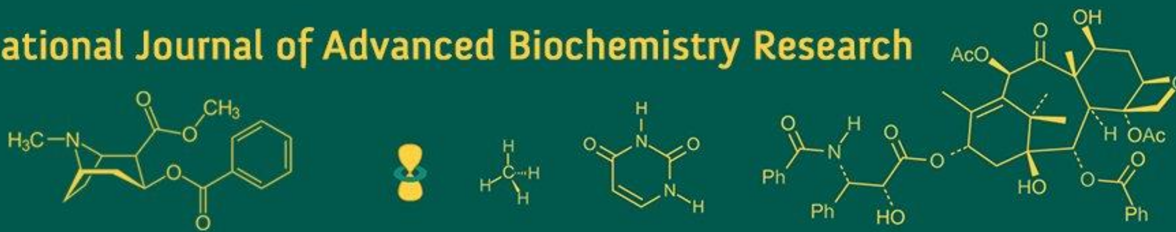

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Preliminary phytochemical evaluation of *Emblica officinalis* and quantification of gallic acid by HPTLC

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Abstract
A contemporary approach was employed to analyze ethanolic and aqueous extracts of *Emblica officinalis* for the existence of phytoconstituents like alkaloids, tannins, glycosides, flavonoids, and saponins. High performance thin layer chromatography was utilized to measure gallic acid. Various compounds including phenols, flavonoids, tannins, alkaloids, carbohydrates, proteins, glycosides, saponins, terpenoids, coumarins, and quinones were identified in both ethanolic and aqueous extracts of *Emblica officinalis*. The ethanolic extract was found to contain 2.43 mg/g gallic acid based on quantitative analysis conducted using HPTLC.

Keywords: *Emblica officinalis* HPTLC, preliminary phytochemical screening

Introduction
Herbal products hold a distinctive place within the pharmaceutical sector. The popularity of plant-based medicines is steadily increasing due to their minimal side effects, cost efficiency, safety, and efficacy. Ayurveda recognizes numerous plants as remedies for kidney and liver disorders. According to the World Health Organisation, more than 30% of pharmaceutical materials are derived from plants, and medicinal plants represents as the primary source of healthcare for 80% of the overall population (Shinwari and Khan, 2014) [10].

Emblica officinalis, commonly known as Amla or Indian gooseberry, is a prominent herbal remedy utilized in the unani and ayurveda medical systems. It belongs to the Euphorbiaceae family. Amla has a long history of use in Indian traditional medicine, and its modern applications are becoming increasingly recognized. The fruits of E.O. contain various physiologically and pharmacologically active compounds such as flavonoids (quercetin), tannoids, gallic acid, and alkaloids. These fruits are also rich in essential minerals, amino acids, and a high concentration of vitamin C (Patel and Goyal, 2012) [8].

Emblica officinalis has been discovered to possess nephroprotective activity against cisplatin-induced nephrotoxicity (Kalra *et al*., 2017) [5] and protective action against carbon tetrachloride-induced hepatotoxicity (Deori *et al*., 2017) [3]. Additionally, Rawal *et al*. (2014) have reported its antioxidant activity against oxidative stress caused by enrofloxacin. The extract derived from *Emblica officinalis* has demonstrated strong anti-inflammatory, anti-diabetic, antioxidant, and chemoprotective properties (Golechha *et al*., 2012) [7].

Materials and Methods
The process of preparing an aqueous extract of *E.O.* can be carried out as follows
The dried fruits of *E.O.* were first combined, ground, and crushed to create powder. Cold extraction method was employed to produce the aqueous extract of *Emblica officinalis* as per Shukla (2006) [11]. The required amount of *Emblica officinalis* fruit powder was weighed, soaked in distilled water, and kept overnight at room temperature. The cold aqueous extract was obtained using filter paper.

Preparation of ethanolic extract by soxhlet Method
The crude extract was prepared using 90% ethanol. Approximately 20 g of the coarsely ground fruit powder was placed in a thimble made of Whatman filter paper No. 1. This thimble was then placed in a 500 mililiter round bottom flask containing 400 ml of solvent,

which was maintained at a temperature of 60–80 °C using a soxhlet apparatus. The extraction process was permissible to proceed for duration of twelve hours. To remove the excess solvent, the extracts were collected in petri dishes and placed in a water bath at 90 °C. The yield percentage was calculated. To preserve the extract for further research, it was stored in an airtight container at 4 °C (Thangaraj, 2016) [12].

Preliminary phytochemical evaluation: In the study conducted by Jeevalatha *et al*. (2022) [4], the ethanolic and aqueous extracts of *Emblica officinalis* were examined using a standard procedure to determine the presence of various phytoconstituents including alkaloids, tannins, glycosides, flavonoids, and saponins.

Test for Phenol
Ferric chloride test
Three millilitres of distilled water and a few drops of a 10 percent aqueous ferric chloride solution were added to one milliliter of the extract. The development of a green tint signifies the existence of phenols.

Test for Flavonoids
Shinoda test
To two milliliters ml of the extract, 1 ml of 1 percent ammonia solution was added. Appearance of yellow colour indicates the presence of flavonoids.

Test for Tannins
Ferric chloride test
One millilitre (1%) of 1% ammonia solution was added to two millilitres of the extract. The presence of flavonoids is indicated by the colour yellow.

Test for Alkaloids
Mayer's test
One millilitre of the extract was combined with one millilitre of 0.008 M potassium ferricyanide and one millilitre of 0.02 M ferric chloride that contained 0.1 N HCl. The presence of tannins is indicated by a blue-black appearance.

Test for Carbohydrates
Fehling's test
Two millilitres of crude extract were added after equal volumes of Fehling A and Fehling B reagents were combined and heated slowly. The test tube's bottom developed a brick-red precipitate, which is an indication that reducing sugars are present.

Test for Proteins
a) Millon's test
When 1 ml of crude extract and 2 ml of Millon's reagent were combined, a white precipitate formed and, when heated gently, became crimson, indicating the presence of protein.

b) Ninhydrin test
After mixing 1 ml of crude extract with 2 ml of a 0.2% ninhydrin solution, the mixture was cooked. There was a violet-colored precipitate that suggested proteins and amino acids were present.

Test for Glycosides
a) Sodium hydroxide test
1 ml of sodium hydroxide and 1 ml of water were used to treat the extract. The development of a yellow hue signifies the existence of glycosides.

b) Keller-Kiliani test for cardiac glycosides
Two millilitres of glacial acetic acid with one drop of ferric chloride solution were added to five millilitres of extract. This was combined with one millilitre of sulfuric acid concentration. A browning of the interface suggests that cardenolides have a deoxy sugar property. Beneath the brown ring, a violet ring can show up, and within the thin layer of acetic acid, a greenish ring might grow very gradually.

Test for Terpenoids
Salkowski test
Three millilitres of strong sulfuric acid were cautiously added to five millilitres of extract and two millilitres of chloroform to create a layer. The interface's reddish-brown coloration suggests the presence of terpenoids.

Test for Coumarin
Coumarins test
Chloroform was added to the extract along with 10% sodium hydroxide. The presence of coumarin is shown by the formation of yellow colour.

Test for Saponins
Foam test
In a test tube, 2 ml of crude extract and 5 ml of distilled water were combined, and the mixture was agitated vigorously. Include a few olive oil drips. It was believed that the production of stable foam indicated the presence of saponins.

Test for Steroids
Salkowski test
A solution of 0.5 ml crude extract containing 2 ml sulfuric acid was mixed with 2 ml of acetic anhydride. When samples' colour shifts from violet to blue or green, steroids are present.

Test for Quinones
Quinone test
One millilitre of crude extract was combined with diluted sodium hydroxide. Quinones are indicated by a red or blue-green colours.

Test for Anthraquinones
Borntrager's test
Each extract weighed 0.5 g, and it was cooked in a water bath with 10% hydrochloric acid for a short while. After filtering, it was left to cool. CHCl3 was added in the same volume to the filtrate. After adding a few drops of 10% ammonia to the mixture, it was heated. The anthraquinones are present when a rose-pink colour forms.

Quantification of Gallic acid by HPTLC
High Performance Thin Layer chromatography was used to quantify gallic acid.

Devices
The components of the High Performance Thin Layer Chromatography system (CAMAG, Muttenz, Switzerland) included a plate warmer, TLC visualizer, derivatization chamber, Hamilton syringe (100µl), twin trough chambers (10 x 10 cm and 20 x 10 cm), and Linomat 5 auto applicator coupled to a nitrogen cylinder. The stationary phase was precoated silica gel 60 F254 TLC plates (20 x 10 cm), 0.2 mm thick (E. Merck KGaA, Darmstadt, Germany). A TLC Scanner 4 with winCATS software was used to perform densitometric analysis (Altan *et al*., 2014) [1].

Standard
Gallic acid (Hi-Media)

Preparation of silica plates
The 60 F254 TLC plates (Merck, Germany) made of silica gel were first created using a standard aqueous Na2EDTA solution, then they were air dried for one hour at ambient temperature and then activated for two hours at 130ºC in a hot air oven.

Standard preparation
By dissolving, a standard solution containing 50 µg/ml of gallic acid, quercetin, and rutin was created. One milligramme of precisely weighed gallic acid, quercetin, and rutin in methanol, with the volume of solution adjusted to one hundred millilitres using methanol. Subsequently, the mixture was passed using a 0.45 µm microfilter. Subsequently, the 50 µg/ml standard gallic acid solution was diluted to 100, 200, 300, 400, and 500 ng.

Preparation of sample solutions
The soxhlet method was used to prepare the samples for the ethanolic extract of *Emblica officinalis*. After precisely weighing 10 mg of extract, it was diluted in 10 ml of ethanol and filtered through a 0.45 µm microfilter.

Mobile phase and migration
The mobile phase for developing silica plates for gallic acid consisted of toluene, ethyl acetate, formic acid, and methanol in a ratio of 3:4:0.8:0.7. The development process was carried out in a linear ascending manner using a 10 x 10 cm twin trough chamber that was equilibrated with the mobile phase. The ideal chamber saturation times for the mobile phase were four hours, one hour, and two hours, respectively. Each development cycle utilized ten millilitres of the mobile phase and allowed migration of 90 mm. Following development, the plates were thoroughly dried.

Densitometric analysis and quantification procedure
A densitometric scanning was performed using the CAMAG TLC scanner in absorbance mode and win CATS planar chromatography version 1.3.4. The radiation source utilized was the deuterium lamp, with a wavelength of 331 nm for spot examination. The analysis was conducted with slit dimensions of 6 mm for length and 0.45 mm for width, at a scanning rate of 20 mm/sec. The scanning encompassed 70–90% of the application band length, approximately 8 mm in this specific case. The concentration of the compound chromatograph was calculated based on the intensity of diffusely reflected light, and then evaluated as peak area against concentrations using a linear regression equation.

Results and Discussion

Phytochemical analysis of *E.O.*
In present research, *Emblica officinalis* was evaluated for antioxidant activity against testicular toxicity produced by sub acute exposure of enrofloxacin in albino rats. Aqueous and ethanolic extract of *Emblica officinalis* were evaluated for phytochemical analysis (Plate 01).

Analysis of phytochemicals using qualitative method
The *Emblica officinalis* aqueous extract was found to contain phenols, flavonoids, tannins, alkaloids, carbohydrates, saponins, coumarins, and quinones during the qualitative phytochemical analysis. However, proteins, quinones, glycosides, saponins, and terpenoids were not detected in the extract. In contrast, the ethanolic extract of *Emblica officinalis* showed the presence of phenol, proteins, glycosides, carbohydrates, saponins, and terpenoids. Flavonoids, glycosides, steroids, and quinones were absent in the ethanolic extract. These findings align with the research conducted by Jeevalatha *et al*. (2022) [4], who also observed the presence of phenols, flavonoids, tannins, carbohydrates, and alkaloids in the ethanolic extract of *Emblica officinalis* obtained through the soxhlet method of extraction (Plate 02, 03).

Table 1: Phytochemical screening of ethanolic extract of *E.O.* obtained by soxhlet method of extraction

S. No.	Phytochemical	Qualitative test	Observation and Result	
1	Phenol	Ferric chloride test	Solution developed green colour	Present
2	Flavonoids	Shinoda test	Solution developed yellow colour	Present
3	Tannins	Ferric chloride test	Solution developed blue-black colour	Present
4	Alkaloids	Mayer’s test	Reddish brown colour precipitate developed	Present
5	Carbohydrates	Fehling’s test	Brick red precipitate developed	Present
6	Proteins	Millon’s test	No specific colour developed	Absent
7	Glycosides	Ninhydrin test	No specific colour developed	Absent
8	Saponins	Sodium hydroxide test	No specific colour developed	Absent
9	Terpenoids	Keller-Kiliani test	No specific colour developed	Absent
10	Coumarin	Foam test	Stable layer of foam developed	Present
11	Steroids	Salkowski test	Solution developed reddish brown colouration of interface	Present
12	Quinones	Coumarins test	No specific colour developed	Absent
13	Anthraquinones	Salkowski test	No specific colour developed	Absent

The results of this investigation are consistent with those of Raja *et al*. (2014) [9]. They reported that the *Emblica officinalis* aqueous extract, which was produced using a cold extraction procedure, included phenols, tannins, carbohydrates, alkaloids, glycosides, and saponins.

Table 2: Phytochemical screening of aqueous extract of *E.O.* obtained by soxhlet method of extraction

S. No.	Phytochemical	Qualitative test	Observation and Result	
1	Phenol	Ferric chloride test	Solution developed green colour	Present
2	Flavonoids	Shinoda test	Solution developed yellow colour	Absent
3	Tannins	Ferric chloride test	Solution developed blue-black colour	Present
4	Alkaloids	Mayer's test	Reddish brown colour precipitate developed	Absent
5	Carbohydrates	Fehling's test	Brick red precipitate developed	Present
6	Proteins	Millon's test	No specific colour developed	Absent
7	Glycosides	Ninhydrin test	No specific colour developed	Absent
8	Saponins	Sodium hydroxide test	No specific colour developed	Present
9	Terpenoids	Keller-Kiliani test	No specific colour developed	Present
10	Coumarin	Foam test	Stable layer of foam developed	Present
11	Steroids	Salkowski test	Solution developed reddish brown colouration of interface	Absent
12	Quinones	Coumarins test	No specific colour developed	Absent
13	Anthraquinones	Salkowski test	No specific colour developed	Absent

Quantitative evaluation of gallic acid by HPTLC

The HPTLC method for quantitatively analyzing gallic acid utilized a mobile phase consisting of ethyl acetate, glacial acetic acid, formic acid, and water in a specific ratio (10:1.1:1.1:2.5). This solvent system effectively separated gallic acid from the matrix, as indicated by their mean retardation factor (RF value) of 0.29. The separation was observed on Plate 5 and 6. The concentration range of 0-60 ng/spot, 0-50 ng/spot, and 0-600 ng/spot was found to be linear in terms of peak area, as demonstrated on Plate 8. After 45 minutes of saturation, the solvent front distance in the chamber was measured to be 80 mm. Under these chromatographic conditions, rutin, gallic acid, and quercetin exhibited well-defined and compact spots with optimal migration. The HPTLC chromatograms were obtained at wavelengths of 254 nm, 375 nm, and 361 nm, and the spectra were compared on Plate 7.The verification of the gallic acid band in the ethanolic extract of *Emblica officinalis*, obtained through the Soxhlet method, was confirmed by overlaying the UV absorption spectrum of the standard gallic acid. The purity of the gallic acid bands was further confirmed by comparing the absorption spectra at the beginning, middle, and end positions of the band spectrum in the ethanolic extract of *Emblica officinalis* (Plate 4).

The concentration of gallic acid in ethanolic extract of *Emblica officinalis* obtained by soxhlet method of extraction is depicted in Table 03.

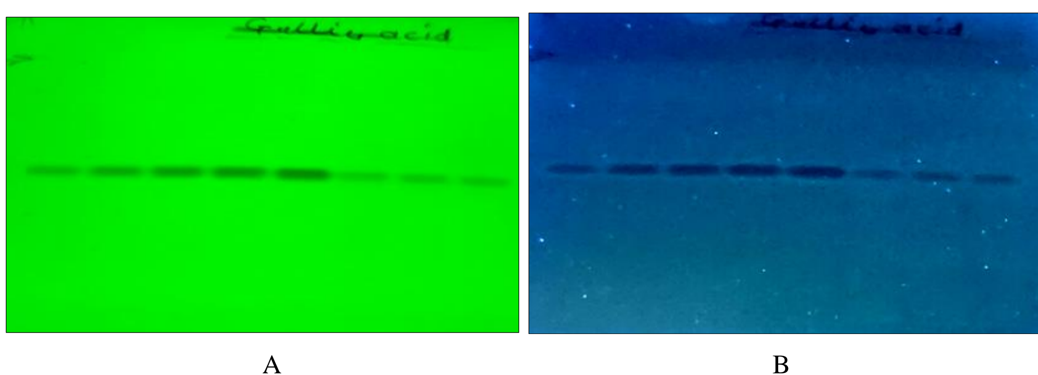
Table 3: Concentration of gallic acid in ethenolic extract of *E.O.*

S. No	Active principle	Concentration (mg/g)
1.	Gallic acid	2.43

Plate 1: (A) fruit powder *of Emblica officinalis* (B) Aqueous extract of *Emblica officinalis*

Plate 2: Preparation of ethenolic extract of *Emblica officinalis* by soxhlet method

Plate 3: Qualitative phytochemical analysis of *Emblica officinalis*



A B

Plate 4: HPTLC finger print of gallic acid (A) Ultraviolet light (254 nm) (B) Ultraviolet light (366 nm)

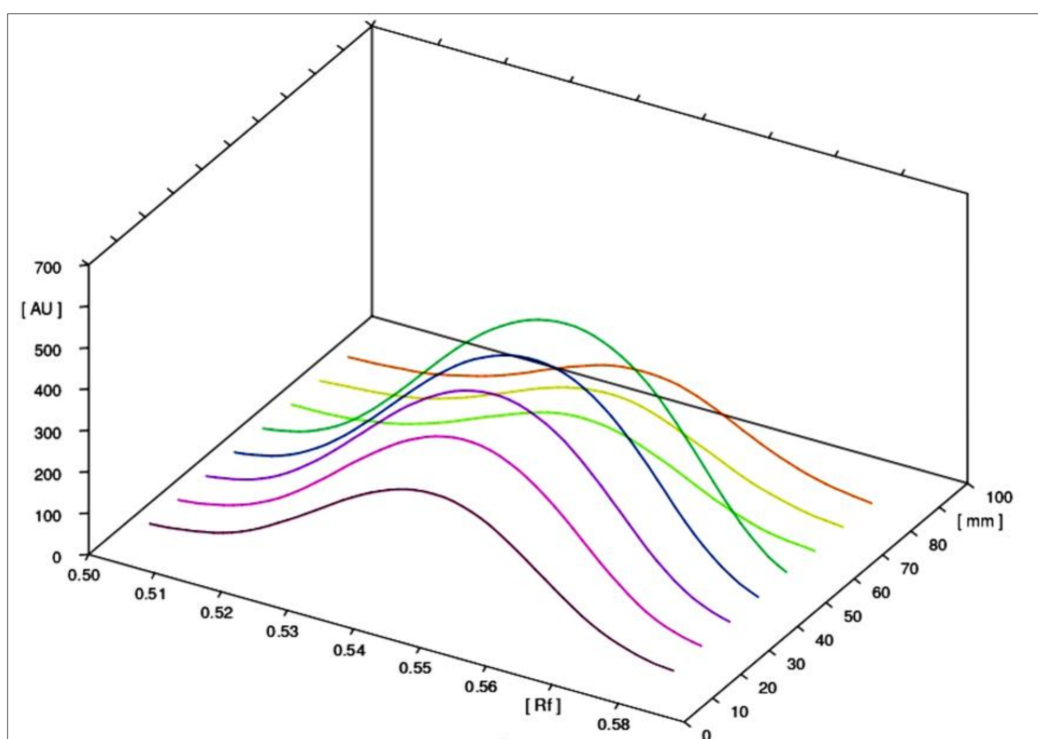


Plate 5: Three dimensional plots of analog curves showing absorbance of gallic acid in all tracks (standard and samples) plotted against Rf value

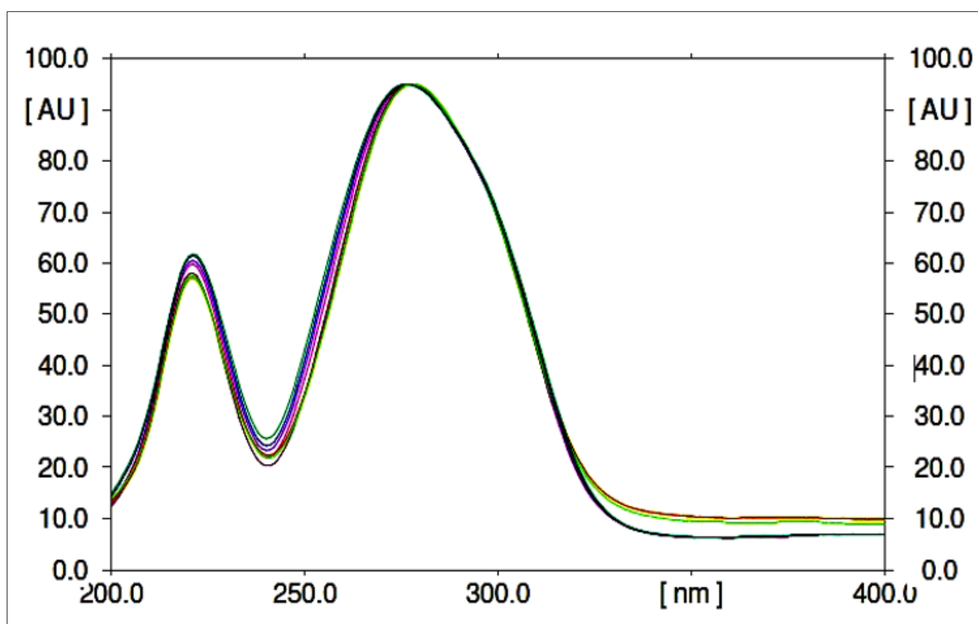


Plate 6: The spectral scanning of gallic acid (showing peak at 254 nm) with absorbance (AU) plotted against wavelength

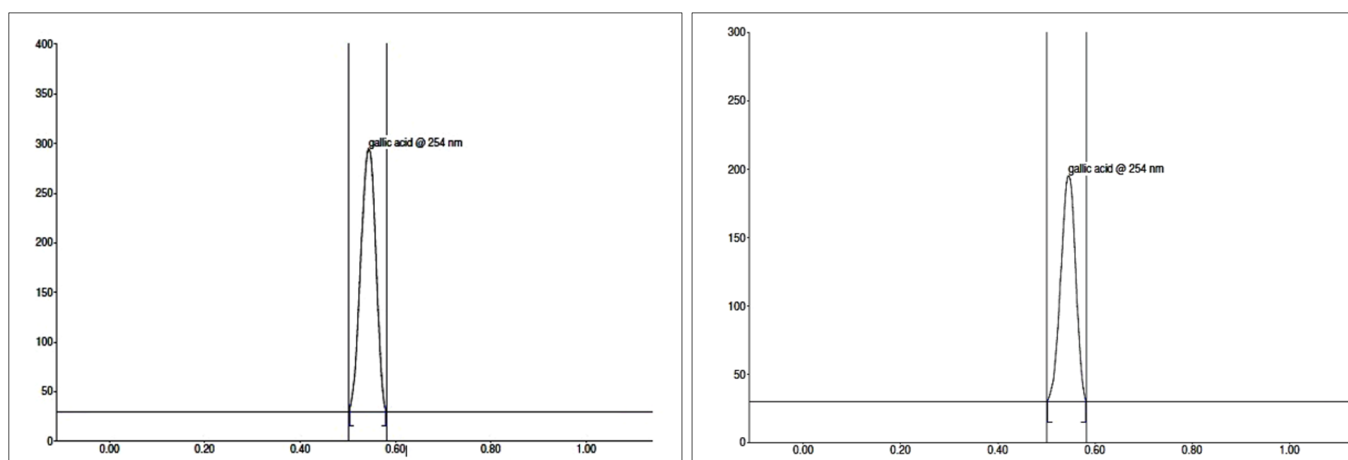


Plate 07: HPTLC chromatogram of gallic acid in ethanolic extract of *Emblica officinalis*

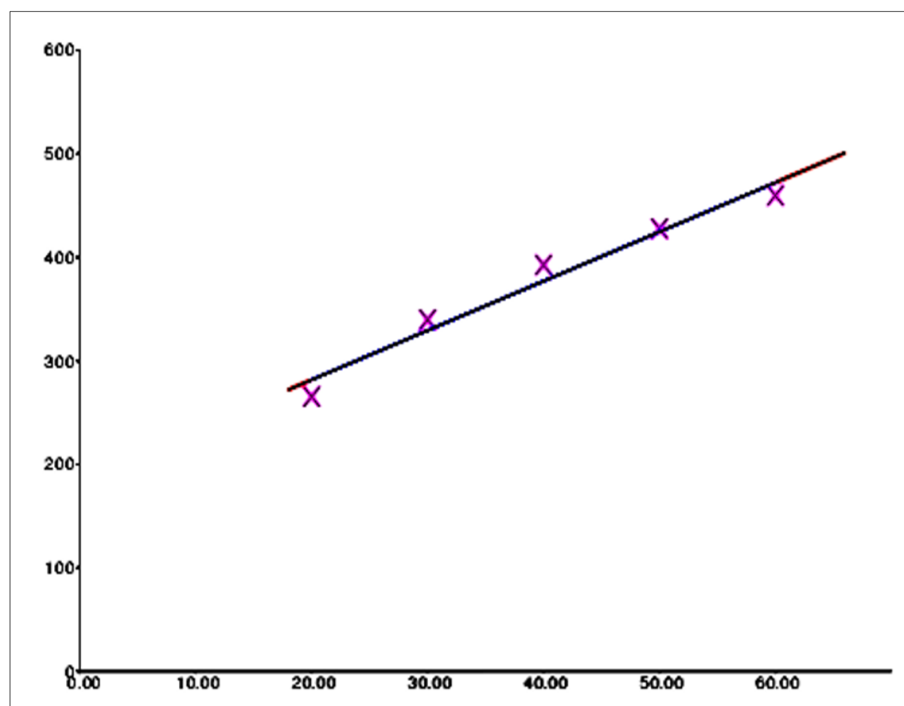


Plate 08: Calibration curve of gallic acid standard

Conclusion
Various compounds such as phenols, flavonoids, tannins, alkaloids, carbohydrates, proteins, glycosides, saponins, terpenoids, coumarins, and quinones were identified in both the aqueous and ethanolic extracts of *Emblica officinalis*. Through HPTLC analysis, it was determined that the ethanolic extract of *Emblica officinalis* contained Gallic acid at a concentration of 2.43 mg/g.

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