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# A detailed characteristic feature of pharmacognostical, physicochemical and phytochemical studies explored in *Emblica officinalis* Gaertn. (Euphorbiaceae)

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**Abstract**
Plants have been used as a medicine even before pre historical period. Traditional medicine is still playing a vital role in curing diseases. Most of the people in developing countries meet their health care needs based on this system. Plants have been scientifically evaluated for its medicinal activities. The objective of the present study is to evaluate the pharmacognostical, physicochemical and phytochemical standardization of the fruits of *Emblica officinalis* Gaertn. (Euphorbiaceae). The pharmacognostical and physicochemical evaluation was performed to proper identity of the plant and to detect the adulterants. Physicochemical analysis like ash value (Total ash – 2.48% w/w, acid-insoluble ash – 1 % w/w, water soluble ash – 2.97 % w/w, sulphated ash – 9.5 % w/w), extractive value (water soluble – 8 % w/w, alcohol soluble - 18 % w/w) loss on drying (3.78 % w/w) was estimated. Phytochemical studies characterize the phytoconstituents. Evaluation of this drug ensures identity and ascertains quality and purity.

**Keywords:** *Emblica officinalis*, Euphorbiaceae, pharmacognostical, phytochemical standardization

## Introduction
Nature is the most precious one for evolution and existence of life. Nature provides us shelter, food, medicine, plants and other resources for us. The Plants have been used as a medicine before pre historical period. In India, there are different traditional systems of medicine such as Ayurveda, Yoga, Unani, Siddha and Homeopathy. The advantage of using traditional medicine is due to low cost, no or less side effects, easily available and prohibitive cost of treatments. Herbs are used to treat acute and chronic diseases like diabetes mellitus, rheumatoid arthritis, liver disorders, cardiovascular diseases, cancer, inflammation, respiratory diseases, etc., *Emblica officinalis* belongs to the family Euphorbiaceae was selected for the study. The objective of the present study is to evaluate the pharmacognostical, physicochemical and phytochemical standardization of the fruits of *Emblica officinalis.* Synonym for *Emblica officinalis* is *Phyllanthus emblica* Linn. Common name is Emblic myrobalan. In Tamil, it is called as Nelli, Nellikkai, Nellimulli. In Hindi, it is called as Amla, Aonla and in Sanskrit as Amalaki. It is indigenous to India, Sri Lanka and distributed throughout India [1-3].

## Taxonomy

| | | |
|---|---|---|
| Kingdom | : | Plantae |
| Subkingdom | : | Tracheobionta |
| Super division | : | Spermatophyta |
| Division | : | Magnoliophyta |
| Class | : | Magnoliopsida |
| Subclass | : | Rosidae |
| Order | : | Euphorbiales |
| Family | : | Euphorbiaceae |
| Genus | : | *Emblica* |
| Species | : | *officinalis* |

A small or moderate size deciduous tree grow up to 10 m height. Fruits are edible, globose, marked with six lobes, yellowish in colour. Fruits are used in the treatment of fever, anaemia, diabetes, jaundice, skin diseases, carminative, flatulence, haemorrhages and carminative [4-6].

## Materials and methods

### Plant Collection

The fresh healthy fruits of *Emblica officinalis* was collected from Vellore District, Tamil Nadu, India.

**Fig 1:** Fruits of *Emblica officinalis*

### Identification and Authentication

Collected fruits of *Emblica officinalis* was identified and authenticated by Dr. Sunil Kumar, Research Officer, Siddha Central Research Institute, Chennai, Tamilnadu, India. Number is E17091101O. A voucher specimen of the plant was deposited in the herbarium for future reference.

### Preparation of powder

Seeds were removed and the collected fruits were dried under shade. Then it was made into coarse powder using a grinding mill and stored in an air tight glass container.

### Chemicals

Ethanol, acetic acid, phloroglucinol, hydrochloric acid, safranin, glycerine, formaldehyde and all other chemicals used were of analytical grade.

### Macroscopical Evaluation

The macroscopical evaluation of the drug was done by organs of sense with macroscopic characterization like appearance, size, colour, odour and taste [7, 8].

### Microscopical Evaluation

Microscopical studies are useful to identify crude and powdered drugs and also to detect adulterants. Characteristic features like cell walls, cell contents and tissues present in the plant were studied. Collected fruits are preserved in fixative solution. Then after 48 hrs, the specimens are dehydrated with tertiary butyl alcohol as per Sass, 1940. Specimens are infiltrated with paraffin wax and cast into paraffin blocks. Thin transverse section was taken from the paraffin embedded specimens and dewaxed as per Johansen 1940. The sections were stained with toluidine blue. Photographs were taken using Nikon ECLIPSE E200 trinocular microscope with digital camera [9-11].

### Powder Microscopical Evaluation

Powdered fruit drug material was mounted with glycerine to study the powder characters of *E. officinalis* fruit [12].

### Quantitative Microscopy

The length and width of fibres and stone cells, diameter of starch grains was studied by the quantitative microscopy linear measurement [13].

### Physicochemical Analysis

As per the standard procedure given in WHO guidelines and Indian Pharmacopoeia, physicochemical analysis for the dried fruit of *E. officinalis* was carried out. Physicochemical analysis like ash values, extractive values, foaming index and loss on drying were performed [14-17].

### Fluorescence Analysis

Powdered drug was subjected to fluorescence analysis with suitable reagents as per Chase and Kokoski methods. The colour changes were observed under UV light (254 and 366 nm) and day light [18-19].

### Determination of heavy metal contamination

Heavy metals are dangerous and toxic to health. While using plants for treating diseases, the toxicity has to be performed to assure the safety of the medicinal plants. Hence, limit test for heavy metals and arsenic was performed as per Indian Pharmacopoeia 1996 [20-22].

### Qualitative analysis and Quantitative estimation of inorganic elements

Inorganic elements like potassium, sodium, phosphate, iron, nitrate, magnesium, sulphate, calcium, carbonate and chloride were qualitatively analysed as per Indian Pharmacopoeia 1996 [20-22].

### Quantitative estimation of iron

Iron content was estimated quantitatively as per Sethi [23].

### Determination of microbial contamination

Total viable count for bacteria and fungi was performed by agar dilution method [20].

### Preliminary Phytochemical Screening

Aqueous extract was subjected to qualitative phytochemical analysis for various phytochemicals like glycosides, alkaloids, carbohydrates, proteins, fats, flavonoids, terpenoids, polyphenols, tannins, saponins, gums and mucilage as per the standard procedure [24].

## Results

### Macroscopy

Fresh fruit is globose having six prominent lines, greenish to yellowish colour, 2.5 to 3.5 cm in diameter. Characteristic odour and taste is sour, astringent then sweet.

### Microscopy

T.S of fruit showed an epicarp with epidermal layer and hypodermis. Epidermal cells are tubular in shape and appear as polygonal, hypodermal cells are elongated thick walled. Mesocorp consists of paenchymatous cells and prismatic calcium oxalate crystals are found in few cells. Xylem and phloem scattered in mesocarp. Elongated xylem fibres are present (Fig. 2A, 2B, 2C and 2D).

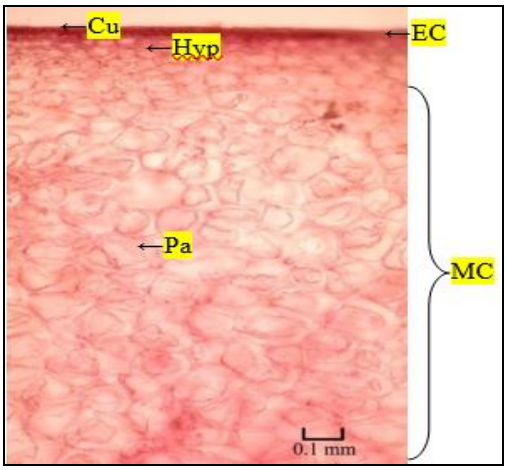


**Fig 2:** A T.S of pericarp

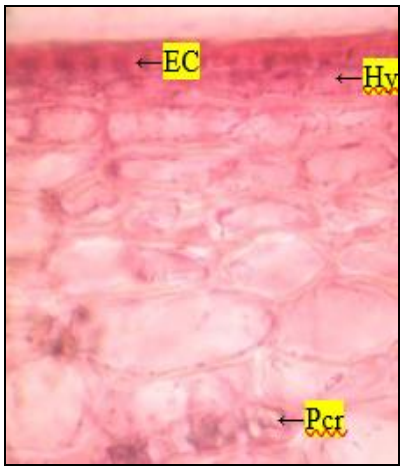


**Fig 2:** B Epicarp and mesocarp

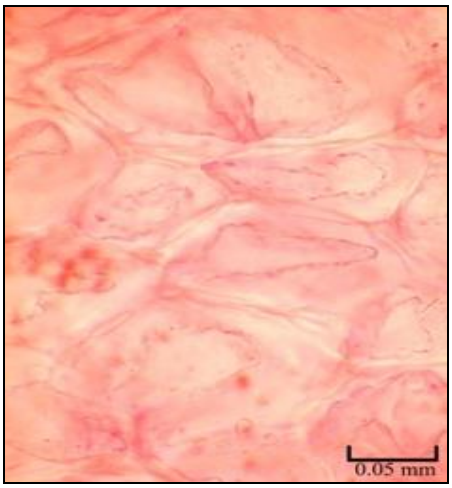


**Fig 2:** C Middle mesocarp

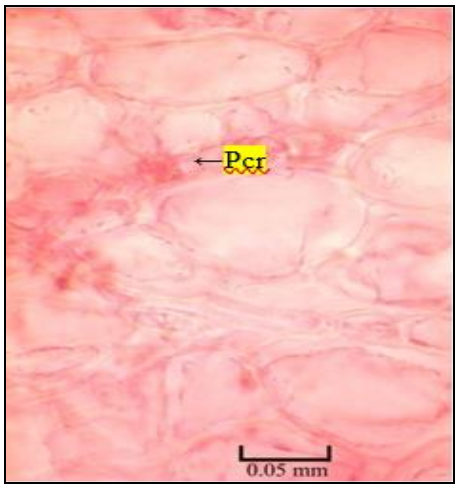


**Fig 2:** D. Inner mesocarp

**Powder microscopy**

Powder microscopic analysis of *E. officinalis* fruit powder showed the following characters epidermis with thickened straight walls, irregular thickened isodiametric parenchyma cells, sclerids, prismatic crystals, starch grains and short fibres (Fig. 3.A, 3.B, 3.C, 3.D, 3.E).

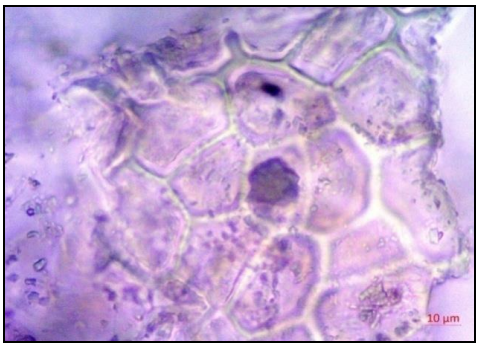


**Fig 3:** A Epicarp

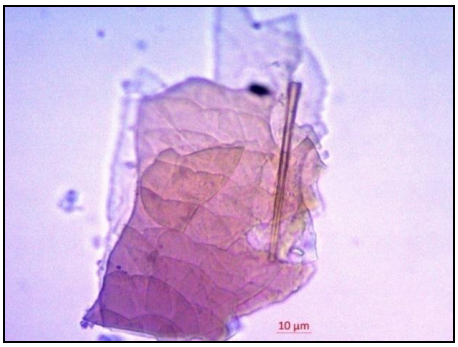


**Fig 3:** B Mesocarp

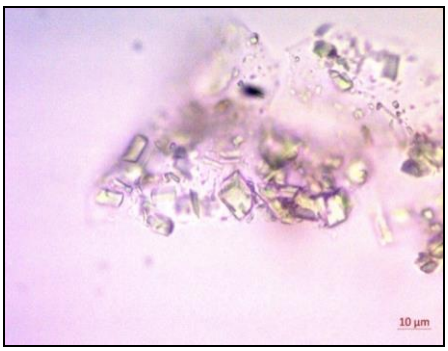


**Fig 3:** C Prismatic crystals


**Fig 3:** D Starch grains


**Fig 3:** E Fibrosclereids

**Table 1:** Determination of length and width of fibres and stone cells of *Emblica officinalis*

| S. No | Parameters | Length (µm) | | | Width (µm) | | |
|---|---|---|---|---|---|---|---|
| | | Max | Avg | Min | Max | Avg | Min |
| 1 | Fibres | 234 | 104 | 52 | 78 | 39 | 26 |
| 2 | Stone cells | 91 | 65 | 39 | 52 | 39 | 26 |

**Table 2:** Determination of diameter of Starch grains

| S. No | Name of the drug | Diameter (µm) | | |
|---|---|---|---|---|
| | | Max | Avg | Min |
| 1 | *Emblica officinalis* | 36 | 24 | 12 |

## Quantitative miroscopy-linear measurements

The results of linear measurements for fibres, stone cells and starch grains were presented in table 1 and 2.

## Physico chemical evaluation

The findings of physicochemical analysis of ash values, extractive values, loss on drying and foaming index were exhibited in tables 3 and 4.

**Table 3:** Determination of Ash values and Extractive values

| S. No. | Name of the Drug | Ash values (% w/w) | | | | Extractive values (% w/w) | |
|---|---|---|---|---|---|---|---|
| | | Total ash | Acid insoluble ash | Water soluble ash | Sulphated ash | Water soluble | Alcohol soluble |
| 1. | *Emblica officinalis* | 2.48 | 1 | 2.97 | 9.5 | 8 | 18 |

**Table 4:** Determination of Loss on drying and Foaming index

| S. No. | Name of the Drug | Loss on Drying (% w/w) | Foaming Index |
|---|---|---|---|
| 1. | *Emblica officinalis* | 3.78 | Less than 100 |

## Fluorescence Analysis

The following table 5. Represented the findings of fluorescence analysis. Fluorescence green was observed with 1N HCl and 1N $HNO_3$ at 254 and 365 nm.

**Table 5:** Fluorescence analysis of *Emblica officinalis*

| S. No | Treatment | Day Light | Short UV (254nm) | Long UV (365nm) |
|---|---|---|---|---|
| 1 | Powder | Light brown | Greenish brown | Dark brown |
| 2 | Powder + water | Yellowish brown | Yellowish green | Brownish black |
| 3 | Powder+1NAlc.NaOH | Yellowish Brown | Greenish brown | Reddish brown |
| 4 | Powder+1N Alc. KOH | Brown | Greenish brown | Greenish brown |
| 5 | Powder + 1N $H_2SO_4$ | Brown | Dark brown | Black |
| 6 | Powder + 1N HCl | Pale brown | Fluorescence green | Reddish brown |
| 7 | Powder + 1N $HNO_3$ | Yellowish Brown | Greenish Black | Fluorescence green |
| 8 | Powder + 1N NaOH | Yellowish Brown | Dark Green | Greenish Black |
| 9 | Powder + 1N KOH | Pale brown | Green | Reddish brown |
| 10 | Powder+Acetic acid | Yellowish Brown | Green | Reddish brown |
| 11 | Powder + Ammonia | Yellowish Brown | Greenish brown | Reddish brown |
| 12 | Powder + Ethanol | Brown | Greenish brown | Dark brown |
| 13 | Powder + $FeCl_3$ | Bluish green | Dark Green | Greenish Black |
| 14 | Powder + Iodine | Bluish brown | Greenish blue | Brownish blue |

## Determination of heavy metal contamination

Limit test for heavy metals and arsenic showed the colour and stain of the sample was less than that of the standard. This confirmed that the dried fruits of *Emblica officinalis* was free from heavy metal contamination and toxicity.

## Qualitative analysis and quantitative estimation of inorganic elements

The results were tabulated in the tables 6 and 7. Qualitative analysis of inorganic elements of the fruits of *Emblica officinalis* showed that potassium and iron was present. Quantitative estimation of iron was found to be 2.9 % w/w.

**Table 6:** Qualitative analysis of essential elements

| S. No | Name of the Drug | Sodium | Potassium | Calcium | Magnesium | Iron | Sulphate | Phosphate | Chloride | Carbonate | Nitrates |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | *Emblica officinalis* | - | + | - | - | + | - | - | - | - | - |

**Table 7:** Quantitative estimation of Iron

| S. No | Name of the Drug | Quantity (% w/w) |
|---|---|---|
| 1 | *Emblica officinalis* | 2.9 |

**Preliminary Phytochemical Screening**

Preliminary phytochemical screening showed the presence of phytoconstituents carbohydrates, alkaloids, glycosides, saponins, phenolic compounds, phytosterols and terpenoids from the aqueous extract of dried fruits of *E. officinalis.*

**Discussion**

The usage of herbal medicine was increased globally for the health care primarily as home remedies and also for the treatment of major diseases. This is due to the less side effects, easily available and low cost. Herbal materials available in the market can be easily adulterated with other species or varities of the drugs. Inorder to ensure safety and efficacy, standardization of the herbal drug is necessary to improve the quality control of the drugs. WHO insisted to evaluate the crude drug for its activity and also to detect the adulterants [25, 26].

The standardization of dried fruits of *E. officinalis* for proper identity is most important to use as herbal medicine and in the preparation of formulations. To include a crude drug in the herbal pharmacopoeias, Pharmacognostical standardization is the primary and utmost important study about the drugs [27]. Pharmacognostical standardization provides us important diagnostic characters of the plants for proper identification and to detect adulterants. Microscopical standardization is the very first simplest method to characterize the correct identity of the herbal drug. According to WHO, macroscopical and microscopical evaluation of plants is the first and basic standards to confirm the identity and purity of crude drugs [28]. Quantitative microscopy – linear measurements are useful to identify and to determine the purity of the crude drug. In this study, the length and width of the fibres, stone cells and diameter of the starch grains are determined. These values are unique to the dried fruits so that it can be easy to detect the adulterants.

Physicochemical evaluation plays an important role in detecting adulterants by establishing fingerprint of few standards of the crude drug *E. officinalis*. Ash value is a constant parameter for individual drug to evaluate quality and purity of the drug. This value varies from one species to another. It gives an idea about the inorganic minerals like oxalate, carbonate, silica, phosphate and siliceous earthy matters present in the drug [29]. Total ash, water soluble ash, acid insoluble ash and sulphated ash values observed in this study from dried fruits of *E. officinalis* showed that these values are different and distinct from others and can be used as identifying marker for authentication of this plant part. It can be used as quality control for the fruits of *E. officinalis.*

Extractive value gives us an information regarding the phytoconstituent substance present in the given solvent from the crude drug. Alcohol soluble extractive value showed high value (18 %) when compared to the water soluble extractive value (8 %) [30].

Loss on drying determines the loss of weight of the drug due to Water and volatile content of the drug under specific conditions. Foaming index determines the foaming ability of the aqueous decoction and the extracts of the drug. Saponins produce foams in the plant drug when it is shaken with water [31].

Fluorescence analysis is one of the parameters to identify the crude drug from adulteration. Powdered drug when react with different chemicals exhibit different colours under day light and UV light. In this study, drug showed fluorescence green with the chemicals 1N HCl and 1N $HNO_3$ under UV light at 254 and 365 nm. This colour change was useful to identify the fruit in powdered form. Fluorescent effects provide evidence for the presence of fluorescent compounds [32].

Plants can be easily contaminated with heavy metals during their growth due to environmental pollution, soil, manures and fertilizers. If the plant contains higher toxic metals can cause toxicity to the humans during the consumption of plant materials. Hence, the limit tests for heavy metals and arsenic were performed and the results showed that they are within the permissible limits. This indicate that the dried fruits of *E. officinalis* are free from toxicity and safe to use as herbal medicine.

Generally, microbial contamination occurs during storage and transport of the plant materials. When the moisture content present in the drug seems to be in higher concentration, then it may be possible to contaminate with the microbes. In this study, there was no bacterial and fungal growth. So, the stability of the crude drug was higher [33, 34].

Preliminary phytochemical evaluation showed the presence of phytoconstituents present in the dried fruits of *E. officinalis.* This will be useful to investigate the pharmacological activities and for further studies. It may also be used as a diagnostic tool for the standardization of dried fruits of *E. officinalis* to improve quality control and identification of the drug and thereby minimize adulteration [35].

**Conclusion**

The present investigation provides the scientific data which highlight the traditional use of this plant to treat various diseases. The pharmacognostical, physicochemical and phytochemical standardization of the dried fruits of *E. officinalis* provides the useful data to create standards to assess the quality and purity of the drug. This study may be useful to the students and researchers to carry out further studies in the fruits of *E. officinalis* and herbal formulations containing fruits of *E. officinalis* as an ingredient.

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**Conflict of interest**

We declare that we have no conflict of interest.

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