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Original Research

GESDAV

Quantification of gallic acid in fruit and leaves of *Careya arborea* by high-performance thin layer chromatography

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ABSTRACT

Objective: The present study was aimed to quantification of gallic acid in *Careya arborea* (*Lecythidaceae*). **Materials and Methods:** High-performance thin layer chromatography (HPTLC) quantification was carried out for various extract of leaf and fruits of *C. arborea*. The thin layer chromatography plate was spotted with gallic acid and fruits and leaves extract of *C. arborea.* The extract of fruit and leaves shows the Gallic acid spot at R_f: value 0.68 using mobile phase ethyl acetate:toluene:formic acid (8:2:0.3, v/v). Hence, the gallic acid was selected as a marker. The gallic acid was quantified using HPTLC technique in *C. arborea* fruit ethyl acetate extract (CFE), *C. arborea* fruit alcoholic extract (CFA), *C. arborea* leave ethyl acetate extract (CLE) and *C. arborea* alcoholic extract of leaves (CLA) of *C. arborea*. **Result:** The percentage (w/w) amount of gallic acid was found to 2.11%, 1.2%, 0.54% and 0.48% in CFE, CFA, CLE, and CLA of *C. arborea,* respectively. **Conclusion:** This HPTLC quantification method useful for standardization of fruits and leaf *C. arborea*.

KEY WORDS: *Careya arborea*, gallic acid and quantification, high performance thin layer chromatography

INTRODUCTION

Quality control of herbal is challenging the task in globe. A plant contains varying number of complex constituents. The concentration of phytoconstituents is also vary depending on climate, season, method of collection, cultivation, etc. It is difficult to differentiate biological marker from chemical marker. Thin layer chromatography (TLC) and high-performance thin layer chromatography (HPTLC) is very useful, viable and essential tool for qualitative and quantitative analysis of plants and herbal products. HPTLC has gain popularity as a routine analytical method due to its sensitivity in nanograms [1]. The time required for demonstration of constituents is very short. TLC and HPTLC provide a chromatographic fingerprint. It is suitable for identity and purity of drug and detection of adulteration and substitution. HPTLC and TLC used for analysis of herbal drugs and separation of phytoconstituents [2].

Careya arborea Roxb (*Lecythidaceae*) is large deciduous tree grows up to 20 m, commonly known as "wild Guava and Kumbhi." It is habitant to from Jammu to West Bengal, Madhya Pradesh and Tamil Nadu. Fruits are large, globose, fleshy, indehiscent, crowned with the calyx limb. Seed numerous, embedded in the fleshy pulp. Leaves are alternating crowned at the branches [3-5]. Leaves are traditionally used in the treatment of dislocated bones, body pain, abdominal pain, myalgia, rheumatic pain, swellings, skin diseases and tongue ulcer [6-10]. In folk medicine fruits are used as cooling agent, diuretic, tonic, aphrodisiac, alternative astringent to the bowels, promote growth of hairs, useful anemia, leprosy, ulcers and vaginal discharge, alopecia, anemia, consumption [11-12]. Leaves reported antileishmanial [13], gastro protective [14], wound healing [15], antitumor and antibacterial activities [16] whereas fruits reported antibacterial [17] and antioxidant activity [18]. Gallic acid is occurring in many plants in the free state or combined. It is a phenolic compound having antioxidant activity [19]. There is no HPTLC analytical method available for quantification of the marker compound in fruit and leaves of *C. arborea*. Hence, the present study was aimed to identification and quantification of marker in fruit and leave extracts of *C. arborea*.

MATERIALS AND METHODS

Plant Material

Fruit and leaves of *C. arborea* were collected from Vadodara in June 2012. Plant was identified and authenticated by Dr. P. S. Nagar at Botany Department of The M. S. University, Vadodara.

Voucher specimen (DC-CA-2) was stored in the herbarium of Pioneer Degree Pharmacy College, Vadodara.

Reagent and Chemicals

All the chemicals and reagents used were of analytical grade. Precoated TLC plates silica gel 60 F 254 was purchased from E. Merck (Darmstadt, Germany), gallic acid from Sigma (chemical Co, St. Louis, MO, USA).

Preparation of Extracts

Methanolic extract: About 20 g of fruit and leaf powder were extracted for 24 h with methanol separately. The extracts were filtered, concentrated by evaporation on the water bath and dried.

Ethyl acetate extract: About 10 g of fruit and leaf powder were extracted for 24 h with ethyl acetate separately. The extracts were filtered, concentrated by evaporation on the water bath and dried.

Selection of marker

The methanol, water, and ethyl acetate extract of leaf and fruits of *C. arborea* were screened with phenolic and flavonoid. Different extract of plants was spotted with gallic acid. Methanolic and ethyl acetate extract shows the presence of gallic acid in fruits and leaf. So, gallic acid was selected as a marker compound. The different solvent system was tried and checked for band separation.

Preparation of Samples

Ethyl acetate extract: A stock solution of extract having concentration 30 mg/ml was prepared in ethyl acetate.

Methanol extract: A stock solution of extract having concentration 40 mg/ml was prepared in methanol.

Standard stock solution: A solution of gallic acid (1 mg/ml) was prepared in methanol.

Chromatographic Condition

HPTLC was carried out using stationary phase; precoated Silica Gel G 60 F 254 plate (20 cm × 10 cm), mobile phase; ethyl acetate:toluene:formic acid (8:2:0.3), band length; 7.0 mm, saturation time; 25 min, solvent front position; 65.0 mm, scanning speed; 20 mm/s, syringe size; 100 μl, instrument; CAMAG Linomat 5, sample applicator; Linomat 5, development chamber; twin trough chamber 20 cm × 10 cm, detection; CAMAG TLC scanner, spraying reagent; alcoholic 1% ferric chloride and data analyze using WinCat Software (CAMAG-Muttenz, Switzerland).

Calibration Curve of Gallic Acid Using HPTLC

Graded concentration of 0.8 mg/ml standard gallic acid solution (2, 4, 6, 8 and 10 μl) were applied on a precoated TLC silica gel 60 F 254 plate (E. Merck) using Camag Linomat V automatic spotter to produce the concentration of gallic acid 1.6, 3.2, 4.8, 6.4 and 8 μg/spots respectively. The plate was developed in an optimised mobile phase and scanned at 254 nm. Data of peak area of each spot of gallic acid was recorded. The calibration curve of gallic acid was obtained by plotting area verses concentration of gallic acid [20,21].

Validation of Method

The developed HPTLC method for estimation of gallic acid was validated for linearity, precision, repeatability, accuracy, limit of detection, limit of quantitation and range. The method was validated as per ICH guideline (CPMP/ICH/381/95 and CPMP/ICH/281/95) [22]. The repeatability was checked by repeated scanning (n = 7) of the same spot of gallic acid (1.6 μg) and expressed as the coefficient of variance (CV %). The accuracy of the method was determined by performing recovery studies at three level (50%, 100%, 150% addition). The percentage average recovery was calculated. The limit of detection and limit of quantification were determined by comparing the peak height of the sample with methanol as a blank on the basis of signal to noise ratio.

Quantification of Marker Compound

The various extract and standard were applied on precoated plate (Silica Gel G 60 F 2540) using sample applicator. The plate was dried for 5 min at 45°C temperature and developed in provisory statured mobile phase (ethyl acetate:toluene:formic acid [8:2:0.3]) in twin trough chamber at constant temperature (25 ± °2). The plate was dried for 10 min. The developed plate was scanned at 254, 366 and invisible mode at 515 nm after derivatization with suitable detection reagents using CAMAG TLC SCANNER-3.

RESULT

Selection of Marker

The *C. arborea* alcoholic extract of leaves (CLA), *C. arborea* leave ethyl acetate extract (CLE), *C. arborea* fruit ethyl acetate extract (CFE), *C. arborea* fruit alcoholic extract (CFA) extracts were applied on TLC plate, run with gallic acid simultaneously. All extract shows presence of gallic acid and hence the gallic acid was selected as a marker [Figures 1 and 2].

Method validation

The repeatability was checked by repeated scanning (n = 7) of the same spot of gallic acid (1.6 μg) and expressed as CV %. The accuracy of the method was determined by performing recovery studies at three level (50%, 100%, 150% addition). The percentage of average recovery was calculated. The limit of detection and limit of quantification were determined by comparing the peak height of the sample with methanol as a blank on the basis of signal to noise ratio. The developed HPTLC method for estimation of gallic acid was validated for linearity, precision, repeatability, accuracy, limit of detection, limit of quantitation and range has shown in Table 1.

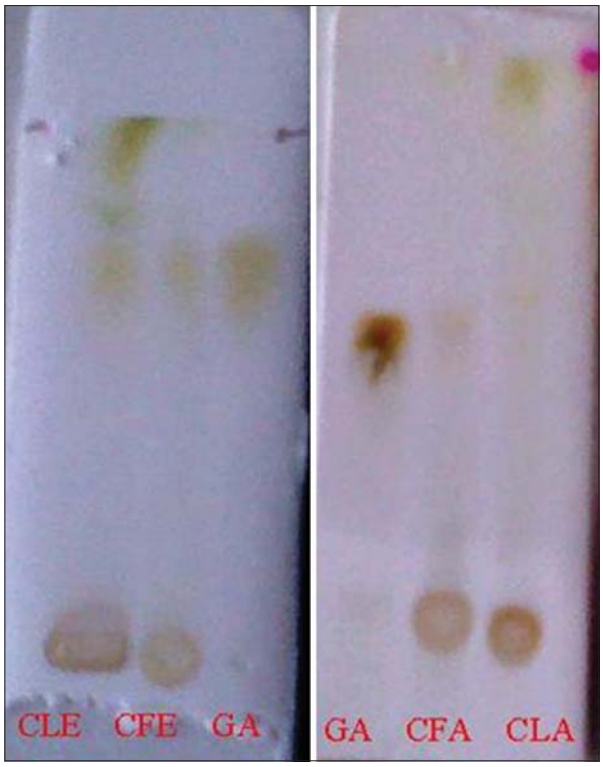

Figure 1: Thin layer chromatography profile of ethyl acetate extract and methanolic extract of fruit and leaves of *Careya arborea* and gallic acid

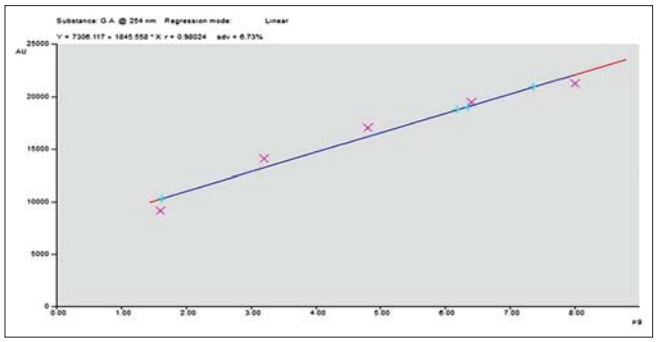

Figure 2: Calibration curve of gallic acid

Table 1: Validation parameter of HPTLC quantification of gallic acid in *C. arborea*

Validation parameter	Result
Accuracy (average % recovery)	98.97%
Precision-coefficient of variance (CV%)	1.794
Limit of detection (μg)	0.12 μg
Limit of quantification (μg)	0.365 μg
Linearity (correlation coefficient)	0.98
Range (μg/spot)	1.6-8 μg
Specificity	Specific

C. arborea: Careya arborea, HPTLC: High performance thin layer chromatography

Quantification of gallic acid using HPTLC in C. arborea extracts

The gallic acid content determined by HPTLC method in methanol and ethyl acetate extracts of leaf and fruits of C. *arborea* reported in Table 2. Gallic acid content in methanol

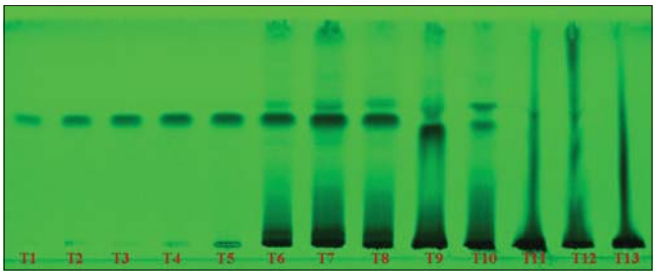

Figure 3: High performance thin layer chromatography profile of gallic acid, *Careya arborea* fruit ethyl acetate extract, *C. arborea* fruit alcoholic extract, *C. arborea* leave ethyl acetate extract, *C. arborea* alcoholic extract of leaves of at 254 nm

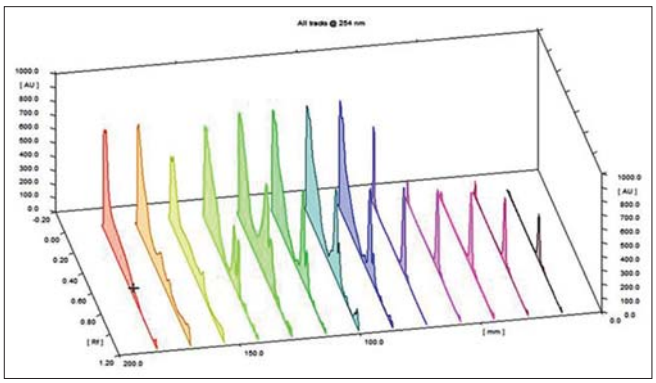

Figure 4: 3-D chromatogram of gallic acid, *Careya arborea* fruit ethyl acetate extract, *C. arborea* fruit alcoholic extract, *C. arborea* leave ethyl acetate extract, *C. arborea* alcoholic extract

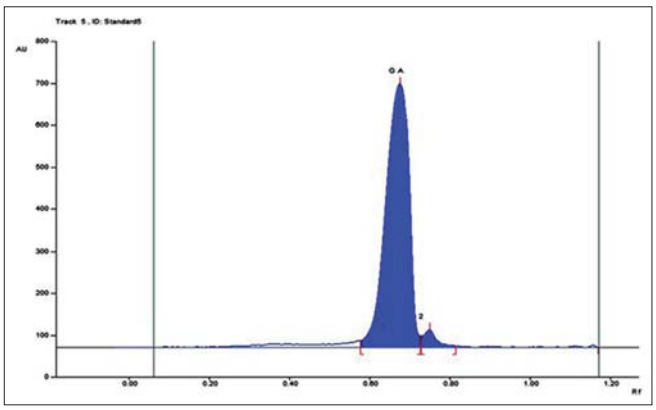

Figure 5: Chromatograph of gallic acid

and ethyl acetate extract of leaf and the fruit of C. *arborea* was carried out. The photographs of TLC plate for estimation of gallic acid in extracts at 254 nm are given in Figures 3 and 4 shows 3-D chromatograph of gallic acid in all tracks. Chromatograph of gallic acid, CFE, CFA, CLE and CLA are recorded in Figures 5-9 respectively. The UV Spectra of gallic acid in all extracts are given in Figure 10.

As shown in Figure 3-TI, T2, T3, T4 and T5 are 1.6, 3.2, 4.8, 6.4 and 8.0 μg concentration of standard gallic acid (μg/ml), respectively. T6, T7; CFE, T8, T9; CFA, T10, T11; CLE, T12, T13: CLA, respectively.

Table 2: Gallic acid content in various extract of leaves and fruits of *C. arborea*

Extract	Gallic acid content (%)
CFE (Ethyl acetate extract [fruit])	2.11
CFA (Methanolic extract [fruit])	1.2
CLE (Ethyl acetate extract [leaves])	0.54
CLA (Methanolic extract [leaves])	0.48

C. arborea: Careya arborea, CFE: *Careya arborea* fruit ethyl acetate, extract, CFA: Careya arborea fruit alcoholic extract, CLE: *Careya arborea* leave ethyl acetate extract, CLA: *Careya arborea* alcoholic extract of leaves

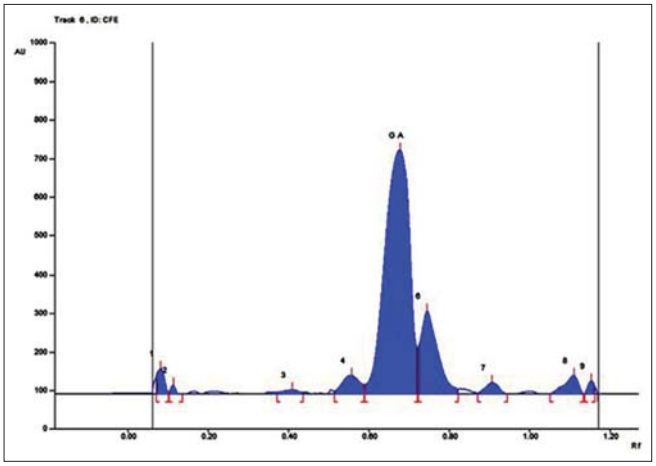

Figure 6: Chromatograph of *Careya arborea* fruit ethyl acetate extract

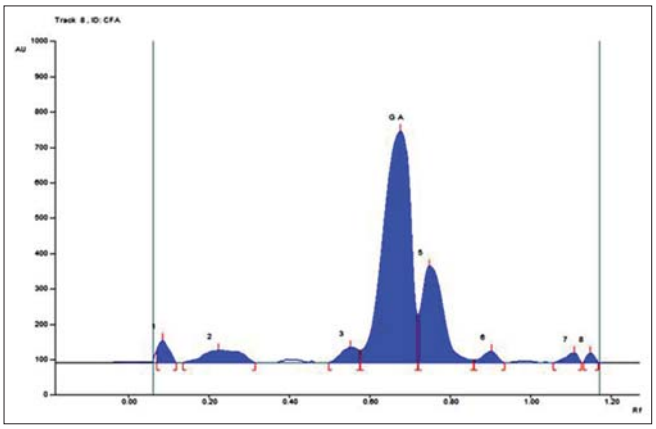

Figure 7: Chromatograph of *Careya arborea* fruit alcoholic extract

DISCUSSION

C. arborea Roxb is known as Kumbhi, because of the fruit giving it somewhat the appearance of a water-pot. The bark, fruits, leaves and flowers of *C. arborea* are widely used in the treatment of various ailments traditionally.

Herbs have been used for treatment of various ailments for several thousands of years. The higher plant species on earth is approximately 250,000. It is reported that 35,000-70,000 species have been used medicinally purpose. Still major population in developing countries depend on plant medicines however modern medicine area available [23].

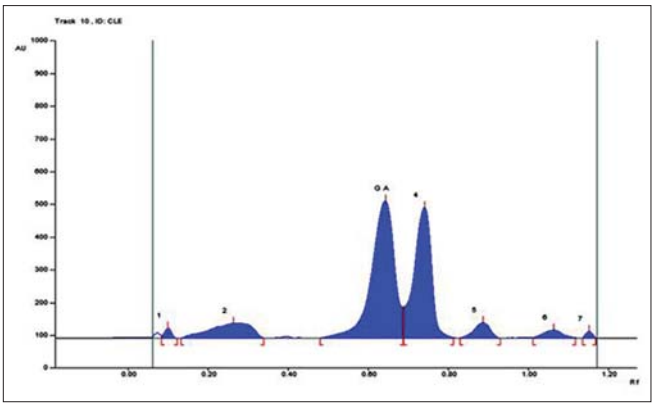

Figure 8: Chromatograph of *Careya arborea* leave ethyl acetate extract

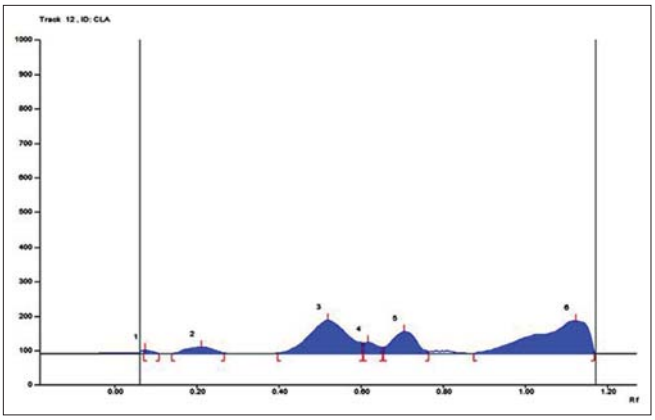

Figure 9: Chromatograph of *Careya arborea* alcoholic extract of leaves

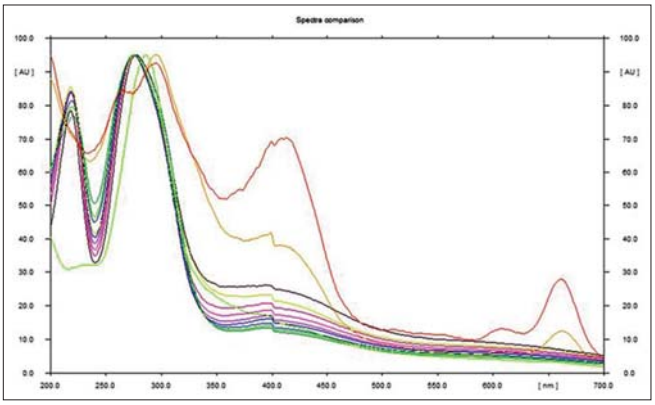

Figure 10: Ultraviolet spectra of gallic acid in all tracks at 254 nm

However, the standardization of herbal drug is difficult due to variation in the environmental factor like rain, temperature, altitude, climate and other factor like time of collection, way of cultivation, etc. affect the quality of the product. So there is a batch to batch variation in herbal formulation. The active constituent present in plant is also varied.

The various parameters like morphological, microscopical, physical constant etc., are used for assessing quality of herbal drugs. Now-a-day various sophisticated instrumental methods are used for standardization of plant drugs. Chromatographic

method like TLC, HPTLC, gas chromatography (GC) and hyphenated techniques like GC-mass spectrometry (MS), liquid chromatography-MS are also used. TLC and HPTLC is mentioned in various such as in American herbal pharmacopoeia, Chinese drug monograph and analysis, pharmacopoeia of peoples republic of china as an analytical tool. TLC and HPTLC are useful in identification, authentification and quality control of herbal drugs. HPTLC is a simple technique, and multiple sample analysis can be performed [24].

In the present study, various extracts were prepared, and qualitative chemical analysis was carried out. Methanolic and ethyl acetate of fruits and leaves of *C. arborea* was showed spot of gallic acid. Different mobile phase viz. ethyl acetate:methanol:formic acid:gallic acid (9:1:0.4:0.2), ethyl acetate:toluene:methanol:formic acid (8:2:1:0.2), ethyl acetate: methanol:formic acid:gallic acid (9:1:0.2:0.2) and ethyl acetate: toluene:formic acid (8:2:0.3) were tried. Among these mobile phase, ethyl acetate: toluene:formic acid (8:2:0.3) was selected. Quantification of gallic acid was carried out using HPTLC and validation was done as ICH guideline.

The quality control of herbal drug is challenging the task due to variation of chemical constitutes. HPTLC densitometry method was developed and validated. The quantification of gallic acid was carried out on fruit and leaves of *C. arborea*. The ethyl acetate extract of the fruit was found to be containing the highest amount of gallic acid. The ethyl acetate and methanolic extract of fruits contain more amount of gallic acid than leaves extracts. The developed method is rapid, accurate and precise. The present study could be useful for identification and standardization of fruit and leaves of *C. arborea*.

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