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RESEARCH ARTICLE

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ESTIMATION OF TWO BIOACTIVE COMPOUNDS FROM *ARTOCARPUS LAKOOCHA* ROXB. LEAVES USING HPTLC.

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ABSTRACT

Sensitive, simple, and accurate high-performance thin layer chromatographic method has been established for determination of β-sitosterol and Lupeol both simultaneously in *Artocarpus lakoocha* Roxb. leaf powder. The chromatographic separation was performed on silica gel 60 F_{254} HPTLC plate, with Toluene: Methanol: Formic acid, 7.0:2.0:0.3 (*v/v/v*), as mobile phase. After development, plates were treated with Methanolic Sulphuric acid Reagent. Detection and quantification were performed by densitometry at 366 nm in fluorescence mode. The developed method was then validated using statistical analysis. This will help in qualitative analysis of plant material using fingerprint pattern and quantitative analysis of β-sitosterol and Lupeol.

KEY WORDS

HPTLC, β-sitosterol, Lupeol, *Artocarpus lakoocha* Roxb.

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 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 anti-inflammatory, antiviral, anticancer and anti-HIV.[8] The literature reveals that there is no High Perform Thin Layer Chromatographic method available for quantitation of β- sitosterol & Lupeol from leaf 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 and Sample Preparation

Leaves of *Artocarpus lakoocha* Roxb. was collected from Madgoan (Goa) region of India. It was authenticated from Botanical survey of India (Pune). After collection, The collected plant material 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. 500 mg of leaf powder of *Artocarpus lakoocha* Roxb. was extracted with 10 mL of methanol. The mixture was vortexed for 5 mins. and it was kept overnight for extraction. It was filtered through Whatman filter paper No. 41 and filtrate was subjected to HPTLC for simultaneous quantitation of β- sitosterol and Lupeol.

Chemicals and standard solutions Preparation

All the chemicals used in the experiments were of analytical grade. Reference standard β-sitosterol and Lupeol (purity 98%) were procured from Sigma Aldrich (Germany). The stock solutions of β- sitosterol and Lupeol (10mg mL^{-1}) each were prepared separately in Methanol. The stock solution were quantitatively transferred to give a solution of appropriate concentration range of β- sitosterol and Lupeol (10 µg mL^{-1} – 40 µg mL^{-1}) respectively. Standard solutions were prepared by dilution of the stock solution.

Instrumentation and Chromatographic Conditions

Chromatographic separation was preformed with CAMAG (Muttenz, Switzerland) Linomat IV sample applicator; equipped with Hamilton (Switzerland) Syringe: 100µl and Camag TLC Scanner II equipped with Wincats 3.0 version software was used for data acquisition. The chromatographic separation was performed on 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: Formic acid, 7.0:2.0:0.3 (*v/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 Methanolic Sulphuric acid 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 corresponding to β-sitosterol and Lupeol in *Artocarpus lakoocha* Roxb. leaf solution was identified by comparing the chromatograms of the sample with that of standards. Chromatographic plate and overlay is shown in Figure 1 and Figure 2 respectively.

Figure 1

Chromatographic plate of leaf powder of Artocarpus lakoocha Roxb. With β- sitosterol and Lupeol.

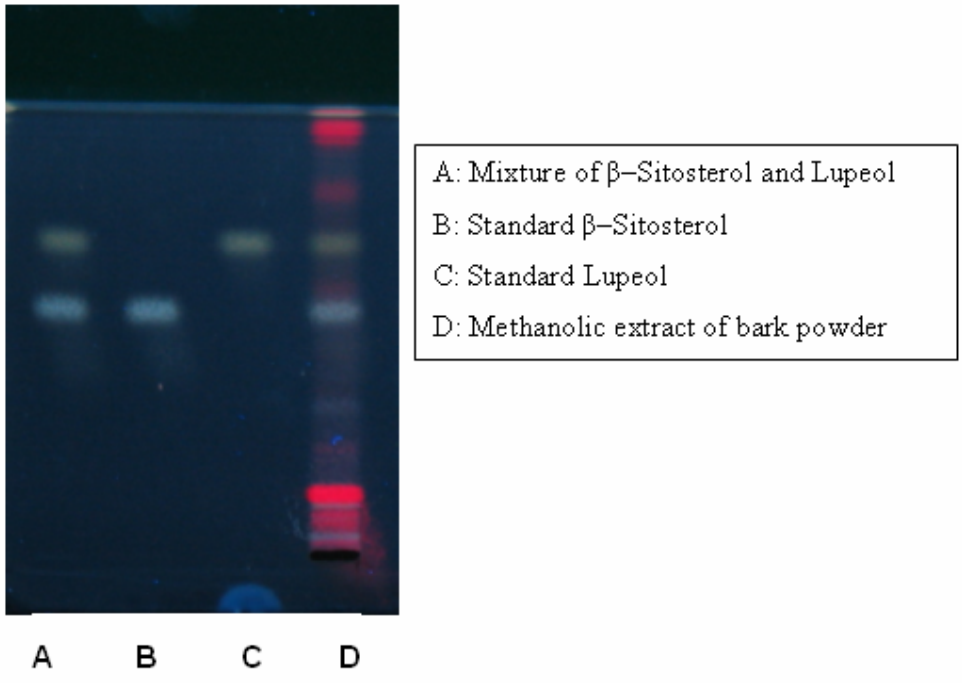
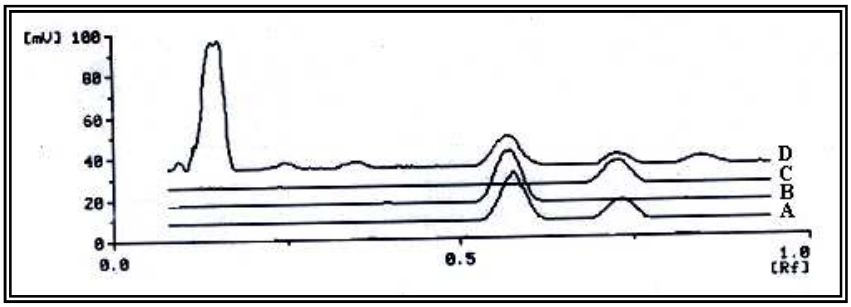


Figure 2
Chromatographic overlay of leaf powder of Artocarpus lakoocha Roxb. with β- sitosterol and Lupeol



Track 1: Mixture of β- sitosterol and Lupeol
Track 2: β-sitosterol
Track 3: Lupeol
Track 4: Leaf powder of Artocarpus lakoocha Roxb.

Method Validation

System Suitability

System suitability tests are used to ensure reproducibility of the equipment. The test was carried out by spotting 10 µL of mixture of standard solution of assay concentration of β-sitosterol and Lupeol six times. The % RSD was found to be 0.69 for β- sitosterol and 1.18 for Lupeol, which was acceptable as it is less than 2%.

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 = 32.98x + 192.5, R^2 = 0.995 for β- sitosterol and y = 69.46x + 96.17, R^2 = 0.991 for Lupeol respectively. The experiment was performed three times and the mean was used for the calculations. The data was analyzed by linear regression least squares fitting. The statistical data obtained is given in Table 1.

Table 1
Method validation summary

Parameters	β- sitosterol	Lupeol
Linearity range [µg mL^{-1}]	10 µg mL^{-1} – 40 µg mL^{-1}	10 µg mL^{-1} – 40 µg mL^{-1}
Slope (*m*)[a)]	32.98	69.46
Intercept(*c*) [a)]	192.5	96.17
Correlation coefficient (R)	0.995	0.991
LOD [µg mL^{-1}]	5	5
LOQ [µg mL^{-1}]	10	10
Intraday precision (n=3 COV)	0.44%	0.65%
Interday precision (n=3 COV)	0.95%	0.52%
System Suitability	0.69%	1.18%

[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.

Limit of Detection and Limits of Quantitation

The signal-to-noise ratio of 3:1 and 10:1 was used to establish LOD and LOQ, respectively. The LOD and LOQ were found to be 5 µg mL^{-1} and 10 µg mL^{-1} for β- sitosterol and Lupeol respectively.

Assay

The developed HPTLC method was used for simultaneous determination of β- sitosterol and Lupeol from leaf powder of *Artocarpus lakoocha* Roxb. The sample working solution (10 µL) was spotted and the area of both β- sitosterol and Lupeol peak was measured. From the calibration curve, the amount of β- sitosterol and Lupeol in dry leaf powder of *Artocarpus lakoocha* Roxb. was calculated. The retention factor (R_f) of β- sitosterol and Lupeol in sample solution was found to be 0.57 and 0.76 and in the standard solution was found to be 0.58 and 0.75 respectively. The mean assay value of β-sitosterol was found to be 0.176 mg per 500 mg of plant powder with % RSD as 0.83 and mean assay value of Lupeol was found to be 0.217 mg per 500 mg of plant powder with % RSD as 0.44. (Table 3)

Table 3
Assay Results

Sample Tested	Content of Marker compound* in mg	
	β- sitosterol	Lupeol
Leaf powder of *Artocarpus lakoocha* Roxb. with β- sitosterol and Lupeol	0.176	0.217

* Mean ± SD, n= 7

Precision and Accuracy

The intra-day and inter-day precision was used to study the variability of the method. The % RSD for intra-day and inter-day precision for β-sitosterol were 0.44 and 0.95%, respectively and Lupeol were 0.65 and 0.52 %, respectively. Accuracy of the method was studied using the method of standard addition. Standard β-sitosterol and Lupeol solutions were added to the extract of the of *Artocarpus lakoocha* Roxb. leaves and the percent recovery was determined at two different levels 25% and 50%. β- sitosterol and Lupeol content was determined and the percent recovery was calculated. The results of recovery analysis are shown in Table 2 for both β- sitosterol and Lupeol.

Table 2
Results of Recovery study

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 (%)
β-sitosterol	0	17.64	0	17.636	0.147	0.831	100.00
	25%	17.64	4.41	22.050	0.232	1.054	99.61
	50%	17.64	8.82	26.610	0.127	0.476	99.90
						Mean	99.84
Lupeol	0	21.67	0	21.674	0.095	0.440	100.00
	25%	21.67	5.42	26.695	0.122	0.458	99.15
	50%	21.67	10.83	31.709	0.169	0.533	98.56
						Mean	99.23

** Mean ± SD, n= 7*

CONCLUSION

The application of a simple, rapid and accurate HPTLC method for the simultaneous quantitation of β- sitosterol and Lupeol in *Artocarpus lakoocha* Roxb. leaf powder. 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, sensitive and can be used for routine quality control of herbal raw materials also for the quantification of these compounds in plant materials.

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