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DETERMINATION OF QUERCETIN IN METHANOLIC EXTRACT OF SESBANIA*SESBAN* (FABACEAE) BY HPTLC METHOD

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ABSTRACT
Sesbania sesban has been used traditionally for different activities such as astringent, anti-inflammatory, purgative, demulcent and anthelmintic. Phytochemical analysis of the plant confirmed the presence of alkaloids, carbohydrates, proteins, phytosterol, phenol, flavonoids etc. In the current study, an effort was made to quantify the flavonoid quercetin in the stem extract. TLC was performed to confirm the presence of quercetin and HPTLC procedure has been developed for quantification of quercetin in the methanolic stem extract. HPTLC separation was carried out on Merck TLC aluminium sheets precoated with silica gel $60F_{254}$ using Toluene: Ethyl acetate: Formic acid: Methanol (5.5: 3: 1: 0.5, v/v/v/v) as mobile phase. Quantitative analysis was carried out in the absorbance at 293 nm. A good linear relationship was obtained in the concentration range of 200-1200 ng band-^{1}.

KEYWORDS: Sesbania sesban, anti-inflammatory, quercetin, TLC, HPTLC, Validation.

INTRODUCTION
Sesbania sesban Linn (Family: Fabaceae) is well known plant widely distributed in India and other tropical countries. The World Health Organization (WHO) estimates that about 80% of people living in developing countries rely almost exclusively on traditional medicines for their primary health care needs. Different parts of the plant (Leaves, seed, and pods) are reputed for their medicinal value. The leaves of Sesbania sesban has traditionally been used as purgative, demulcent, maturant, anthelmintic and for all pains and inflammation.[1] Many researchers have been reported about the presence of carbohydrates, glycosides, proteins, amino acids, saponins, tannins, alkaloid, phenolic compounds, and flavonoids. The flavonoids, 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 phenylpropane units. They are widely distributed in nature but are more common in young 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.[2] The flavonolquercetin (3, 3', 4', 5, 7-pentahydroxyflavone) a phytoalexin, is one of the most potent biomedical agents known. Several types of diseases are inhibited by this bio compound such as cataract, coronary heart disease, diabetes and cancer, especially prostate cancer.[3]

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.[4] High Performance thin layer chromatography is an important tool that can be used qualitatively as well as quantitatively for checking the purity and identity of crude drug and also for quality control of finished product.[5]

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.[6] 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.[7]

Methanolic plant extract of *Sesbania sesban* was subjected to thin layer chromatography and high performance thin-layer chromatography to find out the likely 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 *Sesbania sesban*.

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, Koregaon Park (Pune-411001), 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 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 plant was taken for chromatographic analysis.

Selection of analytical wavelength

From the standard stock solution (1000 µg mL^{-1}), further dilution were done using methanol and scanned over the range of 200-400 nm, the λ_{max} was found to be 293 nm.

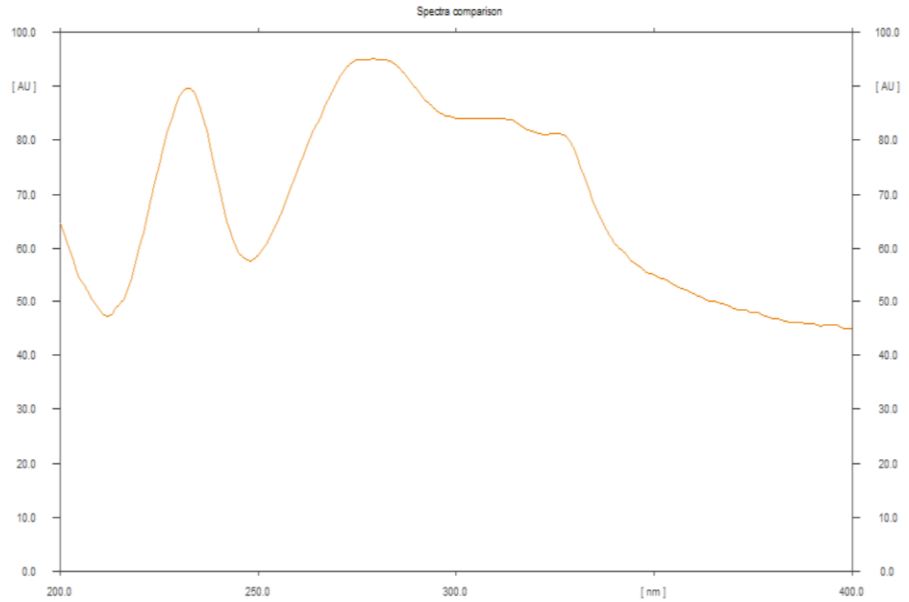

Figure 1: UV Spectrum of quercetin (100 µg mL^{-1}).

Preparation of standard solution

Standard solution of quercetin was prepared by dissolving 10 mg in 10 mL of methanol get concentration of 1000 µg mL^{-1} which was diluted appropriately to obtain final concentration 100 ng μL^{-1}

Preparation of the Sample solution

Sample solution was prepared by dissolving 10 mg in 10 mL of methanol get concentration of 1000 µg mL^{-1} which was diluted appropriately to obtain final concentration 100 ng μL^{-1}.

HPTLC analysis

The chromatographic separation was achieved on aluminum plates precoated with silica gel $60F_{254}$ in (10 cm × 10 cm with 250 µm layer thickness). Sample was applied on the plate as a band of 5 mm width using Camag 100 µL sample syringe (Hamilton, Switzerland) with Linomat 5 applicator (Camag, Switzerland). The mobile phase was composed of Toluene: Ethyl acetate: Formic acid: Methanol (5.5: 3: 1: 0.5, v/v/v/v). CAMAG twin trough glass chamber was used for linear ascending development of TLC plate under 15 min saturation condition and 10 mL mobile phase was used per run, migration distance was 80 mm. Densitometric scanning was performed using Camag TLC scanner 3 in the range of 200-400 nm, operated by win CATS software (version 1.4.3, Camag), slit dimensions were 3.00 × 0.45 mm and deuterium lamp was used as a radiation source. A solution of 100 ng μL^{-1} in methanol of quercetin was prepared and was spotted using Camag Linomat V sample applicator and detection was carried out at 293 nm.

Preparation of calibration curve of quercetin

Aliquots 2, 4, 6, 8, 10 and 12 µL from standard solution (100 ng μL^{-1}) were applied on the TLC plates with sample applicator in nitrogen stream. These deposits are done on bands of 6mm with an interval of 8 mm between them. This plate was then dried at room temperature for 10 min and then was developed on 80 mm in a mobile phase previously saturated during 15 min and composed of Toluene: Ethyl acetate: Formic acid: Methanol (5.5: 3: 1: 0.5, v/v/v/v) contained in a tank of a thin layer

chromatograph (TLC) and separated spots were analyzed densitometrically at 293 nm using Camag TLC Scanner 3 operated by the winCATS software version 1.4.2. 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 quercetin in methanolic plant extract

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

Limit of detection and limit of quantitation

The Limit of detection (LOD) and limit of quantitation (LOQ) were calculated as signal-to-noise ratio of 3:1 and 10:1.

RESULTS AND DISCUSSION

Preliminary phytochemical investigation of methanolic Sesbaniasesban extract indicated the presence of alkaloids, phenols, , terpenoids, carbohydrates, amino acids, sterols, tannins and flavonoids. In order to separate the compounds and to identify one of the phytochemical flavonoid i.e. quercetin in the extract, TLC procedure was optimized . The solvent system comprising of toluene: ethyl acetate: formic acid: methanol (5.5: 3: 1: 0.5, v/v/v/v) was able to give a dense, compact and well-defined peak for quercetin with Rf 0.45 as well as for the extract (Figure 2 and 3). This confirmed the presence of the bioactive compound flavonoid. The identification of the quercetin in sample densitogram was confirmed by compairing the Rf value with that obtained from pure marker. The identity of quercetin in the methanol extract was confirmed by comparing the UV absorption spectra with that of standard using a CAMAG TLC scanner 3.

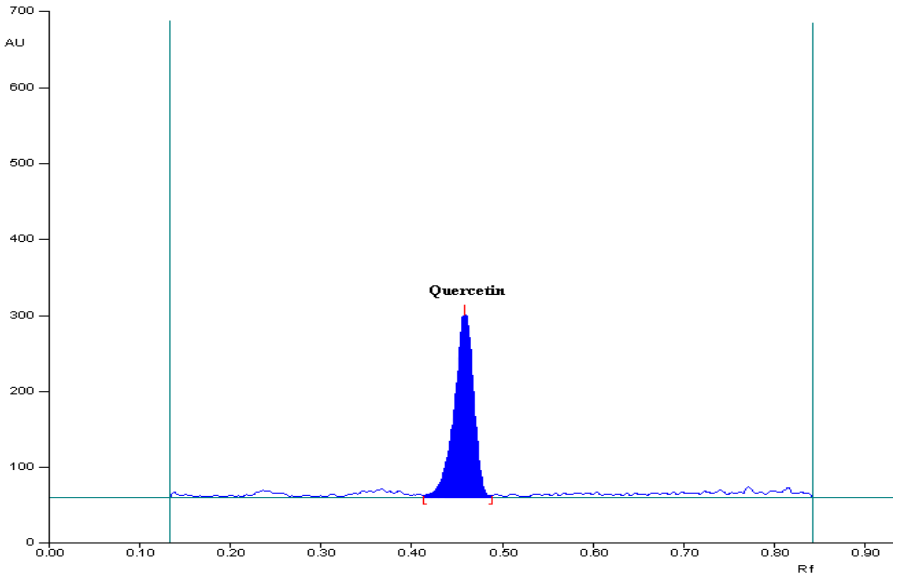

Figure 2: HPTLC densitogram showing the presence of quercetin (Rf =0.45).

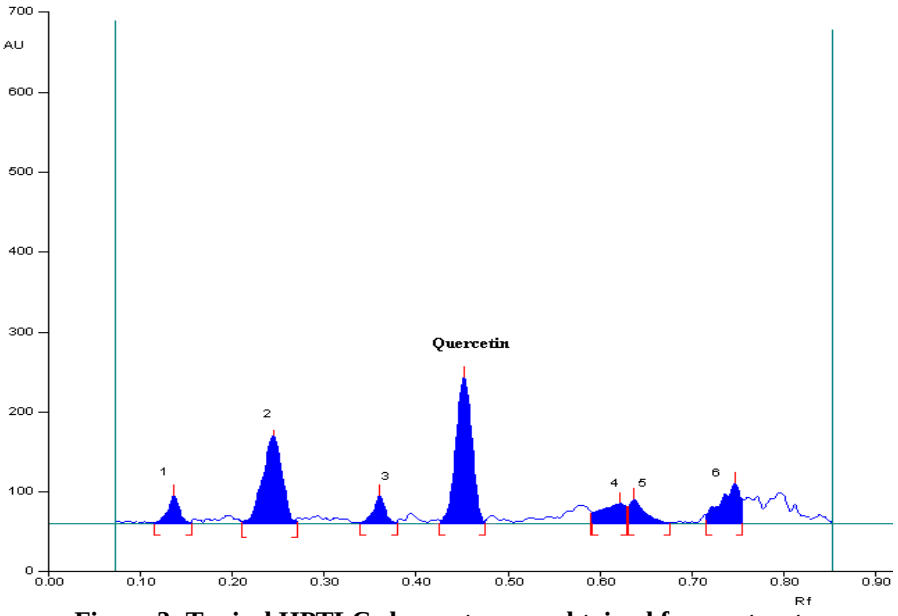

Figure 3: Typical HPTLC chromatogram obtained from extract.

Regression data obtained from calibration curve demonstrated excellent linear relationship over 200-1200 ng band^{-1} concentration range. The linear regression equation was found to be y = 6.985x + 4136 having correlation coefficient 0.993 (Figure 4 and 5).

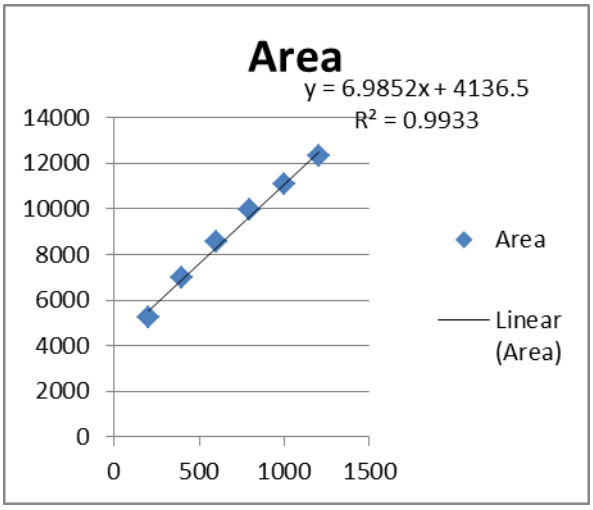

Figure 4: Calibration curve for quercetin.

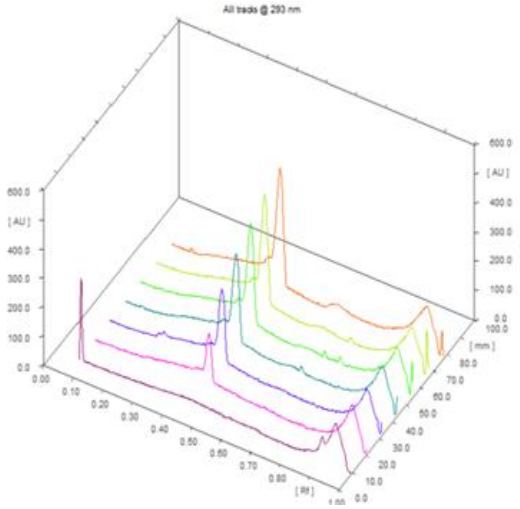

Figure 5: 3D Densitogram of quercetin.

The limit of detection and limit of quantitation was found to be 49.54 ng band^{-1} and 97.23 ng band^{-1}, respectively. With the help of above statistical data, the content of quercetin was determined in the methanolic plant extract which was found to be 102.65 %. The HPTLC data obtained for the quantification of quercetin is summarized in Table 1. HPTLC analysis carried out is proved to be a linear, precise, accurate for herbal identification and can be used further in authentication and characterization of the medicinally important plant.

Table 1: HPTLC data for quercetin.

Sr. No.	Parameter	Quercetin standard
1	Linearity	200-1200 ng band^{-1} R^2 =0.9935
2	Regression equation	y = 6.985x+ 4136
3	Correlation coefficient	0.993
5	Limit of detection	49.54 ng band^{-1}
6	Limit of quantitation	97.23 ng band^{-1}

CONCLUSION

The presence of most of the biologically active compounds in the plant was identified by phytochemical analysis. The chromatographic studies conducted with the methanolic stem extract of *Sesbaniasesban* revealed a significant amount of flavonoid quercetin, which substantiates its medicinal value.

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