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HPTLC method for simultaneous quantification of two biologically active compounds in the bark powder of *Artocarpus lakoocha* Roxb. Plant

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ABSTRACT

Artocarpus lakoocha Roxb. belongs to family of Moraceae. It is commonly called as Monkey jack. *Artocarpus lakoocha* Roxb. is a perennial tree found on west coast from Kokan southwards to Kerala and Tamil Nadu. In present study, a HPTLC method has been developed and validated for simultaneous determination of β-sitosterol and Lupeol in the bark powder of *Artocarpus lakoocha* Roxb. plant. Chromatography was performed on silica gel 60 F_{254} HPTLC plate, with toluene: methanol, 8:1 (*v/v*), as mobile phase. After development, plates were treated with Lieberman-Burchard Reagent, detection and quantification were performed by densitometry at 366 nm in fluorescence mode. The method was validated in terms of its linearity, limits of detection and quantification and precision following standard protocols. β- Sitosterol and Lupeol was found to be linear in the range of 10 μg mL^{-1} – 40μg mL^{-1} for both. Average content of β- Sitosterol and Lupeol in Bark powder of *Artocarpus lakoocha* Roxb. was found to be 0.106 mg and 0.242 mg respectively. The method was found to be simple, faster, precise, sensitive, and accurate and can be used for routine quality control of herbal raw materials and for the quantification of these compounds in plant materials.

KEYWORDS

HPTLC;
Artocarpus lakoocha Roxb.;
β- Sitosterol;
Lupeol.

INTRODUCTION

Herbal medicine continues to be the first preference of about 75 - 80% of the world population, mainly in the developing countries, for primary healthcare[1]. This is primarily because of the general belief that herbal drugs have no side effects besides being cheap and locally available[2]. According to the World Health Organization (WHO), the use of herbal remedies throughout the world exceeds that of the conventional drugs by two to three times[3]. The use of plants for healing purposes predates human history and forms the origin of much modern medicine. In India, the herbal drug market is about $ one billion and the export of plant based crude drugs is around $ 80 million[1]. However, one of the impediments in the acceptance of the Ayurvedic or Herbal formulations is the lack of standard quality control profiles[4]. It seems to be necessary to determine the phytochemical constituents of herbal products in order to ensure the reliability and repeatability of pharmacological and clinical research to understand their bioactivities and possible side effects of active compounds and to enhance product quality control[5].

Artocarpus lakoocha Roxb. belongs to family of

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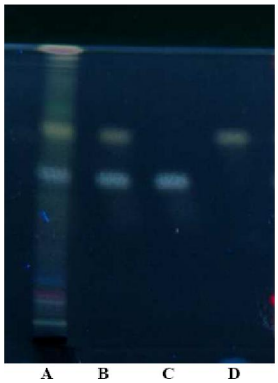

Figure 1 : Chromatographic plate of Bark powder of *Artocarpus lakoocha* Roxb. With β-sitosterol and Lupeol: A: Methanolic extract of bark powder, B: Mixture of β–Sitosterol and Lupeol, C: Standard β–Sitosterol, D: Standard Lupeol

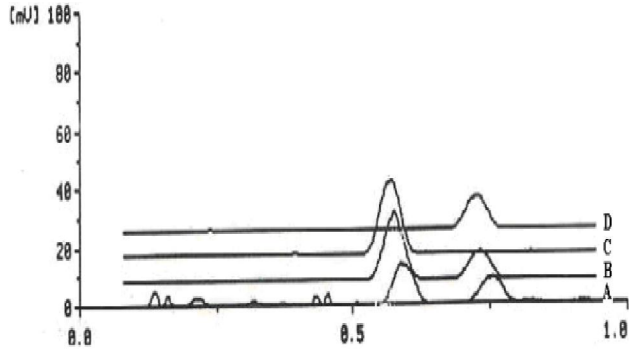

Figure 2 : Chromatographic overlay of Bark powder of *Artocarpus lakoocha* Roxb. With β- sitosterol and Lupeol: Track 1: Whole plant powder of Bark powder of *Artocarpus lakoocha* Roxb., Track 2: β- sitosterol and Lupeol, Track 3: β-sitosterol, Track 4: Lupeol

Moraceae. It is commonly called as Monkey jack. *Artocarpus lakoocha* Roxb. is a perennial tree found on west coast from Kokan southwards to Kerala and Tamil Nadu[6,7]. It has many pharmacological activities such as antiviral, anticancer and anti-HIV[8]. The literature reveals that there is no High Perform Thin Layer Chromatographic method available for quantitation of B-sitosterol & Lupeol from bark of *Artocarpus lakoocha* Roxb. In this paper development and validation of a HPTLC method for the quantitative analysis β-sitosterol and Lupeol is reported. The proposed method has been validated as per ICH guidelines[9-12].

MATERIALS AND METHODS

Plant material

The *Artocarpus lakoocha* Roxb. plant was collected from Sadashivgad (Karwar) District of Karnataka, India during the flowering season. The plant was authenticated by the Botanical survey of India (Pune) Auth 08-137. After collection, the collected bark material of the plant was dried at room temperature in shade and then ground in a mixer to a fine powder, which was passed through an ASTM BSS 85 mesh size and stored in an airtight container at room temperature. The containers were labeled with details such as date of collection, weight of powder, time of collection and the season of collection.

Reagent and standards

Toluene and Methanol used were of analytical reagent grade from S D fine chemicals, Mumbai, India. And Reference standard β-Sitosterol and Lupeol (purity 98%) were procured from Sigma Aldrich (Germany).

TLC conditions

Spotting device: Linomat IV sample applicator; CAMAG (Muttenz, Switzerland); Syringe: 100µl Hamilton (Switzerland); TLC Chamber: Glass twin trough chamber; CAMAG; Densitometer: TLC Scanner in conjunction with CATS software; CAMAG; HPTLC plates: 20x 10 cm^2, 0.2mm thickness precoated with silica gel 60 F_{254}: E. Merck and Solvent System: Toluene: Methanol, 8.0:1.0 (*v/v*).

Sample preparation

500 mg of Bark powder of *Artocarpus lakoocha* Roxb. plant was extracted with 10 mL of methanol. The mixture was vortexed for 1 min and it was kept overnight for extraction. It was filtered through whatmann filter paper No. 41 and filterate obtained was subjected to HPTLC for simultaneous quantitation of β- sitosterol and Lupeol.

Preparation of standard solution

The stock solutions of β-sitosterol and Lupeol (1000µg mL^{-1}) each were prepared separately in methanol. The stock solution was quantitatively transferred to give a solution of appropriate concentration range of β-sitosterol and Lupeol(10µg mL^{-1}-40µg mL^{-1}). Standard solutions were prepared by dilution of the stock solution.

Calibration curves

Standard solution (10 µl) of β-sitosterol and Lupeol (10 µg mL^{-1}-40µg mL^{-1}) were applied in triplicate on

TABLE 1 : Method validation parameter for the quantitation of β-sitosterol and Lupeol

Parameters	β-sitosterol	Lupeol
Linearity range [μg mL^{-1}]	10- 40	10 -40
Slope (*m*)[a)]	31.5	66.4
Intercept(*c*) [a)]	- 0.9	1.5
Correlation coefficient (R)	0.9951	0.9941
LOD [μg mL^{-1}]	5.0	5.0
LOQ [μg mL^{-1}]	10.0	10.0
Intraday precision (n=3 COV)	0.36	0.53
Interday precision (n=3 COV)	0.627	0.363
System Suitability	0.45	0.75

[a)]**of the equation y = mx + c, where y is peak area, m is the slope, x is the concentration and c is the intercept**

precoated silica gel 60 F_{254} HPTLC plates (E. Merck) of uniform thickness of 0.2 mm. The plates were developed in a solvent system of Toluene: Methanol, 8:1 (*v/v*) in CAMAG twin trough chamber up to a distance of 8.5 cm. After development, the plate was dried in air; the plate was derivatized in Liebermann- Burchard reagent and heated for 10 minutes at 105 ± 2°C. The plate was scanned at 366 nm using fluorescence- reflectance mode by CAMAG Scanner II and Wincats software for β-sitosterol and Lupeol. The peak areas were recorded. Respective calibration curves were prepared by plotting peak area versus concentration of β-sitosterol and Lupeol applied.

Simultaneous quantitation of β-sitosterol and Lupeol from the bark powder of artocarpus lakoocha Roxb.

Sample solutions (10 μl) were applied in triplicate on a precoated HPTLC plates with CAMAG Linomat spotter. The band width was 7 mm and the space between two bands was 8 mm. The plate was developed and scanned at 366nm after derivatization in Liebermann- Burchard reagent. The peak areas and absorption spectra were recorded. The amount of β-sitosterol and Lupeol in the sample was calculated using the respective calibration curves.

Method validation

The method was validated for precision, repeatability accuracy. Precision of the method was checked by repeated scanning (n=5) of the same spot of β-sitosterol and Lupeol seven times each. The repeatability of sample application and measurement of peak area were expressed in terms of %CV. Variability of the method was studied by intra-day precision and inter-day precision. In order to estimate limit of detection (LOD) and limit of quantitation (LOQ), blank methanol was spotted to determine signal to noise ratio. LOD was considered as 3:1 and LOQ as 10:1. Accuracy of the method was tested by performing recovery studies at three different levels (0%, 25%, and 50% addition).

RESULTS AND DISCUSSION

In the present study, we have quantitated two marker compounds viz. β-sitosterol and Lupeol from Bark powder of *Artocarpus lakoocha* Roxb. by HPTLC densitometric method using silica gel HPTLC. The developed method was validated as per the ICH guidelines (TABLE 1-3). Mobile phase composition was optimized by introducing small changes in the composition of Toluene: Methanol. It was found that β-sitosterol and Lupeol resolved well at R_f 0.57 and 0.73 (Figure 1) respectively in the solvent system of toluene: methanol (8:1, *v/v*). from other components of the sample extracts.

Linearity

A good linearity was achieved in the concentration ranges of 10 μg mL^{-1} – 40 μg $mL^{-1}$for β-sitosterol and Lupeol. The regression equations and correlation coefficient for the reference were y = 31.5x – 0.9, R^2 = 0.9951 for β-sitosterol and y = 66.4x + 1.5, R^2 = 0.9941 respectively (TABLE 1).

Instrumental precision and intra-day and inter-day precision

Instrumental precision was checked by repeated scanning of the same spot of and β-sitosterol and Lupeol five times each. Standards of β-sitosterol were spotted both at intra-day (spotting each concentration three times within 24 hour) and inter-day (spotting each concentration three times during 3 days intervals) interval to check the precision. The results are expressed as %RSD.

Recovery

The recovery was used to evaluate the accuracy of the method. The present recovery was calculated. Recovery studies at three different levels were done on Bark powder of *Artocarpus lakoocha* Roxb. by accurately spiked with various concentrations of refer-

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TABLE 2 : Recovery study of β- sitosterol and Lupeol from Bark powder of *Artocarpus lakoocha* Roxb. Plant

Standard	Level	Preanalysed Sample in (μg mL^{-1})	Amount of std added to preanalysed Sample in (μg mL^{-1})	Total amount of std Found in (μg mL^{-1})	SD	RSD (%) (n = 7)	Recovery (%)
	0	10.564	0	10.564	0.092	0.869	100.00
β-sitosterol	25%	10.564	2.5	13.006	0.238	1.833	99.55
	50%	10.564	5	15.620	0.261	1.671	100.36
						Mean	99.97
	0	24.151	0	24.151	0.083	0.344	100.00
Lupeol	25%	24.151	6.25	30.017	0.081	0.270	99.43
	50%	24.151	12.5	36.146	0.187	0.516	99.78
						Mean	99.74

ence solutions just prior to the extraction. The percentage recovery at three different levels for β-sitosterol was found to be 99.97% and 99.74% for Lupeol. The results were shown in (TABLE 2).

Limit of detection and limit of quantitation

The LOD and LOQ were found to be 5 and 10 μg ml^{-1} for β-sitosterol and Lupeol.

Quantitative determination

All samples were extracted, as described above and analyzed by HPTLC. The content of each compound was determined by the corresponding regression equation and results are summarized in TABLE 3. The result indicated β-sitosterol and Lupeol were detected in Bark powder of *Artocarpus lakoocha* Roxb. The chromatographic overlay of standards along with plant material is represented in (Figure 2). Whole plant powder of Bark powder of *Artocarpus lakoocha* Roxb. was found to contain 0.106 mg of β-sitosterol and 0.242 mg of Lupeol (TABLE 3).

CONCLUSION

The application of a simple, rapid and accurate HPTLC method for the quantitation of β- sitosterol and Lupeol from Bark powder of *Artocarpus lakoocha* Roxb. The method was validated to track the active principles in the complex mixture of herbal ingredients. The method could be extended for the marker-based standardization of other herbal product containing β-sitosterol and Lupeol. The method was found to be simple, precise, accurate, specific and sensitive and can be used for routine quality control of herbal raw materials and for the quantification of these compounds in plant materials.

TABLE 3 : Marker compounds quantified from Bark powder of *Artocarpus lakoocha* Roxb. plant

Sample Tested	Content of Marker compound* in mg	
	β-sitosterol	Lupeol
Bark powder of *Artocarpus lakoocha* Roxb. plant with β-sitosterol and Lupeol.	0.106	0.242

***Mean ± SD, n= 3**

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