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Development and validation of HPTLC method for analysis of ursolic acid from methanol extract of *mimosoups elengi* L

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Abstract: *Mimusops elengi* L. (ME) is a small to large evergreen tree found all over the different parts of India. Bark of ME possesses cardio tonic, alexipharmic, stomachic, anthelmentic and astringent activity. The present investigation is an attempt to develop and validate the principal chemical constituent like ursolic acid by HPTLC. Linear ascending development with toluene: ethyl acetate: formic acid 14:7:1 (*v*/*v*) as mobile phase was performed and the UA was satisfactorily resolved with R_f value about 0.75 minute. The method is found to be precise as values were found to be 1.23 %, 1.01 % and 1.79 % respectively for system precision, method precision and intermediate precision. The proposed method is found to be accurate at 80%, 100% and 120% with average recovery of 98.25 %, 98.96% and 100.95% respectively. The proposed HPTLC method is simple, rapid, specific and accurate techniques to quantify the Ursolic acid in the presence of other constituents from the methanol extract of ME bark without compromising the accuracy.

Introduction

Mimusops elengi L. (ME) (Sapotaceae) ("Syn.": Bakul, aulsari) is a small to large evergreen tree found all over the different parts of India. It has been used in the indigenous system of medicine for the treatment of various ailments. Bark of *Mimusops elengi* (ME) possesses cardio tonic, alexipharmic, stomachic, anthelmentic and astringent activity [1]. Phytochemical review reveals the presence of ursolic acid, taraxerol, taraxerone, betulinic acid, α-spinosterol, β-sitosterol glycoside, quercitol [2], lupeol [3], alkaloid isoretronecyl tiglate [4] and mixture of triterpenoid saponins [5] in the bark of ME. It is a rich source of tannin, saponin, alkaloids, glycoside, and ursolic acid. ME is appeared to be a rich source of natural antioxidant. The present investigation is an attempt to develop and validate the principal chemical constituent like ursolic acid (UA) by HPTLC method that will help to correlate the chemical constituent with the probable pharmacological activities.

Materials and Method

Bark of ME was collected from Pune (India). The plant was authenticated by the Botanical Survey of India, Pune. The voucher specimen no. is MEGG1. The bark was air-dried at room temperature. Analytical grade solvents toluene, ethyl acetate, methanol, and formic acid were obtained from Merck (Mumbai, India). Standard ursolic acid (Potency 90%, B. No U6753) was purchased from Sigma–Aldrich (Bangalore, India). UA standard was weighed and dissolved in methanol to furnish 50 µg mL−1 stock solutions which was used as working standard for chromatographic analysis.

Sample Preparation

Dried powdered bark of ME (100 gm) was extracted with methanol in a soxhlet apparatus. The extraction was continued until solution from the siphon tube become clear. This extract was concentrated under vacuum by rotary evaporation and the percentage yield was calculated. The extract was used for quantitative analysis and for validation of the HPTLC method.

Chromatographic Conditions

HPTLC was performed on 20 cm × 10 cm aluminium foil plates coated with 0.2 mm layers of silica gel 60 F254 (Merck, India). Samples were applied as bands 6 mm wide by means of a Camag (Switzerland) Linomat 5 applicator equipped with a 100 µL microlitre syringe. Linear ascending development with 20 mL toluene: ethyl acetate: formic acid 14:7:1 (*v*/*v*) as mobile phase was performed in a 20 cm × 10 cm glass twin trough chamber previously saturated

with mobile phase for 15 minute. The development distance was approximately 80 mm from the point of application. After development, the plates were dried in hot air oven at 60°C and then it was sprayed by anisaldehyde reagent. The plate was dried at 105°C for 10 minute. Densitometric scanning at 550 nm was performed by using Camag TLC scanner 3 in absorbance mode operated by WinCATS software (V 1.4.3.6336). The slit dimensions were 5 mm × 0.45 mm and the scanning speed was 20 mm s−1.

Linearity Plot

Linearity was done by peak area with linear regression. The stock solution of UA was diluted to seven different concentrations in the range 50–150% of the working concentration in duplicate, to HPTLC plate. The linearity plot was prepared by plotting the concentration of the standards against average peak area after scanning at 550 nm.

Validation of the HPTLC Method

Preparation of Test Solution of Extract

100 mg of methanol extract of ME was accurately weighed and added to 15 mL methanol. It was allowed to sonicate for 30 minutes. The final volume was adjusted up to 20 mL and the solution was filtered through whatman filter paper no 41. This resulting solution was used as test solution.

Validation of HPTLC Method

International Conference on Harmonization (ICH) guidelines were followed for the validation of the HPTLC method. The proposed HPTLC method was validated in terms of precision, specificity, accuracy, solution stability and robustness as per the guidelines.

Precision

The precision of the system was carried out by six replicate band from the same solution of standard at the working concentration and it was expressed in terms of percent relative standard deviation, % RSD (Acceptance criterion: %RSD should not be more than 2.0%).

Six different samples of the extract were prepared and analyzed for method precision. The percent assay of UA and percent RSD was calculated (Acceptance criterion: %RSD should not be more than 2.0%).

The intermediate precision was carried out on two different systems for six different samples by two different analysts. The UA content and overall percent %RSD was calculated (Acceptance criterion: %RSD should not be more than 2.0%). (Table 1)

Specificity and Accuracy

The specificity of the method was determined by analyzing diluent, standard and extract sample of equivalent concentration to examine the interference of diluent with peak of UA. The specificity of the method was studied by assessment of peak purity of UA using Wincat software (Table 1). The accuracy of the method was determined from recovery studies. A known but varying amount of UA was spiked to pre-analyze sample at 80%, 100% and 120% recovery levels of the working concentration in triplicate. The samples were analyzed according to the proposed method. The percentage recovery was calculated against respective level (Acceptance criterion: % recovery should be in the range of 98%-102%). (Table 2)

Solution Stability and Robustness

The sample solution was prepared as per the proposed method and subjected to stability study at room temperature for 24 hrs. The sample solution was analyzed at initial and at different time intervals up to 24 hrs. The change in peak area response of UA in sample solution with respect to time was calculated as absolute percent difference against initial response. (Table 1)

Robustness was assessed by making small changes to the amount of the polar component of the mobile phase (±0.1 mL) and examining the effect on the results. (Table 1)

Assay of UA from Extract

The percentage of UA in methanol extract of ME barks was determined as per the HPTLC method described under chromatographic conditions. The analysis was carried out in triplicate.

Results and Discussion

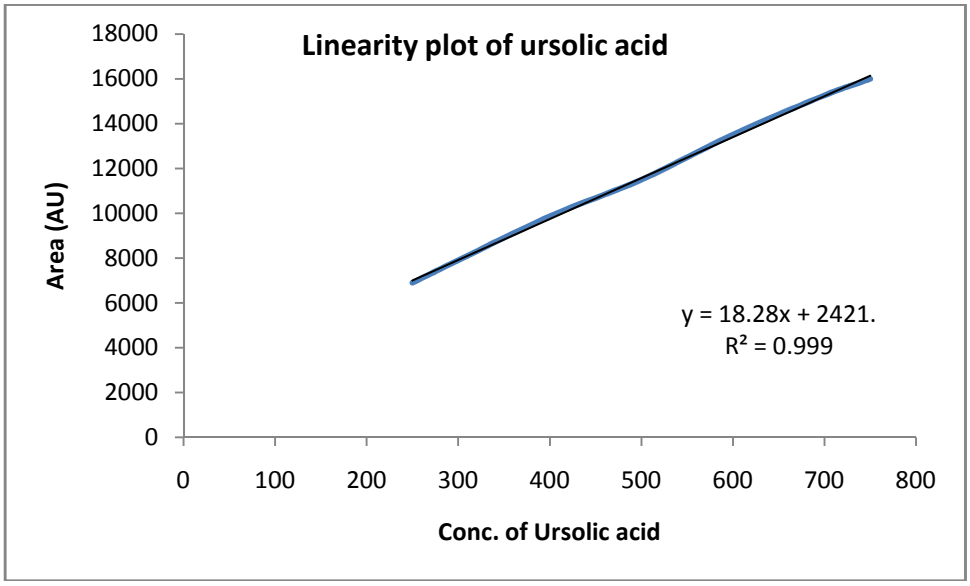
Percent Yield of Methanol Extract

The extraction was carried out by hot soxhlet extraction method and yield was found to be 19% w/w.

Chromatographic Study

The composition of mobile phase in HPTLC method was optimized by testing different solvent compositions of varying polarity and the best results were obtained by using present method which produces highly symmetrical peaks showing good resolution between UA and other peaks. (Figure 2) The scanning wavelength selected was 550 nm for UA. At this wave length the UA showed optimum response. Peak purity was assessed by comparison of overlay spectra of standard UA and peak of UA from extract at the start, apex and end was found satisfactory (Figure 4). The UA was satisfactorily resolved with R_f value about 0.75 minute. (Figure 2, 3)

Figure 1 Linearity plot of ursolic acid



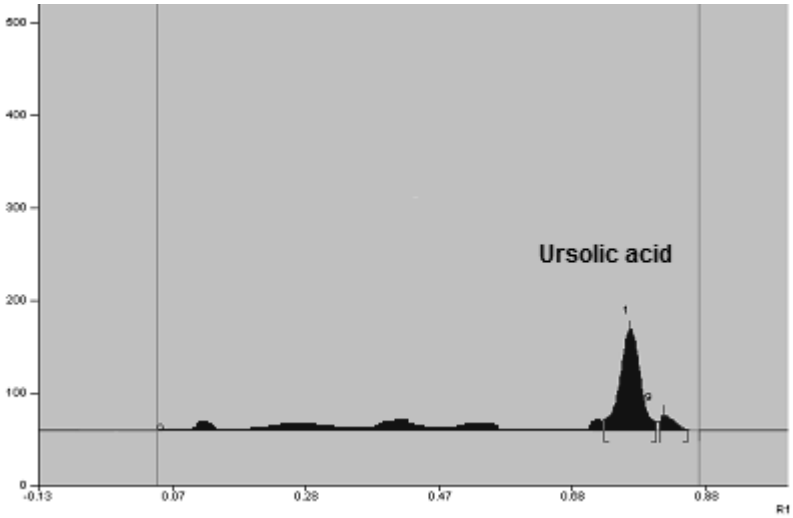


Figure 2 Chromatogram of Standard Ursolic acid

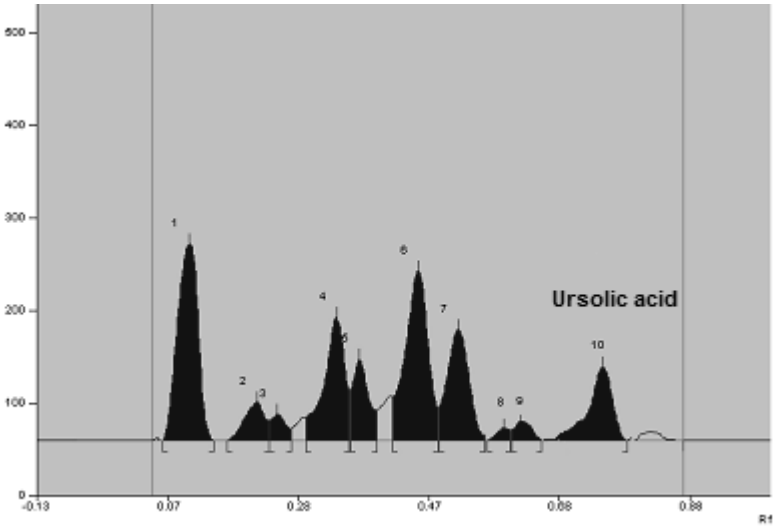


Figure 3 Chromatogram of Methanol extract

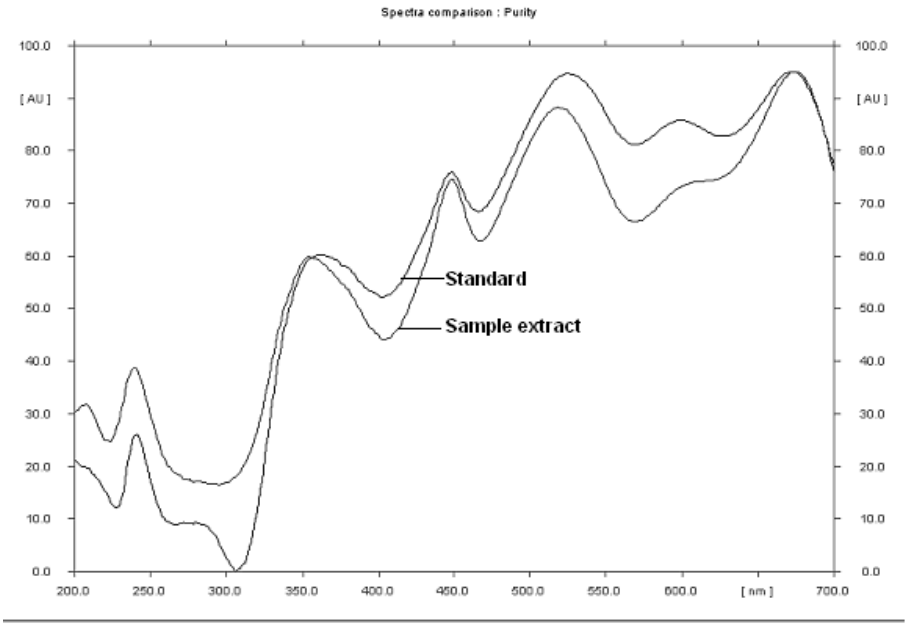


Figure 4 Spectra of Ursolic acid

Linearity Plot

The linearity of peak area against concentration was done and found to be linear in the range 250 -750 ng per band for UA. The plot was represented by linear equation y = 18.28x + 2421. For this equation the correlation coefficient, r^2, was r^2=0.9990**. (Figure 1)**

Precision

The values of system precision, method precision and intermediate precision are given against sample application, scanning of peak area and expressed in terms of %RSD. The values were found to be 1.23 %, 1.01 % and 1.79 % respectively for system precision, method precision and intermediate precision, the % RSD values depicted in **Table 1** showed that the proposed method provides acceptable level of system precision, method precision and intermediate precision.

Table 1: Method validation parameters for determination of Ursolic acid

Sr. No.	Parameters	HPTLC
1.	Specificity	Specific
2.	Linearity (correlation coefficient)	0.9990
3.	System precision. (% RSD) (n=6)	1.23
4.	Method Precision. (% RSD) (n=6)	1.01
5.	Intermediate precision. (% RSD) (n=6)	1.79
6.	Solution stability	Stable
7.	Regression equation	y = 18.28x + 2421

Table 2: Recovery study

Sr. no.	Recovery level	Amount added (mg)	Amount recovered (mg)	% Recovery	% Average Recovery
1	80% -1	0.78	0.768	98.56	100.18
	80% -2	0.78	0.778	99.81	
	80% -3	0.78	0.797	102.19	
2	100% -1	0.98	0.975	99.54	98.84
	100% -2	0.98	0.96	98.01	
	100% -3	0.98	0.969	98.97	
3	120% -1	1.18	1.161	98.31	98.87
	120% -2	1.18	1.175	99.58	
	120% -3	1.18	1.165	98.73	

Specificity

The peak purity of UA was assessed by comparing their respective spectra at peak start, peak apex, and peak end positions of the spot from standard and extracts (Figure 4).

Recovery

The recovery study was performed by spiking standard UA at 80%, 100% and 120% level of working concentration in the preanalysed sample and average recovery was found to be 98.25 %, 98.96% and 100.95% respectively as depicted in Table 1.

Solution Stability

The absolute percentage difference for the area of UA in sample solution to that of initial response does not exceed an acceptable limit upto 24 hours of preparation at room temperature indicating the stability of the sample solution.

Robustness

The given method was optimized by doing robustness and found to be robust for system suitability parameter.

Assay of UA from Methanol Extract

The percent content of UA was found to be 0.98 %w/w from methanol extract of ME bark.

The proposed HPTLC method is simple, rapid, specific and accurate techniques to quantify the Ursolic acid in the presence of other constituents from the methanol extract of ME bark without compromising the accuracy. Method validation proves that method is selective and reproducible for the estimation of Ursolic acid from ME.

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