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Research Article

HPTLC ANALYSIS OF THE ROOTS OF *TAVERNIERA CUNEIFOLIA* (ROTH) ALI

Poonam Mangalorkar[1]*, Sunita Shailajan[2] and Padamnabhi Nagar[1]
[1]Department of Botany, Maharaja Sayajirao University of Baroda, Vadodara, Gujarat, India
[2]Herbal Research Lab, Ramnarain Ruia College, Matunga, Mumbai, Maharashtra, India
*Corresponding Author Email: poonammangalorkar000@gmail.com
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ABSTRACT
Taverniera cuneifolia (Roth) Ali is commonly called as jethimadh (Licorice). High-Performance Thin-Layer Chromatographic analysis was carried out for the regional variation of different regions of India, seasonal variation and species variation. The root powder was extracted with Methanol and further analysis was performed. Quantitation of β-sitosterol and lupeol was carried out for *T. cuneifolia* roots collected from different regions of India like Kutch, Rajkot, Jamnagar (Gujarat), Jodhpur (Rajasthan), Kurnool (Andhra Pradesh), India. The concentrations of β-sitosterol were found to be 1.98 % in Jamnagar, India, 2.64 % in Rajkot, India, 4.45 % in Andhra Pradesh, India and 1.90 % in Jodhpur, India. The concentrations of lupeol were found to be 2.44 % in Jamnagar, India, 2.21 % in Rajkot, India, 2.29 % in Kutch, India and 2.01 % in Jodhpur, India. Seasonal variation of β-sitosterol was found to be highest in monsoon (7.08 %), winter (3.89 %) and summer (2.64 %). Lupeol was found only in summer (1.42 %). Species variation of *Taverniera* roots showed only presence of β-sitosterol where *Taverniera cuneifolia* (10.04 %) and *Taverniera abyssinica* (6.89 %). Quantitation was carried out on HPTLC silica gel 60 F254 pre-coated plates with the mobile phase Toluene: Methanol: 8: 1 (v/v). A TLC scanner set at 366 nm in fluorescence / reflectance mode was used for quantitation.
Keywords: Licorice, *Taverniera cuneifolia*, HPTLC, Regional, Seasonal.

INTRODUCTION

The genus *Taverniera* belongs to the family of Fabaceae and includes twelve species. It is endemic to the Northeast African and Southwest Asian countries[1]. *T. cuneifolia* is often referred to as Indian licorice owing to its sweet taste which is very similar to that of *G. glabra*[2]. *T. cuneifolia* is locally known as Jethimadh and it is used by the tribal's of Barda Hills of Jamnagar in Western India. It is used as a substitute of Licorice or in other words the plant itself is considered to be *G. glabra*[2]. It is traditionally known to be used as an expectorant, blood purifier, anti- inflammatory, wound healing, anti ulcer and in treating spleen tumors[2]. The commercial licorice has a huge demand in the Indian system of medicine, Ayurveda and majority of the requirement of the Ayurvedic drug industry in India is met through import from Afghanistan and Pakistan[3]. Owing to huge demand plants like *A. precatorius* is used as adulterant or as a substitute for *G. glabra*[4,5]. Literature reveals the presence of β-sitosterol and lupeol in *T. cuneifolia*[6]. Hence, geographical variation in different parts of India, seasonal variation and species variation were analysed using β-sitosterol and lupeol.

MATERIALS AND METHODS

Collection, drying and processing of plant samples

The plant material was collected from Munjka village, Near Saurashtra University Campus, Rajkot and Rosy port area, Jamnagar, Tapkeshwari, Bhuj, Kutch, Gujarat, Jodhpur, Rajasthan and Betam cherala, Kurnool, Andhra Pradesh, India. The plant material was authenticated at BSI (Botanical Survey of India) Jodhpur, Rajasthan, India. Ref.no. BSI/AZC/I.12012/ Tech./ 2011-12 (PL.ID)-55. The shade dried roots were used for HPTLC analysis.

Chromatographic Conditions

Instrument

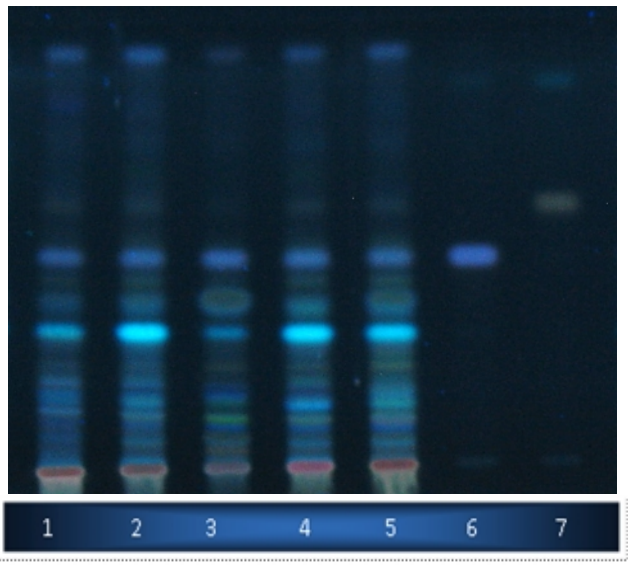
HPTLC plates: Merck, aluminium sheet precoated with silica gel 60 F254 procured from Anchrom laboratories, Mumbai, India. Applicator: CAMAG Linomat 5 sample spotter (Camag Muttenz, Switzerland) equipped with syringe (Hamilton). Developing chamber: CAMAG twin trough chamber, Densitometric scanner: Camag TLC Scanner 4 conjugated with win CATS software, Photo documentation: Camag Reprostar 3.

Reference standards and reagents

Reference compounds (standard) β-sitosterol (98 % purity, Figure 1) and lupeol (95 % purity, Figure 2) was procured from Sigma Aldrich, Steinheim, Germany. Solvents of analytical grade were procured from Merck specialties India Private Limited, India.

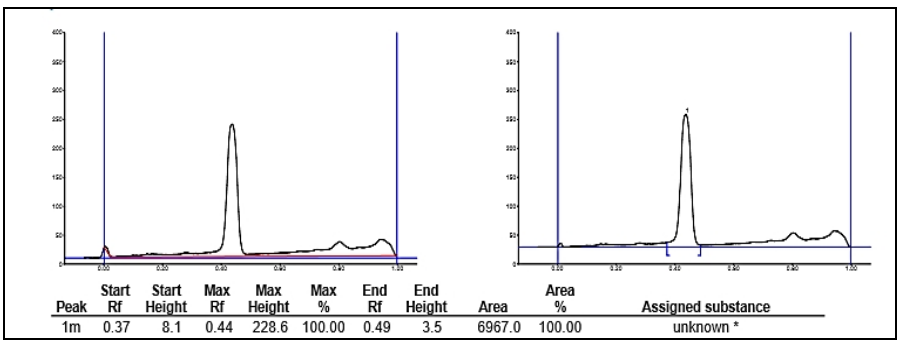
Extraction conditions

Each plant sample was accurately weighed (0.2 g) and extracted in methanol (5.0 mL). The mixture was vortexed for 1 minute and kept standing overnight. The mixture was filtered through Whatmann filter paper no. 41 and the filtrate obtained was diluted with methanol in equal proportion (1:1) and subjected to HPTLC analysis. (Regional, species, seasonal variation) The mobile phase constituted of Toluene: Methanol: 8: 1 (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 minutes. Their respective chromatograms are shown in the Figure.



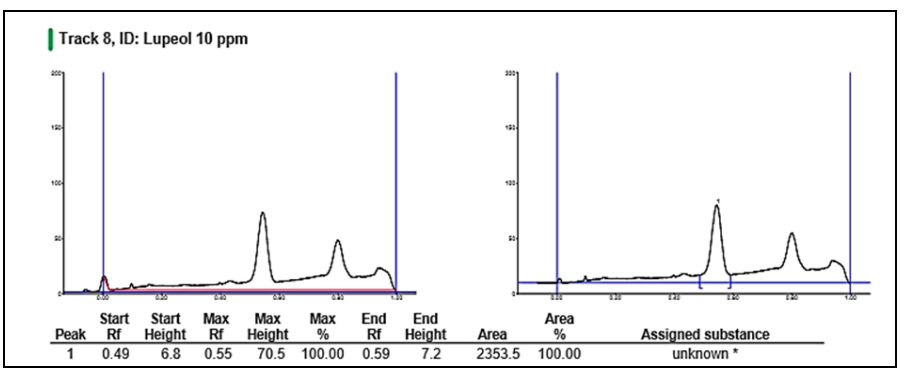
Track 1 Kutch, Track 2 Jamnagar, Track 3 Andhra Pradesh , Track 4 Jodhpur, Track 5 Rajkot, Track 6 Beta-sitosterol, Track 7 Lupeol

HPTLC plate photo representing regional variation of roots of *T. cuneifolia* with standards β-sitosterol and Lupeol Plate Photo at 336 nm



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1m	0.37	8.1	0.44	228.6	100.00	0.49	3.5	6967.0	100.00	unknown *

Figure 1: Chromatogram of β-sitosterol



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.49	6.8	0.55	70.5	100.00	0.59	7.2	2353.5	100.00	unknown *

Figure 2: Chromatogram of lupeol

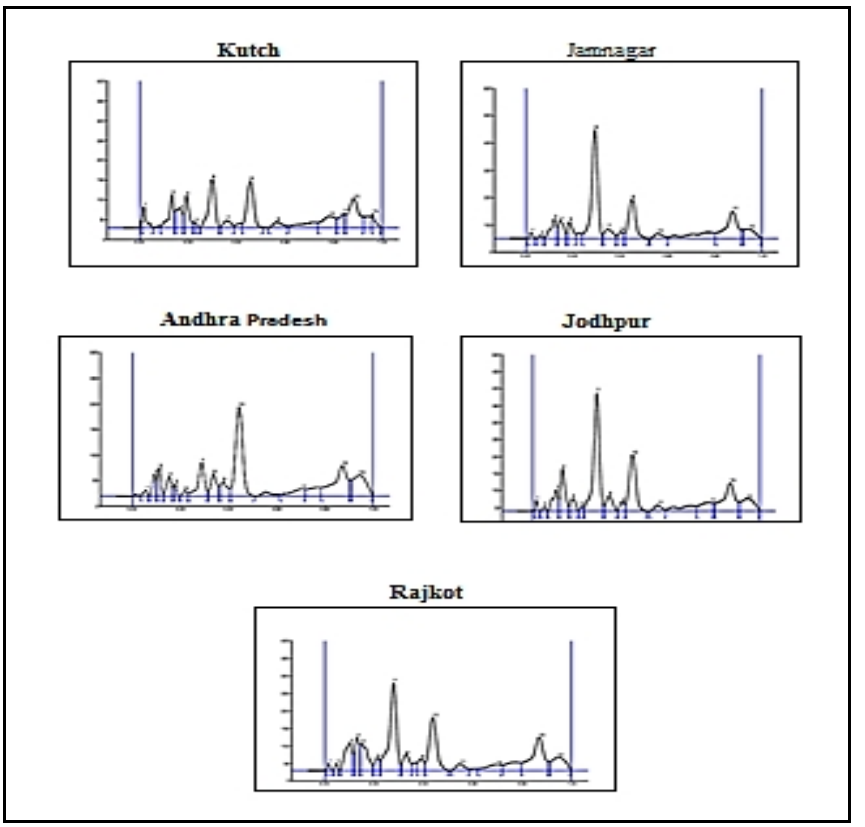
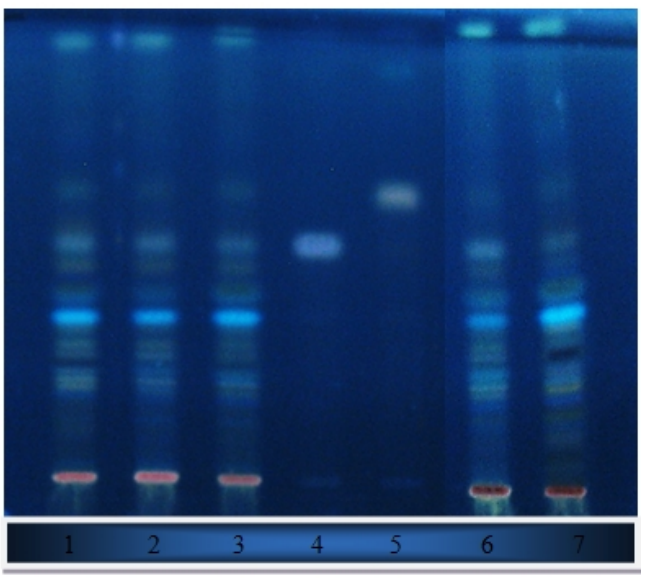


Figure 3: Chromatograms of *T. cuneifolia* roots from different geographical locations

HPTLC: Seasonal variation of *T. cuneifolia* and species variation of the roots of genus *Taverniera*

Plate Photo at 336nm



Track 1 *T. cuneifolia* summer (1:1), Track 2 *T. cuneifolia* monsoon (1:1), Track 3 *T. cuneifolia*winter (1:1), Track 4 betasitosterol 10 ppm, Track 5 lupeol 10 ppm, Track 6 Rajkot (*T. cuneifolia*) (1:1), Track 7 *T. abyssinica* (1:1)

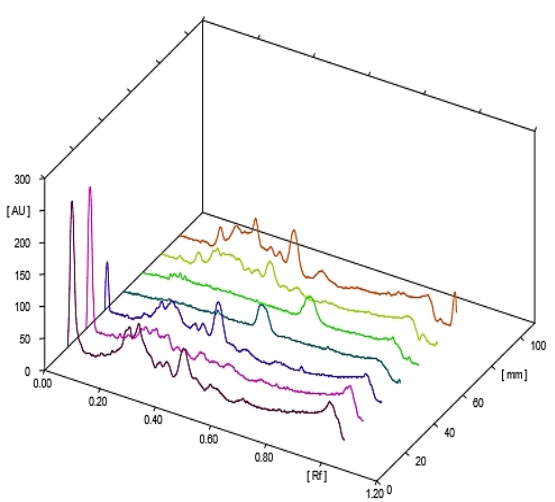


Figure 4: Spectrum of Seasonal variation

RESULTS AND DISCUSSION

Regional Variation

In this section the roots collected from different regions i.e. Kutch, Rajkot, Jamnagar, Jodhpur and Andhra Pradesh, India were compared with the standards of β-sitosterol and lupeol content. In Gujarat, only Kutch, India showed presence of lupeol both the other regions (Rajkot and Jamnagar) showed presence of both β-sitosterol and lupeol. Outside Gujarat, Jodhpur had presence of β-sitosterol and lupeol but Andhra Pradesh showed presence of only β-sitosterol. Andhra Pradesh has the highest amount of area percentage of β-sitosterol with 4.45 % and least is in Jodhpur with 1.90 %. Kutch has highest amount of lupeol (2.99 %) and lowest is in Jodhpur, India with 2.01 %.

Table 1: Regional variation of *T. cuneifolia* roots with β-sitosterol and lupeol

	Jamnagar	Rajkot	Kutch	AP	Jodhpur
β-sitosterol –	1.98 %	2.64 %	-	**4.45 %**	1.90 %
lupeol –	2.44 %	2.21 %	**2.99 %**	-	2.01 %.

Seasonal Variation

In this section of seasonal variation of roots, the study was done on summer, monsoon, and winter. All seasons showed presence of β-sitosterol but only summer season showed presence of lupeol. Monsoon season showed highest amount of β-sitosterol (7.08 %) and least was found in summer season with 2.64 %. Winter showed moderate amount.

Table 2: Seasonal variation of *T. cuneifolia* roots with β-sitosterol and lupeol

	Winter	Summer	Monsoon
β-sitosterol	3.89 %	2.64 %	**7.08 %**
Lupeol	-	1.42 %	-

Species Variation

Roots of two species of *Taverniera* i.e. *T. cuneifolia* and *T. abyssinica* were compared for their β-sitosterol and lupeol content. B-sitosterol concentration is higher in *T. cuneifolia* as compared to *T. abyssinica* while Lupeol was absent in both the species. Though lupeol is present in *T. cuneifolia* in summer, the content of lupeol is not present in the plant material collected during monsoon period.

Table 3: Species variation of *Taverniera* roots with β-sitosterol and lupeol

	T. abyssinica	*T. cuneifolia*
β-sitosterol -	6.89 %	**10.04 %**
Lupeol	-	-

CONCLUSION

Taverniera cuneifolia grows in all kinds of climatic variations and thus β-sitosterol is found to be present in all regions except Kutch. Lupeol is present in all regions except Andhra Pradesh. All seasons showed presence of β-sitosterol but only summer season showed presence of lupeol. B-sitosterol concentration is higher in *T. cuneifolia* as compared to *T. abyssinica* while Lupeol was absent in both the species. Thus, HPTLC analysis of dried roots of *Taverniera cuneifolia* can provide standard fingerprints and can be used as a reference for the identification and quality control of the drug. Such finger printing is useful in differentiating the species from the adulterant and act as biochemical markers for this medicinally important plant in herbal industries and plant systematic studies.

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