticifer, Pharmacognosy

### INTRODUCTION

*Ficus carica* L. popularly known as 'Common Fig' in English and 'Anjir' in Hindi is a tree of moderate size, belonging to the family Moraceae. It is native to Western Asia and is cultivated in many temperate and sub tropical regions.<sup>[1]</sup> The unripe fruits are used as medicine in Homoeopathy. Medicinally, it is useful as aperient, emollient, demulcent, nutritive, for removal of stones in kidney or bladder, cures piles, gout, disorders of liver and removal of

warts.<sup>[1,2,3]</sup> In general, figs are easy to digest, rich in vitamins, fibre and minerals.<sup>[1]</sup> Chemically the plant contains some bioactive compounds such as psoralen, bergapten, chrysin, galangin, anthocyanin,  $\beta$ -amyryn, quercetin, fucosterol, nicotinic acid, riboflavin, ficusin, germacrene D, agelicin, linalool, linalool oxides, cinnamic aldehyde and lupeol acetate.<sup>[1,4,5]</sup> They are good source of flavonoids and polyphenols<sup>[6]</sup> and  $\beta$ -carotenes.<sup>[7]</sup> It is reported to have anti oxidant, antibacterial, hypoglycaemic, cancer suppressive, hypotriglyceridaemic and anthelmintic properties.<sup>[8]</sup>

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The pharmacognostic studies on fruits of *Ficus carica* L. in general is rare<sup>[9]</sup> and on unripe fruit in particular is absent. Considering the medicinal importance of the drug and absence of earlier pharmacognostic studies on unripe fruits, the present study is undertaken.

## MATERIAL AND METHODS

### Pharmacognosy

The unripe fruits of *Ficus carica* L. was supplied by Survey of Medicinal Plants and Collection Unit, Nilgiris, Tamil Nadu, under CCRH. The fruits were slightly boiled and fixed in F.A.A. (Formaldehyde - Acetic acid - Alcohol) and processed for microtomy (Paraffin method), sectioned, stained and permanent slides prepared as per method of Johansen.<sup>[10]</sup> The surface peels of the fruit were obtained by gently scraping with razor blade. The powder microscopy characters were studied by boiling the powdered drug in distilled water, stained in saffranine and mounted with glycerine. Photomicrography was done with Olympus BX 53 trinocular research microscope attached with Sony digital camera.

### Physico-chemical

The air-dried unripe fruits were coarsely powdered to 10/44 (sieve size) and subjected to determination of moisture content (loss on drying at 105°C), total ash, water soluble ash, acid insoluble ash, extractability in different solvents, physico-chemical constants; UV aspects of mother tincture, including its preparation by percolation method given in Homoeopathic Pharmacopeia of India<sup>[11]</sup>.

### High Performance Thin Layer Chromatography (HPTLC) Analysis

25 mL of mother tincture was evaporated on water bath to remove alcohol. The residue was extracted with 3 × 25 mL chloroform. Concentrated chloroform extract was used for the HPTLC study. The concentrated chloroform extract was spotted in the form of band of width 4 mm with a Camag microlitre syringe on pre-coated Silica gel Aluminum plate 60F-254, (5 × 10 cm with 0.25 mm thickness; Merck, Darmstadt, Germany) using a Linomat IV sample applicator (Camag, Muttenz, Switzerland, supplied by Anchrom technologists, Mumbai). A constant application rate of 6 mL/sec was employed. The slit dimension was kept at 4 × 0.45 mm and 20 mm/sec scanning speed was employed. The mobile phase consisted of Chloroform: Methanol (9:1 v/v) and 10 mL of mobile phase was used for chromatography. Linear ascending

development was carried out in a 10 × 10 cm twin trough glass chamber (Camag, Muttenz, Switzerland) saturated with the mobile phase at room temperature (25 ± 2° C) for 20 minutes. The length of the chromatogram run was 8 cm and subsequent to the development, the TLC plates were dried in a current of air with the help of hot air dryer in a wooden chamber with adequate ventilation. Densitometric scanning was performed (Camag TLC scanner III) at 254 nm and 366 nm by reflectance scanning and operated by Wincats software (Camag) present in the system.<sup>[12,13,14]</sup>

## OBSERVATIONS AND RESULTS

### Macroscopy

Unripe fruit is syconium, produced into a globose, clavate structure, 2-8 cm in diameter, bearing pistillate flowers, pale to light green, externally tomentose, with a pore over the top at centre. Plant photograph with fruit branch is given as [Figure 1.1].

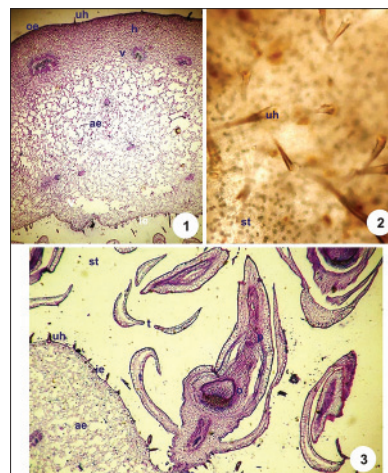
### Microscopy

The epidermal cells over the surface polygonal isodiametric, 5-6 sided, sides thin, straight to curved, cytoplasm slightly dense. Stomata are anomocytic or actinocytic. Trichomes are unicellular conical hair; walls thick, contents dense, surface verrucose [Figure 2.2] Distribution: Common, all over the surface.

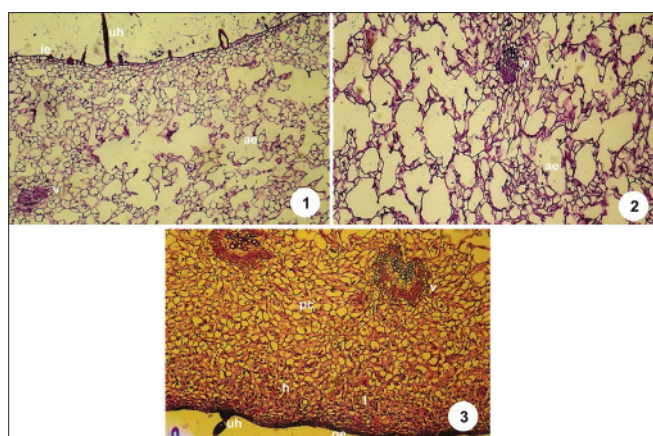
In T.S. the outer most epidermis is 1-layered, cells tabular, radially long, wall thin, contents dense, often interspersed with conical hair and stomata [Figures 2.1 and 3.3]. The epidermis is marked with large hair bases having surrounding cells projecting above the surface [Figures 2.1 and 3.3]. Hypodermal cortex is 8-10 layered with peripheral 2-3 layers with smaller cells which are tabular, arranged in rows and often containing sphaeraphides [Figures 3.3 and 4.2]. The inner cortex is up to 40 layers, aerenchymatous, having oval to spherical and elongated cells with thin walls and dense contents [Figures 2.1, 3 and 3.1, 2]. The cortex is interspersed with laticiferous canals which are unbranched [Figure 3.3 and 3.1]. The vascular bundles arranged in the form of a ring frequently occur towards inside [Figure 4.3]. The vascular bundles are endarch, conjoint, collateral and capped by sclerenchyma. Some bundles occur dispersed irregularly in the aerenchyma or middle cortex [Figures 3.1 and 2]. The innermost layer of receptacle consists of inner epidermis covered with unicellular conical hairs over its surface [Figures 2.1, 3 and 3.1] The inner wall of receptacle possesses female flowers<sup>[1]</sup> [Figure 2.3].



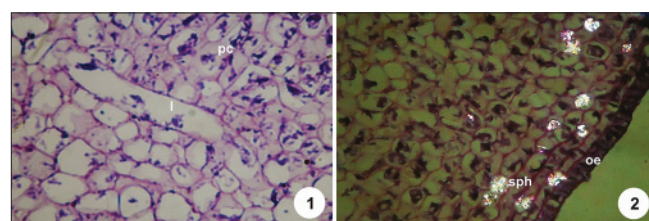
**Figure 1:** Plant with fruiting branch



**Figure 2:** (1) T.S. of receptacle X 33, (2) Fruit epidermis (Surface) X 59, (3) T.S. of receptacle showing L.S. of female flower & floral appendages X 55



**Figure 3:** (1) T.S. of receptacle (inner most side) X 123, (2) T.S. of receptacle (median region) X 107, (3) T.S. of receptacle (peripheral) X 113



**Figure 4:** (1) T.S. receptacle at peripheral with latex tube X 401, (2) T.S. of receptacle at hypodermal region with sphaeraphides X 329

The flowering stalk arises from thalamus. The female flowers consist of outermost tepals in L.S. having 1-layered epithelium like epidermis and underlying mesophyll [Figure 2.2]. The mesophyll is 3-4 layered and with closely packed cells [Figure 2.3]. The central gynoecium is a flask-shaped structure consisting of epidermis and underlying parenchyma with a vascular bundle at its base [Figure 2.3]. Apically the gynoecium is narrowed into a stalk covered by a glandular epithelium.

The fruit in T.S. show many floral appendages mostly the tepals and central area of the female flowers in various planes.

### Powder Microscopy

1. Pieces of thalamus with epidermis, hypodermis and cortex
2. Numerous fragments of conical hair either whole or broken
3. Pieces of cortex with latex tubes

4. Fragments of stalk of gynoecium with dense contents
5. Pieces of ovary with an ovule
6. Pieces of tracheary tissue showing vessels/tracheids with helical thickenings
7. Pieces of epidermis with anomocytic stomata and conical hair
8. Pieces of black tanniferous masses.

### Organoleptic Characters

Colour – Creamish yellow

Touch – Slightly coarse

Odour – Not characteristic

Taste – Not characteristic.

### Physico-Chemical Studies

The determined data under the physico-chemical study for the raw drug is summarised in Table 1 and that of mother tincture preparation and its standardisation in Tables 2 and 3 respectively.

### Qualitative Phytochemical Tests

Moisture content reveals the presence of water in the plant and also some volatile organic matter. Results of physico-chemical studies are summarised in Tables 1-3.

**Table 1: Standardisation of raw drug**

Parameters	Quantitative values (% w/w)
Moisture content (Loss on drying at 105° C)	Not more than 11.44
Total ash	Not more than 7.47
Acid insoluble ash	Not more than 0.21
Water soluble ash	Not more than 5.58
Alcohol soluble extractive	Not less than 10.75
Water soluble extractive	Not less than 21.35
Extractive values in	
Hexane	Not less than 2.42
Chloroform	Not less than 3.80
Methanol	Not less than 13.10

**Table 2: Formulation of mother tincture (percolation technique used)**

Alcohol	65 % v/v (based on MEV*)
Drug strength	1/10
Preparation	
<i>Ficus carica</i> in coarse powder	100 g
Strong alcohol	677 mL
Purified water	350 mL
To make 1000 mL of the mother tincture	

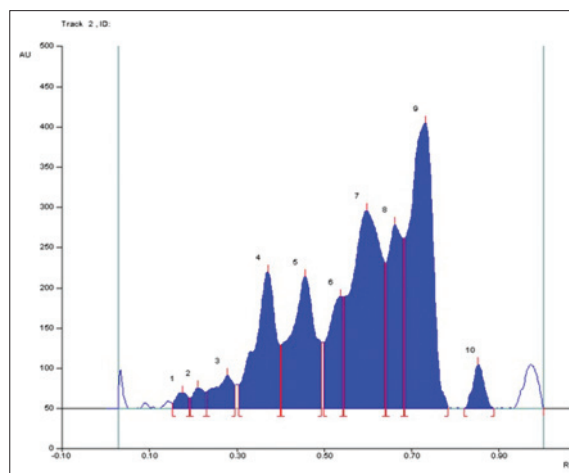
\*MEV: Maximum Extractive Value

**Table 3: Standardisation of mother tincture**

Parameters	Observations
Organoleptic profile	
Appearance	Clear, non-viscous, foaming on shaking
Colour	Greenish brown
Odour	Pleasant, fruity and aromatic
Sediments	Absent
Weight per mL	Not more than 0.89 g
Total solids	Not less than 1.94 % w/v
Alcohol content	62-64 % v/v
pH	6.0-6.5
λ max	290, 326 nm

### HPTLC Finger Printing

The profile of chromatographic separation scanned at 254 nm, reveals 10 spots [Figure 5] out of which 9, 7 and 5 spots possess maximum composition with  $R_f$  at 0.73, 0.59 and 0.45 respectively. While, chromatogram scanned at 366 nm, revealed 6 spots with 4, 5 and 6 spots showing maximum composition at  $R_f$  0.86, 0.70 and 0.60 respectively. It is evident from the data that these are characteristic for the studied drug, which will help in identification and authentication of the mother tincture. This is considered as valuable standard in pharmacopoeia. At 254 nm, ten spots appear at  $R_f$  0.17, 0.21, 0.28, 0.37,



**Figure 5:** HPTLC finger printing of *Ficus carica* mother tincture chloroform extract scanned at 254 nm

0.45, 0.54, 0.59, 0.66, 0.73 and 0.85 (all are in brown colour) [Figure 5] with various concentrations while at 366 nm, five spots at  $R_f$  0.23 (red), 0.43 (light yellow), 0.60 (red), 0.70 (blue) and 0.86 (red).

### DISCUSSION

The unripe fruit is green<sup>[9]</sup> which is presently confirmed. The fruit is a syconium, produced into a globose or pear-shaped structure, 4-8 cm bearing pistillate flowers as reported earlier<sup>[11]</sup> and externally tomentose.

The epidermal surface of the fruit consists of polygonal isodiametric cells with straight to curved anticlinal walls as reported earlier.<sup>[9]</sup> Stomata are reported as anomocytic<sup>[9]</sup> and is presently confirmed, besides a few actinocytic types.

The epidermis in T.S. is single layered with oval to barrel-shaped cells;<sup>[9]</sup> however, it is 1-layered with tabular and radially elongated cells interspersed with conical hairs. Further, the epidermis is intermittently marked with large encircling cells around hair bases projecting over the surface.

Hypodermal region is earlier reported as 3-5 layered collenchymatous tissue but it is 8-10 layered parenchyma tissue with peripheral 2-3 layers having closely packed tabular cells often containing sphaeraphides as was also found.<sup>[9]</sup> The mesocarp region is reported as having large, thin walled oval to polygonal or squarish parenchymatous cells without intercellular spaces.<sup>[9]</sup> But presently the inner cortex is extensive, aerenchymatous with cells possessing dense contents.

The cortex also possesses laticifers which are tabular, elongated with few branched<sup>[9]</sup> which is also presently found except they are unbranched. Vascular bundles occur dispersed in the cortex<sup>[9]</sup> In L.S. vessels were found to possess helical or reticulate thickenings which is presently confirmed. Besides the receptacle on its inner side possess female flowers<sup>[1]</sup> cut in various planes.

The identification of the active principles and investigation of mother tinctures are quintessential in order to ensure safe, effective and constant activity. Chemical profiling is a versatile technique and can be made to good use in standardisation. Pharmacopoeia largely relies on chemical assay and physical constants for routine standardisation. HPTLC finger printing is a modern, precise and accurate tool to authenticate and characterise the drug, which in turn ensures the quality, purity and guarantees uniformity and reproducibility. Thus, the developed chromatogram will be specific with the selected solvent system and R<sub>f</sub> value. In terms of area and height of the peak, help in assigning the identity and specificity. UV-visible study of the mother tincture exhibits two prominent peaks, which serve as characteristic standards. Physico-chemical analysis of *Ficus carica* presented in Tables 1-3, helps substantiate the standards for the drug.

## CONCLUSION

The determined physico-chemical data, spectrophotometric profile along with macro and microscopical characters and methodology employed in the study will help in not only identification, authenticity of the drug but also to ensure batch-to-batch consistency of the products manufactured by

the pharmaceutical companies and provide quality medicines to the consumers.

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**पृष्ठभूमि :** फाइकस केरिका लिन. मौरेसी कुल का पादप है जिसे अंग्रेजी में 'कॉमन फिग' व हिन्दी में 'अन्जीर' नाम से जाना जाता है। अपरिपक्व फल होम्योपैथी में औषधि के रूप में प्रयोग किये जाते हैं।

**उद्देश्य:** सही प्रजाति एवं मानक कच्चे पदार्थ के उपयोग को सुगम बनाने के लिये फार्मकोग्नोस्टिक व भौतिक रसायन अध्ययन किया जाता है।

**सामग्री व विधि:** फाइकस केरिका के अपरिपक्व फल के अधिकृत नमूने का फार्मकोग्नोस्टिक अध्ययन किया गया। अपरिष्कृत औषधि के भौतिक रसायन मापदण्डों जैसे: निष्कर्षण मान, राखीय मान, औषधि निर्माण, मदर् टिक्चर के लिये भार प्रति मिली, कुल ठोस, एल्कोहल सामग्री, उच्च निष्पादन पतली परत वर्णक्रम अध्ययन व पराबैंगनी दृश्यी अध्ययन किये गये।

**परिणाम :** अपरिपक्व फल सायकोनियम, गोलाकार, बाहरी तरफ रोमिल होता है। रन्ध्र अनियम कोशिका व एक्टिनो सायटिक होते हैं। त्वचा रोम एक कोशिकीय शंक्वाकार होते हैं। ट्रान्सेक्शन में एकस्तरीय अधिचर्म, रोम आधार पर बड़ी सुस्पष्ट कोशिकाओं से घिरी दिखाई देती है। अन्तः बल्कुट वायुतकीय तथा दुधीय नलिकाओं युक्त होता है। बल्कुट में सर्वहनी फूल अन्दर की तरफ उपस्थित होता है। ट्रान्सेक्शन में मादापुष्प अन्दर की दीवार पर पाये जाते हैं।

**निष्कर्ष :** औषधि के मानक स्थापित करने के लिये पूर्ण सूक्ष्मदर्शीय लक्षण व ऑर्गेनोलेप्टिक गुण के साथ एवं भौतिक रासायनिक अध्ययन भी नैदानिक हैं।