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QUANTIFICATION OF GALLIC ACID IN *PETIVERIA ALLIACEA* L. BY HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY

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The versatile separation technique High Performance Thin Layer Chromatography (HPTLC) has been developed for the quantification of Gallic acid, a polyphenol compound present in dry plant powder of *Petiveria alliacea* L. Chromatographic analysis was performed using methanol extract on silica gel $60F_{254}$ HPTLC plate. The solvent system consisting of Chloroform, Ethyl acetate and Acetone in the ratio 50:50:1(v/v/v) was used as the mobile phase. The chromatogram showed good separation of Gallic acid at Rf = 0.52. Detection and quantification of Gallic acid was done at λ=254 nm using densitometric scanning. The calibration cure of Gallic acid is linear (200-1200 ng/spot) as concentration against area.

Key words: Gallic acid, HPTLC, *Petiveria alliacea* L. Polyphenol.

Petiveria alliacea L. of Phytolaccaceae is indigenous to India and the family Phytolaccaceae comprises 12 genera and 100 species (Ghosh and Sikdar 1983; Willis 1973). The roots and leaves of *P. alliacea* were long used as herbal remedy for medical conditions (Kim 2006; Lopes *et al*.2002; Pacheco *et* a*l*. 2006). Gallic acid is a polyphenol compound present in *Petiveria alliacea* L. (Sethi and Charegaonkar 1999). In the present study an HPTLC method has been standardised for the quantification of Gallic acid from *P. alliacea* using methanol extract of plant powder. The proposed method using HPTLC technique is simple, precise and sensitive.

MATERIALS AND METHODS

HPTLC was conducted using CAMAG HPTLC System (Switzerland) equipped with Linomat 5 automatic sample spotter, CAMAG glass twin trough chamber and CAMAG TLC Scanner 3 with software Win CATS 1.30 Planar chromatography manager. Silica gel $60F_{254}$ HPTLC pre-coated aluminium sheet plates (Merck, Darmstadt, Germany) were employed (Stahl 1969; Sherma and Fried 1996; Touchstone 1992). Extract of dry plant powder in methanol was used for HPTLC studies.

A stock solution of Gallic acid was prepared in 10ml volumetric flask by dissolving 10mg Gallic acid standard (sigma Aldrich) in 5ml methanol, followed by sonication for 5 minutes and finally making the volume up to mark with methanol, to obtain working standard solution of Gallic acid, in the concentration range of 1000µg/ml.

A stock solution of sample was prepared by making the volume of 100mg extract in methanol to 5ml in a volumetric flask using methanol. The contents of the flask were filtered after sonication for 15 minutes (frequency 50Hz). The filtrate was collected and the volume was adjusted up to mark with methanol.

5µl of methanol extract of plant powder and 0.5 µl Gallic acid standard were applied as band of 6mm width with 12mm spacing between two tracks. Mobile phase used was Chloroform: Ethyl acetate: Acetone (50:50:1 v/v/v). The chamber was saturated with mobile phase for 30-45 min.

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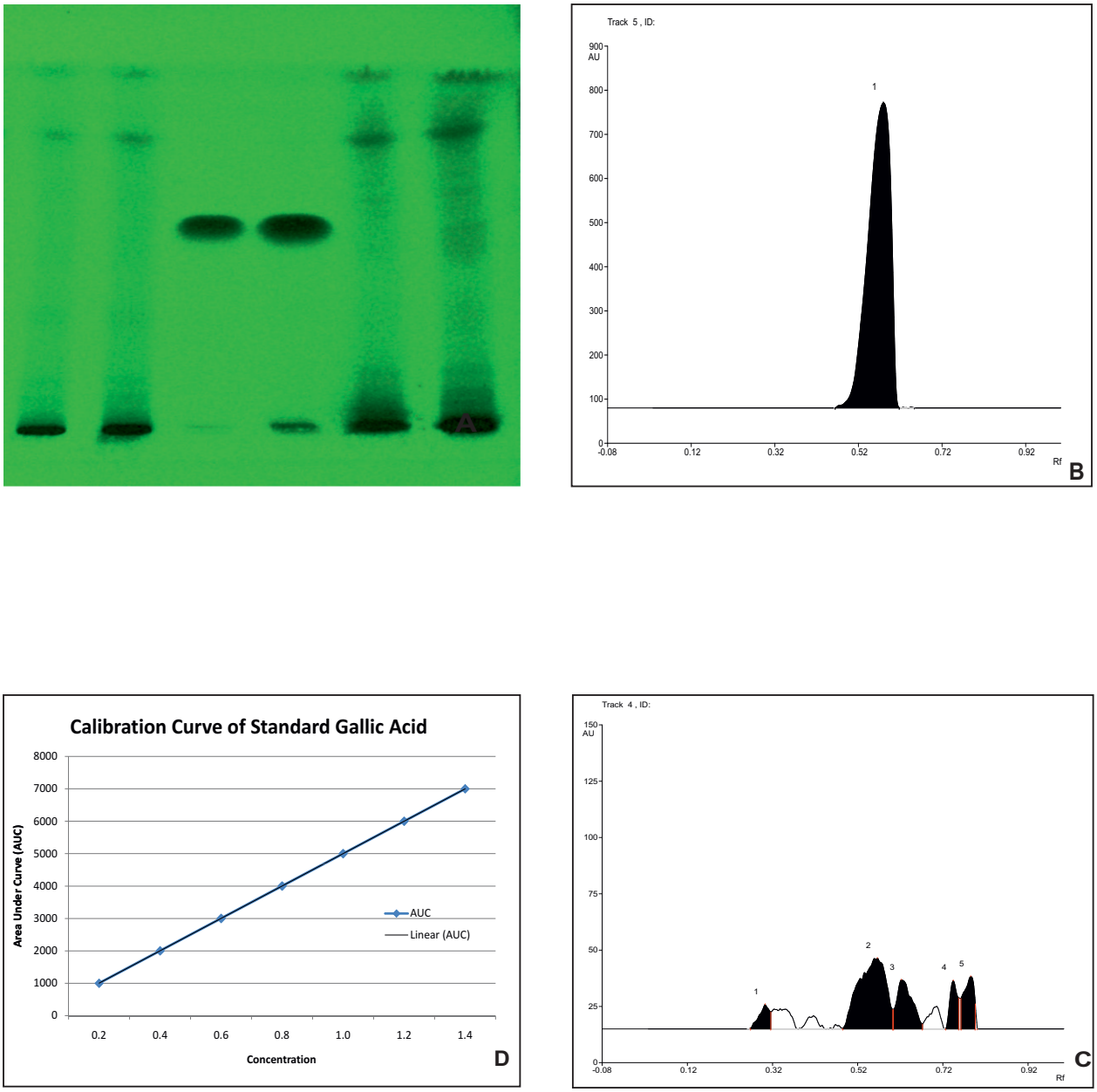

Fig.1: A- HPTLC plate photograph of *Petiveria alliacea* L. Powder extract and standard Gallic acid, B-Densitometric chromatogram of standard Gallic acid, C- Calibration curve of standard Gallic acid, D-HPTLC densitometric chromatogram of *Petiveria alliacea* L. Powder extract

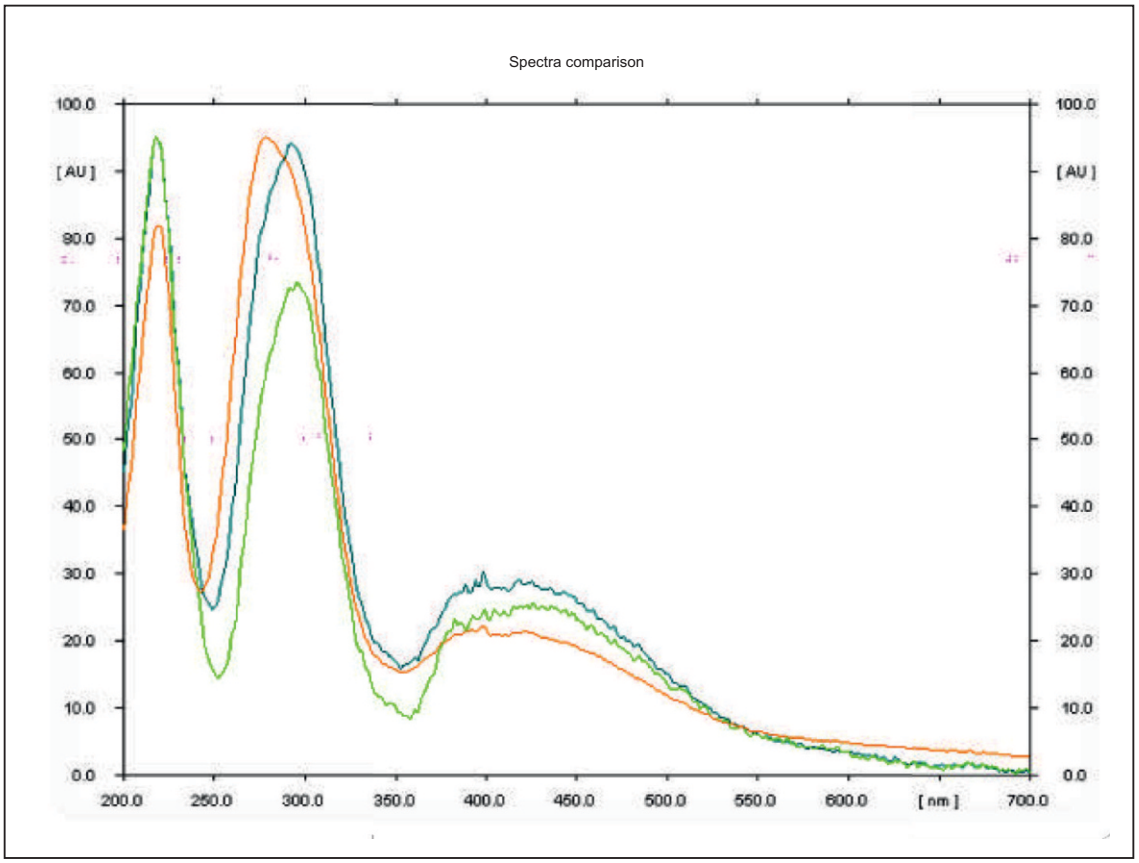

Fig. 2: Spectra comparison by overlay of UV absorption spectrum of Gallic acid standard on corresponding band of *Petiveria alliacea* L. Powder extract

The working standard solution of Gallic acid (1000µg/ml) was spotted to get different concentrations ranging from 200-1200 ng/spot, using a micro syringe. The working standard solutions were spotted as sharp band of 6mm width on pre-coated silica gel aluminium plate $60F_{254}$ (10cm x 10cm) with 200µm thickness using Camag Linomat 5 automatic sample applicator. The plates were developed in a solvent system comprising of Chloroform: Ethyl acetate: Acetone (50:50:1 v/v/v) in a Camag glass twin trough chamber at 25±2^{0}C. The developed plates were dried for 5 min. and densitometric scanning was performed on Camag scanner 3 at 254 nm.

The peaks of Gallic acid for each concentration were recorded and calibration curve was obtained by plotting peak areas of Gallic acid against applied concentration of Gallic acid. The results of linearity range and correlation coefficient showed that within the concentration range indicated there was good correlation between peak area and the corresponding correlation of Gallic acid.

RESULT AND DISCUSSION

The method as described in the present study, utilize silica gel $60F_{254}$ TLC plate as stationary phase and Chloroform: Ethyl acetate: Acetone (50:50:1 v/v/v) as mobile phase gives good separation of Gallic acid at Rf = 0.52 from the other components present in plant powder of *P. alliacea* L. The HPTLC plate was visualized under UV light at 254nm. and photographed (Fig.1 A). The calibration curve of Gallic acid

was found to be linear (200-1200ng/spot) dependent on the concentration against area. The equation of the best fitting line was Y=3639.1X + 130.86 (Fig. 1 C). The concentration of Gallic acid in the methanol extract of dried plant powder of *P. alliacea* by the proposed method was found to be 0.04µg (Fig. 1 B&D). Correlation coefficient 0.9967 indicates good linearity between concentration and peak area.

The identity of the Gallic acid band in the sample extract was confirmed by overlaying the UV absorption spectrum of the sample with that from the reference standard Gallic acid using the Camag TLC scanner 3 (Fig. 2). The HPTLC method proposed developed a good resolution of Gallic acid from other constituents present in the extract of *P. alliacea*.

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