Quantification of β-Sitosterol in *Sesbania grandiflora* Bark using High Performance Thin Layer Chromatography

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Abstract:

An efficient high performance thin layer chromatographic (HPTLC) method was optimized for the purpose of determining the levels of β-sitosterol (BS) in the bark of *Sesbania grandiflora*. The chromatographic separation was finished with a toluene: ethyl acetate: formic acid (50:15:5) mobile phase, and a densitometric scan was performed at 254 nm between 2 and 10 µg/mL. The developed strategy was linear, and the correlation coefficient was 0.9901. This study created and proved a straightforward HPTLC method that is precise, quick, selective, and simple for identifying and quantifying the phytochemical marker BS in *S. grandiflora* bark. The methanolic extract of *S. grandiflora* bark was determined to have a BS content of 153.09 µg/mg upon further investigation. The approach that was described above can be utilized to screen the presence of BS in herbal or pharmaceutical formulations, with potential applications in various industries.

Keywords: High Performance Thin Layer Chromatography; *Sesbania grandiflora*; Bark; Quantification; β-sitosterol.
1. Introduction

According to the Ayurvedic medical system, the plant species *Sesbania grandiflora* (L.) Poir, which is a member of the Fabaceae family and is generally known as "agathi," is considered to be an indigenous medicinal plant in India. The bark of this herbal remedy is used in the treatment of cancer by traditional healers in the state of Chhattisgarh, which is located in the territory of India. Recent research has suggested that it may possess anticancer potential in addition to activities that are anxiety-reducing, cardio-protective, hepatoprotective, anti-uroliothiatic, and antioxidant[^1^,^2^], anticonvulsant[^3^], anti-bacterial and anti-arthritis effects.[^4^,^5^]

There has been reported BS quantification using a variety of different techniques, including liquid chromatography (LC) and tandem mass spectrometry (TMS) with atmospheric pressure photoionization (APPI-LC-MS-MS), LC with evaporative light scattering detection (ELSD), online LC-GC, and gas chromatography (GC).[^6^] In recent years, the HPTLC approach has been applied to the screening of the chemical components variabilities of different plants.[^7^,^8^] HPTLC fingerprinting was used to identify active compounds such as parthenolide and costunolide diepoxide[^9^] and the presence of rosavins and salidroside as adulterants.[^10^] So far, several analytical techniques have been published for the determination of BS alone or in conjunction with other phytoconstituents/drugs in a wide range of formulations and plant extracts. However, HPTLC has only been employed in a few studies to assess quercetin and resveratrol levels in *S. grandiflora*, either alone or in association with other flavonoids (rutin and gallic acid).[^11^-^14^] But right now, there is no report on how HPTLC can be used to measure BS in *S. grandiflora*.

This study was conducted with the intention of developing an HPTLC method for the standardization of BS in methanolic extract of *S. grandiflora* bark that is both the simplest, most cost-effective, and most time-efficient approach currently available. This study will serve as a foundation upon which to build future investigations into such topics as precise identity verification, visual detection of well-separated phytoconstituents, and genuine medical applications. The main new things about this study are how easy it is to prepare samples; how fast this optimized fingerprinting method can give results; how consistent the results are; how cheap the method is; and how only one plate run is needed to evaluate plant parts. The goal of this study was to create a high-performance thin-layer chromatography (HPTLC) method for estimating BS from a single chromatogram. The BS content of the *S. grandiflora* bark extract was estimated using the established HPTLC technique.
2. Materials and methods

Reagents and Chemicals: Pure BS (Gift sample) was procured from Anchrom Lab Mumbai; all other solvents and chemicals used were of AR grade; and TLC plates made of silica gel 60F<sub>254</sub> (20 cm × 20 cm) were purchased from E. Merck (Germany).

Plant Material: The plant was recognized by the Scientist NISCR, New Delhi, India. The voucher sample (04/HSSG) as a herbarium was placed in the Institute of Pharmaceutical Sciences, Bilaspur, Chhattisgarh (C.G.), India.

Crude Extract Preparation: 5 g of coarse powder <i>S. grandiflora</i> bark were accurately weighed and extracted with methanol (4 × 50 mL) on a steam bath at reflux conditions (30 min). The extracts were filtered and concentrated as a whole and shifted to a 25 mL volumetric flask and the volume was adjusted with methanol.

Standard Solution Preparation: A stock solution of BS (10 µg/mL) was made by adding 100 mg of exactly BS in methanol and diluting the solution to 10 mL with methanol.

Phytochemical screening:

A preliminary phytochemical analysis was carried out in accordance with the standard procedures<sup>[15,16]</sup> in order to evaluate the phytochemicals like carbohydrates, phenols, terpenoids, steroids, flavonoids, alkaloids, amino acids, tannins and saponins, especially sterols, that were discovered to be present in the bark extracts.

HPTLC fingerprinting:

Equipment: CAMAG HPTLC system (Muttenz, Switzerland), Linomat V TLC sampler, TLC scanner 3 for HPTLC densitometry, REPROSTAR 3 for photo documentation analysis, and winCATS-4 CAMAG Planer Chromatography software were utilized.

HPTLC method

In order to conduct optimized HPTLC investigations, Reich and Schibli<sup>[17]</sup>; Wagner and Baldt<sup>[18]</sup> guidelines were followed. HPTLC was performed on aluminium foil-backed plates coated with 250 µm layers of silica gel 60F<sub>254</sub>. Samples and standards were applied to plates in 5 mm bands, 10 mm apart, and 15 mm from the bottom and left edge using a CAMAG (Muttenz, Switzerland) Linomat V sample applicator with a 100 µL Hamilton syringe. A nitrogen aspirator maintained a 150 µLs<sup>-1</sup> application rate. Linear ascending development of the plates to a distance of 7 cm with toluene-ethyl acetate: formic acid (50:15:5 v/v) as mobile phase was accomplished in a CAMAG 10 cm ×10 cm twin-trough glass
chambers equilibrated with mobile phase vapour. For each 8-minute development, 10 mL of mobile phase was used and the plates were air-dried. Post-chromatographic derivatization in anisaldehyde-sulphuric acid was followed by 3 minutes at 110°C. Densitometric scanning at 341 nm was done with a CAMAG TLC scanner 3 in reflectance mode and winCATS 1.1.4.0 software. A halogen tungsten lamp radiated. The slit length was 5.00 mm, width was 0.45 mm, and the scanning rate was 20 mm s⁻¹. The monochromator band width was set at 20 nm. From diffusely reflected light, chemical concentrations were determined. Peak area with linear regression was used to evaluate it. To calibrate a TLC plate, 2, 4, 6, 8, and 10 µL of stock solutions were applied in triplicate to yield 2, 4, 6, 8, and 10 µg per band. The sample was put on a plate, developed, and scanned at 254 nm for BS analysis. The analysis was repeated and triple-checked. By extrapolating from a calibration curve, the composition of BS was ascertained. [19, 20]

3. Results and Discussion

After the development of plate with mobile phase toluene: ethyl acetate: formic acid 50:16:4 (v/v), A typical HPTLC Chromatogram of BS and HPTLC chromatogram of S. grandiflora bark extract at 254 nm displayed in Fig.1(a & b) and 2 respectively. After derivatization with anisaldehyde-sulphuric acid delivered a good resolution (Blue colour band) with an Rₜ value of 0.85, shown in Fig.3. When the chamber was drenched with the mobile phase for 15 minutes at room temperature, the well-demarcated area was discovered. BS exhibited a good linear connection with peak height over the concentration range of 2 to 10 µg per spot. [21] With a regression coefficient of 0.99006, linearity was observed. The slopes of the standard curve, as shown in Fig.4, showed no significant changes. By superimposing their UV absorption spectra with those of the relevant reference standards presented in Fig.5, the identity of the band in the sample extract was affirmed. The BS content in S. grandiflora bark was found to be 153.09 µg/mg of methanolic extract.

The preliminary phytochemical study, TLC fingerprinting, and co-TLC investigations with BS of S. grandiflora extract showed the presence of a fair amount of phytosterols, and their positions were similar to those of normal BS. Amongst other methods, such as Rₜ analysis, multi-wavelength imaging, and spectrum mapping, it was successful in proving its authenticity. BS is one of the phytosterols that is created by plants at a higher frequency than any other phytosterol. It has a molecular structure that is quite similar to that of cholesterol. BS has a wide range of health benefits that have been scientifically established to be beneficial, including those for immunological dysfunction, inflammatory disorders, and rheumatoid arthritis; for hypercholesterolemia; for breast cancer; for colon cancer; and for benign prostatic hypertrophy. [22, 23] This substance has been the subject of a significant amount of research due to the numerous therapeutic applications it possesses, the various methods for quantifying it, and its role as
one of the marker ingredients that serve to guarantee the identification of the plant and verify that it is of high quality. A TLC approach that was straightforward, sensitive, particular, and reproducible was used in order to determine the quantity of BS that was present in the bark of *S. grandiflora*.

4. Conclusions

The recommended approach was observed to be basic, exact, precise, delicate, and ideal for the appraisal of β-sitosterol (BS). In addition, in comparison to HPLC approaches, this technique uses significantly less solvent; therefore, it might find application in the Ayurveda and pharmaceutical industries in the near future. A straightforward TLC method was devised for the quantification of BS in *S. grandiflora* extract in light of its extensive medicinal potential, alternative quantification approaches, and as one of the marker ingredients to assure identification and quality of this plant. Henceforth, the proposed technique can be utilized for the assessment of BS in plant extracts or formulations.

Conflict of interest statement:

We declare that we have no conflict of interest.

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References


Fig. 1a HPTLC Chromatogram of β-Sitosterol at 254 nm
Fig. 1b HPTLC chromatogram of methanolic extract of *S. Grandiflora* bark at 254 nm
Fig. 2 Three dimensional HPTLC chromatogram of β-Sitosterol and extract in all tracks.
Fig. 3 Photo document image of derivitized TLC plate for *S. grandiflora* extract and the β-Sitosterol at normal light.
Fig. 4 Standard curve of β-Sitosterol

Fig. 5 UV absorption spectra of β-Sitosterol in the methanolic extract of S. Grandiflora bark