ANALYTICAL AND PHYTOCHEMICAL STUDY OF *EMBLICA OFFICINALIS*

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ABSTRACT

*Emblica officinalis* is an important drug and a widely used medicine in Ayurveda, in folklore and also as a household remedy and food. In the present study bark of *Emblica officinalis* was subjected to pharmacognostical studies such as macroscopic, microscopic and micromeretics parameters were also observed. Physicochemical studies such as ash values, extractive values of plant part were carried out to confirm the identity of plant. Ash values such as total ash, acid insoluble ash and water soluble ash were determined and recorded. Extractive values such as alcohol soluble extractives and water soluble extractive values were also determined. The bark of *Emblica officinalis* Linn shows the presence of phytoconstituents such as alkaloids, terpenoids and tannins.

KEYWORDS: *Emblica officinalis*, Phyto chemical, pharmacognosy.

INTRODUCTION

Indian gooseberry is a wonder herbs and one of the precious gifts of nature to man. It contributes towards health and longevity. It is highly nutritious and is an important dietary source of Vitamin C, minerals and amino acids. The edible fruit tissue contains protein concentration 3-fold and ascorbic acid concentration 160-fold compared to that of the apple. The fruit also contains considerably higher concentration of most minerals and amino acids than apples.

Modern classification: According to Benthem & Hooker (1862-1883)


Kingdom : Planate
Division : Angiospermae
Class : Eudicots
Subclass : Archichlamydeae
Series : Unisexuals
Order : Malpighiales
Family : Euphorbiaceae
Group : Emblica
Species : officinalis Gaertn
Botanical Description

A deciduous small or middle sized tree with crooked trunk and spreading branches.

**Bark:** Greenish grey, peeling off in conchoidal flakes; branch lets glabrous or finely pubescent, 10-20cm long, often deciduous.

**Leaves:** Sub sessile, 10-13 by 2.5-3mm, closely set along the branch lets, distichously, light green, glabrous, narrowly linear, obtuse, imbricate when young, having the appearance of pinnate leaves, stipules ovate, finely acute.

**Flowers:** Greenish yellow, in axillary fascicles on the leaf bearing branchlets, often on the naked portion below the leaves, with fimbriate bracts at the base. Male flowers numerous on short slender pedicels. Sepals 6, oblong, obtuse, 1.2mm long. Disk 0. anther 3 on a short central coloumn. Female flowers few, sub sessile. Sepals as in the male. Disk a lacerate cup. Ovary 3 celled; styles connate at the base, irregularly twice bifid with acute lobes.

**Fruit:** 1.3-1.6cm diameter, fleshy globose with 6 obscure vertical furrows, pale yellow, of three 2 seeded crustceous coci.

**Seeds:** 6, 3- gonous.

**PHARMACOGNOSTICAL STUDY**

**Macroscopic and microscopic features:**

**Macroscopic (Fresh):**
Fruit globose, 2.5-3.5cm in dia, fleshy, smooth and six prominent lines; greenish when tender, changing to light yellowish or pinkish colour when mature, with a few dark specks; taste sour and astringent followed by sweet taste.

**Microscopic (Fresh):**
Transverse section of mature fruit shows an epicarp consisting of single layer of epidermis and 2-4 layers of hypodermis; epidermal cell, tabular in shape, covered externally with a thick cuticle and appear in surface view as polygonal; hypodermal cells tangentially elongated, thick walled, smaller in dimension than epidermal cells; mesocarp forms bulk of fruit, consisting of thin walled parenchymatous cells with intracellular spaces, peripheral 6-9 layers smaller, ovoid or tangentially elongated while rest of cell layers in size, isodiametric and radially elongated, several collateral fibro vascular bundles scattered throughout mesocarp consisting of xylem and phloem. Xylem composed of tracheal elements, fibre tracheids and xylem fibres; and numerous silica crystals.

**Identity, purity and strength:**
Foreign matter Not more than 2%
Total ash  Not more than 7%
Alcohol soluble extractive  Not more than 2%
Water soluble extractive  Not less than 50%
Moisture content  Not less than 30%

**Macroscopic (dried)**

Drug consists of curled pieces of pericarp fruit occurring either as separated single segment; 1-2cm long or united as 3 or 4 segments, highly shriveled and wrinkled external convex surface to somewhat concave, transeversly wrinkled lateral surface, external surface shows a few whitish specks, occasionally some pieces show a portion of stony testa (which should be removed before processing); texture rough, cartilaginous, tough; taste sour and astringent.

**Microscopic (Dried):**

Transverse section of fruit shows epicarp consisting of a single layered epidermis, cell appearing tabular and polygonal in surface view, cuticle present; mesocarp cells tangentially elongated parenchymatous and crushed, difference roughly into a peripheral 8 or 9 layers of tangentially elongated smaller cells, rest consisting of mostly isodiametric larger cells with walls showing irregular thickening.

**Powder:** Fine powder shows epidermis with uniformally thickened straight walled, isodiametric parenchymal cells with irregular thickened walls, occasionally short fibres and tracheids.

**Identity, purity and Strength:**

| Foreign matter (including seed and coat) | Not more than 3% |
| Total ash                              | Not more than 7% |
| Acid insoluble ash                     | Not more than 2% |
| Alcohol soluble extractive             | Not less than 40% |
| Water soluble extractive               | Not less than 50% |

**CHEMICAL CONSTITUENTS:**

Fruits and seed oil contains a good source of vitamin c; carotene, nicotinic acid, riboflavin, D-glucose, D-fructose, myoinositol and a pectin with D-galacturonic acid, D-arabinosyl, D-xyllosyl, L-rhamnosyl, D-glucosyl, D-mannosyl, and D-galactosyl residues, embicol, mucic, indole acetic acid and 4 other auxins-al, a3, a4, and a5, 2 growth inhibitors- R1 and R2; phylembic acid and phylembin and fatty acids .Bark contains leucodelphinide, procyanidin, 3-0-gallated prodelphinidin and tannin .

physical and chemical characteristics.
Sp. Gravity 0.9220  
Acid value 12.7%  
Sap. Value 185%  
Iod value 139.5%  
R.I. value 1.03%  
Acetyl value 2.03%  
Unsapon matter 3.81%  
Sterol content 2.70%  
Unsaturated fatty acids 7.0%

The components of the fatty acids of the oil are:

- Linolenic acid 8.78%
- Linoleic acid 44.0%
- Oleic acid 28.40%
- Stearic acid 2.15%
- Palmitic acid 2.99%
- Myristic acid 0.95%

The enzymes proteolytic and lipolytic are present in the seeds.

<table>
<thead>
<tr>
<th>Activities</th>
<th>Chemical constituents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti microbial</td>
<td>Phyllembin</td>
</tr>
<tr>
<td>Anti oxidative</td>
<td>Emblican a, Emblican B</td>
</tr>
<tr>
<td>Hypolipidemic</td>
<td>Flavonoids</td>
</tr>
<tr>
<td>Hepato protective</td>
<td>Tannoid principles</td>
</tr>
</tbody>
</table>

Analytical study

1. Determination of Moisture content:

Materials: Petriplates, physical balance, dessicator and oven.

Procedure: 2gm of the sample was taken in the previously weiged petriplates. Petriplates were kept in the oven maintained at 110 degree c for drying. After 3 hr’s petriplates were taken out and weighed. This procedure was repeated for 4-5 times until the constant weight was reached.

\[
\% \text{ Moisture} = \frac{\text{Difference in weight}}{\text{weight of sample}} \times 100
\]
2. Determination of Total ash:
Materials: Silica crucible, physical balance, desiccator, bunsen burner and air-dried drug, muffle furnace.
Method: The clean and dry crucible was weighed in which 2 gms of the drug was taken and heated in ignited bunsen burner. Temperature was gradually increased up to 500º C until white colored ash was obtained. The crucible containing ash was cooled in a desiccator and weighed. The procedure was repeated till two consecutive weights did not differ by more than 5mg. The content of total ash in mg per gm of air-dried material was calculated.

3. Determination of Acid insoluble Ash:
Materials: Physical balance, crucible, muffle furnace, desiccator, air dried drug and ash less filter paper.
Method: The crucible containing the total ash, 25 ml of dilute hydrochloric acid was added and covered with a watch glass and boiled gently for 5 minutes. The watch glass was rinsed with 5 ml of hot water and this liquid was added to the crucible. The insoluble matter was collected on an ash less filter paper and washed with hot water until the filtrate was neutral. The filter paper containing the insoluble matter was transferred to the original crucible, dried on a hot plate. The residue was allowed to cool in a desiccators for 30 minutes and weighed again. The content of acid insoluble ash in mg per gram of air-dried material was calculated.

4. Determination of Alcohol soluble extractive:
Materials: Physical balance, powdered air dried drug, glass stoppered conical flask, ethyl alcohol, vacuum filter, hot plate, water bath, beaker and desiccator.
Procedure: 4gm of coarsely powdered air-dried material was accurately weighed, placed in a glass stoppered conical flask. It was macerated with 100 ml of 90% of ethyl alcohol and the flask was weighed. The flask was closed and was allowed to stand for 24 hours, with frequent agitation, this was filtered carefully and 25 ml of the filtrate was taken to a flat-bottomed dish and evaporated on a water bath. Later it was dried, cooled in a desiccator and weighed without delay. The content of alcohol extractive matter in mg/g of air-dried drug was calculated.

5. Water - Soluble extractive value:
Materials: Powdered air-dried drug, physical balance, glass stopper conical flask, water, reflux condenser, filter paper, water bath, beaker and desiccator.
Procedure: 4 gm of coarsely powdered air-dried drug was accurately weighed and placed in a glass stopper conical flask. To this, 100 ml of water was added and weighed to obtain the total weight including the flask. The flask was shaken well and allowed to stand for an hour. The flask
was attached to a reflux condenser and boiled gently for one hour, cooled, weighed and readjusted to the original weight by adding water. This was filtered rapidly through a dry filter. 25ml of the filtrate was transferred to a tarred flat-bottomed dish and evaporated on a water bath. This was dried at 105°C, cooled and weighed without delay. The content of water extractable matter in mg/g of air-dried drug was calculated.

6. Determination of PH:

The PH value of a solution is determined potentiometrically by means of glass electrode, a reference electrode and a PH meter either of the potentiometric or the deflection type.

1. First calibrate the apparatus using buffer solutions PH 4 at 20 degree c.
2. Add the coarsely powdered drug to the solution and stirred for 5 min and adjusted at 25°C.
3. Immerse the electrodes in the solution to be examined and measure the PH at the same temperature as for as the standard solutions.
4. At the end a set of measurements and readings are taken (if the difference between the readings and the original value is greater than 0.5, the set of measurements to be repeated.

Phyto chemical tests:

1. Test for alkaloids: The alcoholic extract of drug was taken with acetic acid. To this a few drops of Dragendorff’s reagent was added. Yellow or orange precipitate shows the presence of alkaloids.
2. Test for Saponins: The water extract of drug was mixed with water and shaken thoroughly; honeycomb froth shows presence of saponin
3. Test for steroids: The chloroform extract was treated with 3 to 4 drops of acetic anhydride, and a drop each of con. H₂SO₄ and acetic acid, and observed for greenish discolouration.
4. Test for sugars: Water extract sample was treated with anthrone and sulphuric acid, and was observed for green and purple discolouration, indicating the presence of sugars.
5. Test for tannin: Water extract of the sample was treated with basic lead acetate solution and was observed for white precipitate, which indicates the presence of tannins.
6. Test for triterpenes: Chloroform extract of the sample was treated with tin and 3 drops of thionyl chloride and observed for violet or purple discolouration, indicating the presence of triterpenes.
7. Test for flavonoids: Alcoholic extract of the sample was treated with a bit of Magnesium and concentrated HCl and heated. Red or orange red colour indicates presence of flavonoids.

Tests for inorganic elements:
Prepare ash of drug material. Add 50%v/v HCL or HNO3 to ash. Keep for 1 hr, filter with filtrate perform the following tests.

**Tests for Iron:**

A) To 5ml test solution, add few drops of 2% potassium ferrocyanide. Dark blue colouration is observed.

B) To 5ml test solution, add few drops of 5% ammonium thiocyanate. Solution turns blood red.

**Tests for Sulphate:**

A) To 5ml filtrate, add few drops 55 BaCl2 solution. White crystalline BaSo4ppt appears.

B) With lead acetate reagent gives white ppt.

**Tests for Phosphate:**

A) To 5ml test solution prepared in HNO3, add few drops ammonium molybdate solution. Heat 10 min, cool, yellow crystalline ppt is observed.

**Tests for Chloride:**

A) To 3ml test solution prepared in HNO3, add few drops 10% AgNO3 solution. White ppt of AgCl3 is observed.

**Test for Carbonate:**

A) with Mercuric chloride solution produces a brownish red ppt.

B) with solution of Magnesium sulphate, white ppt is formed.

**Test for Nitrates:**

A) Liberates red fumes when warmed with sulphuric acid and copper.

**Test for Potassium:**

A) Flame test; Gives violet colour to the flame.

**Test for Sodium:**

A) Flame test; prepare thick paste of ash of drug with concentrated HCl. Golden yellow flame is observed with this.

**Test for Magnesium:**

A) Gives white ppt. with ammonium carbonate solution but not with ammonium chloride solution.

**Test for Calcium:**

A) with solution of ammonium carbonate gives white ppt. which is insoluble in ammonium chloride solution.

**Thin Layer Chromatographic (TLC) study:**
Materials: TLC plates of thickness 0.20 mm, capillary tubes, glass chambers, oven, solvents, spraying agents, extracts.

Method: One gram of the sample was shaken with chloroform (20mL) for 15 minutes and filtered. The filtrate was discarded and the marc was refluxed with 0.5M Sulphuric acid (30mL) and cooled. To this 2 X 20mL of chloroform was added and shaken. Concentrated the chloroform layer and dissolved the residue in 1.0mL of Chloroform: methanol mixture (1:1). The TLC chamber was perfectly cleaned and dried before use. The solvent system was poured into TLC chamber and the glass lid was closed. The chamber was kept undisturbed for about an hour to saturate it. Later the TLC plates were taken and spotted with samples, with the help of capillary tubes, 1cm away from the sides and 2cm away from the base. A space of 2cm was maintained between each spot. The spotted plate was then gently immersed in TLC chamber concentrated with the solvent in such away that the solvent had uniform linear contact with the plate.

Development of plates: The chromatogram developed by the ascending technique. Development was allowed to proceed until the solvent front had traveled the required distance. The plate was then removed from TLC chamber and the solvent front immediately marked with a pencil line. Then the plate was sprayed with spraying reagents and dried and spots were observed. Rf values of the spots were found out by using the formula:

\[
\text{Rf value} = \frac{\text{Distance traveled by the spot}}{\text{Solvent front}}
\]

**TLC extraction procedure:**
Extraction involves the separation of the plant tissues from the inactive components by using selective solvents in standard extraction procedures.

Aqueous Extract: To one part of drug four parts of water was added and reduced to 1/4th the quantity and filtered.

Methanol Extract: To 10gm coarse powder of drug 50ml of methanol is to be added and kept over the water bath till the active constituents of drug get dissolved in methanol. Later the solution is filtered with the help of a filter paper and the extract of methanol collected.

**OBSERVATIONS AND RESULTS**

*Physico-chemical Study Of Emblica officinalis Churna:*

1. Organoleptic description -
   - Colour : Light brown
   - Odour : Characteristic odour
   - Taste : Sour with sweetish
The observed values confirm that *Emblica officinalis* churna is in standard with the values given in Ayurvedic Pharmacopoeia.

**Phyto chemical tests of Emblica officinalis churna:**

<table>
<thead>
<tr>
<th>Sl.no</th>
<th>Phytochemical</th>
<th>Water extract</th>
<th>Alcohol extract</th>
<th>Chloroform extract</th>
<th>Colour change/ppt</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Steroid</td>
<td>+</td>
<td></td>
<td></td>
<td>Greenish</td>
</tr>
<tr>
<td>2.</td>
<td>Triterpene</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Sugar</td>
<td>+</td>
<td></td>
<td></td>
<td>Purple</td>
</tr>
<tr>
<td>4.</td>
<td>Alkaloids</td>
<td>+</td>
<td></td>
<td></td>
<td>Orange ppt</td>
</tr>
<tr>
<td>5.</td>
<td>Saponin</td>
<td>+</td>
<td></td>
<td></td>
<td>Honey comb froth</td>
</tr>
<tr>
<td>6.</td>
<td>Tannin</td>
<td>+</td>
<td></td>
<td></td>
<td>White ppt</td>
</tr>
<tr>
<td>7.</td>
<td>Flavonide</td>
<td>+</td>
<td></td>
<td></td>
<td>Yellowish ppt</td>
</tr>
</tbody>
</table>

**Inorganic Elements:**

<table>
<thead>
<tr>
<th>Inorganic Element</th>
<th>Bhringaraja</th>
<th><em>Emblica officinalis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Sulphate</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Carbonate</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>Sodium</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Potassium</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Nitrates</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>Calcium</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>Phosphate</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Chloride</td>
<td>Present</td>
<td>Present</td>
</tr>
</tbody>
</table>
RF values of HPTLC:

<table>
<thead>
<tr>
<th>Sl.No</th>
<th>Sample</th>
<th>Volume applied (µl)</th>
<th>Rf values @254</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Marker compound</td>
<td>7</td>
<td>0.8, 0.34, 0.49, 0.60.</td>
</tr>
<tr>
<td>2</td>
<td>Aqueous extract</td>
<td>7</td>
<td>0.6, 0.34, 0.52, 0.64</td>
</tr>
<tr>
<td>3</td>
<td>Methanol Extract</td>
<td>7</td>
<td>0.6.</td>
</tr>
</tbody>
</table>

Stationary phase : Silica Gel 60 F_{254},
Mobile Phase : Toluene: Acetic acid (50: 50).
Detection : 254nm.

CONCLUSION

*Emblica officinalis* was identified with their official botanical sources and the drug was confirmed anatomically as typical dicotyledons. Physico chemical characters and HPTLC profile may be used for pharmacopeia standards. *Emblica officinalis*. Indian gooseberry is a wonder herbs and one of the precious gifts of nature to man and a rich source of various elements which are good for human health and well being.

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