

Emblica officinalis



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CONTENTS

1. Brief literature review on *Emblica officinalis* (Pg.1-3)

- a. Classification
- b. Synonym
- c. Vernacular names
- d. Part used
- e. Botanical description
- f. Geographical distribution
- g. Traditional use
- h. Pharmacology and Clinical studies
- i. Safety
- j. Phytochemistry
- k. Active Principles

2. Analytical specifications of *Emblica officinalis* Crude Drug (Pg.4-6)

- a. Macroscopic characterization
- b. Identification of Crude drug by TLC

3. Analytical specifications for *Emblica officinalis* extract. (Pg.7-14)

- a. Identification tests
- b. Identification of extract by TLC profile
- c. Estimation of Tannins in *Emblica officinalis* by Colorimetric method.
- d. Estimation of Gallic acid by HPTLC
- e. Estimation of Gallic acid in *Emblica officinalis* extract by HPLC

7. References (Pg. 15)



Emblica officinalis Gaertn.

(a) Classification:

Kingdom	: Plantae
Division	: Angiospermae
Class	: Dicotyledonae
Order	: Geraniales
Family	: Euphorbiaceae
Genus	: <i>Emblica</i>
Species	: <i>officinalis</i> 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 cocci¹.

(f) Geographical distribution: Found throughout India, the sea-coast districts and on hill slopes upto 200 meters, also cultivated in plains¹.

(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 hair¹⁻⁶.

(h) Pharmacology and clinical studies: Phyllembin, 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 spasmolytic 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 observed¹.

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 symptoms².

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/ml³.

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 diseases⁴.

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 benefited⁵.

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 isoproterenol 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^{6,7}.

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%,



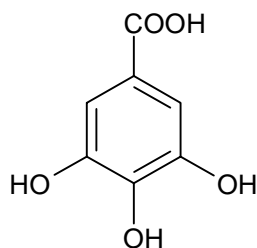
66%, 77% and 90% respectively. Similarly, the tissue lipid level showed a significant reduction following amla juice administration. Aortic plaques were regressed. Amla juice treated rabbits exerted more cholesterol and phospholipids, suggesting that the mode of absorption be affected. Amla juice is an effective hypolipidemic agent and can be used as a pharmaceutical tool in hyperlipidemic subjects⁸.

It is reported to have anti-cancer properties⁹. The crude extract of *Emblica officinalis* was reported to counteract hepatotoxic and renotoxic effects of metals due to anti-oxidant activity¹⁰. Anti-oxidant of the fruit extract is demonstrated in several models¹¹.

(i) Safety: The drug is not reported to have any side effects even after prolonged use¹.

(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 Phyllembin¹⁻³.

(k) Active principle: Tannins and Gallic acid



C₇H₆O₅ Mol. Wt. 170.12

Gallic acid

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



ANALYTICAL SPECIFICATION OF THE CRUDE DRUG

Macroscopic Characters:

Colour & Appearance : The dried fruit is brown to blackish brown in colour.

Odour : Characteristic

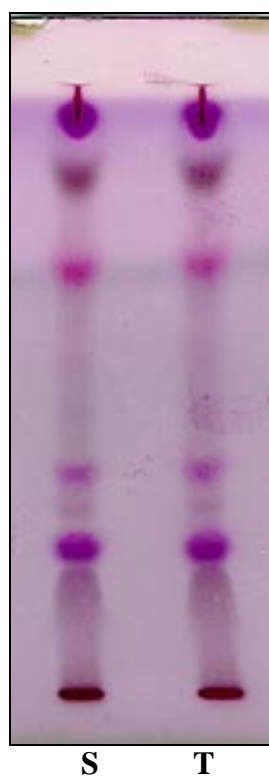
Taste : Sour and astringent

TESTS	LIMITS	PROTOCOLS
<u>Tests for extraneous material</u>		
		Quality Control Methods for Medicinal Plant Materials -WHO
Foreign matter	< 1.0%	-do-
Sand & Silica	Absent	-do-
Insect infestation	Nil	-do-
Rodent contamination	Nil	-do-
<u>Physico-chemical analysis</u>		
Ash content	< 8.0% w/w	-do-
Acid insoluble ash	< 1.0% w/w	-do-
Moisture content	< 8.0% w/w	-do-
<u>Successive extractive value</u>		
Petroleum ether extractive value	0.2 – 0.8% w/w	
Chloroform extractive value	0.6 – 1.5% w/w	-do-
Methanol extractive value	20 – 30% w/w	
<u>Alcohol soluble extractive value</u>		
	22 – 33% w/w	-do-
<u>Water soluble extractive value</u>		
	40 – 60% w/w	
<u>Phytochemical analysis</u>		
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. 10 μ l was applied on different TLC plates.
- Solvent front run upto** : 9 cms
- Application** : CAMAG Linomat IV
- Detection** : Anisaldehyde sulphuric acid (Fig. 1 & 2)



Pet ether fraction

Fig. 1



Chloroform fraction

Fig. 2

S- Standard

T- Test sample



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)



Fig. 3

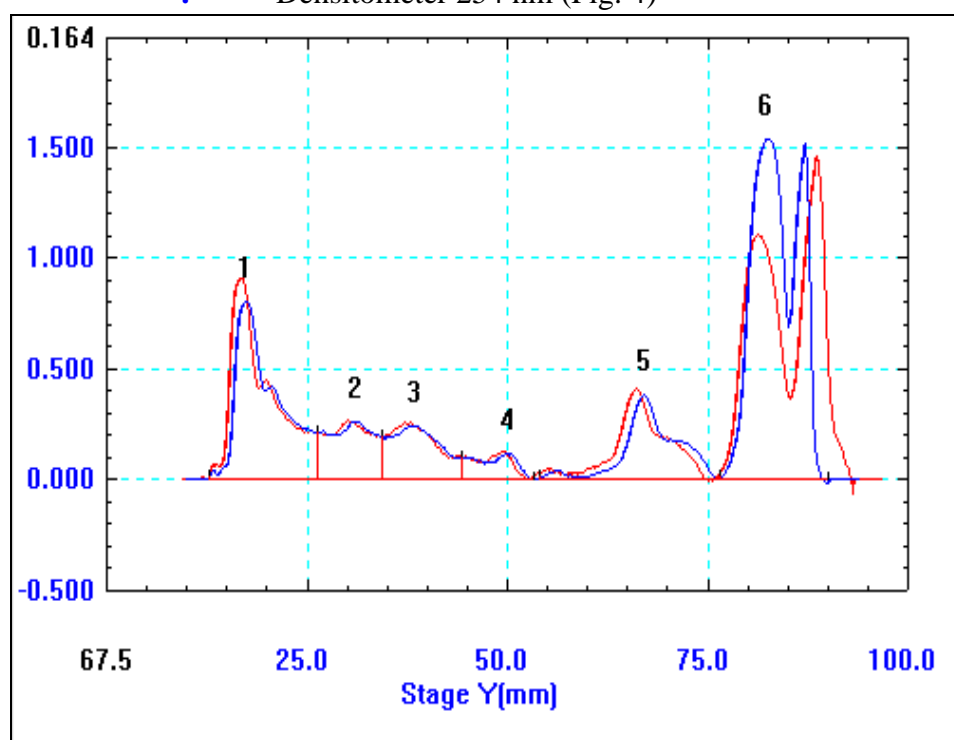


Fig. 4

S- Standard
T- Test sample



ANALYTICAL SPECIFICATIONS FOR THE *EMBLICA OFFICINALIS* - EXTRACT

- Item** : *Emblica 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

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



IDENTIFICATION OF EXTRACT BY TLC

Sample detail	:	<i>Emblica officinalis</i> extract
Adsorbant	:	Silica gel 60 F ₂₅₄
Solvent system	:	Ethyl Acetate : Acetic acid : Formic acid : Water 100 : 11 : 11 : 27
Sample preparation	:	Known amount of <i>Emblica officinalis</i> 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)



Fig.5

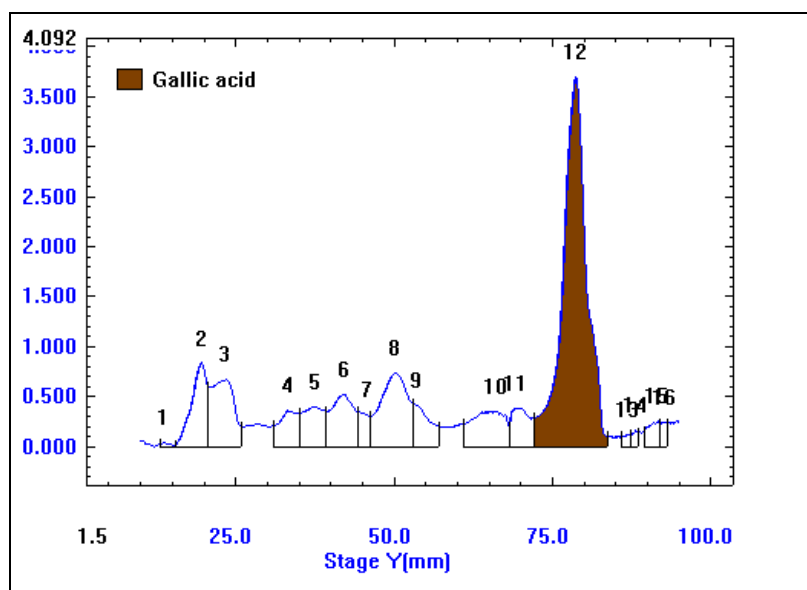


Fig. 6



ESTIMATION OF TANNINS (Colorimetric Method)

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 ($\text{Na}_2\text{WO}_4 \cdot 2\text{H}_2\text{O}$), 20 g of phosphomolybdic acid and 50 ml of 85% phosphoric acid (H_3PO_4). Reflux 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 $70\text{--}80^\circ\text{C}$ 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 Na_2CO_3 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:

1. *Official methods of analysis*, Association of Official Analytical Chemists, Washington, D.C., 11th edn., p. 154 (1970).



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.

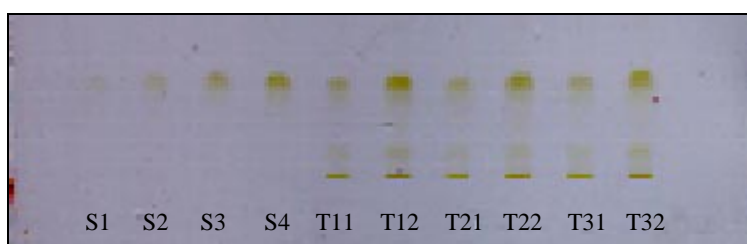
Analytical method:

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 μ l of solution and 2, 4 μ l 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. Sacn 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



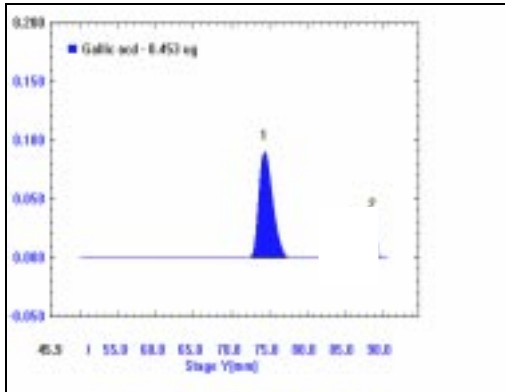
S - Standard Gallic acid (Sigma)

T - Samples

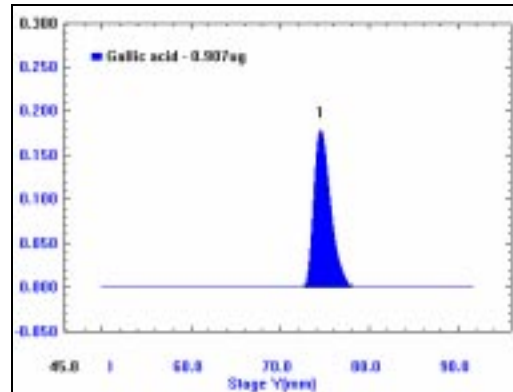


Estimation of Gallic acid by HPTLC

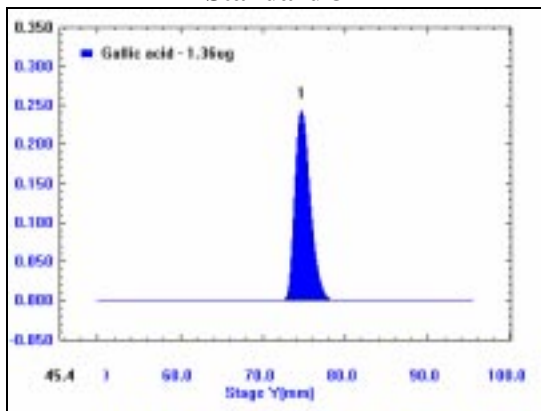
Standard 1



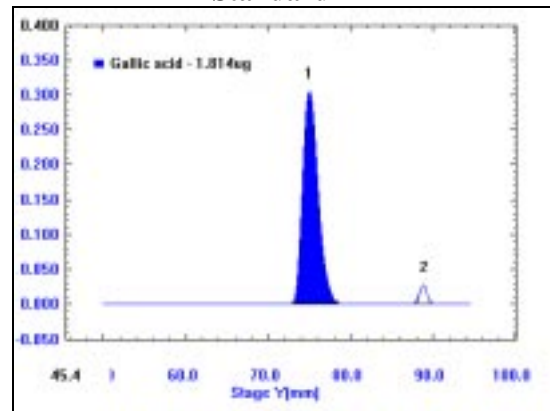
Standard 2



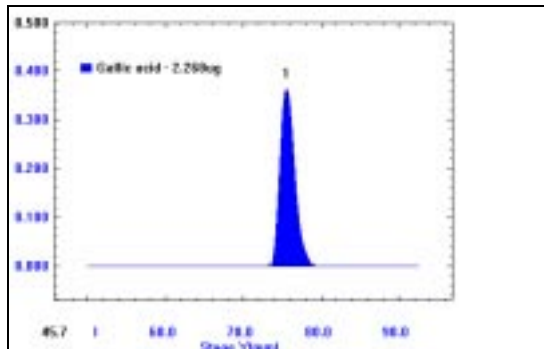
Standard 3



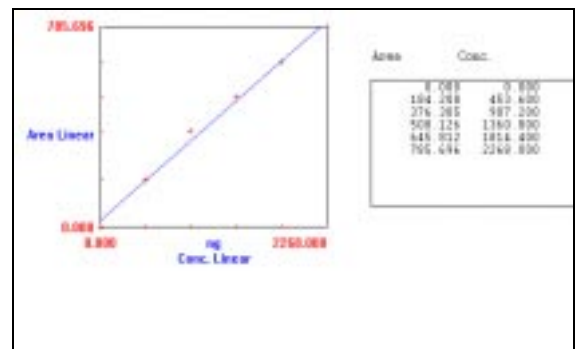
Standard 4



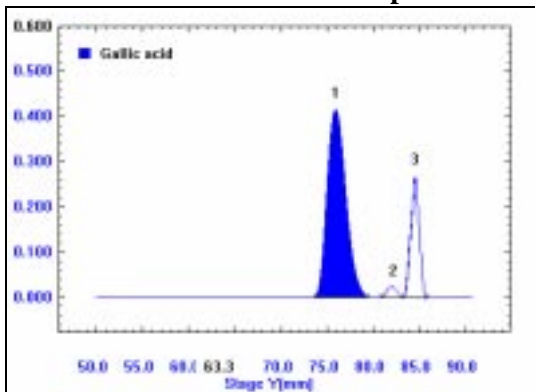
Standard 5



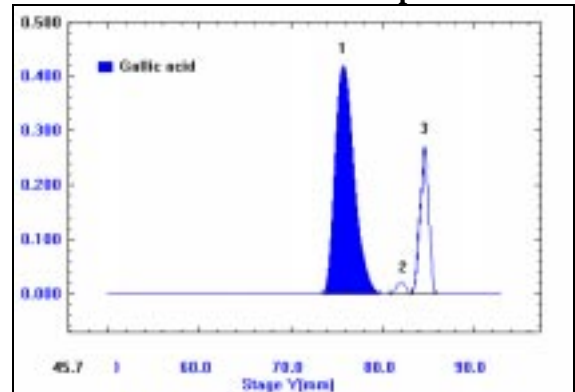
Calibration curve



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, 5 μ 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 : 10 μ l

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.

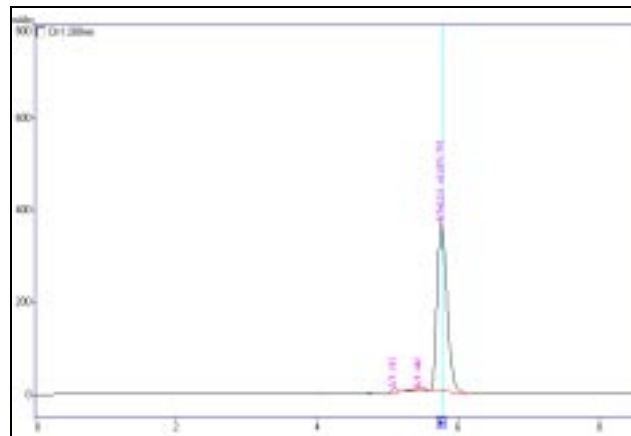
Sample preparation: weigh accurately 250mg of sample (equivalent to 25mg of gallic acid) to a 50ml volumetric flask. Dissolve and make up the volume with methanol. Dilute 5ml to 50ml with mobile phase.

Procedure: Set the instrument as per the chromatographic condition prescribed above. By means of suitable syringe inject 10 μ l 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 10 μ l of sample preparation and record the chromatogram.

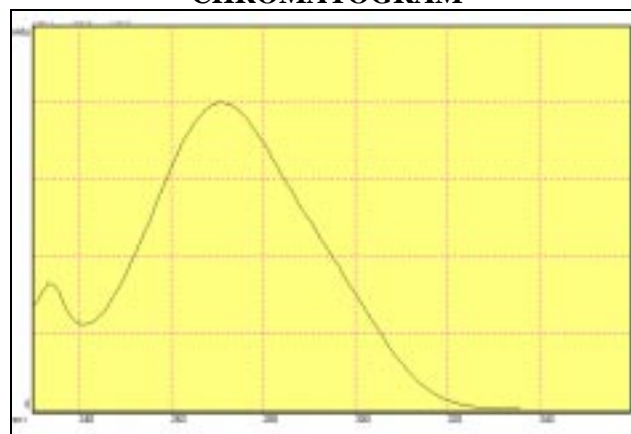
Calculate the percentage of Gallic acid content from the peak areas.



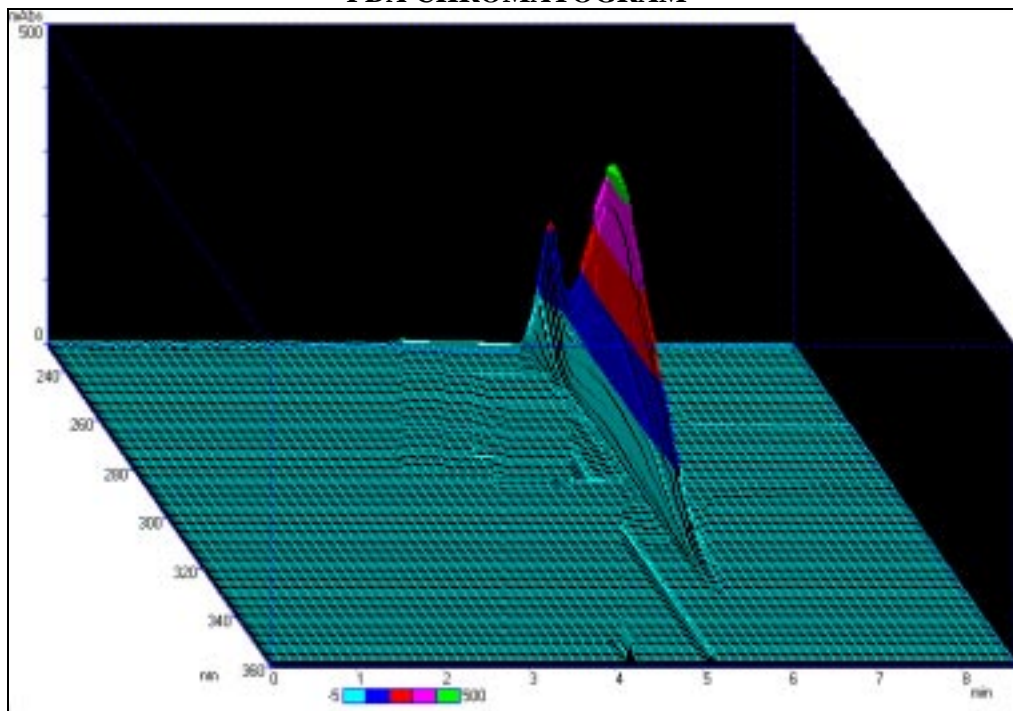
STANDARD



CHROMATOGRAM



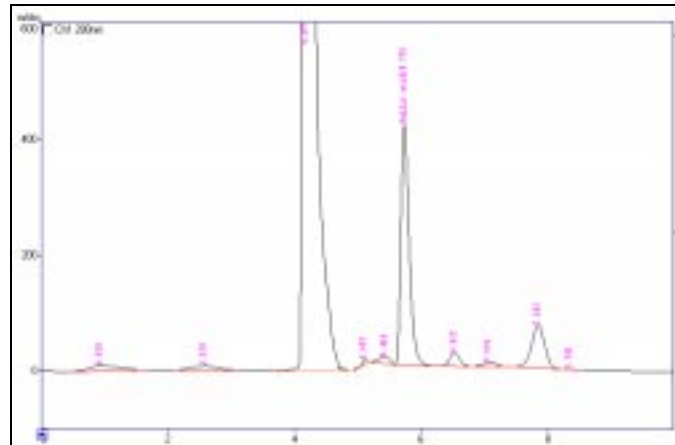
PDA CHROMATOGRAM



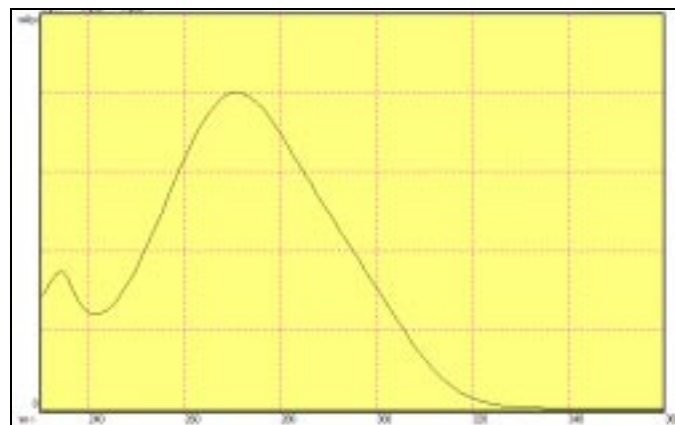
3D VIEW



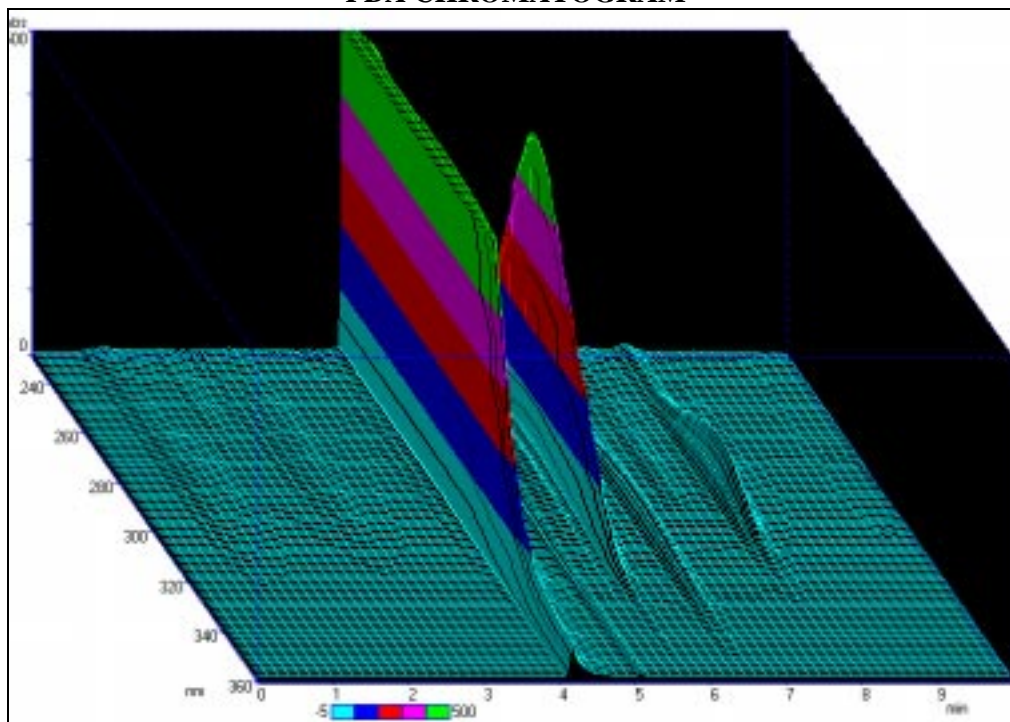
SAMPLE



CHROMATOGRAM



PDA CHROMATOGRAM



3D VIEW



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Botanical description:

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