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DETERMINATION AND FINGERPRINTING OF GALLIC ACID IN HYDROALCOHOLIC EXTRACT OF *HELICTERUS ISORA* (MALVACEAE) BY HPTLC METHOD

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Abstract:

The medicinal plants are widely used by the traditional medicinal practitioners for curing various diseases. Helicteres isora plant is used traditionally for different activities like antimicrobial, antioxidant, anticancer, antiulcer, hepatoprotective, antineoplastic, antimicrobial, Anti-inflammatory. Phytochemical analysis of the plant confirmed the presence of carbohydrate, proteins, polyphenols, tannins, flavonoids, alkaloids, saponin, and steroid. In the current study, an effort was made to quantify the phenolic acid gallic acid in the hydroalcoholic extract of Helicteres isora. High performance thin layer chromatographic method has been used to determine the presence and to quantify gallic acid in Helicteres isora extract. Separation was carried out with the use of silica gel 60F_{254} precoated TLC plates as stationary phase and mixture of Chloroform: Ethyl acetate: Methanol: Formic acid (12:8:1.5:1.5, v/v/v/v) as mobile phase. Densitometric evaluation was carried out at 275 nm. A good linear relationship was obtained in the concentration range of 100-600 ng band^{-1}.

Keywords: *Helicteres isora,, Gallic acid, HPTLC, Anti-inflammatory*

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INTRODUCTION:

The medicinal plants having a great importance in human being show diverse pharmacological properties. Medicinal plants originated from India possess a plethora of therapeutic compounds useful for treating various diseases (Badgujar and Jain, 2009). Helicteres isora Linn (Malvaceae) is a plant growing gregariously throughout India. The plant is commonly called as 'Mrigashringa' in Sanskrit is an important medicinal plant described in indigenous system of medicine (Pagi et al., 2010]. It contains hairy, ovate shaped leaves. Flowers are orange-red in colour. The fruits are compound pod, twisted like screw with pointed end (Shah, 2015). The plant is traditionally used as antimicrobial (Varghese and Narayanan, 2012), anti-diabetic, anti-diarrheal, anti-oxidant (Chaudhary and Sharma, 2016), antispasmodic and anti-inflammatory (Dayal and Singh, 2015). Well identified active phytoconstituents includes cucurbitacin b, isocucurbitacin b (steroids), gallic acid, caffeic acid, vanillin, p-coumaric acid (Sajeeth, 2010). Gallic acid is phenylpropanoid and chemically it is 3, 4, 5,-Trihydroxybenzoic acid, and possess anti-inflammatory and astringent activity (Patil and Kurhade, 2012). HPTLC is becoming a routine analytical technique due to its advantages of low operating cost, high sample throughput and need for minimum sample clean-up. The major advantage of HPTLC is that various samples can be run simultaneously using a small quantity of mobile phase unlike HPLC, thus lowers analysis time and cost per analysis (Sadia, 2015).

Methanolic plant extract of *Helicteres isora* was subjected to high performance thin-layer chromatography to find out the likely number of compounds present in them. Consequently, the present study was focused on the quantitative estimation of the phenylpropanoid gallic acid by high performance thin-layer chromatography (HPTLC) in the herbal species *Helicteres isora.*

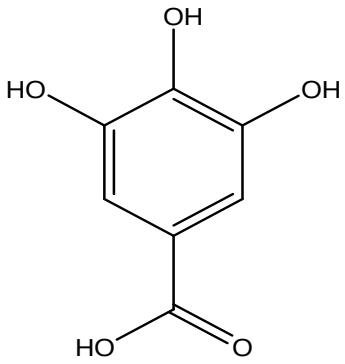

Fig. 1: Chemical structure of Gallic acid

MATERIALS AND METHODS:

Plant material:

The plant was identified and collected, the plant sample was washed thoroughly with tap water, dried at room temperature away from sun light, cut into small pieces, and then powdered. Hydroalcoholic extract was prepared by cold maceration of powder in ethanol and water for 7 days. The extract was filtered, concentrated under reduced pressure.

Selection of Analytical Wavelength

Working standard solution of gallic acid having concentration of 1000 µg mL^{-1} was prepared by dissolving 10 mg in 10 mL of methanol. The resulting solution was diluted further to get solution having final concentration 10 µg mL^{-1} and its absorbance was recorded in range of 200-400 nm using UV visible spectrophotometer. Gallic acid showed maximum absorbance at 275 nm and was chosen as detection wavelength.

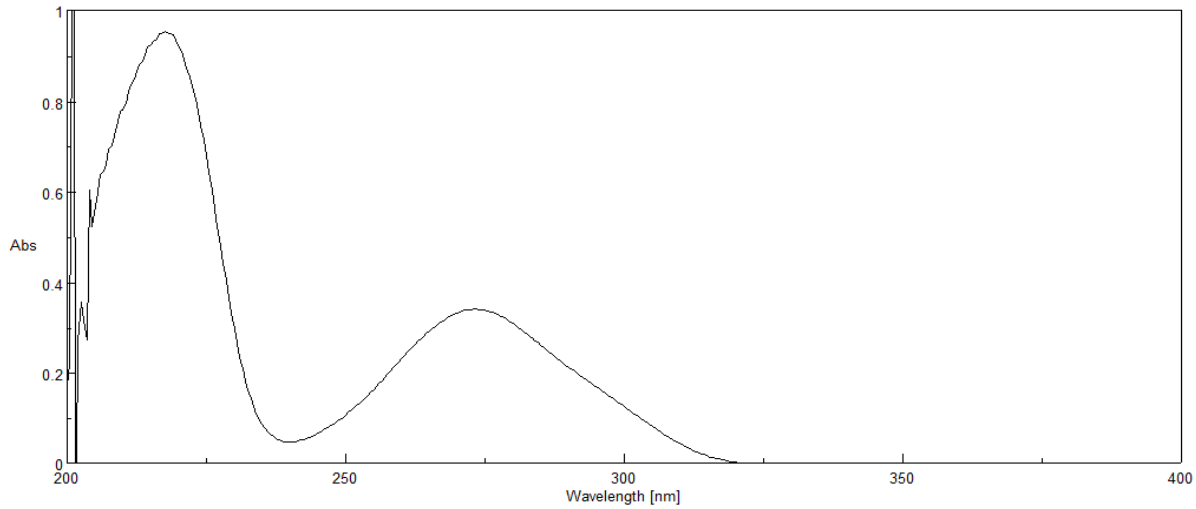

Fig. 2: UV Spectrum of Gallic acid (10 µg mL^{-1})

Preparation of standard solution

Standard solution of gallic acid was prepared by dissolving 10 mg in 10 mL of methanol to get concentration of 1000 µg mL^{-1} which was further diluted appropriately with methanol to obtain final concentration 100 ng μL^{-1}.

Preparation of the Sample solution

Sample solution was prepared by dissolving 10 mg of *Helicteres isora* extract in 10 ml of methanol to get concentration of 1000 µg mL^{-1} which was further diluted appropriately to obtain final concentration 100 ng μL^{-1}.

HPTLC analysis

The chromatographic separation was achieved on aluminium plates precoated with silica gel $60F_{254}$ in (10 cm × 10 cm with 250 µm layer thickness). Sample was applied on the plate with band width of 6 mm using Camag 100 µL sample syringe (Hamilton, Switzerland) with Linomat V applicator (Camag, Switzerland). The optimised mobile phase consisted of chloroform: ethyl acetate: methanol: formic acid (12:8:1.5:1.5 v/v/v/v). The development was carried out by linear ascending TLC using Camag twin trough glass chamber saturated previously with mobile phase for period of 15 min. Densitometric scanning was performed at 275 nm using Camag TLC scanner III operated by win CATS software version 1.4.2. A solution of 100 ng μL^{-1} of gallic acid in methanol and extract was prepared and was spotted using Camag Linomat V sample applicator and detection was carried out at 275 nm.

Preparation of calibration curve of gallic acid

Volumes 1, 2, 3, 4, 5 and 6 µL from standard solution (100 ng μL^{-1}) were applied on the TLC plates with sample applicator in nitrogen stream. The mobile phase utilized consisted of chloroform: ethyl acetate: methanol: formic acid (12:8:1.5:1.5 v/v/v/v) and separated spots were analysed densitometrically at 275 nm using Camag TLC Scanner III operated by the winCATS software version 1.4.2. The calibration curve of the standard drug concentration (X-axis) against the average peak area (Y-axis) was prepared to get a regression equation.

Estimation of gallic acid in hydroalcoholic plant extract

The mean peak area of the sample was calculated and the content of gallic acid was estimated using the regression equation obtained from the standard Calibration curve.

Limit of detection and limit of quantitation

The Limit of detection (LOD) and limit of quantitation (LOQ) were calculated as signal-to-noise ratio of 3:1 and 10:1 [10].

RESULTS AND DISCUSSION:

Preliminary phytochemical examination of hydroalcoholic *Helicteres isora* extract indicated the presence of alkaloids, phenols, , terpenoids, carbohydrates, amino acids, sterols, tannins and flavonoids. In order to separate the compounds and to identify one of the phytochemical phenolic acid i.e. gallic acid in the extract, TLC procedure was optimized. The solvent system comprising of chloroform: ethyl acetate: methanol: formic acid (12:8:1.5:1.5 v/v/v/v) was able to give a dense, compact and well-defined peak for gallic acid with Rf 0.25 as well as for the extract (Figure 3 and 4). This confirmed the presence of the bioactive compound phenolic acid.The identification of the gallic acid in sample densitogram was confirmed by compairing the Rf value with that obtained from pure marker.

Regression data obtained from calibration curve demonstrated excellent linear relationship over 100-600 ng $band^{-1}$ concentration range. The linear regression equation was found to be y = 23.215x + 3023.8 having correlation coefficient 0.996 (Fig. 5 and 6). The limit of detection and limit of quantitation was found to be 9.31 ng $band^{-1}$ and 28.23 ng $band^{-1}$, respectively. With the help of above statistical data, the content of gallic acid was determined in the hydroalcoholic plant extract which was found to be 91 %. The HPTLC data obtained for the quantification of gallic acid is summarized in Table 1. HPTLC analysis was carried out to determine the content and to quantify the gallic acid present in *Helicteres isora* plant extract.

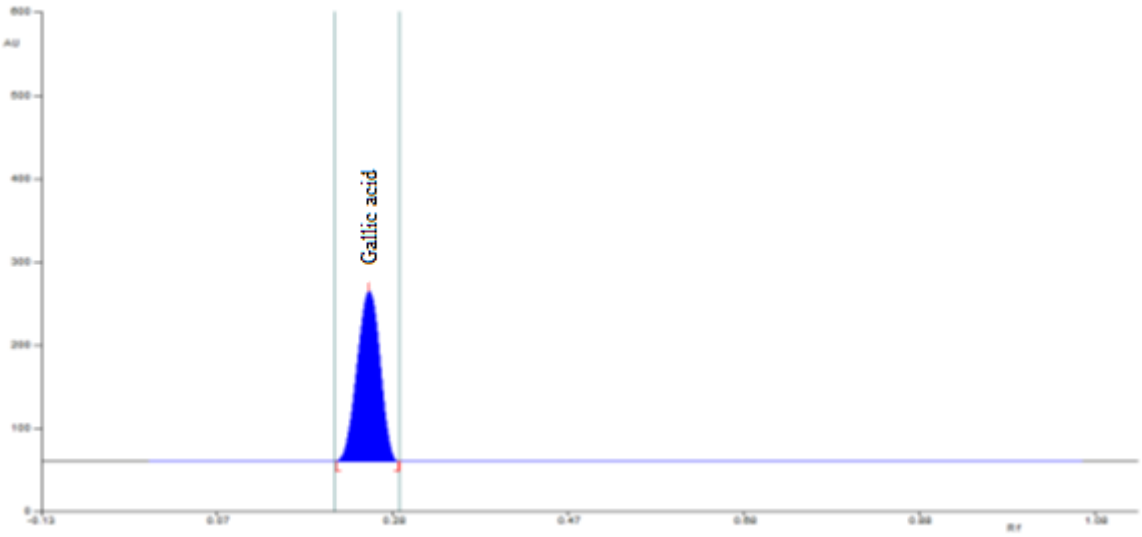

Fig. 3: HPTLC Densitogram showing the presence of gallic acid (Rf =0.25)

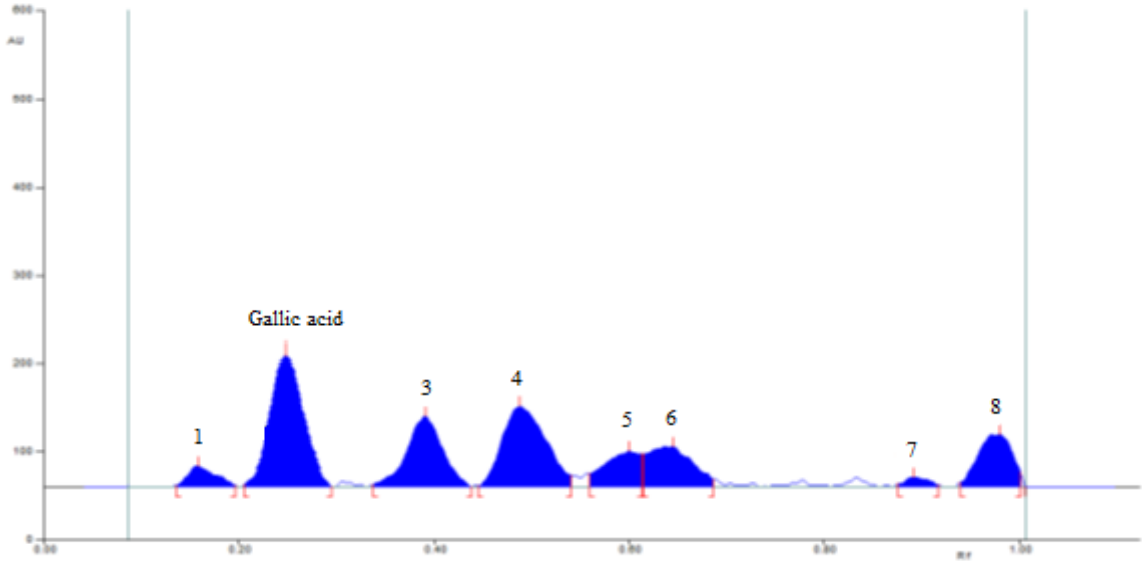

Fig: 4. Densitogram of *H.isora* extract showing the presence of gallic acid (Rf =0.25)

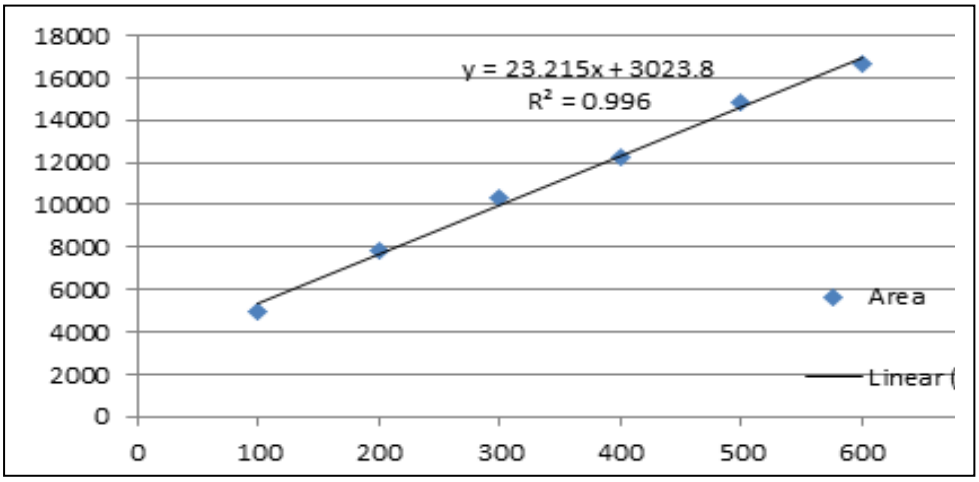

Fig. 5: Calibration curve for Gallic acid

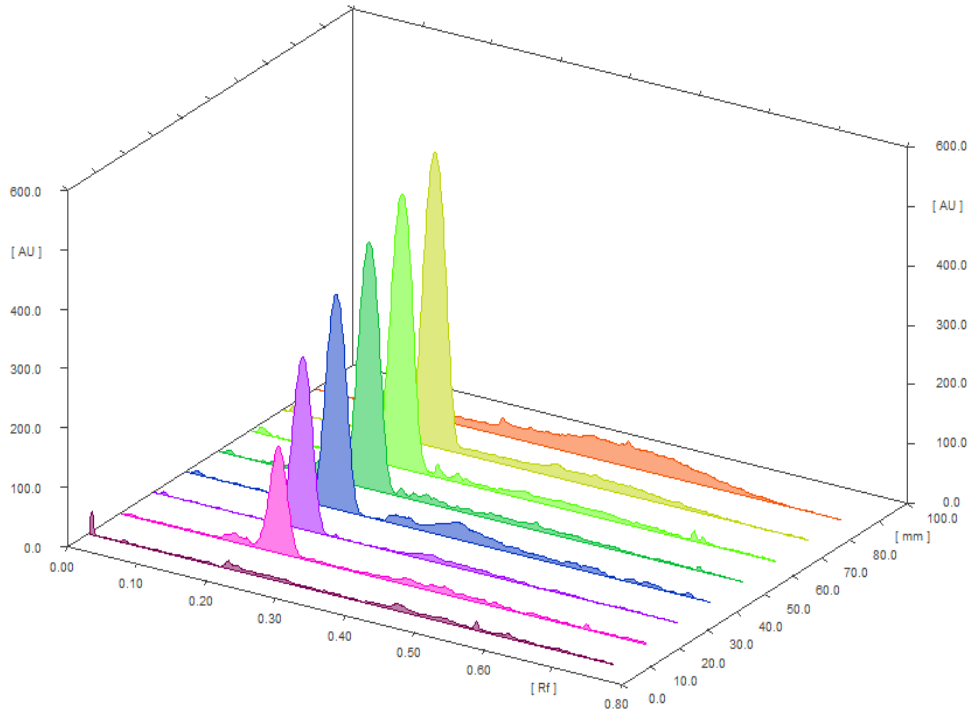

Fig. 6: 3 D spectra of gallic acid in the range of 100-600 ng band^{-1}

Table 1: HPTLC data for Gallic acid

Sr No.	Parameter	Value
1	Linearity	100-600 ng band $^{-1}$
2	Correlation Coefficient	R^2 = 0.996
3	Regression equation	y = 23.215x + 3023.8
4	Limit of detection	9.31 ng band $^{-1}$
5	Limit of quantitation	28.23 ng band $^{-1}$

CONCLUSION:

The presence of most of the biologically active compounds in the plant was identified by phytochemical analysis. The chromatographic studies conducted with the hydroalcoholic plant extract of *Helicteres isora* revealed a significant amount of phenolic acid (tannins) gallic acid, which possess its distinct medicinal value.

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