QUANTITATIVE ESTIMATION OF GALLIC ACID IN *ROSA SINENSIS*, *EMBLICA OFFICINALIS* AND *SYZYGium AROMATICUM* BY HPTLC

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ABSTRACT

A number of plants are rich source of gallic acid, a pharmacologically important molecule which exists either in free form or as a part of the tannin molecule. The present study was designed to identify and compare the presence of gallic acid in the ethanolic extracts of three Indian medicinal plants, *Embllica officinalis*, *Syzygium aromaticum* and *Rosa sinensis* by high performance thin layer chromatography. The pulp of *Embllica officinalis* had the highest content of gallic acid, 27.36 mg / g of dry weight of extract. The gallic acid content in the petals and buds of *Rosa sinensis* and *Syzygium aromaticum* was 12.76 mg / g and 25.98 mg / g of dry weight respectively. The R_f value of gallic acid was found to be 0.29. This study significantly compares the presence of gallic acid in the three extracts which are a potential source of antioxidant activity.

Keywords: Gallic acid, HPTLC, *Rosa sinensis*, *Syzygium aromaticum*, *Embllica officinalis*.

INTRODUCTION

High performance thin layer chromatography (HPTLC) can be used qualitatively and quantitatively for the estimation of chemical constituents present in the plant materials¹. Gallic acid is found in tea, red wine, fruits and beverages and in various other medicinal plants². Chemically it is 3, 4, 5-trihydroxybenzoic acid. It possess astringent activity, anti-inflammatory, cardio-protective, antioxidant activity and are proven to show beneficial effects on human health³. They also have anti-carcinogenic, anti-hepatotoxic, anti-ischaemic, anti-allergic, anti-ulcerative activities⁴. All these properties make gallic acid a very important molecule with medicinal value, therefore the study was undertaken to identify and compare the presence of gallic acid in ethanolic extracts of three Indian medicinal plants, *Embllica officinalis*, *Syzygium aromaticum* and *Rosa sinensis* by HPTLC.

MATERIALS AND METHODS

Collection and Identification of the Plants

Petals of *Rosa sinensis* were collected from Amity Institute of Organic Agriculture Farm, Noida, India. The fruits of *Embllica officinalis* were collected from Bharmar village, Himachal Pradesh, India and the buds of *Syzygium aromaticum* were purchased from a local market, Noida, India. All the plant materials were identified by Z.Y. Rizvi, Senior horticulturist, Government of India.

Preparation of the Plant Extracts

Ethanolic extracts of the plants were prepared as per the extraction procedure mentioned earlier by Mehrotra et al.⁵.

Standard and Reagents

Standard gallic acid was obtained from Sigma-Aldrich Chemie (Steinheim, Germany). Analytical grade chloroform, ethyl acetate and formic acid used as solvent system were obtained from Qualigens Fine Chemicals, India.

Preparation of Standard Solution of Gallic Acid

For preparation of standard curve 0.5 mg / ml solution of standard gallic acid was prepared by dissolving gallic acid in 100 % methanol.

High Performance Thin Layer Chromatography

Three levels (0.3 µg, 0.4 µg and 0.5 µg) of standard gallic acid were used for the calibration curve for which 0.6 µl, 0.8 µl and 1.0 µl of standard solution was applied in duplicate as bands of 5 mm using a Camag microlitre syringe on a precoated silica gel plate 60F₂₅₄ [10 cm X 10 cm with 0.2 mm thickness, E. Merck] using a semi automatic Linomat V sample applicator. The plates were developed in a solvent system in CAMAG glass twin through chamber [20 cm X 10 cm] previously saturated with the solvent (chloroform: ethyl acetate: formic acid = 7.5: 6: 0.5) for 30 minutes. (Figure 1). The TLC plates were air dried and scanning was performed on a Camag TLC Scanner III at 254 nm using Wincats software 4.03 version1 (Figure 2 and 3). The mean peak height / area of duplicate samples was calculated and the content of gallic acid was quantified using the regression equation obtained from the standard curve⁶.

RESULT AND DISCUSSION

The standard gallic acid has R_f value of 0.29 [Figure 2(A)]. A good linear relationship ($r^2 = 0.99612$ and 0.99771 with respect to peak area and height, respectively) was observed between the concentration ranges of 0.05 µg to 0.5 µg. The regression equation was found to be $Y = 6.764 + 0.3748 * X$ with respect to height and $Y = 176.3 + 10.46 * X$ with respect to area, where Y is the peak height / area and X is the concentration of standard gallic acid. Out of the three ethanolic extracts (*Rosa sinensis* petals, *Embllica officinalis* pulp and *Syzygium aromaticum* buds), the pulp of *Embllica officinalis* had the highest content of gallic acid, 27.36 mg / g of dry weight of extract [Figure 2(C)] and similar observation was also reported by Borde et al.²

Figure 1: HPTLC chromatogram of gallic acid, *Rosa sinensis* (petal), *Emblica officinalis* (pulp) and *Syzygium aromaticum* (bud) extracts at 254 nm using Chloroform: Ethyl acetate: Formic acid = 7.5: 6: 0.5 as solvent system.
The details of the individual tracks are listed as tabular form in the right

Figure 2: HPTLC chromatograms of A. standard gallic acid ($R_f = 0.29$); B. ethanolic extract of *Rosa sinensis* (petal); C. ethanolic extract of *Emblica officinalis* (pulp); D. ethanolic extract of *Syzygium aromaticum* (bud); mobile phase: chloroform:ethyl acetate: formic acid (7.5: 6: 0.5),
X axis corresponds to R_f and Y axis corresponds to absorbance

Figure 3: HPTLC three-dimensional image of the calibration curve of gallic acid (tracks 1 to 12; 0.1 μ l - 1 μ l) from *Rosa sinensis* petal (tracks 13 and 14; 0.5 μ l), *Emblica officinalis* pulp (tracks 15 and 16; 1 μ l) and *Syzygium aromaticum* bud (tracks 17 and 18; 1 μ l) extracts at 254 nm. X axis corresponds to R_f and Y axis corresponds to absorbance

The gallic acid content in the petals of *Rosa sinensis* was 12.76 mg / g of dry weight of extract [Figure 2(B)] and in the buds of *Syzygium aromaticum* was 25.98 mg / g of dry weight of extract [Figure 2(D)]. The UV spectrum of the plant extracts was super-imposable (Figure 3) with that of the standard gallic acid indicating the purity of the peak and the presence of the standard (gallic acid) tested (Figure 3). Simplicity, specificity and sensitivity of the developed HPTLC method makes it a suitable method for monitoring the gallic acid content for standardization of raw materials at the time of formulating a preparation using the crude extract as well as for the quality control of the finished product.

CONCLUSION

The standard gallic acid was quantitatively determined in ethanolic extracts of three different plants. *Emblica officinalis* (pulp) contains the highest gallic acid content, followed by *Rosa sinensis* (petal) and then by *Syzygium aromaticum* (bud). The R_f value of gallic acid was found to be 0.29. This study is important as it quantitatively compares the presence of gallic acid in three extracts which is a potential source of antioxidant activity by a very simple and reliable-HPTLC.

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