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Research Article

DETERMINATION OF LUPEOL, B-SITOSTEROL AND QUERCETIN FROM ETHYL ACETATE EXTRACT OF *RHIZOPHORA MUCRONATA* BARK BY HPTLC TECHNIQUE

ROHINI RM*, AMIT KUMAR DAS

Department of Pharmaceutical Chemistry, Krupanidhi College of Pharmacy, Bangalore, India.
E-mail: rm_rohini@rediffmail.com

ABSTRACT

Rhizophora mucronata bark is tropical plant growing in saline water and maintains the ecosystem of the environment. In India the plant was very much used in leather industry because of its rich tannin content. Quantitative determination of lupeol , β–sitosterol, and quercetin was done by HPTLC using toluene, ethyl acetate and formic acid in different ratio. The detection and quantification was performed at 296 nm, 300 nm & 320 nm respectively. The correlation coefficient obtained for the linearity was 0.98526 for β–sitosterol, 0.98966 for lupeol, and 0.99526 for quercetin. The amount of lupeol, β–sitosterol, and quercetin was found to be 3.34 µg/ mg, 3.25 µg/ mg and 2.352 µg/ mg of ethyl acetate extract of *R mucronata* bark.

Key words: *R mucronata* , quercetin , lupeol, sitosterol , HPTLC.

INTRODUCTION

Rhizophora mucronata a tropical plant commonly known as mangrove helps to maintain marine life and balances the ecosystem. Traditionally the bark of this plant has been used to treat heamaturia , diabetes[1] , diarrhea[2] and inflammation[3]. The plant was used in leather industry because of its rich tannin content. The polysaccharides have been reported for anti-HIV activity[4]. Phytochemical analysis of the bark is found to contain terpenoids , sterols, saponins , flavanoids and phenol acid[2] . The present study includes to identify and quantify marker compounds (lupeol, sitosterol, quercetin) from HPTLC .

MATERIALS AND METHOD

Materials

Rhizophora mucronata bark *(RMB*) was collected from Kundapur of Mangalore district, India.Authentication of the bark was done from Bangalore University. Marker compounds lupeol, β sitosterol, and quercetin were obtained from NBRI, Lucknow India. All the solvents used in the experiment were of analytical grade. Linomat V Automatic sample Spotter; CAMAG (Switzerland). Syringe: 100 µl Hamilton;TLC Chamber: glass twin trough chamber (20 x10 x 4 cm); Densitometer; TLC scanner 3 linked to Wincats software; Experimental condition: temperature 25 ± 2 ^{0}C, relative humidity 40 %. HPTLC plates: 20 x10 cm, 0.2 mm thickness pre-coated with silica gel F_{254} (E.Merck).Stationary phase**:** Pre-coated TLC plates of silica gel 60 F_{254} (E.Merck) of uniform thickness (0.2 mm).

HPTLC determination of quercetin , lupeol, and β- sitosterol

The dried *RMB* powder was extracted from ethyl acetate by soxhlet extraction. The ethyl acetate extract was used to quantify the marker compounds.

Preparation of the standard solutions

10 mg of respective standards (marker compounds) lupeol, β sitosterol and quercetin were dissolved in 10 ml of methanol separately, to obtain 1mg/ml concentration.

Preparation of Test solution

10 mg of the ethyl acetate extract was dissolved in 1 ml of methanol.

Procedure*:* Various solvent systems were used to get appropriate combination for the separations of desired components from the extract, Toluene: ethyl acetate 8:2(lupeol and β -sitosterol), Toluene: Ethyl acetate: formic acid: 7:3:1(quercetin), were found appropriate as developing solvents for the above standard compounds. The plates were developed in their respective mobile phase in CAMAG twin trough chambers. The chambers were saturated with the respective mobile phases for a period of 30 minutes before the development. 10 µl each of the test solution were applied on a pre-coated silica gel 60 F_{254} TLC plate of uniform thickness of 0.2 mm. The plates were allowed to develop in the solvent system to a distance of 8.5 cm. The plates were observed under UV light at 254 nm and 366 nm or the plates were sprayed with anisaldehyde-sulphuric acid reagent and heated at 110ºC for 10 minutes to visualize the spots. The R_f values of the resolved bands was recorded.

Preparation of calibration curve

10 µl of the standard solutions of the respective marker compounds were applied on a pre-coated silica gel 60 F_{254} TLC plates. The plates were developed in the respective solvent system in twin trough chamber to a height of 8.5 cms. Evaluations of the separated bands were visualized at λ 254 nm and 366 nm in standards and test samples and were densitometricaly scanned at λ 325 nm 300 nm and 296 nm respectively. The peak area was recorded and the calibration curve was generated from peak area Vs concentration of standards.

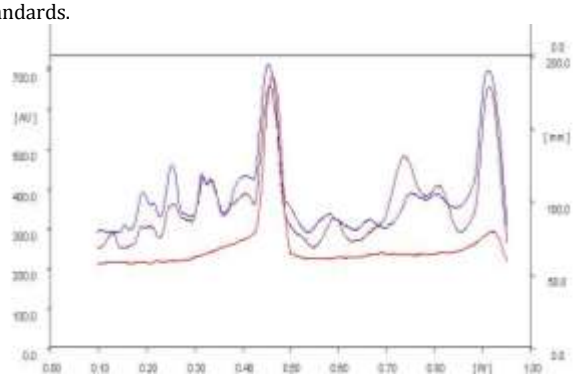

Figure -1a

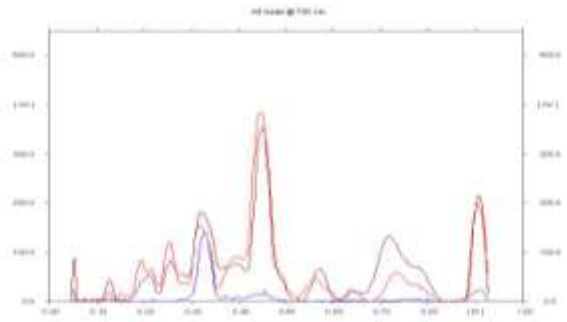

Figure -1b

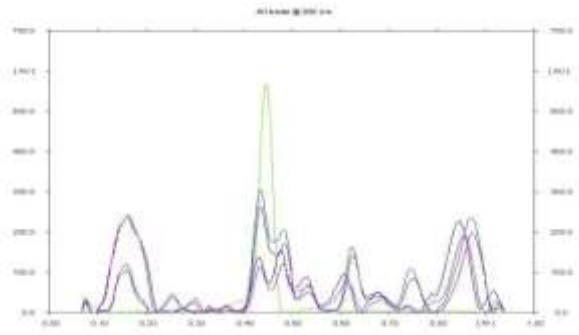

Figure -1c

Figure -1a, 1b, 1c: Densitometer Scans of Standard Lupeol B-Sitosterol, Quercetin And Ethyl Acetate Extract of *RMB*

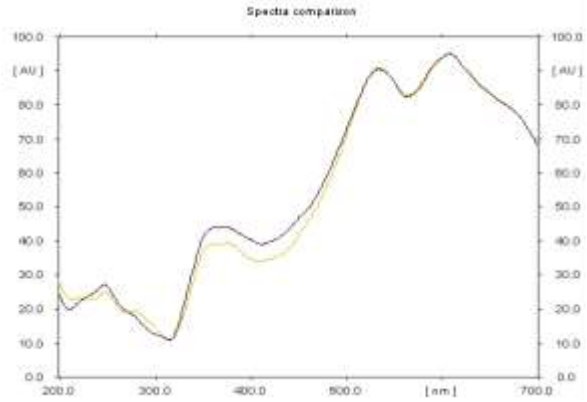

Figure -2a

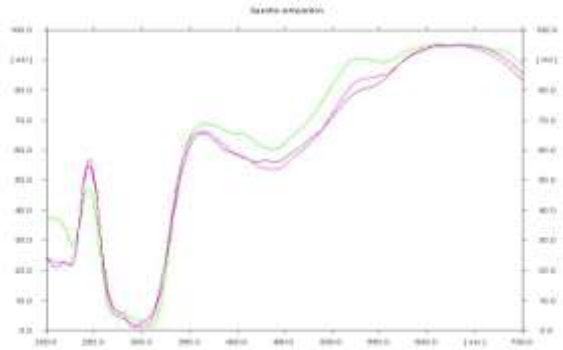

Figure -2b

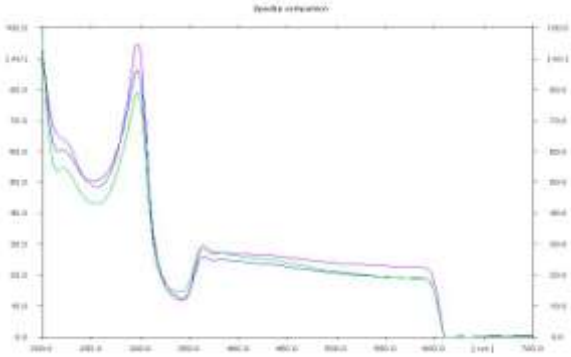

Figure -2c

Figure -2a, 2b, 2c: Absorption Spectra of Lupeol, Sitosterol and Quercetin in Standard Solution and Ethyl Acetate extract of R Mucronata Bark At 320 nm , 300 nm AND 296 nm

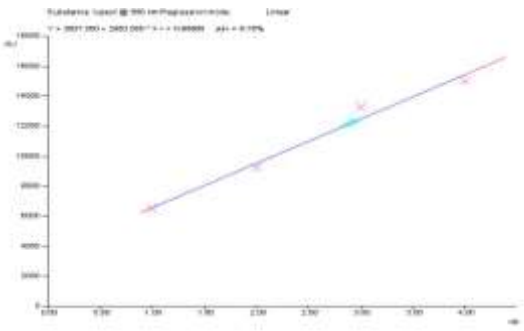

Figure -3a

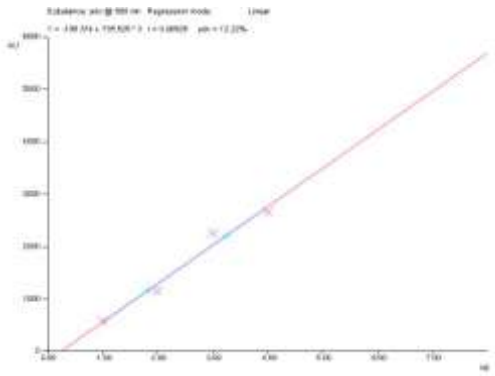

Figure -3b

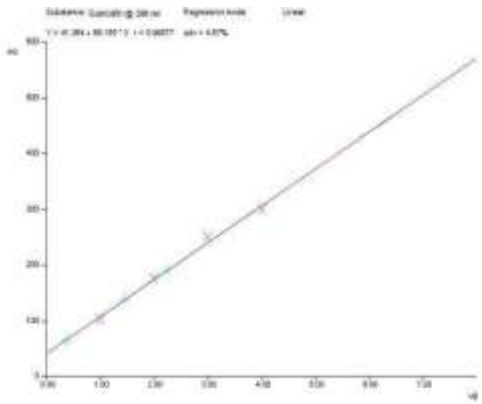

Figure -3c

FIigure -3a, 3b, 3c: Calibration Curve for Standard Lupeol, Sitosterol and Quercetin Solution

Table -1: Regression Coefficient of the Tested Compounds in Ethyl Acetate Extract of R *Mucronata* Bark

Identified marker compound	Y	r	Std dv (%)
Sitosterol	186.374+ 735.525	0.98526	12.22
Lupeol	360.050 + 2953.890	0.98966	6.16
Quercetin	41.384+ 66.155	0.99526.	4.67

$Y = a_0 + a_1X$, r = correlation coefficient, Std dv = standard déviation

RESULTS

HPTLC analysis of lupeol β -sitosterol and quercetin in ethyl acetate extract of *R mucronata* bark

The densitogram for the standard, lupeol , β -sitosterol and quercetin and tested ethyl acetate extract in duplicates are shown in FIG:1a,1b and 1c, a retention factor of 0.45 was observed. Comparison of the absorption spectra of lupeol , β-sitosterol and quercetin in the standard solution and ethyl acetate extract of *R mucronata* matched at λ 320 nm, 300nm and 296 nm are depicted in FIG: 2a ,2b, 2c respectively. Calibration curve obtained at concentration 1.0 µg/ml, 2.0 µg /ml, 3.0 µg /ml, 4.0 µg /ml and 5.0 µg /ml for the standard Lupeol and β- sitosterol and quercetin solutions are shown in FIG: 3a ,3b and 3c.The regression analysis of the linearity of the graph is represented in table 1 . Amount of lupeol and β -sitosterol present in 10µl ethyl acetate extract was calculated from the calibration curve.

DISCUSSION

TLC densitometry methods were developed using HPTLC for the identification and quantification of three marker compounds for the ethyl acetate extract *RMB*. Solvent systems were optimized to achieve best resolution of the marker compounds from the other compounds of the sample extracts. Of the various solvents tried hexane: ethyl acetate (8:2) gave best resolution for lupeol (Rf = 0.45) and β -sitosterol (Rf = 0.47) and solvent system Toluene: Ethyl acetate: formic acid (7:3:1) gave good resolution for quercetin (Rf =0.42)(fig 1a,1b,1c). The identity and purity of the bands in the sample extracts were confirmed by comparing the Rf values and by overlaying their UV absorption spectra with their respective standard. Using CAMAG TLC scanner 3 (figure 2a, 2b & 2c) in the concentration 1 µg/ml to 6 µg/ml obtained for marker compounds the linear relationship between the concentration of the standard solutions and peak response was expressed as $Y = a_0 + a_1X$ type, where Y is the response and X is the amount of the marker compounds identified from the extract of *RMB* , a_0 is the intercept of the plot on the y-axis, a_1 is its slope.From HPTLC data Quantification of lupeol, sitosterol, quercetin in the ethyl acetate extract were established from the calibration curve of test solution contains and found Lupeol 3.034 µg/mg, β- sitosterol 3.252 µg/mg and, 2.352 µg/mg Quercetin to be present in ethyl acetate extract of *R mucronta* bark .

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