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Research Article

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A PERSPECTIVE STUDY OF HARITAKI

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ABSTRACT
Haritaki is a common herbaceous plant, which is very extensively used in the preparation of many Ayurvedic medicines. It is found throughout India chiefly in deciduous forests and areas of light rainfall. It grows on variety of soils but thrives best in clay and sandy soils. The fruits ripe from November to March depending upon the locality. The extract obtained from Haritaki fruit contains a substance which has antibacterial and anti fungal properties. This substance inhibits the growth of bacteria and fungi such as *E. coli*. It is also used in the treatment of leucorrhoea, chronic ulcers, pyorrhea and other types of fungal infections of the skin. In this article, an attempt has been done to review the Ethno-botanical, Biochemical, Ayurvedic and Pharmacognostical aspects of Haritaki.
Key Words: Haritaki, Triphala, Ayurveda, *Terminalia chebula*.

INTRODUCTION

Haritaki is widely used medicinal plant in Ayurveda. Haritaki is used as a natural cleanser of the digestive system. It improves the functioning of the liver, spleen and the colon and hence it is widely used as a digestive tonic. The extract of Haritaki fruit is also used widely in many Ayurvedic formulations. Many research studies indicated that the oil obtained from the kernel of the Haritaki had certain substances which increased the motility of the gastro intestinal tract. This action was similar to that of castor oil. The extract obtained from Haritaki fruit also reported to contain a substance which has antibacterial and anti fungal properties. Haritaki is also used in combination with two more herbs to prepare a formulation known as Triphala. This medicine is widely used for Anti aging activity. It is also used for increasing the immunity of the body.

Taxonomical Description

Botanical Name: *Terminalia chebula* Retz.
Botanical Classification
Family**:** Combretaceae
Kingdom: Plantae
Division : Phanerogams
Subkingdom: Angiosperms
Class: Monocotyledons
Subclass : Epigynae
Order: Scytaminiales
Family: Combretaceae
Genus: Terminalia
Species: chebula

Vernacular Names

Eng.- Chebulik myrobalan. Hindi-, Harara, Harad, Beng.- Haritaki. Guj.- Hardo. Kan.- Harra, Karakkayi, Aalekayi. Mal.- Katukka. Mar.- Hirda, Hirda-phula, Bala hirade. Punj.- Har, Halela, Hurh, Harrar. Tam.- Katukkay. Tel.- Karakkaya, Karitaki. Arab.- Halilaj, Halilaje-asfar, Haliaje-asvad. Assam- Silikha, Hilikha. Gond.- Karka, Harro, Hir. Kash.- Halela. Kon.- Ordo. N.W.P.- Har, Haraira, Harara. Oriya- Haridra, Horitoli, Jonghihorida, Karedha, Harira. Pers.- Halilah, Halilahe-sard, Halilahe-siyah. Santhal- Rola, Hadra. Sind.- Har. Urdu- Haejarad.

Classical Synonyms

Haritaki, Abhaya, Pathya, Kayastha, Putana, Haimavati, Avyatha, Chetaki, Putana, Shiva, Vayastha, Rohini.

Parts Used: Fruit

Botanical Description

A tree, 15-24 m high. Leaves ovate or elliptic with a pair of large glands at the top of the petiole. Flowers yellowish white, in terminal spikes. Drupes ellipsoidal, obovoid or ovoid, yellow to orange-brown, sometimes tinged with red or black and hard when ripe, 3-5 cm long, 5 ribbed on drying. Seeds hard, pale yellow.

Distribution

It is found throughout the greater parts of India chiefly in deciduous forest and areas of light rainfall, also in slightly moist forest ascending to an altitude of 1500 m in the Himalayas, also in West Bengal, Assam, Bihar, Orissa, Madhya Pradesh, Maharashtra, Deccan and South India.

Propagation and Cultivation

It grows on variety of soils but thrives best in clay and sandy soils. The fruits ripen from November to March depending upon the locality. Mostly fallen fruits are collected in first half of January, they are dried and the seeds can be stored for one year. The germination of seeds is low because of hard cover and seed requires pre-sowing treatment. Best germination is obtained when the seeds are chipped at their broad end without damaging the embryo and then soaked in water for 36 hours, before they are sown in nursery beds. Germination starts after 15 days and continues for 3 to 4 weeks. The tree can be successfully raised by directly sowing the seed or by transplanting the seedlings or by stem cuttings. It is observed that transplanting of 1 year seedling grows better than cutting or direct seed sown plants. The young plants require watering during 1st hot weather. Shelter is desirable in early stages in nursery and also after transplanting. The general growth of the plant is slow.

Macroscopic & Microscopic Studies

Fruit yellowish – brown, ovoid, generally 20-35 mm long, 13-25 mm wide, wrinkled and ribbed longitudinally. Pericarp is fibrous, 3-4 mm thick, non-adherent to the seed. Taste astringent.

Transverse section of pericarp shows epicarp consisting of one layer of epidermal cells, inner tangential and upper portions of radial wall thick. Mesocarp consists of 2-3 layers of collenchymas, followed by a broad zone of parenchyma in which fibres and sclereids in group of vascular bundles are scattered. Fibres have peg like out growth and simple pitted walls. Sclereids are of various shapes and sizes but mostly elongated. Epidermal surface view reveal polygonal cells, uniformly thick walled several of them divided into two by thin septa. Starch grains simple, rounded or oval in shape measuring 2-7µ in diameter, found in plenty in all cells of mesocarp. Powdered drug is brownish in colour and shows a few fibres, vessels with simple pits and groups of sclereids under microscope.

Powder

Brownish in colour, under microscope shows a few fibres, vessels with simple pits and groups of sclereids.

Physico-Chemical Analysis

Foreign matter: Not more than 2 per cent.
Total Ash: Not more than 5.5 per cent.
Acid-insoluble ash: Not more than 0.5 per cent.
Alcohol-soluble extractive: Not less than 40 per cent.
Water-soluble extractive: Not less than 56.0 per cent.
Heavy metals: 1.0g complies with the limit test for heavy metals.
Loss on drying: Not less than 9.0 per cent by drying in an oven at 105^0C

Phytochemistry

Haritaki contains not less than 5 percent of Chebulagic acid and not less than 12.5 percent of Chebulinic acid, calculated on the dried basis.

Major

Tannins (20 to 40 percent), which on hydrolysis give chebulic acid and a D-galloyl glucose.

Others

Chebulagic acid, chebulinic acid, ellagic acid; a tannin terchebin, an ellagitannin terchebulin, syringic acid, gallic acid (1.21 percent) (Figure 1-4)

Assay/Instrumental Analytical Standard

Determination by thin layer chromatography

Mobile phase

A mixture of 35 volumes of toluene, 50 volumes of acetone, 15 volumes of glacial acetic acid and 5 volumes of formic acid.

Test solution

To 1 g of the coarsely powdered substance being examined, add 50-75 ml of methanol and reflux for 15 minutes, cool and filter. Reflux the residue further for two times with 75 ml of methanol, cool and filter. Combine all the filtrates and concentrate under vacuum to 100 ml.

Reference solution

To 0.1 g of the Haritaki RS, add 50-75 ml of methanol and reflux for 15 minutes, cool and filter. Reflux the residue further for two times with 75 ml of methanol, cool and filter. Combine all the filtrates and concentrate under vacuum to 10 ml.

Apply to the plate 10µl of each solution as bands 10 mm by 2 mm. Allow the mobile phase to rise 8 cm. Dry the plate in air, spray with solution of 10 percent w/v ferric chloride solution in water. Examine the plate in day light. The chromatographic profile of the test solution is similar to that of the reference solution.

Determination by Liquid Chromatography

Test Solution

Weigh accurately about 0.5 g of coarsely powdered substance under examination, add 50 ml of water, sonicate for 3 minutes and heat on a boiling water bath for 15 minutes, cool and dilute to 100.0 ml with water and filter. Dilute 10.0 ml of the solution to 25.0 ml with water.

Reference solutions

Weigh 0.5 g of Haritaki RS, add 50 ml of water, sonicate for 3 minutes and heat on the boiling water bath for 15 minutes, cool and dilute to 100 ml with water and filter. Dilute 10.0 ml of the solution to 25.0 ml with water.

A 0.01 percent w/v solution of chebulagic acid RS in water.

A 0.01 percent w/v solution of chebulinic acid RS in water.

Chromatographic system (Table. 1)

A stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5µm).

Mobile phase filtered and degassed gradient mixtures of acetonitrile and a buffer solution of pH 2.5 prepared by dissolving 0.136 g of potassium di-hydrogen orthophosphate in 500 ml of water, add 0.5 ml of orthophosphoric acid and make up to 1000 ml with water.

Flow rate, 1.5 ml per minute.

Spectrophotometer set at 270 nm,

A 20µl loop injector.

Inject the reference solution (b) and (c). The relative standard deviation for the replicate injections is not more than 2.0 per cent.

Inject the test solution and reference solution (a).

Calculate the content of chebulagic acid and chebulinic acid.

Estimation of total phenolics

Preparation of calibration curve for Gallic acid

Weigh accurately 10 mg of standard Gallic acid and dissolve in 100 ml distilled water in a volumetric flask (100 µg/ml of stock solution). From the above stock solution pipette out aliquots of 0.5 to 2.5 ml into 25 ml volumetric flasks. Add 10 ml of distilled water and 1.5 ml of Folin Ciocalteu reagent, diluted according to the label specification, to each of the above volumetric flasks. After 5 min add 4 ml of 20 percent sodium carbonate solution, make up the volume to 25 ml with distilled water and incubate for 30 min. Record the absorbance at 765 nm and plot a standard curve of absorbance vs concentration.

Test Solution

Weigh accurately 0.5 g of powdered drug and extract with (3 x 25 ml) of 50 percent aqueous methanol by cold maceration for 2 h with intermittent shaking. Filter and make up the volume to 100 ml.

Procedure

From the above extract, pipette out 0.1 ml into a 25 ml volumetric flask and follow the above procedure for developing colour using Folin Ciocalteu's reagent. Calculate the amount of total phenolics using the standard curve of Gallic acid. The percentage of total phenolics ranges from 36 to 45 (reported in ICMR, Vol-I).

High Performance Thin Layer Chromatographic (HPTLC) Analysis of Gallic Acid

Solvent system

Toluene : Ethyl acetate: Formic acid: Methanol (6:6:1.8:0.25)

Sample solution

Take 1 g of the powder drug in a conical flask and extract twice with 25 ml of 2 M hydrochloric acid under reflux on a water bath for 2 h Filter and extract the filtrate with 5x40 ml diethyl ether. Pool the diethyl ether extracts and make up the volume to 100 ml with diethyl ether in a volumetric flask. Take 2 ml volumetric flasks and adjusting the volume to 10 ml with methanol.

Calibration curve for Gallic acid

Apply 10 µl of each of the standard solutions on precoated silica gel 60 F_{254} TLC plate. Develop the plate in the solvent system to a distance of 8 cm. Dry the plate in air and scan at 280 nm. Record the peak area and prepare the calibration curve by plotting peak area vs concentration of Gallic acid applied.

Estimation of Gallic acid in the drug

Apply 10 µl of each of the sample solution on silica gel 60 F_{254} TLC plate of uniform thickness. Develop the plate in the solvent system and record the chromatogram as described above for the calibration

curve. Calculate the amount of Gallic acid present in the sample from the calibration curve of Gallic acid. (Figure 5)
The percentage of gallic acid ranges from 0.9 to 2.5 as reported in ICMR, Vol. I

HPTLC Method for the Quantitative Determination of Gallic Acid

Preparation of plant extract

The sample was dried in the shade, finely powdered and was passed through 80 mesh sieve and stored in airtight container at room temperature (30 ± 2^0 C). About 300 gm of the powder was taken in a Soxhlet extractor and extracted with hydroalcohol. The solvent recovered by quick fit glass distillation. The powdered material of dried Sample was treated with hydroalcohol (70% ethyl alcohol and 30% water). Hydro-alcoholic extract of test sample was obtained by continuous heat extraction. The extract was collected and was subjected to freeze drying at - 30^0C for 3 days (72 hrs) and again lyophilized at temperature - 40^0C and pressure to dryness. The dried extract was properly stored in the desiccator for further experiment and analysis.

Preparation of stock solutions

Preparation of gallic acid standard solution

A stock solution of standard gallic acid (40µg/ml) was prepared by transferring 4 mg of gallic acid, accurately weighed, into a 100 ml volumetric flask, dissolving in 50 ml methanol. It was then sonicated for 10 minutes and the final volume of the solutions was made up to 100 ml with methanol to get stock solutions containing 40 µg/ ml.
The peak purity of gallic acid was assessed by comparing the spectra at peak start, peak apex and peak end positions of the spot of the sample. Chromatogram of Standard gallic acid is given in Fig.6.

Preparation of sample solution

Accurately weighed 125 mg of dried hydro alcoholic extract of sample was transferred to a 100 ml volumetric flask dissolving in 80 ml of methanol. It was then sonicated for 10 minutes and the contents of the flask were filtered through Whatman No. 1 paper (Merck, Mumbai, India). The final volume of the solution was made up to 100 ml with methanol to get stock solution containing 1.25 mg/ ml.

Instrumentation and chromatographic conditions

HPTLC was performed on 20 cm × 10 cm aluminum backed plates coated with silica gel 60 F_{254} (Merck, Mumbai, India). Standard solution of gallic acid and sample solution were applied to the plates as bands 8.0mm wide, 30.0 mm apart, and 10.0 mm from the bottom edge of the same chromatographic plate by using a Camag (Muttenz, Switzerland) Linomat V sample applicator equipped with a 100-L Hamilton (USA) syringe. Ascending development to a distance of 80 mm was performed at room temperature (28 ± 2°C), with mobile phase, in a Camag glass twin-trough chamber previously saturated with mobile phase vapor for 20 min. After development, the plates were dried with a hair dryer and then scanned at 292 nm with a Camag TLC Scanner with WINCAT software, using the deuterium lamp. The method was validated according to the ICH guide lines.

Method Validation

Linearity

A stock solution of standard gallic acid (40µg/ml) was prepared in methanol. Different volume of stock solution 2, 4, 6, 8 and 10 µl, were spotted on TLC plate to obtained concentration 80, 160, 240, 320 and 400 ng/spot of gallic acid, respectively. The data of peak areas plotted against the corresponding concentration. Calibration curve of gallic acid is given in Fig.7.

Precision

Instrumental precision, intra-assay precision, and intermediate precision of the method were determined. Instrumental precision was measured by replicate (n =10) application of the same gallic acid standard solution (Concentration 80 ng). Intra assay precision was evaluated by analysis of three replicate applications of freshly prepared standard solutions of same concentration, on the same day. Intermediate precision was evaluated by analysis of three replicate applications of standard solution of same concentration on three different days.

Robustness of the method

By introducing small changes in the mobile phase composition, mobile phase volume and duration of mobile phase saturation, the effects on the results were examined. Robustness of the method was done in triplicate at a concentration level of 160 ng/spot and the % R.S.D. of peak area was calculated.

Ruggedness

A solution of concentration 160 ng/ spot was prepared and analyzed on day 0 and after 6, 12, 24, 48 and 72 h. Data were treated for % R.S.D. to assess ruggedness of the method.

Limits of Detection and Limit of Quantitation

The limits of detection (LOD) and (LOQ) were determined as the amounts for which the signal-to-noise ratios were 3:1 and 10:1, respectively.

Recovery studies

The accuracy of the method was established by performing recovery experiments at three different levels using the standard addition method. In 2 µl (1.25 mg/ ml) of sample known amounts of gallic acid standard (80, 120 and 160 ng/spot) were added by spiking. The values of percent recovery and average value of percent recovery for gallic acid.

Specificity

The specificity of the method was ascertained by analyzing the standard drug and extract. The spot for gallic acid in the sample was confirmed by comparing the Rf values and spectra of the spot with that of the Standard. The peak purity of the gallic acid was assessed by comparing the spectra at three different levels, viz. peak start, peak apex and peak end positions of the spot.

System Suitability

System suitability tests are performed to verify whether resolution and repeatability were adequate for the analysis. System suitability was determined by applying freshly prepared standard solution of gallic acid, concentration 160.00 ng/ spot, five times to the same chromatographic plate. The plate was developed under the optimized chromatographic conditions then scanned and the densitograms were recorded. The measured peak areas for gallic acid and their retention factors were noted for each concentration of gallic acid and values of the mean peak area, the standard deviation (SD) and the relative standard deviation (%RSD) were calculated.

Pharmacology

The fruit pericarp of *T. chebula* showed cytoprotective activity, cardiotonic activity, ant mutagenic activity and antifungal activity.

Therapeutic category

Purgative

Experimental Studies (Pharmacology)

Safety study: LD_{50} value of Chebulin is reported to be 550mg/Kg in mice.

Biological activity study

The ethanolic extract of Haritaki (*Terminalia chebula*) was investigated for the cardioprotective activity against isoproterenol (200mg/Kg sub-cutaneously) induced myocardial damage in rats.
Triphala, a combination of Haritaki (*Terminalia chebula*), Vibhitaki (*Terminalia bellerica*) and Amalaki (*Emblica officinalis*) in equal parts is evaluated for anti-inflammatory activity in adjuvant induced right hind paw oedema in Swiss albino mice.
Haritaki (*Terminalia chebula*) at the dose of 140 mg/Kg were studied against cisplatin induced changes in gastric motility and

intestinal transit. Results showed a significant increase of gastric emptying in treated group.

Clinical Studies

Studies showed that *T. chebula* in 2g dose twice a day significantly reduced S. cholestrol, triglycerides, total lipids, LDL, VLDL.

Triphala Guggulu at the dose of 1g twice daily was administered to 30 Diabetic retinopathy subjects (both IDDM and NIDDM patients were studied). Triphala Guggulu showed 46.67% improvement in visual acuity field.

An Aqueous extract of *T. chebula* used as a mouth rinse in 50 subjects was found to be an effective anticaries agent.

Classical Formulations

Abhayarishta, Agastya Haritaki Rasayana, Chitraka Haritaki, Danti Haritaki, Dashamula Haritaki, Brahma Rasayana, Triphala Churna, Vyaghri Haritaki, Triphala Kvatha Churna, Pathyadi Kvatha Churna, Phalatrikadi Kvatha Churna, Abhaya Vati, Triphala Mandura, Shothari Mandura, Amavatari Rasa, Jalodarari Rasa, Nityananda Rasa.

Dose

3-6g, in powder form.

Substitutes and Adulterants

Terminalia citrine Roxb. ex Flem., found in the foothills of Himalayas from Nepal east words to Assam is also called Haritaki in Bengali language and its fruits have medicinal properties similar to that of *Terminalia chebula* and they are also used medicinally as those of *T. chebulla*

Ayurvedic Properties

Rasa : Madhura, Amla, Katu, Tikta, Kashaya
Guna : Laghu, Ruksha
Virya : Ushna
Vipaka : Madhura
Prabhava: Tridoshahara
Doshaghnata: Tridoshahara, Vatahara
Rogaghnata: Shotha, Arsha, Aruchi, Hridroga, Kasa, Udavarta, Vibandha, Jirnajvara, Vishamajvara, Shiroroga, Tamaka Shvasa, Gulma, Udararoga, Kushtha, Krimi, Svarabheda, Grahani, Adhmana

Action (karma) of Haritaki according to Ayurveda

Deepaniya :Increases appetite
Rasayana: Rejuvenative
Brimhana: Nourishing
Yogavahi : Catalyst enhancing the action of other herbs
Pachana : Digestive
Grahi : Absorbs fluids from the intestines
Lekhaniya : Scrapes accumulations from the tissues and channels
Chakshushya : Improves the eyesight
Anuloma : Corrects the flow of vata downwards
Stanyashodhana : Purifies breast milk
Rechana : Purgative
Vibandha hara : Alleviates constipation
Medhya : Improves intellect
Ayuvardhak : Increases longevity
Arshoghna : Anti-haemorrhoidal

Ethanobotanical Importance

It is commonly used in the form of Triphala**.** According to Ayurveda, proper digestion is the base of health. If the digestive system functions properly then it helps the other systems of the body to perform well. Triphala has tridoshahara property; it can be mixed with other herbs in compound formulations. Triphala has the quality to nourish the skin both directly and indirectly.

TRADE & COMMERCE

Retail market price
Unripe fruit powder: Rs. 300/- Kg
Ripe fruit powder : Rs. 150/- Kg. (2000).

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TABLE-1: GRADIENT MIXTURES OF ACETONITRILE AND A BUFFER SOLUTION FOR THE DETERMINATION BY LIQUID CHROMATOGRAPHY

Time (min.)	Buffer solution (percent v/v)	Acetonitrile (percent v/v)
0	95	5
18	65	35
25	45	55
28	45	55
35	95	5

SAFETY & QUALITY STANDARDS

TABLE-2: PERMISSIBLE LIMITS OF AFLATOXINS

S. No.	Aflatoxin	Limit as per Ayurvedic Pharmacopoeia of India (API) (ppm)
1.	B_1	0.5
2.	B_2	0.1
3.	G_1	0.5
4.	G_2	0.1

TABLE-3: PERMISSIBLE LIMITS OF MICROBIAL LOAD AND PATHOGEN

S.No.	Microbial Load	Permissible Limit as per WHO/API		
		For contamination in the crude plant materials (g^{-1})	For plant materials that have been pretreated (used as topical dosage form) (g^{-1})	For other plant materials for internal use (g^{-1})
1	Total Viable Aerobic Count	-	$<10^7$cfu	$<10^5$cfu
2	E.coli	10^4	10^2	10
3	Total Yeast and mould count	10^5	10^4	10^3
4	Total enterobacteriaceae	-	10^4	10^3
5	Salmonellae sp.	-	None	None
6	S. aureus	Absent	Absent	Absent
7	Pseudomonas aeruginosa	Absent	Absent	Absent
8	Coliforms	Absent	Absent	Absent

TABLE-4: LIMITS FOR PESTICIDE RESIDUES

S. No.	Name of Pesticides/ insecticides	Limit as per FDA/ EP/API (ppm)
1	Quinolphos	0.01
2	DDE	1.00
3	Alderin	0.05
4	Dieldrin	0.05
5	DDT	1.00
6	DDD	1.00
7	HCH (Hexa chlorocyclohexane)	0.30
8	Malathion	0.10
9	Parathion	0.30

TABLE-5: LIMITS FOR HEAVY/TOXIC METALS

Test for heavy/ toxic metals	Permissible limit as per Ayurvedic Pharmacopoeia of India (API) (ppm)
Lead	10.0
Cadmium	0.30
Mercury	1.00
Arsenic	3.0

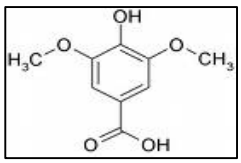

Figure 1. Chemical configuration of Syringic acid

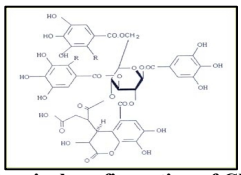

Figure 2. Chemical configuration of Chebulinic acid

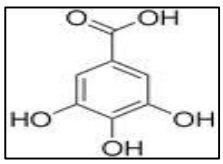

Figure 3. Chemical configuration of gallic acid

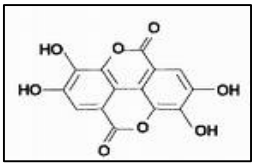

Figure 4. Chemical configuration of Ellagic acid

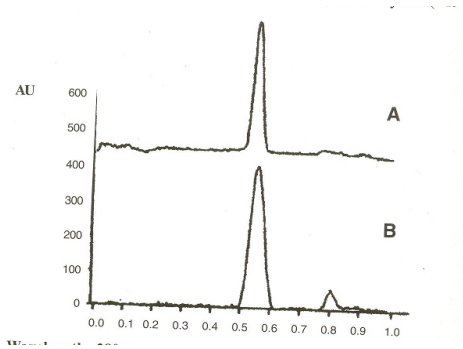

Figure 5. TLC densitometric chromatograms scan at Wavelength 280nm of test solution of *Terminalia chebula* fruit pericarp (excluding endocarp): A. Gallic acid; B. Test solution.

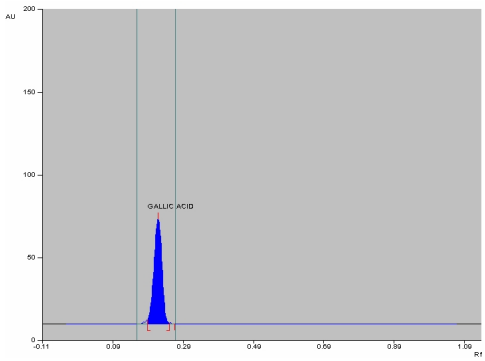

Figure 6. HPTLC chromatogram of standard gallic acid for quantitative determination

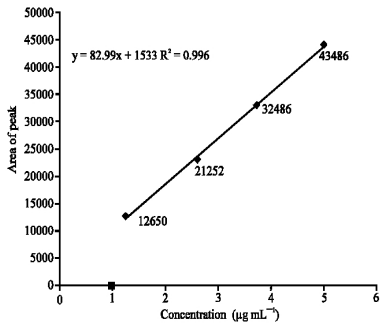

Fig.7. Calibration curve of gallic acid

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