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Research Article

Determination of Quercetin by HPTLC Method in *SesbaniaSesban* (L.) Merr. Stem Extract

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ABSTRACT

*Sesbaniasesban*has traditionally been used as astringent, anti-inflammatory, carminative purgative, demulcent and anthelmintic. Phytochemical analysis of the plant showed thepresence of alkaloids, carbohydrates, proteins, phytosterol, phenol, flavonoidsetc.In the present study, an attempt was made to quantify the flavonoid quercetin in the stem extract. TLC was done to confirm the presence of quercetin and HPTLC method has been developed for quantification of quercetin in the methanol stem extract. TLC silica gel 60 F 254 plate was used as stationary phase and the solvent system toluene: ethyl acetate: formic acid: methanol (5.5:3:1:0.5) as the mobile phase. Quantitative analysis was carried out in the absorbance at 386 nm. A good linear relationship 0.99926 was obtained between the concentration ranges of 100-600 ng.

Keywords: *Sesbaniasesban*,anti-inflammatory,quercetin, TLC, HPTLC.

INTRODUCTION

The flavonoids, which occur both in the free stateand as glycosides, are the largest group of naturallyoccurring phenols. They are formed from threeacetate units and phenylpropane units. They arewidely distributed in nature but are more common inyoung tissues, where they occur in cell sap. Flavonoids have been referred to as nature's biological compound because of their inherent ability to modify the reaction taking place in the body due to allergies, virus and carcinogens (Aiyelaagbe*et al.*, 2009). They havebeen used extensively as chemotaxonomic markersand are abundant in the Polygonaceae, Rutaceae,Leguminosae, Umbellifarae, and Compositae. Whilemost are *O*-glycosides, a considerable number of C-glycosidesare also known. Many flavonoids-containing plants arediuretics or antispasmodics and some flavonoids have antitumor, antifungal and antibacterial propertiesas well as antihepatotoxic activity.

The flavonolquercetin (3, 3', 4', 5, 7-pentahydroxyflavone)a phytoalexin, is one of themost potent biomedical agents known. Several typesof diseases are inhibited by this biocompound such ascataract, coronary heart disease, diabetes and cancer, especially prostate cancer(Andrade-Filho*et al.*, 2009).

Quantitative estimation of these compounds is important for current research and a variety of methods are required for this. TLC and HPTLC are the methods primarily used for separation, qualitative identification and semi-quantitative visual analysis of the samples (Avijeet Jain*et al.*, 2009).High Performance thin layerchromatography is an important tool that can beused qualitatively as well as quantitatively forchecking the purity and identity of crude drug andalso for quality control of finished product. (GunoSindhuChakraborthy*et al.*, 2010).

Sesbaniasesban was one of the non-conventional plants, not studied for biologically active compound and was not considered as a crop of medicinal importance. Traditionally the plant is used in the treatment of inflammatory rheumatic conditions, diarrhea, in excessive menstrual flow, to reduce enlargement of spleen and in skin diseases (Nirmal*et al.*, 2012).Various phytochemical studies of crude leaves and flowers of this plant showed the presence of sterol, saponin and flavonoids. These chemical constituents are well known for their potential health benefits and have been reported to possess valuable biological

activities such as astringent, anti-inflammatory, antimicrobial, antifertility, demulcent and purgative etc. (Sheikh Sajid*et al.*, 2012).

Methanol stem extract of *Sesbaniasesban*was subjected to thin layer chromatography and high performance thin-layer chromatography, to find out the probable number of compounds present in them. Consequently, the present study was focused on the quantitative estimation of the flavonoid quercetin by high performance thin-layer chromatography (HPTLC) in the herbal species *Sesbaniasesban*.

MATERIALS AND METHODS

Plant materials

The plant parts (leaf, stem and root) of *S.sesban* were collected and the authenticity of the plant was confirmed by the Botanical Survey of India, Coimbatore, India.

Preparation of the extract

The plants were cleaned, washed, shade dried and powdered for the phytochemical study. The parts used were leaf, stem and root. The solvents used were hexane, chloroform, methanol, ethanol and water. The extracts obtained through the cold percolation method were subjected to preliminary phytochemical qualitative screening for the presence or absence of various primary or secondary metabolites. Based on the results obtained in the qualitative phytochemical analysis, the methanol extract of the stem was taken for chromatographic analysis.

Preparation of the Standard

1mg/ml of the standard,quercertin() was prepared with methanol.From this 50µl was diluted with 950µl of methanol andhence the concentration of the standard was 50 µg/ml.

Preparation of the Sample

10 mg of the sample was diluted with 1 ml of methanol. Thus, the concentration of the sample was 10mg/ml.

TLC study

Thin layer chromatography was conducted to study the number of compounds present in the extract. The adsorbent used for thin layer chromatography was silica gel 60 F 254. The pre-coated TLC plate (Merck, Germany) was heated in an oven for activation. 1ml of the standard flavonoid quercetinand the methanol stem extract were applied dried and then kept in the developing tank. The chamber was saturated with the solvents for 20 min at room temperature.Several solvent systems were tried to identify a suitable developing solvent system for the separation of compounds.After the development of the plate, it was air-dried then the numbers of spots were noted and Rf values were calculated.

HPTLC Analysis

Instrument: CAMAG Automatic TLC Sampler 4 (ATS4) "ATS4_140608" S/N 140608 (1.02.13) with win CATS software.
Stationary phase: TLC plates silica gel 60 F 254 pre coated layer (20 cm X 10 cm), thickness 0.2 mm.,
No. of tracks: 16, band length: 8 mm.
Mobile phase: Toluene: Ethyl acetate: Formic acid: Methanol (5.5:3:1:0.5)
Standard: Brown powder
Sample : Brown powder
Solubility: Methanol
Standard concentration: 50µg/ml
Standard Injection volumes (µl): 1, 2, 4,6,8,10,12,14,16,18
Sample concentration: Stem - 10 mg/ml
Sample application volumes (µl): 1, 2,4,8,12,16
Development chamber: Twin trough chamber (20 X 10)
Development mode: Ascending mode
Distance run: 75 mm
Scanning wavelength: 386 nm
Lamp: D2
Slit dimensions 4.00 x 0.30 mm, Micro
Measurement mode: absorbance.

Preparation of the plates

The plates used for HPTLC was silica gel 60 F 254 (E.MERCK KGaA).50 µg/ml of the standard was applied in the form of bands usingLINOMAT IV applicator. The volumes appliedwere 1, 2, 4, 6, 8, 10, 12, 14, 16 and 18 µl.The sample concentration was 10mg/ml and the different volumes were 1, 2, 4, 8, 12 and 16 µl. The mobile phase used was toluene: ethyl acetate: formic acid: methanol (5.5:3:1:0.5).The chromatograph was developed for15 minutes, dried at room temperature and scanned at 386 nm.Average peak area of the standard was calculated. The calibration curve of the standard drug concentration (X-axis) over the average peak height / area (Y-axis) was prepared to get a regression equation by Win Cats software.

Estimation of quercetinin methanol stem extract of *Sesbaniasesban*

The mean peak height / area of the sample were calculated and the content of quercetin was quantified using the regression equation obtained from the standard curve.

Limits of Detection and Limit of Quantification

The limit of detection (LOD) was the lowest amount of the analyte in the sample which can be detected. The limit of quantification was the lowest amount of the analyte in the sample which can be

quantitatively determined. The signal-to-noise ratios were 3:1 and 10:1 respectively.

RESULTS AND DISCUSSION

Preliminary phytochemical analysis of methanol extract of the stem of *Sesbaniasesban*revealed the presence of alkaloids, amino acids, phenols, sterols, terpenoids, carbohydrates, flavonoids, and tannins.

The TLC procedure was optimized with a view to separate the compounds and to identify one of the phytochemical flavonoid in the extract. Initially toluene: ethyl acetate: formic acid: methanol in varying ratios was tried along with several combinations of other solvents. The developing system consists of toluene: ethyl acetate: formic acid: methanol (5.5:3:1:0.5 v/v/v/v) gave a sharp and well-defined band with Rf = 0.71 for quercetin(Table-1). This showed the presence of the bioactive compound flavonoid.The identity of the quercetin bands in sample chromatograms was confirmed by the chromatogram obtained from the sample with that obtained from the reference standard solution (Sachin U. Rakesh*et al.*, 2009).

HPTLC analysis of the methanol stem extract of *Sesbaniasesban* was carried out along with the standard flavonoid quercetin and toluene: ethyl acetate: formic acid: methanol (5.5:3:1:0.5) as the mobile phase. The number of bands obtained was three to four (Fig.1 A, B). The identity of the bands of quercetin in the methanol extract was confirmed by comparing the UV-Vis absorption spectra with those of standards using a CAMAG TLC scanner 3 (Fig.2). The standard quercetin has Rf value of 0.49 (Table-2).

A good linear relationship (r^2 = 0.99826 and 0.99926 with respective to height and peak area, respectively) was observed between the concentration ranges of 100-600 ng/ spot (Fig.3). The use of standard ensures the concentration and ratio of the test compound in the stem (Fig.4, 5). This result coincides with the study of Sachin U. Rakesh*et al.*, (2009) who finds a good correlation (r = 0.9998) between the standard and the sample of quercetin in the dried flowers of *Nymphaeastellata.*

The limit of detection and limit of quantification was found to be 100 ng and 300 ng respectively. The regression equation was found to be $Y= 29.69+0.8307^{*}X$ withrespective to height and $Y=290.5+19.31^{*}X$ withrespect to area, where Y is the peak height / areaand X is concentration of quercetin. With the help of above statistical data, the content of quercetin was determined in the methanol stem extract of *Sesbaniasesban*which was found to be 198.2 mg/100gm (Table-3).The peak purity of quercetin was assessed by comparing the spectra at peak start, peak apex and peak end positions of the spot.HPTLC fingerprinting is proved to be a liner, precise, accurate method for herbal identification and can be used further in authentication and characterization of the medicinally important plant (DuraisamyGomathi*et al.*, 2012). HPTLC technique could be considered as an accurate and precise method for the determination of flavonoid in *Clerodendrumvicosum vent* root samples (Prasanth*et al.*, 2012).

CONCLUSION

The phytochemical studies showed the presence of most of the biologically active compounds in the plant.It is generally realized that for monitoring quality, HPTLC fingerprinting is ideal which involves comparison between a standard and a sample. The chromatographic studies conducted with the methanol stem extract of *Sesbaniasesban* revealed an appreciable amount of flavonoid quercetin, which confirms its medicinal value.

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Table 1: Thin layer chromatography results of methanol stem extract of *Sesbaniasesban*and the standard quercetin

Band No.	Rf (Std. flavonoid-quercetin)	Rf (methanol stem extract)	Colour of the band
1	-	0.35	Brown
2	-	0.50	Brown
3	-	0.57	Fluorescent yellow
4	0.71	0.71	Yellow
5	-	0.79	Fluorescent pink
6	-	0.85	Fluorescent pink

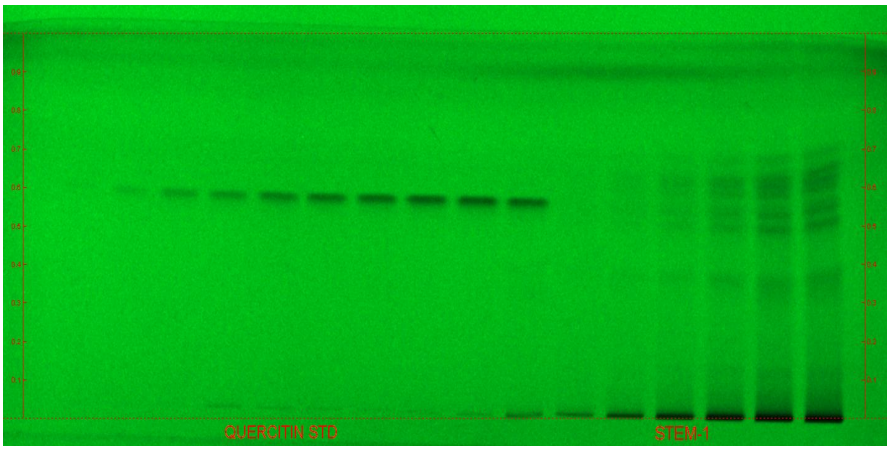
Table 2: HPTLC results of methanol stem extract of *Sesbaniasesban*and the standard quercetin

Track	Peak	Rf	Height	Area	Assigned substance
2	1	0.49	109.8	2209.9	Quercetin
4	1	0.48	279.1	6017.2	Quercetin
5	1	0.48	373.3	8197.7	Quercetin
6	1	0.48	436.5	9844.0	Quercetin
16	1	0.28	72.4	4137.9	unknown
16	2	0.45	117.0	4864.0	unknown
16	3	0.50	120.0	3133.1	Quercetin
16	4	0.58	188.2	6810.6	unknown

Table 3: HPTLC data for quantification of quercetin

Parameter	Stdquercetin
Limit of detection (LOD)	100 ng/spot
Limit of quantification (LOQ)	300 ng/ spot
Linearity	0.99926
Range (ng /spot)	100-600 ng
Reproducibility	< 2.0
SDV	2.37

A



B

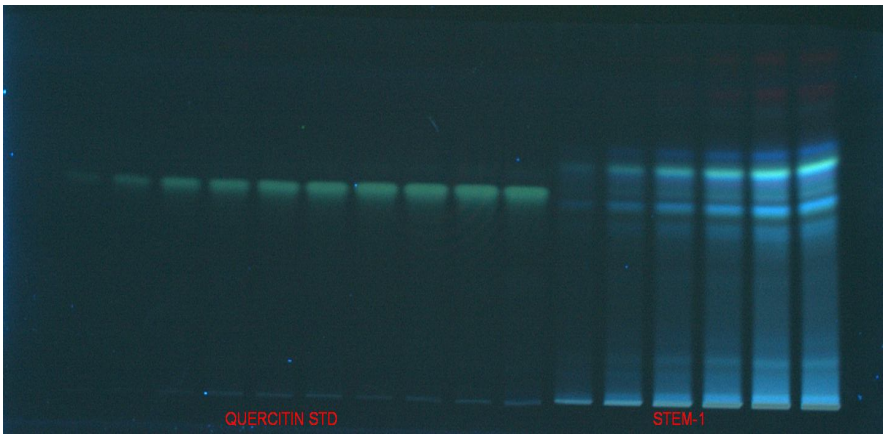


Fig. 1: HPTLC. Track 1-10 represent quercetinand Track 11-16 represent stem extract A- 254 nm, B- at 366nm

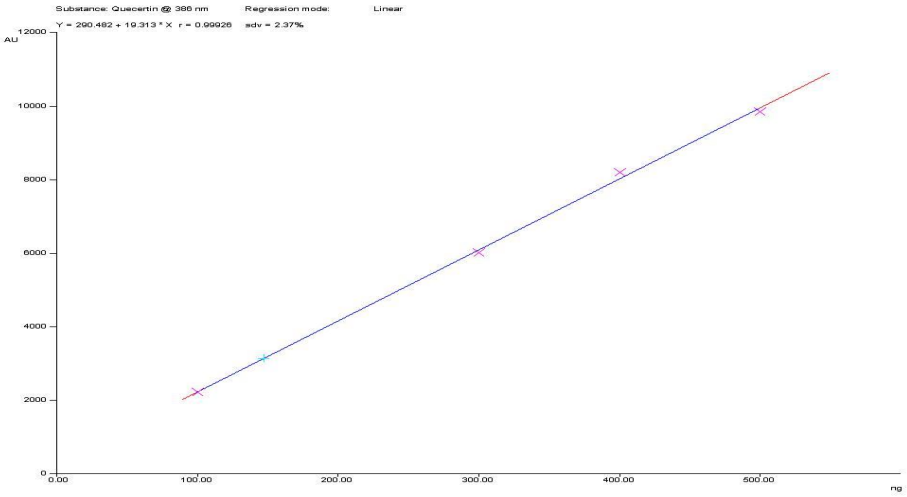


Fig. 2: Calibration curve

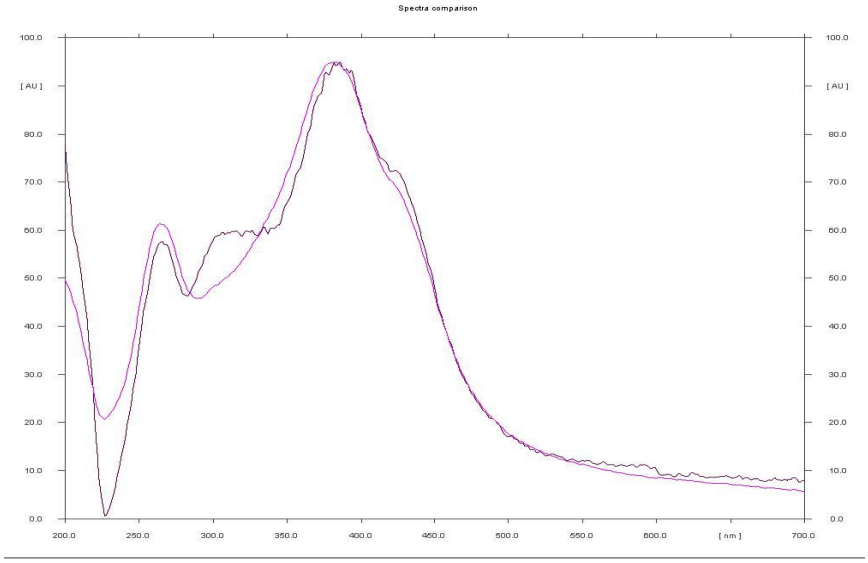


Fig. 3: Standard and Stem extract Peak

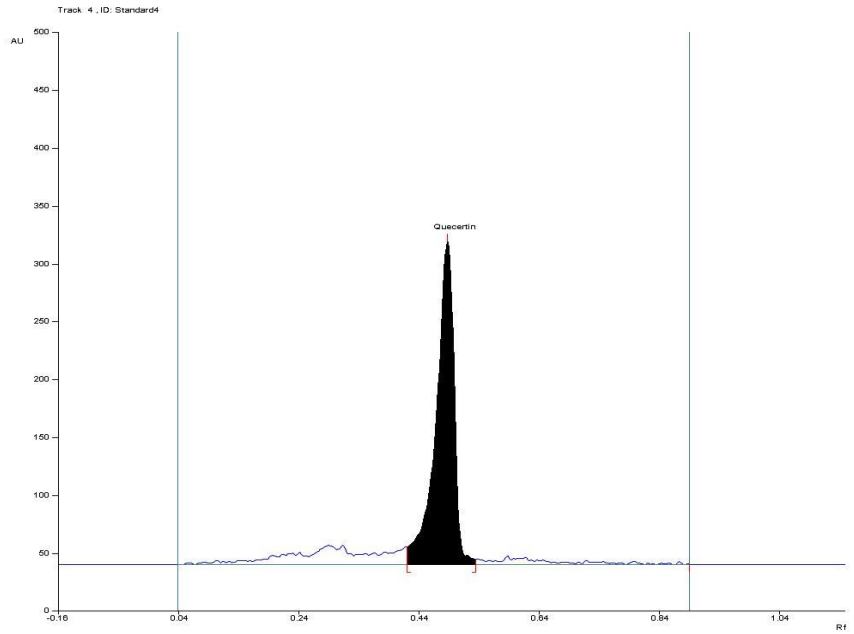


Fig. 4: Chromatogram of Standard Quercetin

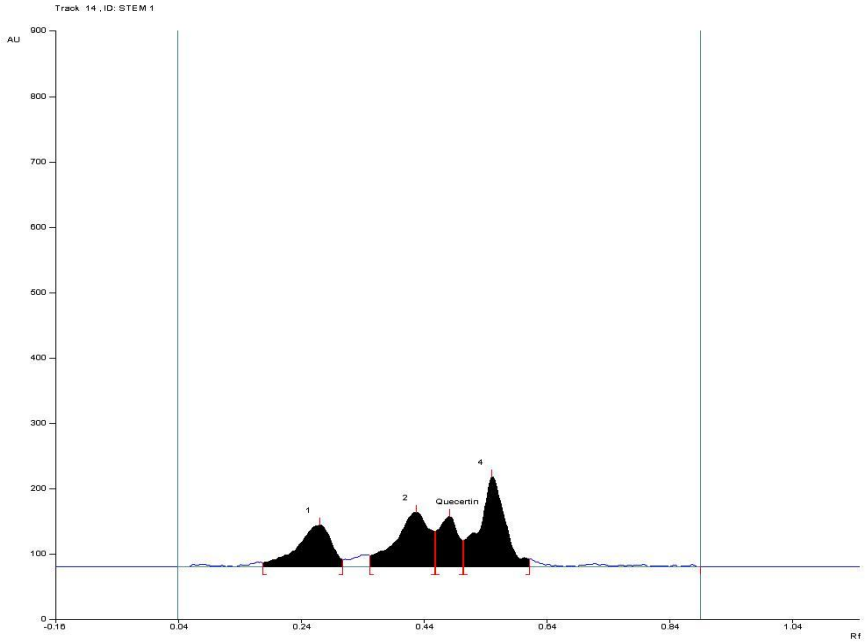


Fig. 5: Chromatogram of *Sesbaniasesban*stem extract

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