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Original Article

Variation of Ursolic Acid Content in Four *Ocimum* Species from Four Different places of Maharashtra by HPTLC

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ABSTRACT

Ursolic acid is a very important compound due to its biological potential as an important drug. This study presents the HPTLC analysis of ursolic acid (UA) content in four different *Ocimum* species: *O.tenuiflorum, O. Basilicum, O. kilimandscharicum and O. gratissimum* collected from four different agriculture colleges of Maharashtra. UA was detected in different yields, with *O. Tenuiflorum* of *Akola* showing the highest content (0.14 mg/g).

Keywords: Ursolic acid; *Ocimum; Lamiaceae;* HPTLC.

INTRODUCTION

Ursolic acid (3β-hydroxy-urs-12-en-28-oic acid) is an ursane type triterpene (Figure 1) found in plants and has important biological activities like anti-inflammatory, trypanocidal, antirheumatic, antiviral [1], antioxidant, anti-tumoral properties [2-6].

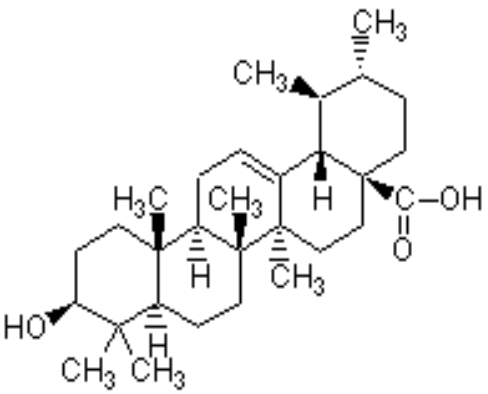

Figure1: Chemical structure of Ursolic acid.

Genus *Ocimum* of the Lamiaceae family is important, due to the extensive use of many of its species as economically important medicinal and culinary plants. Ursolic acid was earlier identified in only two species of *Ocimum*: *O. Basilicum* and *O. tenuiflorum* [7- 9]. This study presents the variation of ursolic acid content in four species of the genus *Ocimum*: *O. basilicum, O. gratissimum, O. tenuiflorum and O.kilimandscharicum* collected from different geographical places determined by HPTLC analysis.

METHODS

Preparation of methanolic extracts of *Ocimum* species: To the accurately weighed plant (leave) powders of *O.tenuiflorum., O. basilicum, O.kilimandscharicum and O. gratissimum* (500mg each) collected from 4 Agriculture colleges of Maharashtra "Dr. Panjabrao Deshmukh Krishi Vidyapeeth Akola(A), Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth Dapoli (B), Mahatma Phule Krishi Vidyapeeth Rahuri (C) and Keshav Srushti, Uttan krishi sanshodhan sanstha Bhayander, Mumbai (D). 10 ml of methanol was added, vortexed for one minute and the mixture was kept standing overnight. These mixtures were filtered using whatman paper no. 41 and the filtrates were used for HPTLC analysis.

Preparation of standard stock solution: 10.0mg of ursolic acid was accurately weighed and transferred to a volumetric flask of 10 ml (1000 µg/mL).

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Preparation of working standard solutions: Aliquots of ursolic acid in concentration range of 0.5 µg/mL to 250.0 µg/mL were prepared from stock solution in 10 ml volumetric flask and diluted with methanol. Quantity control sample 5.0 µg/mL (LQC), 35.0 µg/mL(MQC), and 100.0 µg/mL (HQC) were used for precision and recovery studies.

Table 1: Optimized chromatographic conditions for quantification of ursolic acid

Parameters	**Description**
Sample application volume	10µ
Band and space width	7 and 8 mm
Mobile phase/ solvent system	**Cyclohexane: chloroform: ethyl acetate 20:5:8.**
Development distance	8.5 cm
Chamber saturation time	20 minutes
Scanning detection lamp (wavelength)	Mercury (366nm)
Derivatizing reagent	Anisaldehyde sulphuric acid reagent

Validation of the Methods: ICH guidelines were followed for the validation of the analytical procedures (CPMP/ICH/281/95 and CPMP/ICH/381/95). The methods were validated for precision, repeatability, and accuracy. Instrumental precision was checked by repeated scanning (n = 3) of the same spot of ursolic acid and expressed as relative standard deviation (RSD).

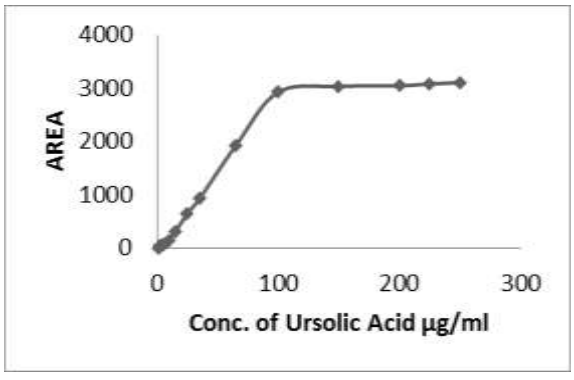
Specificity: The spot for ursolic acid in the samples was confirmed by comparing the R_f and color of the spot with that of an ursolic acid Std.

System suitability: System suitabitity was determined by spotting (10µ L) of ursolic acid *(*35 µg/mL) six times on the HPTLC plate and the plate was developed. The mean value of % CV for the AUC and R_f of ursolic acid was found to be 0.37 and 0.90 % respectively which is less than 2%, thus, proving that the System was suitable for the chromatographic analysis.

Sensitivity: The LOD and LOQ for ursolic acid were determined by measuring the signal to noise ratio (S/N). LOD and LOQ were considered at S/N of 3:1 and 10:1 respectively. The values for LOD and LOQ for ursolic acid were found to be *2.5µ*g/mL and *5.0*µg/mL respectively.

Linear Dynamic Range (LDR): The mean values of AUC, S.D and the percentage of coefficient of variation (% CV) for respective concentration (1 to 225 µg/mL) of ursolic acid is represented in Fig. 2. The acceptance criterion for % CV for AUC was considered to be ≤ 2% (Bansal and De Stefano, 2007). The graph of concentration of ursolic acid (X-axis) v/s their respective AUC values (Y-axis) was plotted and is represented in Figure 2. The R_f value of ursolic acid was found to be 0.45.

Figure 2: Graphical representation of linear dynamic range of ursolic acid



Linear Working Range (LWR): Seven aliquots from conc. 5 to 100 µg/mL were found linear. The analysis was carried out in triplicates. The mean values of AUC, S.D. and % CV for respective concentration of ursolic acid is determined. The graph of concentration of Ursolic acid (X.axis) v/s their respective AUC values (Y-axis) was plotted and is represented in Figure 3. A straight line fit was made through the data points by least square regression analysis and coefficient of determination (r^2) was determined.

Regression analysis: The regression analysis of the calibration data (5 to 100µg/mL) was carried out to determine the relationship between the dependent variable (peak area ratio) and independent variable (drug concentration). Regression equation is represented as y= mx+c, Where, y = dependent variable (peak area), m= slope of the regression line, x= independent variable (concentration) and c= intercept on y-axis. The regression equations and correlation coefficient for ursolic acid:
y= 30.80x + 132.5, r^2 = 0.999.The results of the regression analysis are represented in Table 2.

Figure 3: Graphical representation of linear working range of ursolic acid

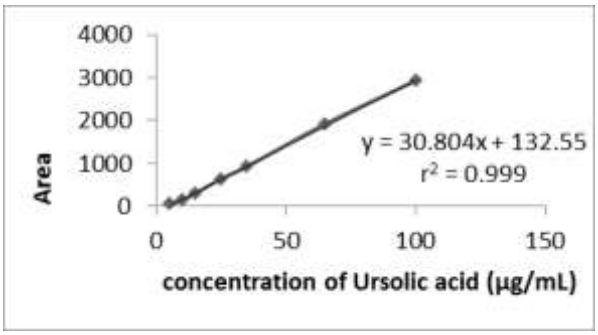


Table 2: Results of regression analysis for ursolic acid

Slope (m)	30.80
Intercept (c)	132.5
Coefficient of determination (r^2)	0.999

Precision (intra and inter day): The concentration of ursolic acid obtained after precision study was calculated by substituting the AUC values in the regression equation and its mean, S.D and % CV were calculated. Form this observed concentration of ursolic acid, % normal value for each of the level was calculated.

Formula: % Nominal = $\frac{\text{Observed value}}{\text{Actual value}} \times 100$

Recovery: Quality control samples of ursolic acid were spiked into Ocimum sample and extracted. Four different extracts namely blank (unspiked), LQC-spiked, MQC-spiked and HQC-spiked were obtained, the % recovery of the quality control samples was as:

$$\% \text{ Recover} = \frac{\text{Amount found}}{\text{Original amount}} \times 100$$

Table 3 : Result of recovery of ursolic acid from O.tenuiflorum, *O. basilicum*, *O. kilimandscharicum* and ***O. gratissimum***

Samples	Mean recovery
O.tenuiflorum	**98.80**
O. basilicum	**98.85**
O. kilimandscharicum	**98.41**
O. gratissimum	**98.28**

Assay: It provides result which allows an accurate statement on the content or potency of the analyte in any sample. The method was finally tested to evaluate the content of ursolic acid from ***O. tenuiflorum, O. basilicum, O. kilimandscharicum*** and ***O.gratissimum*** collected from different geographical regions using regression equation obtained from LWR. The assay experiment was repeated three times and the amount of ursolic acid present in one gram of plant samples, its S.D. and % CV were calculated (Table 4).

RESULT AND DISCUSSIONS

HPTLC fingerprinting is proved to be a linear, precise, accurate method for herbal identification and can be used further in authentication and characterization of the medicinally important plants. In the current work HPTLC fingerprint was developed form *O.tenuiflorum, O.basilicum, O.gratissimum and O.kilimandscharicum*. It revealed several peaks. The procedure was optimized for quantification of ursolic acid in methanolic extracts of *Ocimum* species. The mobile phase **cyclohexane: chloroform: ethyl acetate with ratio** (**20:5:8**V/V/V) gave good resolution, intense, compact and well separated spot of ursolic acid after derivatization using ASR reagent. The R_f value of ursolic acid was found to be 0.45 ± 0.03 respectively. Further developed HPTLC method for ursolic acid was validated as *per* ICH guideline. The content of ursolic acid was quantified by using regression equation from 4 *Ocimum* species collected from four different Agriculture colleges. Maximum content of ursolic acid was found in *O.tenuiflorum* species collected from different regions while least in *O.kilimandscharicum*. This clearly indicates that there is variation in phytoconstituents content due to graphical locations where these plants grow.

Figure 4. HPTLC-densitogram at 530 nm of *O. Tenuiflorum* with ursolic acid standard after derivatization with anisaldehyde-sulfuric acid reagent: (A) Sample Solution (Akola); (B) Sample Solution (Rahuri); (C) Sample Solution (Dapoli); (D) ursolic acid standard; (E) Sample Solution (Mumbai).

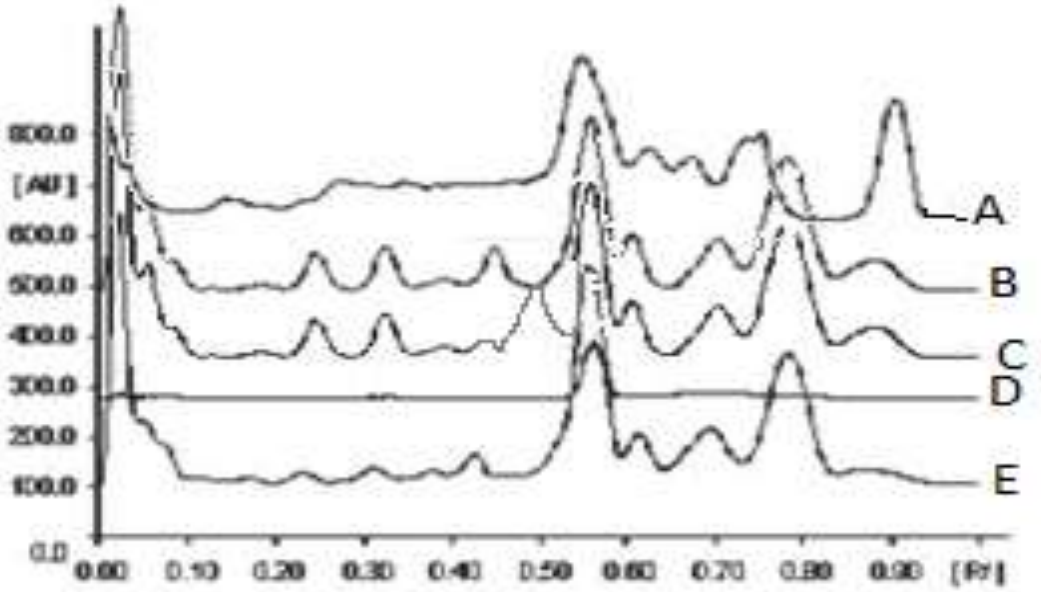
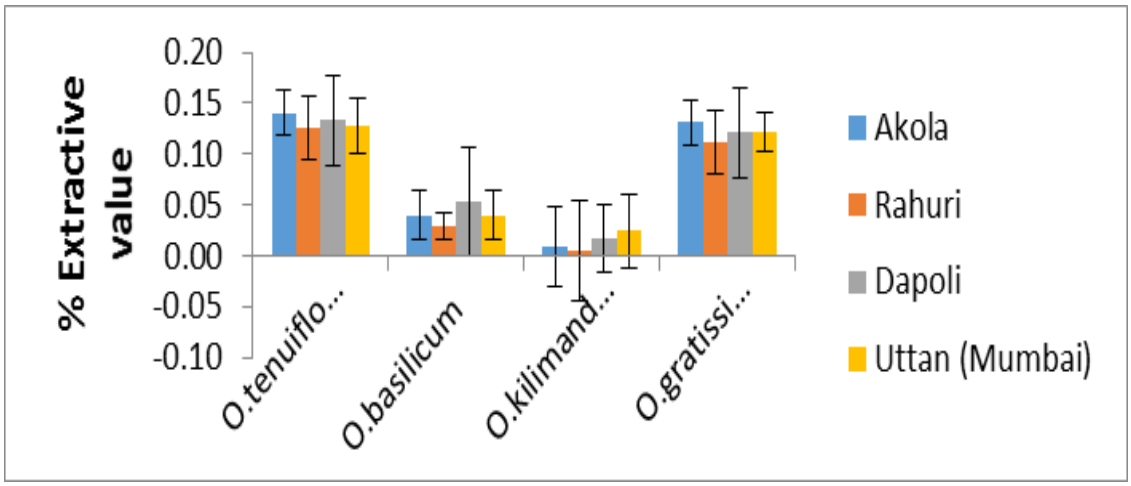


Table 4: Ursolic acid content in *O.tenuiflorum, O. basilicum, O.kilimandscharicum and O.gratissimum* collected from four different geographical regions

Amount of Solvent (mL)	Akola		Rahuri		Dapoli		Uttan (Mumbai)	
	%Mean±S.D.(n=3)	**% CV**	%Mean±S.D.(n=3)	**% CV**	%Mean±S.D.(n=3)	**% CV**	%Mean±S.D.(n=3)	**% CV**
OT	0.14±0.02	**1.92**	0.13±0.03	**1.42**	0.13±0.05	**1.20**	0.13±0.03	**1.29**
OB	0.04±0.02	**1.22**	0.03±0.01	**1.41**	0.05±0.05	**1.27**	0.04±0.02	**1.81**
OK	0.01±0.04	**1.96**	0.01±0.05	**1.24**	0.02±0.03	**1.52**	0.02±0.04	**1.64**
OG	0.13±0.02	**1.92**	0.11±0.03	**1.42**	0.12±0.05	**1.20**	0.12±0.02	**1.75**

Figure 5: Graphical representation of ursolic acid content in *O.tenuiflorum, O. basilicum, O.kilimandscharicum and O.gratissimum* collected from different regions.



CONCLUSION

In the current research work, HPTLC fingerprints were developed for each plant material. The proposed HPTLC method is simple, rapid, accurate, selective and economical and can be used for routine quality control analysis and qyantitative determination of ursolic acid in different *Ocimum* species as well as their polyherbal formulation.

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