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Research Article

DETERMINATION OF GALLIC ACID IN *ACACIA NILOTICA LINN.* BY HPTLC

V Leela *, L Kokila, R Lavanya, A.Saraswathy, P.Brindha
Department of CARISM, SASTRA University, Thanjavur, Tamilnadu.
Captain Srinivasa Murti Drug Research Institute for Ayurveda and Siddha (CCRAS), Arumpakkam, Chennai-106, Tamilnadu.
Email: leelevadivelu@gmail.com

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Abstract

The stem bark of *Acacia nilotica* is used as a powerful astringent and the decoction is used as gargle for throat troubles and stomatitis.q[3] Bark contains several polyphenols such as catechin, epicatechin, dicatechin, quercetin and tannin. Gallic acid has been reported to have antidiabetic, antithrombotic, anti inflammatory and anticarcinogenic activities. A simple, precise and accurate high-performance thin layer chromatographic method has been established for the determination of Gallic acid in the bark powder of *Acacia nilotica* Linn. The acetone extract of the bark powder was used for the experimental work. Separation was performed on Silica gel 60 F_{254} HPTLC plates with Toluene: Ethyl acetate: Formic acid (6:4:0.8 v/v), as mobile phase. The plate was scanned in the densitometric absorbance mode at 280 nm. Gallic acid response was linear over the range 2-7 µg ml^{-1}. The HPTLC method was validated in terms of sensitivity, accuracy, precision and reproducibility. The concentration of gallic acid in the bark powder was found to be 0.86%.

Keywords

Acacia nilotica Linn, Gallic acid, HPTLC

Introduction:

Acacia nilotica (L). Karuvelam, Karuveal (Fabaceae) is a moderate sized tree usually up to 15m high. Leaves are bipinnate with spinescent stipules. Flowers are golden yellow, fragrant crowded in long stalked globose heads, forming axillary clusters of 2 to 5 heads. It is found throughout the warmer regions of India ascending to 800 m. Barks are medicinally important and contains several polyphenols such as catechin, epicatechin, dicatechin, quercetin and tannin [1,2]. The bark is a powerful astringent and the decoction is used as gargle for throat troubles and stomatitis, in chronic dysentery and in diarrhoea, and also for cleansing in haemorrhagic ulcers and wounds. Juice of the bark mixed with breast milk is instilled into the eyes in conjunctivitis. Burnt bark mixed with burnt almond and salt are used as a tooth powder [3-6]. Quality control and quantitative assay for marker compounds in traditional herbal medicines have become a necessary for authentication as well as for determining the strength of the drug source. The present study is aimed as to determine the percentage of Gallic acid present in the drug by employing HPTLC technique.

Materials and Methods:

The stem bark of *Acacia nilotica* was collected from Thanjavur. Standard Gallic acid was purchased from M/s Sigma chemicals. Aluminum plates precoated with silica gel 60 F_{254} of 0.2mm thickness (E. Merck, Darmstadt, Germany) were used without pretreatment. All chemicals and solvents used were of analytical and HPLC grade (E.Merck, Mumbai, India).

Standard stock solution

Standard solution containing 1mg mL^{-1} of Gallic acid was prepared by dissolving 10 mg of gallic acid in 10 ml of methanol.

Sample preparation

Sample solution was prepared from dried and coarsely powdered bark (1g) of *A.nilotica*. The powder was extracted with acetone (4x10ml) at ambient temperature for 16-20 hours, filtered and concentrated under vacuum and the yield was 35%. 100 mg extract was taken and dissolved in methanol (10ml) and filtered through 0.45µm filter for HPTLC analysis.

HPTLC Instrumentation

A camag HPTLC system equipped with an automatic TLC sampler (Linomat 5), TLC scanner 3 (WINCATS version 1.3.4) with UV cabinet and twin through glass tank (20x10cm) was used for the analysis. The samples were applied using automated TLC sampler in 6 mm bands at 10 mm from the bottom, both sides and 12 mm space between the two bands.

Calibration

Standard Gallic acid solution of different concentrations (100,150,200,250,300 and 350 ng $spot^{-1}$ were prepared in methanol. Standard solution of 2, 3, 4, 5, 6, 7 µl were applied to the HPTLC plate for preparing six point linear calibration curve. Sample solution (10 mg /ml) applied on the HPTLC plate in duplicate with similar band pattern. The experimental parameters were identical for all the above analysis. The data obtained is presented in Table 1.

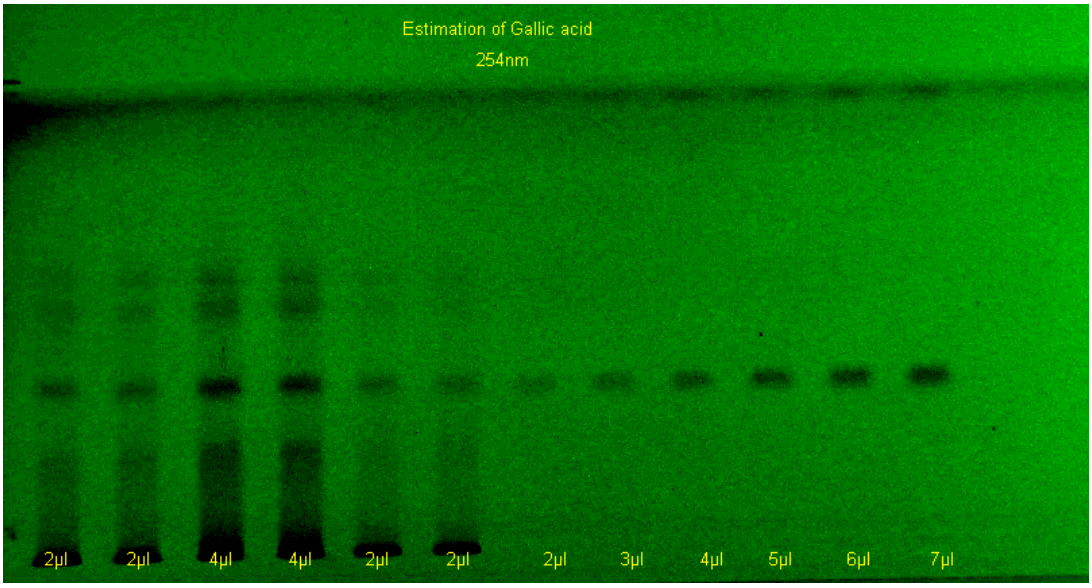
Table: 1

S.No	Parameter	Results
1	Linearity range	100-350ng $spot^{-}$
2	Correlation coefficient	0.9992
3	Limit of detection	0.05µg/ml
4	Limit of Quantification	0.07µg/ml
5	Recovery	97.50%
6	Specificity	Specific

Development of the chromatogram

The TLC plate was developed in a camag twin –trough glass tank which was presaturated with developing solvent Toluene: ethyl acetate: Formic acid (6:4:0.8 v/v/v). The composition of the developing solvent was optimized using varying polarity of solvents. The plate was developed to a height of about 8 cm from the base of application. After development, the plate was air dried and spots were visualized under UV light at 254 nm. Quantitative evaluation of the plate was performed in the remission /absorbance mode at 280 nm, with the following conditions slit width 6.00 X 0.45 mm, micro scanning speed 20 mm and data resolution 100µm per step. The HPTLC chromatogram of standard Gallic acid and test solution is shown in Fig. 1.

Fig-I



Recovery

For percent recovery, known concentration of standard was added to a pre- analyzed sample of *A. nilotica* bark. The spiked sample was then analyzed by similar method in duplicate. The data obtained is presented in Table 1.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The compounds were identified on the basis of R_f values and UV- VIS spectral overlaying of the standard compounds. Standards were diluted and applied on HPTLC plate to plot the calibration curves. LOD was determined based on the lowest concentration detected by the instrument from the standards while the LOQ was determined based on the lowest concentration quantified in the sample. The data obtained were presented in Table 1

Specificity

The specificity of the method was determined by analyzing the sample along with the standard gallic acid. The band for gallic acid from sample solution were confirmed by comparing the Rf and spectra of the band to those of the standards. The peak purity of gallic acid was assessed by comparing the spectra at three different levels that is peak start, peak apex, and peak end position of the spot. The data obtained is presented in Table 1 (Fig.2)

Fig II: Spectra comparison purity

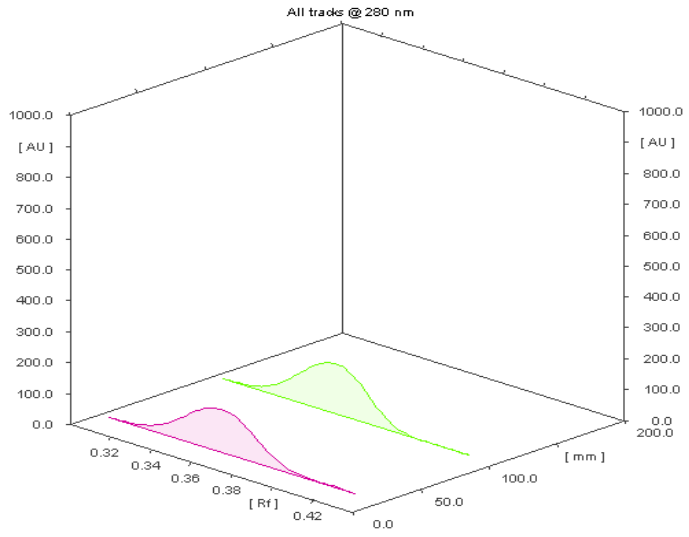


Fig II

Results and Discussion

The mobile phase Toluene: Ethyl acetate: Formic acid 6:4:0.8 (v/v/v) gave good separation of gallic acid (R_f 0.36) from the other phytochemicals of *A.nilotica* (Fig.3). The identity of gallic acid in the plant extract was confirmed by overlaying the gallic acid in plant with that of the gallic acid standard both obtained with the camag TLC scanner. The detector response of gallic acid was found to be linear in the range 100-350ng $spot^{-1}$ with correlation coefficient of 0.9992. The concentration of gallic acid in the whole bark powder was found to be 0.86%. The mean percent recovery of 97.50% indicated the accuracy of the proposed method.

Fig III (all tracks at 280nm)

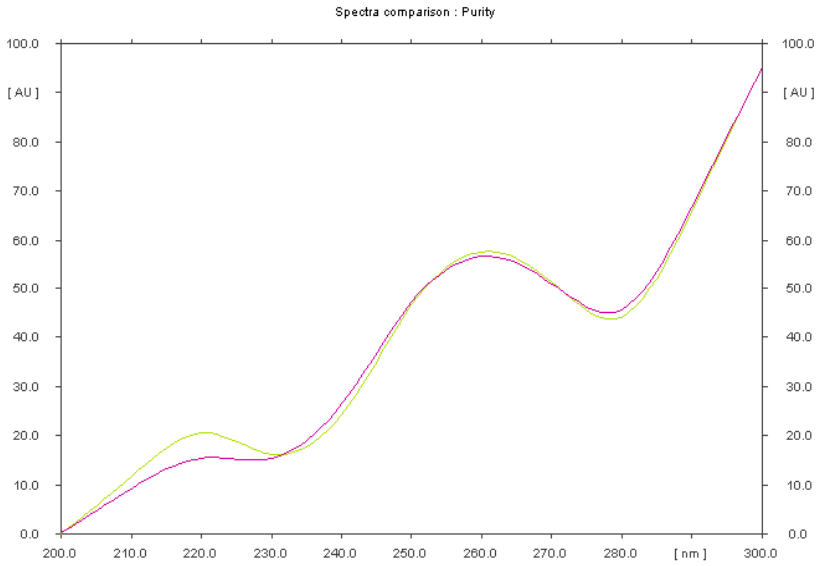


Fig III

The robustness of the method was studied, during the method development, by determining the effects of small variation in (i) mobile phase composition (±2%), (ii) chamber saturation period for development distance and (iii) scanning time (10% variation of each). No significant change of R_f or response to Gallic acid was observed which indicated the robustness of the method adopted.

The present method provides a lower limit of detection for the calibration curve (1-5µgml^{-1}) which is comparable with the existing report method [7] .The RSD and correlation coefficient values indicate high reproducibility of the method. The developed method is specific for determination of gallic acid from *A. nilotica* and can be readily profiled.

Conclusion

Quantitative estimation of Gallic acid in stem bark of *A.nilotica* was reported by employing simple, precise, accurate and sensitive HPTLC method. This method could be used in all Quality control studies of *A. nilotica* Linn.

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Corresponding Author:

V.Leela M.Pharm (PhD,)
Asst.Professor
Department of Pharmacy
CARISM SASTRA University
Thanjavur