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Simultaneous quantitation of lupeol and β -sitosterol from the whole plant powder of *Asteracantha longifolia* Nees

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ABSTRACT

A simple, sensitive and accurate high performance thin layer chromatographic method has been established for simultaneous quantitation of Lupeol and β -sitosterol in the whole plant powder of *Asteracantha longifolia* Nees. This method was also used for estimation of β - sitosterol content in herbal formulations containing *Asteracantha longifolia* Nees. as an ingredient. A methanolic extract of the powder was used for the experimental work. Separation was performed on aluminium HPTLC plates coated with silica gel 60 F₂₅₄ with toluene- ethyl acetate- methanol, 7.5+ 1.5+ 0.7 (v/v/v), as mobile phase. After development, the plates were treated with Liebermann-Burchard reagent and detection and quantitation was performed by densitometry at 366 nm. The concentration of Lupeol and β - sitosterol were found to be 0.162 mg g⁻¹ and 0.045 mg g⁻¹ respectively in the whole plant powder of *Asteracantha longifolia* Nees., while the concentration of β -sitosterol was found to be 0.048 mg g⁻¹ and 0.039 mg g⁻¹ in Lukol and Speman formulations respectively. Both the methods were validated for linearity, precision, accuracy, robustness and can be used for routine quality control of *Asteracantha longifolia* Nees. whole plant powder to be used in the herbal formulation. © 2008 Trade Science Inc. - INDIA

KEYWORDS

Asteracantha longifolia Nees.;
Lupeol;
 β -Sitosterol;
HPTLC;
Derivatization.

INTRODUCTION

Asteracantha longifolia Nees. (known as Kokilaksa in Sanskrit and Talmakhana in Hindi, Family- Acanthaceae) is widely distributed in India and is popularly used in Ayurvedic and herbal formulations^[4]. It has been reported to contain chemical constituents such as β -sitosterol, Lupeol, and linoleic acid and oleic acid as main constituents; fatty acids, polysaccharides histidine, phenyl alanine^[4], Lupeol and β - sitosterol both have been reported for many pharmacological activities such as antipyretic^[1] hepatoprotective^[4,7,8,12], antioxidant^[1], anticancer^[1], macrofilaricidal^[3]. Both the con-

stituents are used as a gargle for odontopathy^[1]. Lithotropic and antifertility^[3] activity of the drug is attributed to Lupeol^[3]. Lupeol is also a potential phytochemical in controlling arthritis^[13]. The structure of β - sitosterol and Lupeol is shown in figure 1. In the present investigation chromatographic fingerprint of *Asteracantha longifolia* Nees. Whole plant powder was developed by HPTLC method. The method is found to be rapid, precise, accurate, can be applied for routine quantitation of Lupeol and β -sitosterol simultaneously from the whole plant powder of *Asteracantha longifolia* Nees. β - sitosterol has been quantitated from two herbal formulations containing *Asteracantha longifolia* Nees. as an ingredient.

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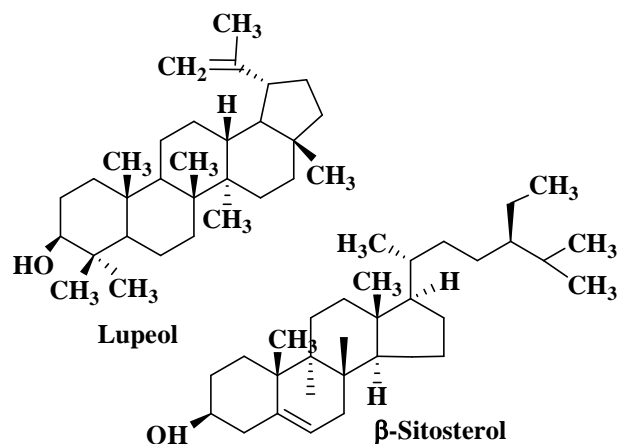


Figure 1: Structure of lupeol and β -sitosterol

EXPERIMENTAL

Materials

Whole plants of *Asteracantha longifolia* Nees. were collected in winter (October – November 2007) during flowering season from ‘Thane’, Maharashtra, India. Herbaria of the whole plant were authenticated from National Institute of Science Communication (NISCOM), New Delhi and National Botanical research Institute (NBRI), Lucknow. Lukol and Spemen containing *Asteracantha longifolia* Nees. were purchased from local market (manufactured by Himalaya drug Co.). Standard β -sitosterol (99 % purity) and Lupeol (97 % purity) were procured from Sigma-Aldrich Chemie GmbH (Aldrich Division, Steinheim, Federal Republic of Germany). The solvents toluene, ethyl acetate, and methanol of analytical grade purchased from Qualigens Fine Chemicals, Mumbai, India were used for the analysis.

A TLC scanner with computer system and Cats 3 Version Software were obtained from Camag (Muttenez, Switzerland). The source of radiation was mercury lamp. Camag Linomat IV was used as applicator. Separation was done on silica gel F₂₅₄ HPTLC precoated plate procured from Merck (Darmstadt, Germany).

Standard and sample preparation

A stock solution of Lupeol (1000 μ g mL⁻¹) was prepared by dissolving 25.0 mg of accurately weighed Lupeol in methanol and diluting to 25.0 mL with methanol. Aliquots (0.3 mL to 0.7 mL) of this stock solution

were transferred to 10 mL standard volumetric flasks and the volume of each was adjusted to 10 mL with methanol, to obtain working standard solutions containing 30 μ g mL⁻¹ to 70 μ g mL⁻¹. And same for the stock solution of β -sitosterol (1000 μ g mL⁻¹) was prepared by dissolving 25.0 mg of accurately weighed β -sitosterol in methanol and diluting to 25.0 mL with methanol. Aliquots (0.1 mL to 0.7 mL) of this stock solution were transferred to 10 mL standard volumetric flasks and the volume of each was adjusted to 10 mL with methanol, to obtain working standard solutions containing 10 μ g mL⁻¹ to 70 μ g mL⁻¹.

Whole plants of *Asteracantha longifolia* Nees. were collected, washed, shade dried, powdered, sieved through an 80- mesh (BSS) sieve and stored in an airtight container at 25°C, 1000 mg of the dried powder was accurately weighed and placed in a stoppered tube and 10 mL of methanol was added, the sample was vortexed for 1- 2 minutes and left to stand overnight at room temperature (28 \pm 2°C). the contents of the tube were filtered through Whatmann No. 41 paper (E. Merck, Mumbai, India) and the filtrate was used for experimental work. The formulations containing *Asteracantha longifolia* Nees. were extracted separately using the same method.

Chromatography

Procedure

Chromatography was performed on silica gel F₂₅₄ HPTLC pre-coated plate^[5]. Samples (10 μ L) were applied on the plates as band of 7mm width with the help of a Camag Linomat IV sample applicator at the distance of 14 mm from the edge of the plates. The mobile phase constituted of toluene- ethyl acetate-methanol, 7.5 + 1.5 + 0.7 (v/v/v). The plates were developed to a distance of 80 mm in a Camag twin- trough chamber previously equilibrated with mobile phase for 30 minute. The chromatographic conditions had previously been optimized to achieve the best resolution and peak shape. After development, plate were derivatised in Liebermann- Burchard reagent and heated at 105°C for 15 minute and densitometric evaluation of the plates was performed at 366 nm in fluorescence/ reflectance mode using mercury lamp with a Camag Scanner II in conjunction with Cats 3 Version Software. A typical HPTLC chromatogram of Lupeol and β -sitosterol stan-

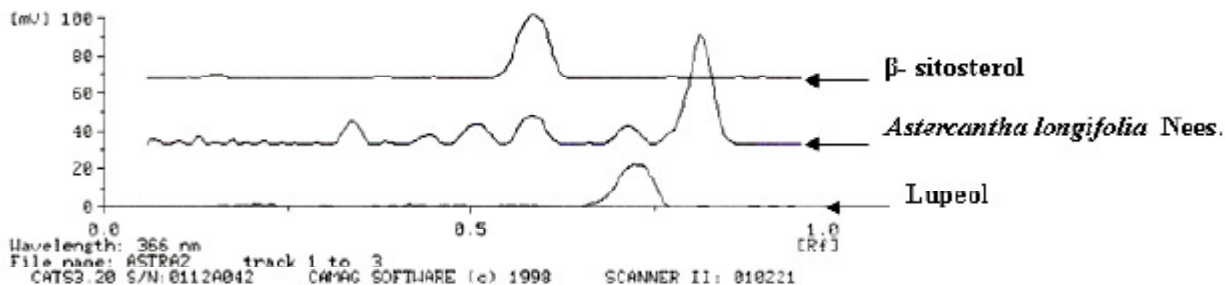


Figure 2: HPTLC densitometric scan at 366 nm of Lupeol and β -sitosterol standard. with methanolic extract of *Asteracantha longifolia* Nees. whole plant powder

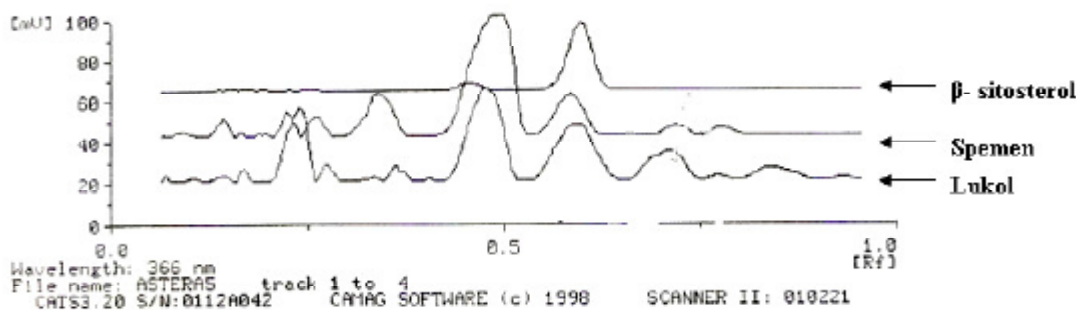
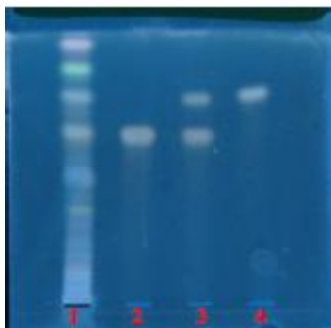


Figure 3: HPTLC densitometric scan at 366 nm of β -sitosterol standard. with methanolic extract of Lukol and spemen



Methanolic extract of whole plant powder of *Asteracantha longifolia* Nees; T2- β -sitosterol ; T3- standard β -sitosterol + Lupeol; T4- Lupeol

Plate 1: Chromatographic plate of standard Lupeol and β -sitosterol with methanolic extract of *Asteracantha longifolia* Nees. whole plant powder



T1- Lukol formulation; T2- Spemen formulation; T3- β -sitosterol

Plate 2: Chromatographic plate of standard β -sitosterol with methanolic extract of Lukol and spemen formulations

standard with plant is shown in figure 2 and same of β -sitosterol with both the formulation is shown in figure 3. The chromatographic plate of Lupeol and β -sitosterol standard with *Asteracantha longifolia* Nees. is shown in Plate 1, and with formulations, it's shown in Plate 2.

Linearity of detector response

Each standard solution (10 μ l, for Lupeol corresponding to 30, 40, 50, 60, 70 μ g mL⁻¹ and for β -sitosterol corresponding to 10, 20, 30, 40, 50, 60, 70 μ g

mL⁻¹) were prepared in methanol. Each of these solutions (10 μ L) was applied to a plate, the plates were developed, and the detector response for the different concentrations was measured. A graph was plotted of drug peak area against concentration of Lupeol and β -sitosterol respectively. The plot of Lupeol was linear in the range 30 to 70 μ g mL⁻¹ and for β -sitosterol; the plot was linear in range 10 to 100 μ g mL⁻¹. The experiment was performed three times and the mean was used for the calculations. The linearity data is given in TABLE 1.

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TABLE 1: Linearity data of standard lupeol and β -sitosterol

	Lupeol	β -Sitosterol
Linearity range [$\mu\text{g mL}^{-1}$]	30 to 70	10 to 70
Slope (m)	16.96	29.98
Intercept (c)	83.37	61.57
Correlation coefficient (R)	0.999	0.994
LOD [$\mu\text{g mL}^{-1}$]	2	2
LOQ [$\mu\text{g mL}^{-1}$]	5	4
Instrument precision RSD% (n=5)	0.21	0.14
Intraday precision RSD% (n=3)	0.15	0.16
Interday Precision RSD% (n=3)	0.16	0.17

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

TABLE 2: Results of assay of lupeol in whole plant powder of *Asteracantha longifolia nees*

Sample	Weight of Sample in mg	Amount of Lupeol present in sample in mg g^{-1}	RSD (%) n = 3	Average % content of Lupeol
Whole plant powder of <i>Asteracantha longifolia</i> Nees.	1000	0.162	0.26	0.162

Results of assay for β -sitosterol in whole plant powder of *Asteracantha longifolia nees.*, lukol and spemen formulations

Sample	Weight of sample in mg	Amount of β -sitosterol present in sample in mg g^{-1}	RSD (%) n = 3	Average % content of β -sitosterol
Whole plant powder of <i>Asteracantha longifolia</i> Nees.	1000	0.045	0.33	0.045
Lukol formulation	1000	0.048	0.32	0.048
Spemen formulation	1000	0.039	0.20	0.039

Assay procedure

The solution of 0.5 ml of 50 ppm ($50\mu\text{g mL}^{-1}$) Lupeol was mixed with 0.5 ml of 50 ppm ($50\mu\text{g mL}^{-1}$) β -sitosterol and $10\mu\text{L}$ of methanolic extract of *Asteracantha longifolia* Nees. were spotted on a HPTLC plate. The amount of Lupeol and β -sitosterol present in the plant extract was calculated by comparison of area measured for the sample to that for the respective standard. Similarly the amount of standard β -sitosterol was calculated from Lukol and Speman formulations. The assay procedure described earlier was repeated three times. The results of assay are given on

TABLE 2. The mean assay values of Lupeol and β -sitosterol were found to be (0.162 mg g^{-1}) and (0.045 mg g^{-1}) in the plant extract respectively. The value of β -sitosterol in Lukol formulation and Speman formulation was found to be (0.048 mg g^{-1}) and (0.039 mg g^{-1}) respectively.

RESULT AND DISCUSSION

Lupeol and β -sitosterol standards were detected and quantitated in an accurate manner using silica gel F_{254} HPTLC pre-coated plates with the mobile phase made of toluene-ethyl acetate-methanol, 7.5+1.5+0.7 (v/v/v). The identity of band of Lupeol and β -sitosterol in the plant extract was confirmed by overlaying the chromatogram obtained from the standards Lupeol and β -sitosterol and by comparing their R_f (0.71 and 0.58). Formulations, Speman and Lukol are polyherbal in composition with 9 and 23 ingredient respectively. The identity of band of β -sitosterol in the formulation was confirmed by overlaying the chromatogram obtained from the standard β -sitosterol and by comparing their R_f (0.58). Though the identity of Lupeol from the formulation could be confirmed by overlapping the chromatogram obtained from the standard Lupeol, quantitation was not accurate, probably due to the interfering components from the other ingredients present in the formulation.

The linearity range of Lupeol was observed over a concentration of 30 to $70\mu\text{g mL}^{-1}$ with correlation coefficient of 0.999. And for β -sitosterol it was 10 to $70\mu\text{g mL}^{-1}$ with correlation coefficient of 0.994. The concentration of Lupeol and β -sitosterol were found to be 0.162 mg g^{-1} and 0.045 mg g^{-1} respectively in the whole plant powder of *Asteracantha longifolia* Nees. While the concentration of β -sitosterol was found to be 0.048 mg g^{-1} and 0.039 mg g^{-1} in Lukol and Speman formulations respectively.

Instrument precision, intraday precision, interday precision were measured to evaluate the precision of the method. The % RSD values were found to be less than 2% indicating that the selected method is precise and reproducible.

The robustness of the method was studied, during method development, by determining the effects of small variation, of mobile phase composition ($\pm 2\%$), cham-

ber saturation period, development distance and scanning time (10% variation of each). No significant change of R_f response to Lupeol and β -sitosterol were observed, indicating the robustness of the method.

CONCLUSION

In this study HPTLC fingerprint of and further quantitation of two photochemical markers namely Lupeol and β -sitosterol from the whole plant powder of *Asteracantha longifolia* Nees. has been established. The simultaneous determination of Lupeol and β -sitosterol provides a better method for quantitation of *Asteracantha longifolia* Nees. content in the herbal raw materials and polyherbal formulation. This is more advantageous than quantifying the contents using a single phytochemical marker. The HPTLC method could be successfully used to quantitate β -sitosterol from two polyherbal formulations. But quantitation may be influenced by the interference from other raw materials present in the formulation. High Performance Thin Layer Chromatography as reported in this study provides a chromatographic fingerprint of *Asteracantha longifolia* Nees. whole plant and is useful in identifying the herb as a raw material to be used ingredient in a polyherbal formulation.

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