Emblica officinalis

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7. References (Pg. 15)
**Emblica officinalis** Gaertn.

(a) **Classification:**
- **Kingdom:** Plantae
- **Division:** Angiospermae
- **Class:** Dicotyledonae
- **Order:** Geraniales
- **Family:** Euphorbiaceae
- **Genus:** Emblica
- **Species:** officinalis Gaertn.

(b) **Synonym:** *Phyllanthus emblica* Linn.

(c) **Vernacular names:**
- **English:** Emblic myrobalan, Indian Goose berry
- **Sanskrit:** Aamalaki
- **Hindi:** Amla
- **Kannada:** Nelli Kayi
- **Marathi:** Amla
- **Gujarati:** Ambla
- **Malayalam:** Nelli Kayi
- **Tamil:** Nelli
- **Telugu:** Usirikaya
- **Kashmir:** Aonla

(d) **Part used:** Dried fruit

(e) **Botanical description:** A small to medium sized deciduous tree, 8-18 meters height with thin light grey bark exfoliating in small thin irregular flakes, leaves are simple, subsessile, closely set along the branchlets, light green having the appearance of pinnate leaves; flowers are greenish yellow, in axillary fascicles, unisexual, males numerous on short slender pedicels, females few, subsessile, ovary 3-celled; fruits globose, fleshy, pale yellow with six obscure vertical furrows enclosing six trigonous seeds in 2-seeded 3 crustaceous cocci.

(f) **Geographical distribution:** Found throughout India, the sea-coast districts and on hill slopes upto 200 meters, also cultivated in plains.

(g) **Traditional use:** The fruits are sour, astringent, bitter, acrid, sweet, cooling, anodyne, ophthalmic, carminative, digestive, stomachic, laxative, alterant, aphrodisiac, rejuvenative, diuretic, antipyretic and tonic. They are useful in vitiated conditions of tridosha, diabetes, cough, asthma, bronchitis, cephalalgia, ophthalmopathy, dyspepsia, colic, flatulence, hyperacidity, peptic ulcer, erysipelas, skin diseases, leprosy, haematogenesis, inflammations, anemia, emaciation, hepatopathy, jaundice, strangury, diarrhoea, dysentery, hemorrhages, leucorrhoea, menorrhagia, cardiac disorders, intermittent fevers and greyness of hair.

(h) **Pharmacology and clinical studies:** Phylllembin, isolated from the ethanolic extract of the fruit pulp has been found to potentiate the action of adrenaline in vitro and in vivo. It showed a mild
depressant action on Central Nervous System and also had a spasmodic activity. The drug also revealed mild stimulant action on isolated frog heart, short and insignificant rise in cat’s blood pressure, contraction of the nictitating membrane, the reduction of outflow of the perfusate in the hind limb of the rat and ear of rabbit, mild cerebral depressant action and anti-spasmodic activity. Of the indirect actions, potentiation of the action of adrenaline on the blood pressure of cat, isolated frog heart, and nictitating membrane of cat and the prolongation of the hypnosis were observed.

Further studies on the action of phyllemblin revealed that the drug antagonized the spasmogenic effect of acetylcholine, bradykinin and serotonin on the guineapig ileum. It also antagonized serotonin and acetylcholine-induced contractions of oestrogenised rat uterus. It increased the amplitude of cardiac contraction and heart rate transiently. An increase in coronary flow was followed by persistent decrease. On perfused rat hind limb and rabbit ear preparation, phyllemblin in small doses, increased the amount of perfusate whereas in larger doses it decreased the flow significantly. A triphasic response that is initial transient rise, followed by a transient fall and then sustained rise in blood pressure was seen in anaesthetized albino rats. The sustained rise was blocked by phentolamine (1mg/kg.). The drug produced 80 percent protection against leptazol seizures in mice. It protected effectively against tremors and clonic and tonic convulsions induced by nicotine. It also antagonized tremorine-induced tremors and other cholinergic symptoms.

The ether extract and 80 percent alcoholic extract of fruits acidified with hydrochloric acid, were found to have antibacterial activity. The other extract of acidified alcoholic extract showed the highest activity, inhibiting the growth of *M. pyogenes* var. *S. typhosa* and *S. paratyphi* at a concentration of 0.21mg /ml and that of *M. pyogenes* var. *albus*; *S. schottmellari* and *S. dysenteriae* at a concentration of 0.42mg/ml.

The effect of crude amla (traditionally known as amalaki rasayana) on total serum protein and its fractions was studied in rabbits. The drug had no significant effect on the levels of serum protein fractions, but it raised the total protein level and increased the body weight. The studies indicated that the increase in the body weight was due to positive nitrogen balance. The drug was found to have only anabolic effect without affording resistance against diseases.

Clinical studies were conducted to investigate the effect of crude amla in gastritis syndrome. The crude amla was given in 20 cases in a dose of 3 gms, 3 times a day for 7 days. The drug was found effective in 85% of the cases. It was observed that the drug did not have any significant beneficial effect in cases of hypochlorhydria. Only cases of hyperchloridia with burning sensation in abdominal and cardiac regions and epigastric pain were benefited.

Alcoholic extract of a plant (1g/kg) has shown an increase in the cardiac glycogen and a decrease in serum GOT, GPT and LDH in isoprotenol pretreated rats, suggesting a cardioprotective action. It showed a reduction in serum cholesterol levels and a significant antiatherogenic effect. This study suggest that Vitamin C content alone may not responsible for the antiatherogenic effect of the plant in animals.

The lipid lowering and antiatherosclerotic effects of amla fresh juice were evaluated in cholesterol fed rabbits (rendered hyperlipidemic by atherogenic diet and cholesterol feeding). Amla fresh juice was administered at a dose of 5ml/kg body weight per rabbit per day for sixty days. Serum cholesterol, Triglycerides, phospholipid and Low-density lipoprotein levels were lowered by 82%,
66%, 77% and 90% respectively. Similarly, the tissue lipid level showed a significant reduction following amla juice administration. Aortic plaques were regressed. Amla juice treated rabbits exerted more cholesterol and phospholipids, suggesting that the mode of absorption be affected. Amla juice is an effective hypolipidemic agent and can be used as a pharmaceutical tool in hyperlipidemic subjects.

It is reported to have anti-cancer properties. The crude extract of *Emblica officinalis* was reported to counteract hepatotoxic and renotoxic effects of metals due to anti-oxidant activity. Anti-oxidant of the fruit extract is demonstrated in several models.

(i) **Safety**: The drug is not reported to have any side effects even after prolonged use.

(j) **Phytochemistry**: The fruits of *Emblica officinalis* are rich in tannins. The fruits have 28% of the total tannins distributed in the whole plant. The fruit contains two hydrolysable tannins Emblicanin A and B, which have antioxidant properties, one on hydrolysis gives gallic acid, ellagic acid and glucose wherein the other gives ellagic acid and glucose. The fruit also contains Phyllemblin.

(k) **Active principle**: Tannins and Gallic acid

![Gallic acid structure](image)

\[
\text{Gallic acid} \quad \text{C}_7\text{H}_6\text{O}_5 \quad \text{Mol. Wt. 170.12}
\]

*The information provided herein has been collected from sources considered reliable, but has not been independently verified by Natural Remedies Pvt. Ltd.*
**Emblica officinalis**

**ANALYTICAL SPECIFICATION OF THE CRUDE DRUG**

**Macroscopic Characters:**

- **Colour & Appearance**: The dried fruit is brown to blackish brown in colour.
- **Odour**: Characteristic
- **Taste**: Sour and astringent

<table>
<thead>
<tr>
<th>TESTS</th>
<th>LIMITS</th>
<th>PROTOCOLS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tests for extraneous material</td>
<td>Quality Control Methods for Medicinal Plant Materials -WHO</td>
<td></td>
</tr>
<tr>
<td>Foreign matter</td>
<td>&lt; 1.0%</td>
<td>-do-</td>
</tr>
<tr>
<td>Sand &amp; Silica</td>
<td>Absent</td>
<td>-do-</td>
</tr>
<tr>
<td>Insect infestation</td>
<td>Nil</td>
<td>-do-</td>
</tr>
<tr>
<td>Rodent contamination</td>
<td>Nil</td>
<td>-do-</td>
</tr>
</tbody>
</table>

**Physico-chemical analysis**

- **Ash content**: < 8.0%w/w -do-
- **Acid insoluble ash**: < 1.0%w/w -do-
- **Moisture content**: < 8.0%w/w -do-

**Successive extractive value**

- **Petroleum ether extractive value**: 0.2 – 0.8%w/w -do-
- **Chloroform extractive value**: 0.6 – 1.5%w/w -do-
- **Methanol extractive value**: 20 – 30%w/w -do-

**Alcohol soluble extractive value**

- **22 – 33%w/w -do-**

**Water soluble extractive value**

- **40 – 60%w/w**

**Phytochemical analysis**

- **Total tannins**: 12 –18 % w/w By Spectrophotometer
- **Gallic acid**: 4-6% w/w By HPLC
IDENTIFICATION OF CRUDE DRUG BY TLC

**Sample detail** : *Emblica officinalis* crude drug (dried fruit)

**Adsorbent** : Precoated silicagel (Al - Sheet)

**Mobile Phase** : Toluene: Ethyl Acetate 93 : 7

**Sample preparation** : 2 gms of *Emblica officinalis* dried fruit powder was extracted with petroleum ether and the mark was further extracted with chloroform and both the extracts were concentrated and diluted with chloroform. 10µl was applied on different TLC plates.

**Solvent front run upto** : 9 cms

**Application** : CAMAG Linomat IV

**Detection** : Anisaldehyde sulphuric acid (Fig. 1 & 2)
**IDENTIFICATION OF CRUDE DRUG BY TLC**

**Sample detail**: *Emblica officinalis* crude drug (dried fruit)

**Adsorbent**: Precoated silicagel (Al - Sheet)

**Mobile Phase**: Ethyl Acetate : Formic acid : Acetic acid : Water  
100 : 11 : 11 : 27

**Sample preparation**: 2 gms of *Emblica officinalis* dried fruit powder was successively extracted with petroleum ether and chloroform. The mark obtained from chloroform extract was further extracted with methanol. 10µl was applied on TLC plate.

**Solvent front run upto**: 9 cms

**Application**: CAMAG Linomat IV

**Detection**: Anisaldehyde sulphuric acid (Fig. 3)

**Scanning**: Densitometer 254 nm (Fig. 4)

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**Fig. 3** & **Fig. 4**

*S* - Standard  
*T* - Test sample
## Emblica officinalis

### ANALYTICAL SPECIFICATIONS FOR THE EMBLICA OFFICINALIS - EXTRACT

**Item** :  *Embla officinalis* extract (≥10% Gallic acid)

**Description** : Light brown to very dark brown powder with characteristic odour and taste.

**Identification:**
1) Comparison with the standard TLC profile.
2) Positive for Tannins and Gallic acid

<table>
<thead>
<tr>
<th>TESTS</th>
<th>LIMITS</th>
<th>PROTOCOL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Physico-chemical analysis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Loss on drying (Moisture)</td>
<td>&lt; 9.0% w/w</td>
<td>As per I.P / B.P</td>
</tr>
<tr>
<td>pH of 5% solution</td>
<td>2.5 - 3.8</td>
<td>As per I.P / B.P</td>
</tr>
<tr>
<td>Ash Content</td>
<td>&lt;10.0% w/w</td>
<td>As per I.P / B.P</td>
</tr>
<tr>
<td>Acid insoluble ash</td>
<td>&lt; 1.0% w/w</td>
<td>As per I.P / B.P</td>
</tr>
<tr>
<td>Bulk density</td>
<td>0.2 - 0.6 g/cc</td>
<td></td>
</tr>
<tr>
<td><strong>Heavy metal analysis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lead</td>
<td>&lt; 10ppm</td>
<td>By A.A.S.</td>
</tr>
<tr>
<td>Cadmium</td>
<td>&lt; 2ppm</td>
<td>By A.A.S.</td>
</tr>
<tr>
<td>Arsenic</td>
<td>&lt; 2ppm</td>
<td>As per U.S.P</td>
</tr>
<tr>
<td><strong>Microbiological tests</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Viable Aerobic Count</td>
<td>&lt; 10^4 cfu g⁻¹</td>
<td>As per I.P / B.P</td>
</tr>
<tr>
<td>Total Fungal count</td>
<td>&lt; 10^3 cfu g⁻¹</td>
<td>As per I.P / B.P</td>
</tr>
<tr>
<td>Total Enterobacteriaceae</td>
<td>&lt; 10^2 cfu g⁻¹</td>
<td>As per B.P.</td>
</tr>
<tr>
<td>E. coli</td>
<td>Absent</td>
<td>As per I.P / B.P</td>
</tr>
<tr>
<td>Salmonella typhii</td>
<td>Absent</td>
<td>As per I.P / B.P</td>
</tr>
<tr>
<td>S. aureus</td>
<td>Absent</td>
<td>As per I.P / B.P</td>
</tr>
<tr>
<td><strong>Mycotoxin analysis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aflatoxins (Total B₁,B₂,G₁,G₂)</td>
<td>&lt; 5 ppb</td>
<td>As per A.O.A.C</td>
</tr>
<tr>
<td><strong>Phytochemical analysis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total tannins</td>
<td>≥ 30.0% w/w</td>
<td>Spectrophotometer</td>
</tr>
<tr>
<td>Gallic acid</td>
<td>≥10.0% w/w</td>
<td>HPLC(High Performance Liquid Chromatography)</td>
</tr>
</tbody>
</table>

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IDENTIFICATION OF EXTRACT BY TLC

Sample detail : *Emblica officinalis* extract

Adsorbant : Silica gel 60 F$_{254}$


Sample preparation : Known amount of *Emblica officinalis* was dissolved in methanol and applied on TLC plate.

Solvent front run upto : 8 cms

Application : CAMAG Linomat IV

Detection : By spraying Ferric chloride (Fig. 5)

Scanning : Densitometer 254 nm (Fig. 6)

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Fig. 5

Fig. 6
Colorimetric estimation of tannins is based on the measurement of blue colour formed by the reduction of phosphotungstomolybdic acid by tannin like compounds in alkaline solution.

Reagents:

(a) Folin-Denis reagent: To 750 ml of water, 100 g of sodium tungstate (Na₂WO₄·2H₂O), 20 g of phosphomolybdic acid and 50 ml of 85% phosphoric acid (H₃PO₄). Reflex the mixture for 2 hr, cool to 25°C and dilute to 1000 ml with water. Alternatively use readymade solution.

(b) Saturated sodium carbonate solution: To 100 ml of water, add 35 g of anhydrous sodium carbonate, dissolve at 70-80°C and cool overnight. Decant the clear liquid before the use.

(c) Tannic acid standard solution: Dissolve 100 mg of tannic acid in 1 litre of water. Prepare fresh solution for each determination (1 ml = 0.1 mg of tannic acid).

Preparation of standard curve: Pipette 0 to 10 ml aliquots of the standard tannic acid solution into 100-ml volumetric flasks containing 75 ml of water. Add 5 ml Folin-Denis reagent and 10 ml Na₂CO₃ solution into each of the volumetric flasks and make up to 100 ml with water. Mix well and measure the colour after 30 min at 760 nm against experimental blank adjusted to absorbency.

Preparation of sample: Dissolve 1 g of sample with 80 ml of water, transfer to 100 ml volumetric flask and dilute mark. Shake well and filter.

Determination: Use an aliquot of the filtrate containing not more than 0.1 mg of tannic acid. Proceed as in standard, and obtain mg tannic acid from the standard curve.

Reference:

ESTIMATION OF GALLIC ACID BY HPTLC

**Principle:**

Gallic acid in *Emblica officinalis* extract is separated from other compounds by TLC. The separated spots are compared with the standard Gallic acid which are simultaneously spotted along with sample.

The Gallic acid spots are scanned in a Densitometer to calculate the percentage.

**Analytical method:**

**Standard preparation:** Weigh accurately 50mg of Gallic acid Reference standard (Sigma) in to a 100 ml volumetric flask. Dissolve and make up the volume with methanol. Transfer 5 ml of this dilution to a 25ml volumetric flask and make up the volume with methanol.

**Sample preparation:** Weigh accurately 400mg of the sample into a 100 ml volumetric flask. Add 50ml of methanol and shake for 15 mins. Dilute to 100ml with methanol. Filter the solution before applying to TLC plate.

**Application of solution in TLC plate:**

In a 100 x 100mm precoated Silica gel GF254 (E. Merck) TLC plates apply 2, 4, 6, & 8 µl of solution and 2, 4 µl sample solution in 3 mm band at 10cm height and at 5 mm distance. Develop the plate in Toulene : Acetic acid (70 : 30) mobile phase. Run the mobile phase upto 9cm of the plate. Remove the plate and dry in air. Scan the tracks in densitometer (Shimadzu CS-9301PC) at 273 nm. Make a calibration curve for standard gallic acid and calculate the percentage of gallic acid in the sample from the calibration curve. TLC profile enclosed

S - Standard Gallic acid (Sigma)
T - Samples
Estimation of Gallic acid by HPTLC

Standard 1

Standard 2

Standard 3

Standard 4

Standard 5

Calibration curve

Emblica officinalis extract - Sample id. no. 1

Emblica officinalis extract - Sample id. no. 2
ESTIMATION OF GALLIC ACID IN
EMBLICA OFFICINALIS EXTRACT BY HPLC

ANALYSIS:

Chromatographic system: High Performance Liquid Chromatographic system equipped with LC8A pump, SPD-M 10vp Photo Array Detector in combination with Class LC 10A software.

Chromatographic conditions:

Mobile phase: water : Acetonitrile : Acetic acid
90 : 10 : 0.2

Column: ODS (Octadecyl silane) C18, 5µ size, 250 x 4.6mm (Merck) RP-18, Lichrocart® 250-4

Detector: SPD-M 10Avp Photo Array Detector

Wave length for recording the chromatogram: 273nm
Flow rate : 1ml/min
Inject volume : 10µl

Standard preparation: Weigh accurately 25mg of Gallic acid to a 50ml volumetric flask. Dissolve and make up the volume with methanol. Dilute 5ml to 50ml with mobile phase.

Sample preparation: weigh accurately 250mg of sample (equivalent to 25mg of gallic acid) to a 50ml volumetric flask. Dissolve and make up the volume with methanol. Dilute 5ml to 50ml with mobile phase.

Procedure: Set the instrument as per the chromatographic condition prescribed above. By means of suitable syringe inject 10 µl of standard solution. Record the chromatograms repeat the injections for another 4 times and calculate the RSD of the area. It should not be more than 2%. Inject 10µl of sample preparation and record the chromatogram.

Calculate the percentage of Gallic acid content from the peak areas.
Emblica officinalis

STANDARD

CHROMATOGRAM

PDA CHROMATOGRAM

3D VIEW
SAMPLE

CHROMATOGRAM

PDA CHROMATOGRAM

3D VIEW
**Emblica officinalis**

**References:**

**Botanical description:**

**Geographical distribution:**

**Traditional uses:**
3. Sharma P. V. Charaka samhita, Chikitsa stana – 26th Chapter (English).

**Pharmacology and Clinical studies:**
2. Khurana, S.C; Gupta, S.K; Sharma, R.C; Arora, R.B; "Study of pharmacodynamic properties of emblica officinalis" *Ind. J. Physiol. Pharmacol.*, 1970; 14 : 39
6. Thakur, C.P; "Emblica officinalis reduces serum, aortic and hepatic cholesterol in rabbits" *Experientia*, 1985; 41 : 423
8. Ritu Mathur; Arti Sharma; Dixit, V.P; Mira Varma; "Hypolipidaemic effect of fruit juice of Emblica officinalis in cholesterol-fed rabbits" *J. Ethnopharmacology*, 1996; 50 : 61-68

**Safety:**

**Phytochemistry:**

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