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Research Article

HPTLC METHOD FOR THE DETERMINATION OF LUPEOL FROM *CALOTROPIS PROCERA* ROOT BARK AND *NYCTANTHES ARBORTRISTIS* LEAVES

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ABSTRACT

Objective: Development of a simple, rapid, selective and quantitative HPTLC method for determination of lupeol from root bark of *Calotropis procera* R. Br. and leaf of *Nyctanthes arbortristis* Linn.

Methods: Standard curve for lupeol was obtained by applying different concentration of standard lupeol solution. Different concentration of standard lupeol along with the hexane extracts of *Calotropis procera* root bark and *Nyctanthes arbortristis*. Linn. leaf were applied on TLC aluminium plate precoated with Silica gel60 GF254 and developed using benzene : chloroform (1:1) v/v as a mobile phase. The plate was sprayed (derivatized) with 5% v/v aqueous sulphuric acid reagent followed by heating for 10 min. Detection and quantification were carried out by densitometry at wavelength of 366 nm. Percentage of lupeol in both samples was calculated using standard curve. The developed method was validated in terms of linearity, precision, repeatability and accuracy.

Results: Percentage of lupeol found in the root bark of *C. procera* and leaf of *N. arbortristis* was 0.165 % w/w and 0.503 % w/w respectively. A good linearity was obtained for lupeol in the range of 0.1-0.6 µg/spot. The mean recovery was close to 100%, which indicates the accuracy of the method proposed. The method was also found specific and reproducible. The limit of detection and limit of quantification were found to be 12.4 ng/spot and 37.57 ng/spot respectively.

Conclusion: The method was found to be simple, precise, accurate, specific, reproducible and can be used for routine quality control of *C. procera* root bark and *N. arbortristis* Linn. leaf powder, also for the quantification of lupeol in plant materials.

Keywords: Lupeol, *Calotropis procera*, *Nyctanthes arbortristis*, HPTLC

INTRODUCTION

The genus *Calotropis* is distributed in tropical and subtropical region of Asia and Africa, while in India it is represented by two species viz. *Calotropis procera* and *Calotropis gigantea* [1]. *Calotropis procera R.Br* Syn. *Calotropis hamiltonii,* wight or *Asclepias procera,* willd (Family- *Asclepediaceae*) known vernacularly as Ak, Akado, Nani rui is a small, erect, compact shrubs, covered with cottony tomentum up to 5.4 m in height found growing throughout india in cooperatively drier and warmer areas up to altitude of 1.050 m. Traditionally, the plant has been used as antifungal, antipyretic and analgesic [2-4]. The root bark powder is effective in diarrhea [5] and asthma [6]. The previous pharmacological studies include reports of anticancer [5]. and antifungal activity [7] of *C. procera*. The flowers of the plant are also reported for its hepatoprotective activity, anti-inflammatory, antipyretic, analgesic, antimicrobial properties and larvicidal activity [8-10]. Phytochemically, the plant contains triterpenoids, calotropursenyl acetate and calopfriedelenyl; a norditerpenyl ester, calotropternyl ester, oleanene triterpenes like calotropoleanyl ester, proceroleanenol-A and B [11], cardiac glycosides calotropogenin, calotropin, uscharin, calotoxin calactin, anthocyanins [5] and cardenolides [12]. The root bark also found to possess α-amyrin, lupeol, β-sitosterol, β-amyrin and flavanols like quercetin-3-rutinoside [13-15].

Nyctanthes arbortristis Linn. (Family- *Oleaceae*) commonly known as night jasmine or coral jasmine occurring wild in the sub-himalayan region from chennai to nepal up to 1500 m and in chota nagpur, rajasthan, madhya pradesh and south to godavari and vernacularly known as Harsinghar, Seoli or Jayaparvati [16]. The leaves decoction of the plant used by ayurvedic physicians for the treatment of arthritis, obstinate sciatica, malaria, cholagogue, laxative and as a tonic [16]. In India fresh leaf of the plant is being used as a remedy for intestinal worms in ayurvedic and unani medicine for the treatment of ringworm and other skin diseases [17]. The plant has also been proved for its leishmanicidal, analgesic, antipyretic, anti-allergic, antimalarial, amoebicidal and antiimflamatory activity scientifically [18-23]. Phytochemically, the plant has been investigated for the presence of flavanoids, triterpenes, iridoid monoterpenes, steroids, tannins and alkaloids. Plant showed presence of iridoid glucosides, arbortristosides A, B, C, and 6-b-hydroxyloganin [24], a phenyl propanoid glycoside and nyctoside-A (18) [25]. Leaves of plant contain iridoid monoterpenes, arborside A, B, C [26] and D [27], nyctanthoside, triterpenes like lupeol, friedelin, nyctahnthic acid and steroid like β-sitosterol [28].

Lupeol is the common active principle present in both the plant samples and along with other phytoconstiuents it may attributes hepatoprotective and antioxidant activity of both plants. Literature survey reveals that no HPTLC method as yet is reported for the determination of lupeol in *Calotropis procera* R.Br. and leaf of *Nyctanthes arbortristis* Linn. In the present study a simple, rapid, economical, precise and accurate HPTLC method has been established for the determination of lupeol in root bark of *C. procera* and leaf of *N. arbortristis*. This method can be used for phytochemical profiling of *C. procera* root bark, leaf of *N. arbortristis* and quantification of lupeol.

MATERIAL AND METHOD

Plant material

Fresh, well-developed plants of *C. procera* and *N. arbortristis* were collected from Rajkot, Gujarat, in the month of september-2007. The authenticity of plants was confirmed by a taxonomist of Gujarat Ayurveda University, Jamnagar, Gujarat. Voucher specimen (HNS 11) and (HNS 12) were deposited in the department of Pharmacognosy, Shri H. N. Shukla Institute of Pharmaceutical Education and Research, Rajkot, Gujarat.

Equipments

Camag Linomat IV (semiautomatic spotting device), Cammag Twin Trough Chamber (size 20x10 cm) with SS lid, Cammag Dipping Chamber, TLC, TLC Scanner 3, Aluminium pre-coated plate with Silica gel 60 GF254 (size 10X10cm; 0.2 mm thick) E. Merck.

Chemicals

Analytical grade; Methanol, n-Hexane, ethyl acetate, Benzene, Chloroform, Sulphuric acid were used, obtained from S. D. Fine

Chem. Ltd. (Mumbai, India). TLC aluminium pre coated plate with Silica gel 60 GF254 (10X10 cm2; 0.2 mm thick) used were obtained from E. Merck Ltd. (Mumbai, India) and standard lupeol obtained as a gift sample from S. J. Thakkar college of pharamacy, Rajkot, India.

Experimental

Sample preparation

Sample solutions were generated using root bark and leaf powder (*C. procera* and *N. arbortristis*) as per the following procedure. 100 g of dried powder of above plants extracted exhaustively using methanol (250 ml × 2). The marc and filter paper are washed with 10 ml of methanol each time. The methanolic extract of the root bark of *C. procera* (MCP) and leaves of *N. arbortristis* (MNA) obtained were concentrated and dried. Further, phytoconstituents directed fractionation was carried out using concentrated MCP and MNA extracts, suspended in water, acidified with 2N sulphuric acid and sequentially partitioned with n-hexane (HCP and HNL respectively) and ethyl acetate (ECP and ENL respectively) separately. The acidic layer was basified with dilute ammonium hydroxide (pH-10) and extracted with chloroform (CCP and CNL). The n-hexane and ethyl acetate fractions of both the plant materials were concentrated and dried and used for analysis. 100 mg of the n-hexane extracts of both the samples weighed accurately and dissolved in 10 ml of n-hexane in two separate volumetric flasks (10 mg/ml).

Standard preparation

5mg of standard lupeol dissolved in 5ml of n- hexane and made up to 5 ml in standard volumetric flask. From this 1 ml was diluted up to 10 ml with n- hexane in a volumetric flask to give a final concentration of the standard solution (100µg/ml).

Calibration curve of lupeol

From standard lupeol solution (100 µg /ml), 1, 2, 3, 4, 5 and 6 µl per spot were spotted on methanol washed silica gel G 60 F_{254} TLC plates (E. Merck) with camag Linomat IV automatic spotter.

Estimation of lupeol in *C. procera* and *N. arbortristis*

HPTLC method was developed to determine lupeol content and to develop fingerprinting for root bark of *C. procera* and leaves of *N. arbortristis*. 10 µl of test sample solutions of root bark of *C. procera* and leaves of *N. arbortristis* were used for spotting.

Chromatography

Cammag Twin Trough Glass Chamber (20 x 10cm2) with SS lid was used for development of TLC plate. The twin trough glass chamber was saturated with mobile phase for 30 minutes. TLC plate was developed in mobile phase Benzene: Chloroform (1:1) V/V. The plate was removed from the chamber and air dried at room temperature. This plate was sprayed (derivatized) with 5% sulphuric acid reagent followed by heating at 110^0C for 10 minutes and the plate was scanned at 366 nm after derivatization using Camag TLC scanner III. Calibration curve of peak area vs. concentration of lupeol was plotted. Concentration of lupeol in root bark of *C. procera* and leaves of *N. arbortristis* was calculated by using standard curve.

Validation of HPTLC method

The method was validated in terms of linearity, precision, repeatability and accuracy. The range of the concentration of the lupeol was determined for the linearity and expressed in terms of correlation co- efficient (r^2) of the linear regression analysis. The intra-day precision was determined by analyzing lupeol for three times on the same day. The inter-day precision was determined by analyzing lupeol daily for 3 days. Results were expressed as co-efficient of variance (% CV). Instrumental precision was checked by repeated scanning of the same spot of lupeol seven times and expressed as % CV. The repeatability of the method was affirmed by analyzing 3 µl/spot of lupeol after application on TLC plate (n=7) and was expressed as % CV. Accuracy of the method was determined by performing recovery studies at three levels (50, 100, and 150 % addition) and the percent recovery was calculated. The LOD and LOQ were separately determined based on the calibration curves. The standard deviation of the y-intercepts (σ) and slope of the regression lines (S) were used. These values were calculated using following formula,

$$LOD = 3.3 \times \sigma / S, LOQ = 10 \times \sigma / S$$

RESULTS AND DISCUSSION

In the present study, lupeol was detected and quantified in *C. procera* root bark and *N. arbortristis* Linn. leaf using HPTLC. Chromatographic plate is shown in Fig.1 with its respective chromatogram in Fig.2. Lupeol resolved as a light blue colour band at Rf. 0.25 very efficiently from the other components in n-hexane extracts of *C. procera* root bark and *N. arbortristis* Linn. leaf. The identity of the band of lupeol in both the plant extracts was confirmed by overlaying the chromatogram of plants with that of the standard lupeol and by comparing their Rf value. The spectra of standard lupeol spot and lupeol in samples were also found to be similar (Fig.3).

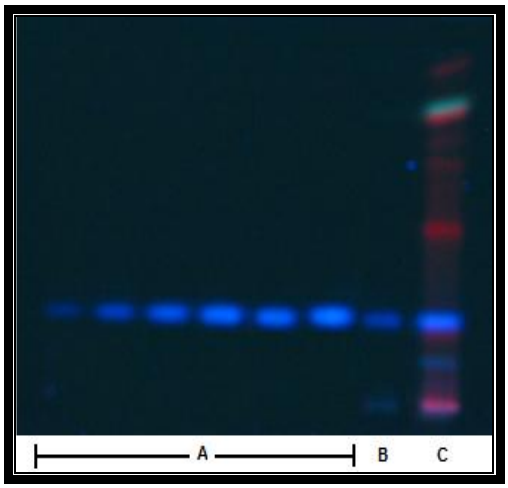

Fig. 1: Chromatographic plate of lupeol and extracts

A: Standard lupeol solution (0.1 to 0.6 µg /spot)

B: n-hexane extract of *C. procera* root bark

C: n-hexane extract of *N. arbortristis* leaves

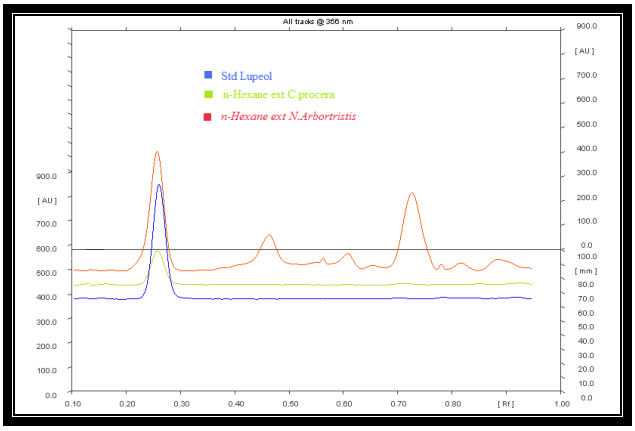

Fig. 2: Chromatogram of lupeol and extracts.

Calibration curve of standard lupeol was obtained. Densitometric chromatogram of calibration curve is shown in Fig.4. As shown in Fig.5, graph was plotted using the peak area against concentration of lupeol and the percentage of lupeol found in samples via graph was 0.165 % w/w and 0.503 % w/w in the root bark of *C. procera* and leaf of *N. arbortristis* respectively. The proposed method was validated in terms of linearity, precision, repeatability and accuracy. The detection of lupeol was observed to be linear over a concentration range of 0.1-0.6 µg/ with a good correlation coefficient (r^2) value, linearity data is shown in Fig.5.

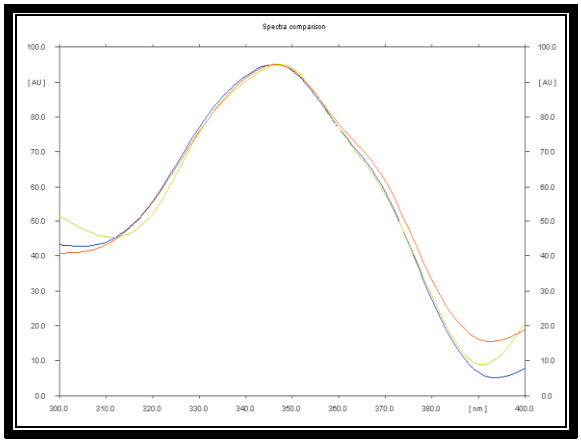

Fig. 3: Spectra of lupeol and extracts at 366nm

Blue: Standard lupeol, Yellow: n-hexane extract of *C. procera,* Red: n-hexane extract of *N. arbortristis*

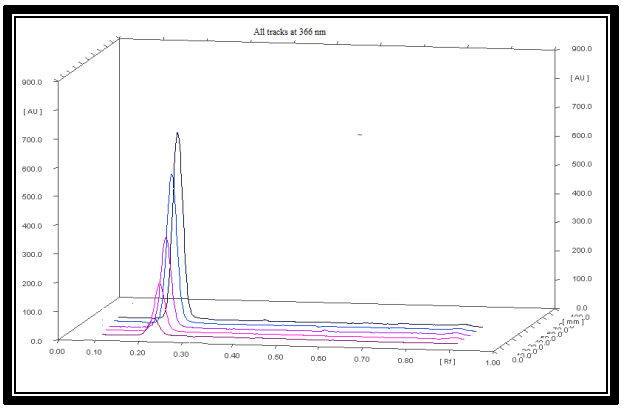

Fig. 4: Chromatogram of calibration curve of lupeol

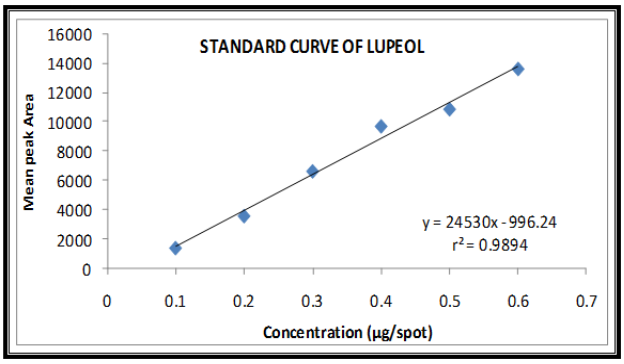

Fig. 5: Concentration vs mean peak area

Instrument precision, intraday assay precision, interday assay precisions were measured to evaluate the precision of the method and the % CV values were found to be less than 2% (Table 1), indicating that the selected method is precise and reproducible. The accuracy of the method was established by means of recovery experiment. The mean recovery was close to 100%, which indicates that the method is efficient. The mean recovery of lupeol of both the samples is given in Table 1. It was observed that the other constituents of root bark and leaf did not interfere with the peak of lupeol. Therefore the method was specific. The limit of detection and limit of quantification were also calculated separately based on calibration curve and shown in Table 1.

Table 1: Summary of validation parameters

S. No.	Parameters	Results
1	Linearity	0.9894
2	Precision (% C.V.)	
	▪ Repeatability of Measurement	0.143 0.206
	▪ Repeatability of Application	0.54 – 0.80%
	▪ Interday	0.21 – 0.74%
	▪ Intraday	
3	Limit of Detection	12.4 ng/spot
4	Limit of Quantification	37.57 ng/spot
5	Accuracy	*C. procera:* 99.2– 100.1% *N. arbortristis:* 98.11–99.8%
6	Specificity	Specific

CONCLUSION

The proposed HPTLC method is simple, rapid and accurate, reproducible and economic for the quantitation of lupeol in *C. procera* root bark and *N. arbortristis* Linn. leaf powders. The method can be used for routine quality control of *C. procera* root bark and *N. arbortristis* Linn. leaf powders and also for the quantification of lupeol in plant materials.

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