INTERNATIONAL RESEARCH JOURNAL OF PHARMACY

www.irjponline.com ISSN 2230 – 8407

Research Article

QUANTIFICATION OF PHARMACOLOGICALLY ACTIVE MARKERS GALLIC ACID, QUERCETIN AND LUPEOL FROM ACACIA LEUCOPHLOEA WILLD FLOWERS BY HPTLC METHOD

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Article Received on: 18/12/12 Revised on: 01/01/13 Approved for publication: 10/02/13

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ABSTRACT

TLC densitometric method for quantification of gallic acid, quercetin and lupeol using HPTLC is developed. This is the first report of quantification of these three bioactive compounds viz Gallic acid, quercetin and lupeol using HPTLC from this plant. Quantification of gallic acid and quercetin was carried out from the methanolic extract using the solvent system of Toluene: Ethyl acetate: Formic acid (6:4:0.8 v/v/v). Lupeol was quantified from chloroform extract using the solvent system of Toluene: Ethyl acetate (7:3 v/v). The R_f values of gallic acid, quercetin and lupeol are 0.22, 0.37 and 0.70 respectively. The linearity ranges for gallic acid (100 to 600ng), quercetin (2000 to 7000ng) and lupeol (100 to 1200ng) with correlation coefficients (r-values) of 0.99968, 0.99708, 0.99971 respectively. The amount of gallic acid, quercetin and lupeol was 124.31, 580.4, 24.89 µg/ml respectively. Quantification of gallic acid, quercetin and lupeol showed good resolution and separation from other constituents of extract. Its main advantages are its simplicity, accuracy, and selectivity. This method can also be used for the estimation of these compounds in other herbal preparations and may be useful for standardization purposes.

Keywords: *Acacia leucophloea*, HPTLC, Gallic acid, Quercetin, Lupeol

INTRODUCTION

Herbs contain different biologically active phytoconstituents which exhibit therapeutic effects [1]. Among the various class of phytoconstituents, flavonoids and triterpenoids are widely distributed in plants. Flavonoids are phenolic compounds which occur both in the free state and as glycosides are the largest group of naturally occurring phenols. They are formed from three acetate units and phenyl propane units[2]. Gallic acid is phenyl propanoid (Fig1), chemically it is 3, 4, 5-Trihydroxy benzoic acid and possess astringent activity. Quercetin (Fig2) constitutes phenolic compounds which are structurally derived from the parent substance flavones. Triterpenoids lupeol (Fig3) are compound with a carbon skeleton based on six isoprene units which are derived biosynthetically from the acyclic C_{30} hydrocarbon squalene. The above three biologically active phytoconstituents are reported to have several pharmacological activities. Quercetin is reported for its antiallergic[3], antitumor[4], immunomodulatory[5] activities. Lupeol is reported to show antimalarial[6] and hepatoprotective[7] activities. These two major groups of phytoconstituents largely differ in polarity due to their structural difference.

A.leucophloea is a medicinal plant employed in the Indian indigenous system of medicine[8-9] . The stem bark is an astringent[10-17],used in ulcers and wound healing[18-19] as an antidote to snake bite[20], in dysentery[21] and for tooth ache[22]. Phytochemical evaluation is one of the tools for the quality assessment which includes preliminary phytochemical screening, chemo profiling and marker compound analysis using modern analytical techniques. High performance thin layer chromatography method is suitable method for estimation of chemical constituents present in plant materials. Hence *A. leucophloea* contain gallic acid, quercetin and lupeol are important active constituents and is estimated by HPTLC method.

MATERIALS AND METHOD

Reagent and Materials

The flowers of *A. leucophloea* were collected from Thanjavur, Tamilnadu. Shade dried and coarsely powdered. Standard quercetin, gallic acid and lupeol were purchased from M/s Sigma chemicals. Aluminium plates precoated with silica gel $60F_{254}$ of 0.2mm thickness (E.merck, Darmstadt, Germany) were used without pretreatment. All chemicals and solvents used were analytical and HPLC grade (E.Merck, Mumbai, India). Derivatizing reagent is Anisaldehyde sulphuric acid was prepared as per the procedure described in (Reich 2006)[23].

Preparation of extract

Air dried *A. leucophloea flowers* (1g) were chopped into small pieces and exhaustively extracted using methanol (4X10ml) for 16-20hrs. The solvent was evaporated under reduced pressure producing the crude extract (8%w/w).

Preparation of standard and stock solution

Standard solution of gallic acid

A stock solution of gallic acid was prepared by dissolving 10mg of accurately weighed gallic acid in methanol and making up the volume to 10ml with methanol. Then 1ml was pipetted out from stock solution and made up to the volume 10ml with methanol to get the final concentration of 100µg/ml.

Standard solution of quercetin

A stock solution of quercetin was prepared by dissolving 10mg of accurately weighed quercetin in methanol and making up the volume to 10ml with methanol to get final concentration of 1000µg/ml.

Standard solution of lupeol

A stock solution of lupeol was prepared by dissolving 10mg of accurately weighed lupeol in chloroform and making up the volume to 10ml with chloroform. Then 1ml was pipette out from stock solution and made up to the volume 10ml with chloroform to get the final concentration of 100µg/ml.

Preparation of sample solution for gallic acid and quercetin estimation

Sample solution was prepared by dissolving 100mg of methanolic extract in methanol and making up the volume to 10ml to get the concentration of 10mg/ml. The solution was filtered through Whatman no. 41 filter paper and used for further chromatographic analysis.

Preparation of sample solution for lupeol estimation

Sample solution was prepared by dissolving 100mg of the methanolic extract in chloroform and making up the volume

to 10ml to get the concentration of 10mg/ml. The solution was filtered through Whatman no. 41 filter paper and used for further chromatographic analysis.

Development of HPTLC Technique

The samples were spotted in the form of bands with CAMAG microlitre syringe on a precoated silica gel GF_{254} plates (20cmX20cm with 0.2mm thickness, E.Merck) using camag linomat V. Automatic sample spotter of band width 7mm. The plates were developed in a solvent system in CAMAG glass twin trough chamber previously saturated with the solvent for 30min. The distance was 8cm subsequent to the scanning, TLC plates were air dried and scanning was performed on a CAMAG TLC scanner in absorbance at 254 nm, 280 nm and 538 nm operated by Wincats software 4.03version.

Specificity

The specificity of the method was ascertained by analyzing standards, blank and sample extract by simultaneously applying on the same TLC plate. The spots of gallic acid, quercetin and lupeol in the sample extract were confirmed by comparing the R_f values and spectra of the spots with those of respective standards. The peak purity was assessed by comparing the spectra at three different levels, i.e. peak start. Peak apex and peak end.

Calibration curve for gallic acid quercetin and lupeol

The standard solution of gallic acid (100µg/ml), quercetin (1000µg/ml) and lupeol (100µg/ml) in different volumes were located on the different TLC plate for preparation of calibration curve (1-6 µl of gallic acid, 2-6 µl of quercetin and 1-12 µl of lupeol) checked for reproducibility. The calibration curve was prepared by plotting the concentration of standard vs. average peak area after scanning at 254 nm, 280 nm and 538 nm.

Quantification of gallic acid, quercetin and lupeol

Gallic acid and quercetin estimation in *A.leucophloea*

Stationary phase: Silica gel GF254 plates
Mobile phase: Toluene: Ethyl acetate: Formic acid (6:4:0.8)
Standard: Gallic acid (100µg/ml), quercetin (1000µg/ml)
Sample: Methanolic extract of *A.leucophloea* flower (10mg/ml)
Migration distance: 80mm
Scanning wavelength: 254nm and 280nm
Mode of scanning: Absorption (deuterium)

Lupeol estimation in *A.leucophloea*

Stationary phase: Silica gel GF254 plates
Standard: Lupeol (100µg/ml)
Sample: Methanolic extract of *A.leucophloea* flower dissolved in chloroform (10mg/ml)
Migration distance: 80mm
Scanning wavelength: 538nm
Mode of scanning: Absorption (deuterium)

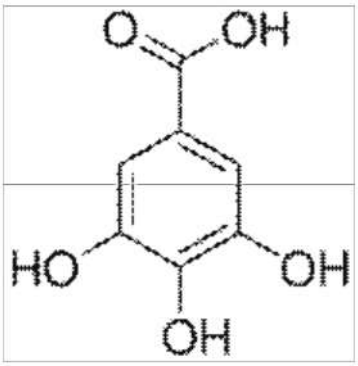


Figure 1 Chemical structure of Gallic acid

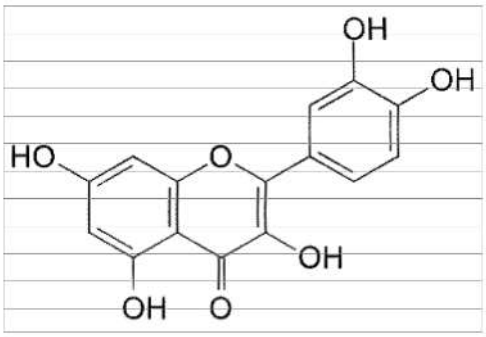


Figure 2 Chemical structure of Quercetin

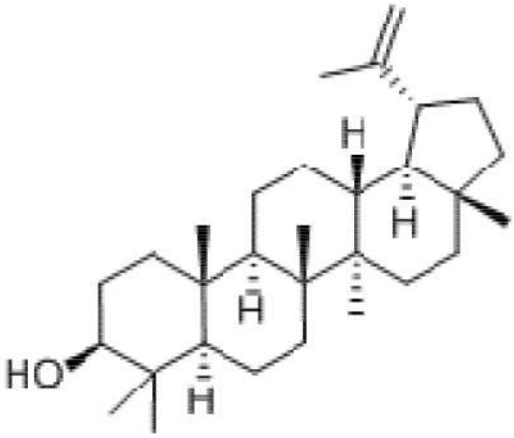


Figure 3 Chemical structure of Lupeol

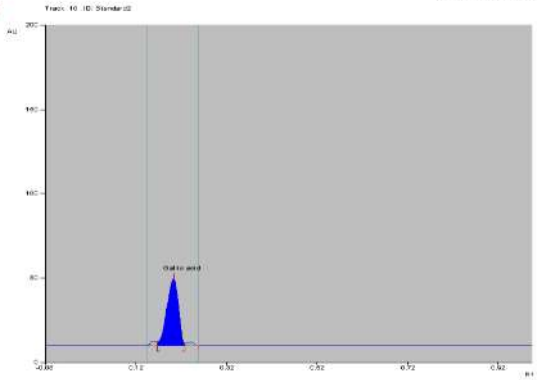


Figure 4 HPTLC chromatogram of Gallic acid

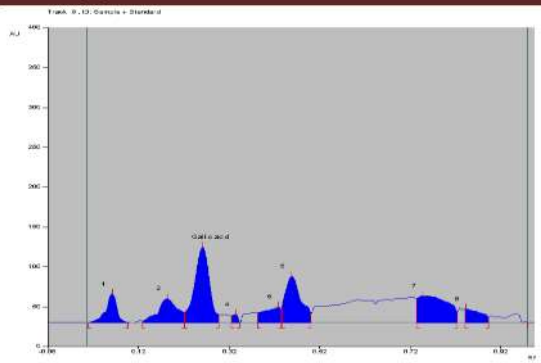


Figure 5 HPTLC chromatogram of sample containing Gallic acid

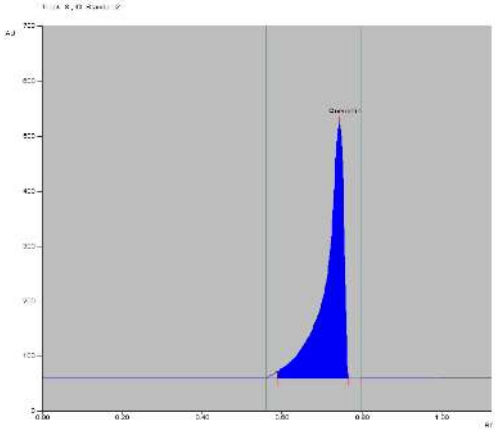


Figure 6 HPTLC chromatogram of Quercetin

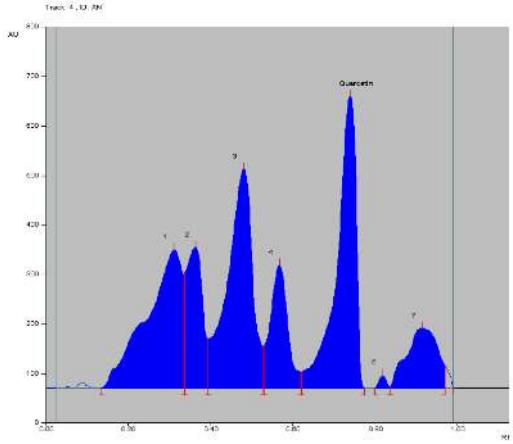


Figure 7 HPTLC chromatogram of sample containing Quercetin

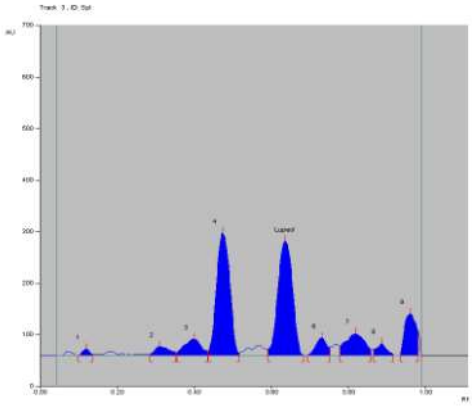


Figure 8 HPTLC Chromatogram of sample containing Lupeol

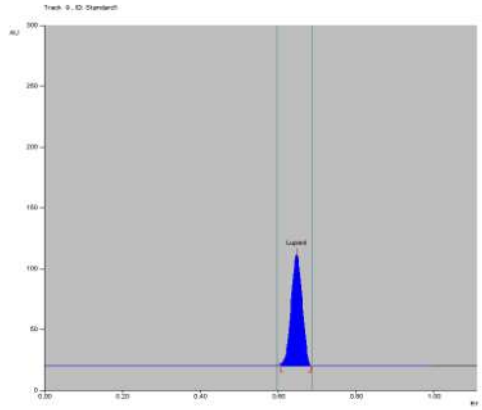


Figure 9 HPTLC Chromatogram of Lupeol

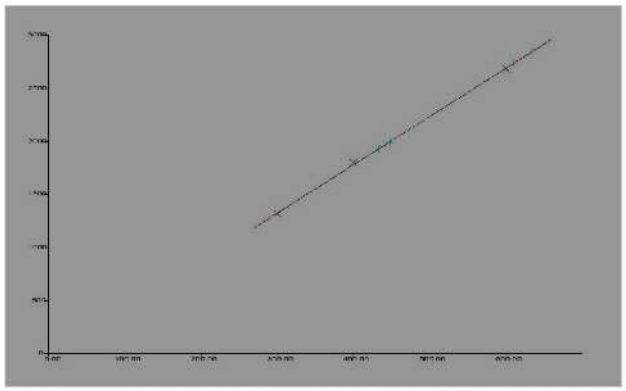


Figure 10: Calibration plot obtained by chromatography of marker compound Gallic acid. Regression via Area regression mode = Linear

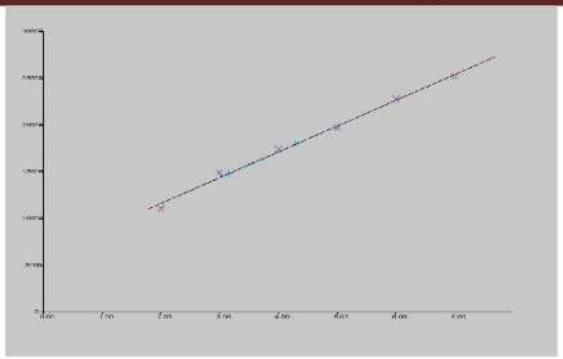


Figure 11: Calibration plot obtained by chromatography of marker compound Quercetin. Regression via Area regression mode = Linear

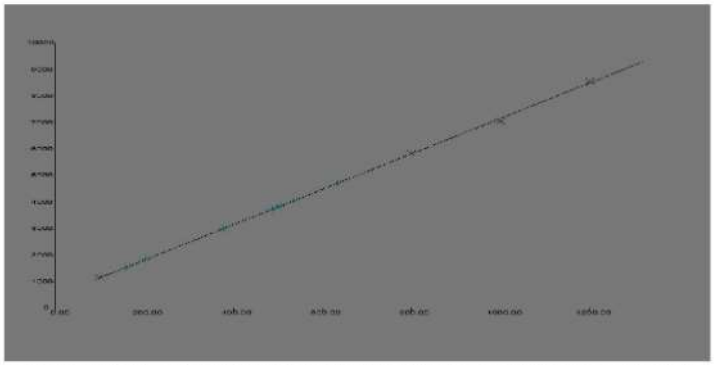


Figure 12: Calibration plot obtained by chromatography of marker compound Lupeol. Regression via Area regression mode = Linear

RESULT AND DISCUSSION

Toluene: Ethyl acetate: Formic acid (6:4:0.8 v/v/v) as mobile phase gave the best resolution of gallic acid and quercetin (R_f 0.22, 0.36) respectively. Toluene: Ethyl acetate (7:3v/v) as mobile phase for lupeol with R_f values 0.70 from the other components of the methanolic extract of *A.leucophloea*. The identity of band of gallic acid, quercetin and lupeol in plant matrix was confirmed by overlay in UV absorption spectra with those of the standards gallic acid and quercetin, while identity of bands of lupeol in plant matrix was confirmed by overlay in visible spectra with those of the standard lupeol using CAMAG TLC scanner 3. The purity of bands of gallic acid, quercetin and lupeol in plant extract was confirmed by overlaying the absorption spectra at the start, middle and end position of the band (Fig 4-9).The linearity ranges for gallic acid (100 to 600ng/spot), quercetin (2000ng to 7000ng/spot) and lupeol (100 to 1200ng/spot) with correlation coefficient (r values) of 0.99968, 0.99708, 0.99971 respectively (Fig 10-12). The amount of gallic acid, quercetin and lupeol was 124.31, 580.4, 24.89µg/ml respectively.

CONCLUSION

In conclusion, an HPTLC method has been developed with some modifications and it can be used for the quantitative determination of gallic acid, quercetin and lupeol in *A.leucophloea* flower; its main advantages are its simplicity, accuracy, and selectivity. This method can also be used for the estimation of these compounds in other herbal preparations and may be useful for standardization purposes.

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Source of support: Nil, Conflict of interest: None Declared

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