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Research Article

IMPACT OF REGIONAL VARIATION ON LUPEOL CONTENT IN *CARISSA CARANDAS* LINN. FRUITS: EVALUATION USING VALIDATED HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY

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ABSTRACT

The present work is an attempt to evaluate the impact of regional variation on lupeol content in *Carissa carandas* Linn. fruits collected from different geographical regions of India using a validated HPTLC method. Chromatographic separation was achieved on TLC plates pre-coated with silica gel 60 F_{254} using toluene: methanol (8:1, v/v) as a mobile phase. Detection of lupeol was carried out by derivatizing the plates with 10 % methanolic sulphuric acid reagent followed by its densitometric scanning using CAMAG TLC scanner 4 at 366 nm. The method was validated as per ICH guidelines. Statistical analysis reveals that, the content of lupeol in different geographical region varied significantly.

Keywords: *Carissa carandas*, fruits, HPTLC, lupeol, regional variation.

1. INTRODUCTION

C. carandas L. (Apocynaceae) commonly known as Karvanda is a tall, highly branched thorny woody shrub distributed throughout India. Fruits of *C. carandas* have been reported as an anti-scorbutic agent and as a remedy for biliousness [1]. Pharmacological studies on fruits of *C. carandas* revealed its potential use as antipyretic, analgesic, anti-inflammatory, anti-diabetic agent [2-4]. Fruits of *C. carandas* have been reported to contain carisol, epimer of α-amyrin, linalool, β-caryophyllene, carissone, carissic acid, carindone, ursolic acid, carinol, ascorbic acid, lupeol and β-sitosterol [1, 5, 6]. Lupeol has been reported to possess anticancer, anti-inflammatory, immunomodulatory, hepatoprotective and antimicrobial properties [7].

Since, *C. carandas* is distributed in different regions of India; contents of its bioactive markers vary greatly depending on their geographical locations, climatic conditions and other factors. HPTLC technique is reported to be useful for identification of morphological and geographical variations in terms of chemical markers from various medicinally important plants [8-11].

Variation in the phytochemical profile of *C. carandas* fruits using HPLC has been recently reported by our group [12]. In this research work, an HPTLC method has been developed and validated to study the phytochemical diversity in *C. carandas* fruits collected from different geographical regions of Maharashtra in terms of lupeol content.

2. MATERIAL AND METHODS

2.1. Collection, drying and storage

Ripe fruits of *C. carandas* were collected from different provinces in Maharashtra. Plant specimens were authenticated by Agharkar Research Institute (Voucher Specimen No. Auth 11-123), Pune, India and deposited at Department of Botany for future reference. Fruits were oven dried at 45°C, powdered, sieved (BSS 85-mesh) and stored in air tight containers.

2.2. Reference standards and reagents

Lupeol standard (95% purity, Fig. 1) was procured from Sigma Aldrich chemical company, (Steinheim, Germany). Solvents of analytical grade were procured from Merck specialties Private Limited, India.

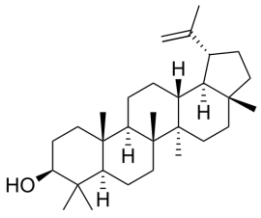


Fig. 1: Structure of lupeol

2.3. Extraction conditions

Each plant sample was accurately weighed (0.4 g) and extracted in methanol (10.0 mL). This mixture was vortexed for 1 minute, kept standing overnight and then filtered through Whatman filter paper No. 1 (E. Merck, India). The filtrates were subjected to chromatographic analysis.

2.4. Chromatographic and Instrumental conditions

Chromatographic separation was achieved on TLC plates pre-coated with silica gel 60 F_{254} (E. Merck) of 0.2 mm thickness with aluminium sheet support. Samples (10.0 µL) were spotted using CAMAG Linomat 5 sample spotter (Camag Muttenz, Switzerland) equipped with syringe (Hamilton). Plates were developed in a twin trough chamber (CAMAG) pre-saturated for 30 minutes with toluene: methanol (8:1, v/v) and scanned with Camag TLC Scanner 4 conjugated with winCATS software. The plate was derivatized with 10 % methanolic sulphuric acid reagent and scanned at 366 nm. Camag Reprostar 3 system was used for photo documentation. The experimental condition was maintained at 20 ± 2 °C.

2.5. Preparation of standard solutions

A stock solution of lupeol (1000.0µg/mL) was prepared in methanol. Seven calibrant samples ranging from 5.0 µg/mL-75.0 µg/mL and three quality control samples of lupeol namely low, mid, and high (LQC, MQC and HQC; 6.5, 20.0, 60.0 µg/mL respectively) were prepared in methanol using the stock solution.

2.6. Method validation

The developed HPTLC method was validated as per ICH guidelines [12] in terms of its specificity, system suitability, sensitivity (LOD and LOQ), linearity, precision, stability, recovery and ruggedness.

2.7. Estimation of lupeol in *C. carandas* fruits

Relative response for the characteristic band of lupeol in *C. carandas* fruit samples was obtained and the content of lupeol in each sample was determined using the regression equation obtained from regression analysis of the calibration curve.

2.8. Statistical evaluation

Statistical analysis of the data was carried out using Microsoft Excel - 2007.

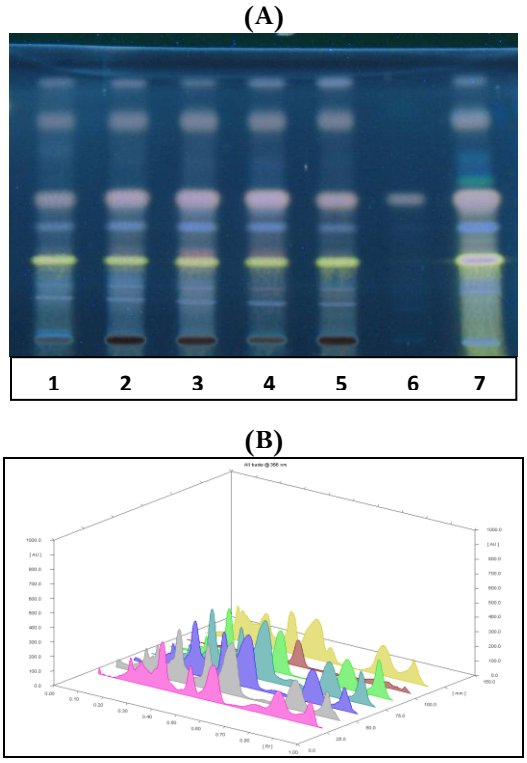
3. RESULTS AND DISCUSSION

C. carandas fruits have been used as medicinal food or dietary supplement for centuries and are of increasing interest to consumers [5, 13]. Hence, there is a concern about authenticity and quality of *C. carandas* fruits. In the current work, quality of *C. carandas* fruits was evaluated on the basis of lupeol content using a validated HPTLC method. Samples collected from different provinces of Maharashtra were subjected to the estimation of lupeol using HPTLC.

So far, there is no report on the evaluation of the lupeol content in fruits of *C. carandas* using HPTLC. Estimation of lupeol from plant matrices using validated HPTLC methods has been previously reported by our group [14-18] But, the mobile phases used consisted of three or more than three solvents including toluene, chloroform, ethyl acetate, glacial acetic acid, cyclohexane, formic acid, methanol, acetone, dichloromethane etc which did not resolve lupeol from the fruits of this plant satisfactorily which may be due to the high content of latex in fruits [19]. Hence, we further modified the mobile phase which consisted of only two solvents and resulted in its proper separation from plant matrix.

Briefly, the separation of lupeol was achieved from the methanolic extract of *C. carandas* fruits on TLC plates using toluene: methanol (8:1, v/v) as a mobile phase and 10 % methanolic sulphuric acid as a derivatizing regent. HPTLC analysis of *C. carandas* fruits collected from different geographical regions with lupeol at 366 nm along with the 3-D overlay of chromatograms is shown in Figure 2.

Fig. 2: HPTLC analysis of C. carandas fruits collected from different geographical regions with lupeol at 366 nm, Plate photo (A), 3-D Overlay of Chromatograms (B).

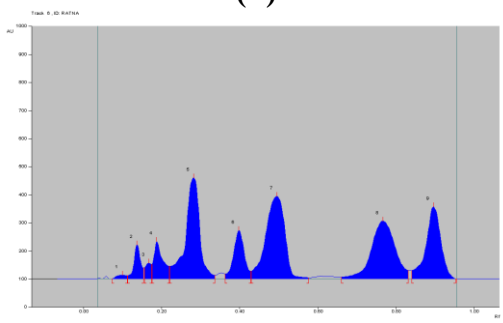


Track details: Sample from, a: Lonavala, b: Matheran, c: Kalyan, d: Malshej, e: Ratnagiri, f: Lupeol (40 µg/mL), g: Baroda

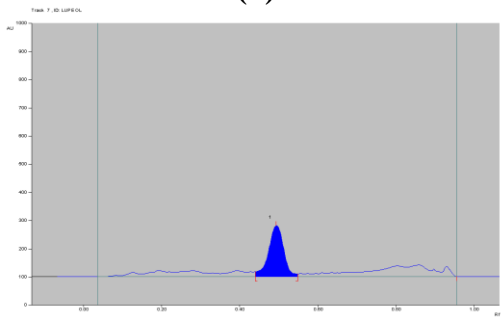
Lupeol was detected at R_f = 0.49 and its identity in the matrix of *C. carandas* fruits was confirmed by overlay and colour with that of the standard lupeol. The representative TLC densitometric chromatograms of lupeol and *C. carandas* fruit is represented in Fig. 3.

Fig. 3: Representative HPTLC chromatograms of C. carandas fruits (A) and lupeol (B)

(A)



(B)



The HPTLC method was validated as per ICH guidelines and was found to be rapid, specific, precise, sensitive and rugged during the validation experiment (Table 1).

Table 1: The results of method validation parameters for lupeol using HPTLC technique

Parameters	Results
LOD (µg/mL)	2.0
LOQ (µg/mL)	5.0
Linear range (µg/mL)	5.0 – 75.0
Regression equation	y = 24.81 x + 35.10
Mean coefficient of determination (r^2)	0.995
System suitability (% CV, n = 5)	
R_f	1.68
Area	1.03
Precision (% CV, n = 3)	
Intraday	0.09 - 1.01
Interday	0.19 - 1.34
Recovery (%, n = 7)	
Low (6.5 µg/mL)	95.14
Mid (20.0 µg/mL)	96.03
High (60.0 µg/mL)	97.04
Stability	
Long-term stability	
Standard Stock Solution stability (for 10 days)	Stable at (4 ± 1^{0}C)
Short-term stability	
Bench top stability (For 6.00 hours)	Stable at (25 ± 2^{0}C)
Auto sampler stability (For 12.00 hours)	Not applicable
Ruggedness	Rugged

The method was found applicable to evaluate the impact of regional variation on the content of lupeol in *C. carandas* fruits collected from different provinces of Maharashtra. Using the regression equation (y = 24.81 x + 35.10), the exact content of lupeol in the samples was determined (Table 2). The impact of regional variation on the lupeol content of *C. carandas* fruits was clearly evident from HPTLC analysis (Figure 2 and Table 2) and the results were in compliance with the other published reports [8-12]. The content of lupeol was found maximum in the sample collected from Baroda while sample from Ratnagiri showed minimum content.

Table 2: Assay results showing the content of lupeol in C. carandas fruits collected from different provinces of Maharashtra

Sample	Concentration of lupeol in mg/g (Mean ± SD, n=3)
Lonavala	1.36 ± 0.02
Matheran	0.86 ± 0.01
Kalyan	1.62 ± 0.02
Malshej	0.75 ± 0.03
Ratnagiri	0.45 ± 0.01
Baroda	3.38 ± 0.05

4. CONCLUSION

The impact of regional variation was clearly evident from the chromatographic analysis indicating that the geographical locations, soil and climatic conditions have a direct effect on the content of chemical phytoconstituents. This method can be utilized as routine quality control tool for the quality evaluation of *C. carandas* fruits and can be applied to various plant matrices and polyherbal formulations containing lupeol.

5. ACKNOWLEDGEMENTS

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