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Evaluation of content of β-sitosterol from stem bark and flowers of *Salmalia malabarica* collected from different regions

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ABSTRACT

A simple, rapid, selective and quantitative HPTLC method has been developed for the quantitation of β-sitosterol from *Salmalia malabarica* stem bark, flower collected from different regions and its formulations. The method was carried out using the methanolic extract of stem bark, flower and formulation of *Salmalia malabarica* and was chromatographed on silica gel 60 F_{254} plates with Toluene- Ethyl Acetate- Methanol 7.0+1.0+0.5 (v/v/v) as mobile phase. Detection and Quantitation were performed by densitrometric scanning at λ= 366 nm using flourescence lamp. The plate was derivatised using 10% Methanolic sulphuric acid reagent followed by heating at 110°C for 10 minutes. The accuracy of the method was checked by conducting various validation parameters according to ICH guidelines. This method quantitates bioactive marker β-sitosterol as well as discriminates *Salmalia malabarica* stem bark from flowers which can be used as a quality control method to know the botanical identity of the plant raw material individually and in polyherbal formulation.

KEYWORDS

Salmalia malabarica;
Stem bark;
Flower;
HPTLC;
β-sitosterol;
ICH.

INTRODUCTION

Salmalia malabarica Linn syn. *Bombax ceiba* belonging to Family Bombacaceae is a medium-sized deciduous tree, mainly grows in tropical areas such as Southern China, India and Northern Australia[1]. Stem bark is used in dysentery, bleeding disorders and in the treatment of acne vulgaris, skin eruptions and boils. Flowers are diuretic, laxative, astringent and are applied to cutaneous afflictions[8]. Stem bark of *Salmalia malabarica* contains β-sitosterol, shamimicin and lupeol[8,12]. Flower of *Salmalia malabarica* contains β-D-glucoside of β-sitosterol, hentraicontane, hentriacontanaol, traces of essential oil, quercetin, kaempferol and free β-sitosterol[7]. In recent chemical research on the flower of *Salmalia malabarica*, three new compounds, bombasin, bombasin 4-O-b-glucoside and bombalin were isolated. The three known compounds were identified as dihydrodehydrodiconiferyl alcohol 4-O-b-d glucopyranoside, trans-3-(p-coumaroyl) quinic acid and neochlorogenic acid[1-5]. The presence of amino acids, lysine, arginine, alanine, glutamic acids, glycocol, leucine and the sugars like fructose, glucose, galactose, sucrose, lactose and arabinose have been reported in the flower of *Salmalia malabarica*[7]. Stem bark as well as flower of *Salmalia malabarica* contains β-sitosterol as a common constituent. β-sitosterol is reported to help in curing Hyperlipidemia, Choles-

terol absorption, Breast Cancer, immune disorders, Aging Prostrate gland and Gynecological disorders[9,10].

Several herbal industries have been using *Salmalia malabarica* stem bark and flower in various Ayurvedic, Unani and herbal formulations which are used in management of various disorders like diarrhoea, fever, chronic inflammation and female reproductive disorders[1-3]. Literature survey reveals that there hasn't been any method reported for the quantitation of β-sitosterol from Stem bark and flowers of *Salmalia malabarica* collected from different regions and from the formulations. Present investigation describes the development of HPTLC technique for the quantitative estimation of β-sitosterol from Stem bark as well as flower of *Salmalia malabarica* collected from different regions and checking the accuracy of the method by conducting various validation parameters like linearity, precision, repeatability and recovery as per ICH guidelines[11].

EXPERIMENTAL

Plant material

Stem bark and flower of *Salmalia malabarica* were collected from Lonavla, Mumbai, Panvel and Raigad was authenticated by Dr. Sunita Shailajan, Associate Professor in Botany, Herbal Research Lab, Ramnarain Ruia College. Specimens of the plant materials are deposited in Herbal Research Lab. Kalpak formulation and Shalmali pimple pack were procured from Khadi Gram Udyog.

Chemicals

The solvents Toluene, Ethyl Acetate, Methanol, Concentrated sulphuric acid were of analytical grade and were purchased from Qualigens Fine Chemicals, Mumbai, India, were used for the analysis. Standard β-sitosterol (98% purity) was procured from Sigma Aldrich Chemie (Steinheim, Germany). Figure 1

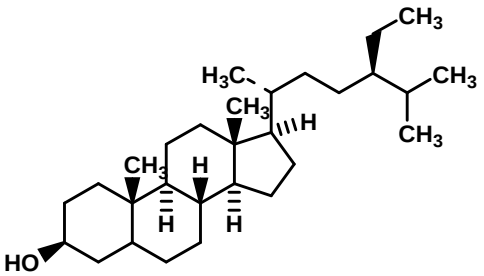

Figure 1 : Structure of β-sitosterol

Standard preparation

A stock solution of β-sitosterol (1000 μg mL^{-1}) was prepared by dissolving 10.0 mg of accurately weighed β-sitosterol in 10.0 mL Methanol. Aliquots of this stock solution were prepared to obtain working standards containing 5 μg mL^{-1} to 50 μg mL^{-1}.

Sample preparation

Stem bark and flower of *Salmalia malabarica* were collected, shade dried, powdered and sieved through an 80 mesh (BSS) sieve and stored in an airtight container. 1.0 gm of the dried powder was accurately weighed, placed in a stoppered tube and 10 mL of Methanol was added. The sample was vortexed for 1-2 min and kept overnight. The contents of the tube were filtered through Whatmann filter paper No. 41 (E. Merck, Mumbai, India). The clear supernatant was used for Quantitation and Validation. For marketed formulations the above procedure was followed for sample preparation.

Preparation of derivatizing reagent

Derivatizing reagent was prepared by adding 30 mL concentrated Sulphuric acid with 270 mL methanol slowly while cooling in ice. The plate was dipped in the reagent using a derivatizing chamber.

Chromatographic conditions

Chromatography was performed on HPTLC silica gel 60 F_{254} pre-coated plates. Samples (10 μL) were applied on the plates as bands of 10 mm width with the help of a Camag Linomat IV sample applicator at the distance of 15mm from the edge of the plates. The mobile phase constituted of Toluene- Ethyl Acetate-Methanol 7.0+1.0+0.5 (*v/v/v*). The plates were developed up to a distance of 85 mm in a Camag twin-trough chamber previously equilibrated with mobile phase for 30 min. The chromatographic conditions had previously been optimized to achieve the best resolution and peak shape. After development, plates were dried at room temperature, derivatised with freshly prepared 10 % Methanolic Sulphuric acid reagent in a derivatisation chamber for 20 secs and dried at room temperature. After drying, plates were heated in oven at 105°C for 10 mins before densitometric scanning. Densitometric evaluation of the plates was performed

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at 366 nm in fluorescence/reflectance mode using Mercury lamp with a Camag Scanner II in conjunction with Cats 3 version software.

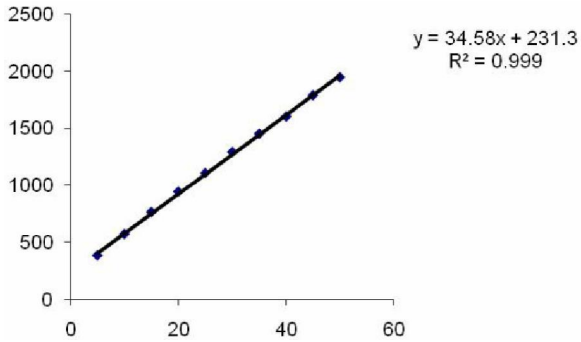
METHOD VALIDATION AND SAMPLE ANALYSIS

Linearity of detector response

Solutions containing β-sitosterol at ten different concentrations (5 to 50 μg mL^{-1}) were prepared in Methanol. Each of these solutions (10 μL) was applied on a plate, the plate was developed and the detector response for different concentrations was measured. A graph was plotted using the peak area against concentration of β-sitosterol. The plot was linear in the range 5 to 50 μg mL^{-1}. The experiment was performed three times and the mean was used for the calculations. The linearity data is given in TABLE 1. Figure 2

TABLE 1 : Method validation

Parameters	Result
Linearity range	5 to 50 μg mL^{-1}
Slope (m)	34.588
Intercept (c)	231.35
Correlation coefficient (R)	0.999
LOD	0.5 μg mL^{-1}
LOQ	1 μg mL^{-1}
System Suitability (n =5 % CV)	0.09
Instrument Precision (n=6 % CV)	0.11
Intraday (precision) (n=3 % CV)	0.06
Interday (precision) (n=3 % CV)	0.12



(y = mx + c, where, y= peak area, m= slope, x= concentration, c= intercept)

Figure 2 : Linearity graph

Assay procedure

The standard solution of β-sitosterol (20 μg mL^{-1}) and 10μL of sample solutions were spotted on a TLC silica gel 60 F_{254} pre-coated plates. The amount of β-sitosterol present in this solution was calculated by comparison of area measured for the sample to that for the standard. The assay procedure described earlier was repeated seven times. The results of assay are given in TABLE 2 & 3. Figure 3, 4 & 5 & 6.

TABLE 2 : Results of assay with *Salmalia malabarica* flower and stem bark

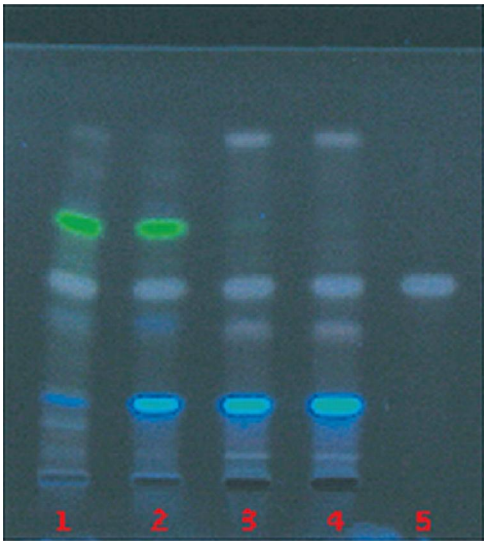
Regions	Weight of sample (mg)	Amount of β-sitosterol *Salmalia malabarica* (stem bark) mg/g	Amount of β-sitosterol *Salmalia malabarica* (flower)mg/g
Lonavala	1000	0.0686 ± 0.0978	0.2111 ± 0.0345
Panvel	1000	0.0813 ± 0.0134	0.2198 ± 0.0786
Mumbai	1000	0.1579 ± 0.0765	0.2378 ± 0.0876
Raigad	1000	0.1315 ± 0.0456	0.1805 ± 0.0567

*** Mean ± standard deviation (SD, n= 3).**

TABLE 3 : Results of assay with formulation containing *Salmalia malabarica* flower and stem bark

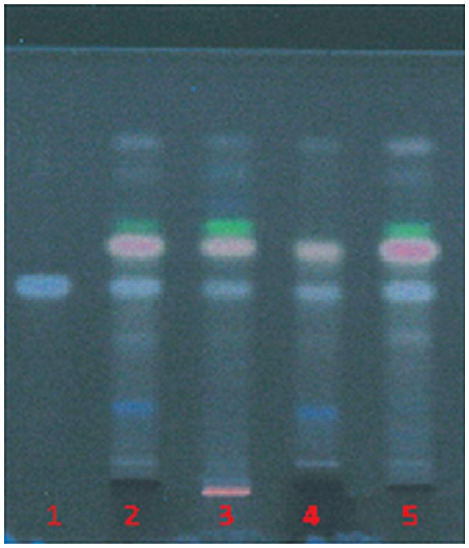
Formulations	Weight of sample in mg	Amount of β-sitosterol present in plant sample in mg/gm
Kalpak Formulation	1000	0.0119 ± 0.0675
Shalmali Pimple Pack	1000	0.0848 ± 0.0375

***Mean ± SD, n= 3**



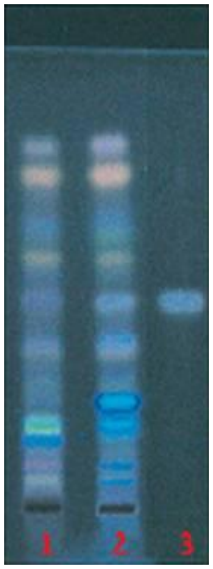
Track 1: *Salmalia malabarica* flower Raigad
Track 2: *Salmalia malabarica* flower Lonavla
Track 3: *Salmalia malabarica* flower Panvel
Track 4: *Salmalia malabarica* flower Mumbai
Track 5: β-sitosterol

Figure 3 : Chromatographic plate of flower of *Salmalia malabarica* with β-sitosterol



Track 1: β-sitosterol
Track 2: *Salmalia malabarica* stem bark Raigad
Track 3: *Salmalia malabarica* stem bark Lonavla
Track 4: *Salmalia malabarica* stem bark Panvel
Track 5: *Salmalia malabarica* stem bark Mumbai

Figure 4 : Chromatographic plate of stem bark of *Salmalia malabarica* with β-sitosterol



Track 1: *Salmalia malabarica* Kalpak formulation
Track 2: *Salmalia malabarica* Shalmali pimple pack
Track 3: β-sitosterol

Figure 5 : Chromatographic plate of formulations containing *Salmalia malabarica* flower and stem bark with β-sitosterol

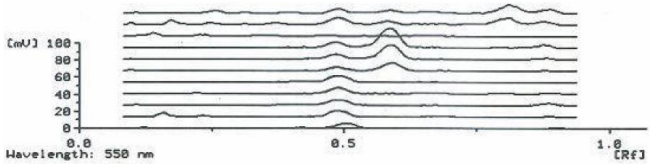


Figure 6 : Overlay of *Salmalia malabarica* flower, stem bark and formulation

Accuracy/recovery

The accuracy of the method was established by performing recovery experiments by the standard addition method. Recovery of standard β-sitosterol added to the extract of *Salmalia malabarica* stem bark and *Salmalia malabarica* flower collected from Mumbai was studied at two different levels, each being analyzed in a manner similar to that described for the assay. The β- sitosterol content and the percent recovery was calculated. The results are given in TABLE 4.

TABLE 4 : Results of accuracy /recovery analysis

Excess standard added to the analyte (%)	*Salmalia malabarica* (stem bark) Recovery (%)	*Salmalia malabarica* (flower) Recovery (%)
0	---	---
10	100.08 ± 0.0615	99.17 ± 0.5623
20	100.07 ± 0.0561	98.58 ± 0.0235
30	100.05 ± 0.0451	99.74 ± 0.0663
Mean Recovery	100.06 ± 0.1255	99.16 ± 0.0134

***Mean ± SD, (n = 6)**

RESULTS AND DISCUSSION

A good linearity was achieved in the concentration ranges of 5 to 50 μg mL^{-1} for β-sitosterol. The regression equation and correlation coefficient for the reference standard was y = 34.588x -231.35 and R^2 = 0.999 respectively (TABLE 1). The relative standard deviation for repeatability of sample application, intra-day and inter-day analysis was found to be less than 2%. The LOD with signal/noise ratio of 3:1 was found to be 0.5 μg mL^{-1} and LOQ with signal/noise ratio of 10:1 was found to be 1 μg mL^{-1} (TABLE 1). The sample of *Salmalia malabarica* stem bark and flower was spiked with 10, 20 and 30 % of the standard solution and the mixtures were analyzed by the proposed method. The experiment was conducted six times which yielded recovery of 100.06±0.01% for *Salmalia malabarica* stem bark and *Salmalia malabarica* flower 99.1±0.50% (TABLE 3). Ruggedness of the method was determined by making changes in the mobile phase composition and time for which the RSD was determined and found to be less than 2%.

The method was validated to trace the active principle β-sitosterol from the stem bark and flower of

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Salmalia malabarica. This method could be recommended for fingerprinting and quality assurance to establish the authenticity of stem bark and flower of *Salmalia malabarica* using β-sitosterol as a marker. The method could be extended for the marker-based standardization of other herbal product containing β-sitosterol as a marker compound.

ABBREVIATIONS

HPTLC : High Performance Thin Layer Chromatography
ICH : International Conference on Harmonization
CV : Co-efficient of Variation
LOD : Limit of Detection
LOQ : Limit of Quantitation

CONCLUSION

The developed HPTLC method is simple, reliable and fast to establish the quality of raw material with respect to β-sitosterol, which plays an important role in the therapeutic efficacy of the plant. Such reproducible modern techniques can make the traditional medicines more acceptable in the local and global market. Thus rationally designed, carefully standardised, synergistic traditional herbal formulations and botanical drug products with robust scientific evidence can also be alternative to modern medicine.

So far there hasn't been any reference available on quantitation of β-sitosterol from stem bark and flowers of *Salmalia malabarica* collected from different regions. The developed HPTLC method plays an important role in quantitation of β-sitosterol as well as identification of the plant raw material and formulation containing the plant material due to distinct and unique pattern of bands.

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