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HPTLC evaluation of oleanolic acid and ursolic acid from the methanol extract of *Wattakaka volubilis*

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ABSTRACT

Objective: To find out the secondary metabolites present in the methanol extract of *Wattakaka volubilis* (*W. volubilis*). **Methods:** High performance thin layer chromatography method for the quantification of triterpenoids in soxhlet methanol extract of *W. volubilis* is described by densitometric scanning. The linear regression data from the calibration curve was plotted over the range of 10-25 μ g/mL, r^2 = 0.992 46, 0.950 42 respectively. A mixture of toluene: methanol (9:1) was used as mobile phase for oleanolic acid were petroleum ether: chloroform: ethyl acetate: methanol (4:1:0.1:0.1) were used for ursolic acid. **Results:** The results showed that the presence of oleanolic acid and ursolic acid in methanol extract. The content found to be 218.30 ng and 509.99 ng/10 mg of extract. **Conclusion:** For conclusion, above study scientifically validated as a useful traditional medicine with the identification of bioactive secondary metabolites.

1. Introduction

There has been an increasing interest in the study of medicinal plants as natural products to different parts around the world[1]. The medicinal value of plants depends on bioactive phytochemical constituents who produce definite physiological action in the human body. Based on World Health Organization recommendations, plant origin is important for use in traditional medicine[2].

Since ancient times, the medicinal properties of plants have been investigated in the recent scientific developments throughout the world. Although the original hopes regarding their therapeutic usefulness were not immediately realized, recent researchers have demonstrated their involvement in the medicinal action of a number of plant drugs. It is very probable that a number of herbal remedies, the constituents of which are still unknown. Quantitative estimation of these compounds is important for current research, and a variety of methods are required for this. *Wattakaka volubilis* (*W. volubilis*) is a medicinal member of the family Asclepiadaceae well-known for their ethnobotanical importance and widely used in Indian traditional medicines.

In detailed chemical investigation of the *W. volubilis* were reported that phenolic acids (chlorogenic acid and hydroxycinnamic acids), flavonol glucosides (rutin, kaempferol3-rutinoside, isorhamnetin 3-rutinoside) and triterpenoids[3-5]. GC-MS analysis of ethanol extracts of *W. volubilis* identified octadecatrienoic acid, *n*-hexadecanoiacid tetracosahexaene, diiooctylester, phytol, benzenedicarboxylic acid, 3-0-methyl-d-glucose, and octadecatrienic acid[6], multiflor-7-en-12, 13-ether and multiflor-7-en-12α-ol[7] were reported as the major components.

Consequently, present study was focused on the quantitative estimation in the leave methanol extract of *W. volubilis* for the identification of ursolic acid and oleanolic acid by high performance thin layer chromatography.

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2. Materials and methods

2.1. Chemicals and reagents

Toluene, methanol, petroleum ether, chloroform, ethyl acetate. The TLC plates $60F_{254}$, E. Merck were used without any pretreatment. All the chemicals and reagents procured were of A.R. grade.

2.2. Plant material and extraction

Leaves of *W. volubilis* were collected from Ooty, Tamil Nadu, India. It was identified by the Dr. Rajan, field botanist, Ooty. The leaves was dried and powered for soxhlet method of extraction (72 h) using methanol. The extract was then filtered and concentrated under vacuum to obtain crude form of methanol extract with this extract was taken for HPTLC analysis.

2.3. Standard stock solutions and sample preparation

Stock solutions of all standards (1 mg/mL) were prepared by dilution in ethanol, with the obtained working standard solutions were then applied in volumes of 10, 15, 20 and 25 μg/mL to RP-TLC plates to prepare linear calibration curves.

2.4. HPTLC analysis

Chromatography was performed on pre-activated (100 ℃) silica gel $60F_{254}$ HPTLC plates (10 cm × 10 cm; 0.25 mm layer thickness; Merck). The CAMAG densitometry (Camag Model-3 TLC scanner equipped with Camag CATS 4 software), a reflectance pectrometer of monitoring range 190-700 nm was employed for the analysis. The slit was set to 8 mm × 0.4 mm and data acquisition and processing were performed using the software win CATS.

Samples (10 μL) were applied to the layers at 8 mm wide bands, positioned 10 mm from the bottom of the plate, using a Camag (Mutten, Swizterland) Linomat IV automated TLC applicator with nitrogen flow providing delivery from the stringe at a speed of 10 mL/s was maintained for all analyses. TLC plate development was performed using a Camag twin-trough glass tank, which had been pre-saturated with mobile phase for 2 h. Solvent was allowed to run up the plate to a height of 8 cm.

TLC analyses were made under room temperature. A mixture of toluene: methanol (9:1) was used as mobile phase for oleanolic acid were petroleum ether: chloroform: ethyl acetate: methanol (4:1:0.1:0.1) were used for ursolic acid.

After development, the layers were dried and the components were visualized by UV light at 525 and 366 nm. The quantitative determination was performed by win CATS software program using the external calibration method[8].

3. Results

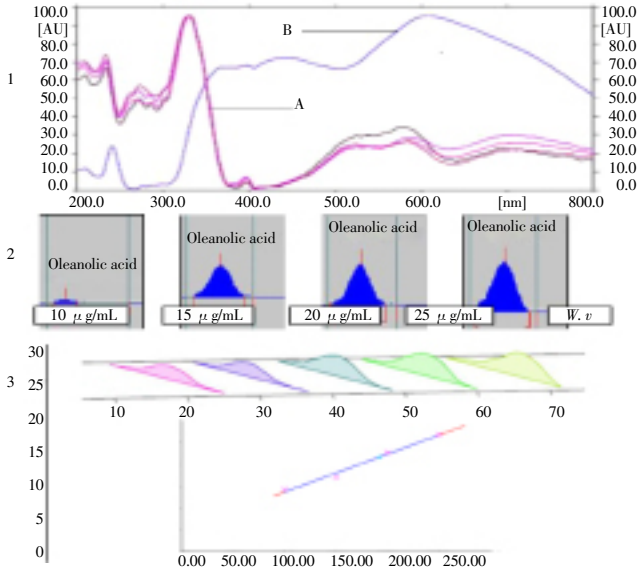

Figure 1. HPTLC profile: (1) UV-Visible spectrum of standard oleanolic acid (A) and *W. volubilis* (B); (2) chromatogram; (3) standard calibration curve of oleanolic aicd. Eluent: Toluene: Methanol (9:1 v/v); Detection: 525 nm.

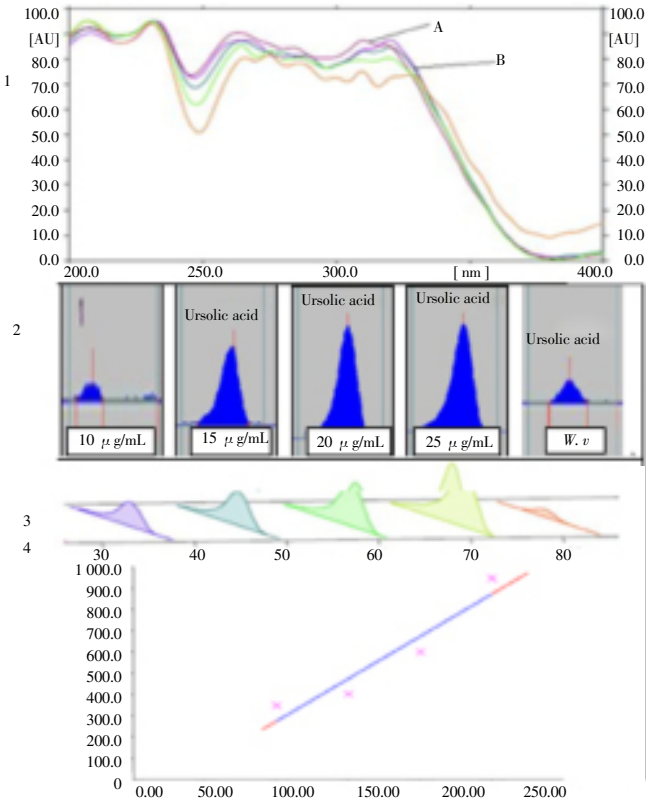

Figure 2. HPTLC profile: (1) UV-Visible spectrum of standard ursolic acid (A) and *W. volubilis* (B); (2) chromatogram; (3) standard calibration curve of ursolic acid. Eluent: petroleum ether: chloroform: ethyl acetate: methanol (4:1:0.1:0.1); Detection: 366 nm.

The identity of the bands of triterpenoids in the methanol extract was confirmed by comparing the UV-Vis absorption spectra with those of standards using a CAMAG HPTLC

Table 1
System precision, linear regression equation and R_f values of the developed method.

Standards	R_f	Regression equation	r*	Sdv	Standards	Oleanolic acid (Area)	ursolic acid (Area)	Amount of standards in crude fraction (ng)
Oleanolic acid	0.31	Height: Y=5.012+0.0867	0.992 46	4.19	100 ng	389.9	348.16	218.30
		Area: Y=186.100+2.004	0.996 18	2.59	150 ng	489.8	403.05	
Ursolic acid	0.49	Height: Y=-4.322+0.129	0.952 99	17.76	200 ng	570.6	600.67	509.99
		Area: Y=-123.700+3.992	0.950 42	17.96	250 ng	679.0	947.61	
					W. volubilis	592.9	1 933.12	

r* : Correlation coefficient; Sdv: standard deviation.

scanner. Comparison of the spectral characteristic of the peaks for standards and sample revealed the identity of standards in the methanol extract, qualitatively similar HPTLC fingerprints were obtained for the methanol extract from different locations giving reliable indication of the same identity Figure 1 & 2, is the illustration of the HPTLC scanned chromatogram of standard markers. The methanol extract was able to resolve 2 compounds in the developing solvent system by using mobile phase, which produced good separation with R_f values of methanol extract is 0.31 and 0.49. The desired resolution of two terpenoids, which showed the presence of oleanolic acid and ursolic acid peak were detected. The standard oleanolic acid has R_f value of 0.31, a good linear relationship (r^2 = 0.992 46 and 0.996 18 with respective to peak height and area respectively) was observed in 10-25 μg/mL, Linear regression equation, R_f, and standard deviation are given in Table 1.

The regression equation was found to be Y= 5.012+0.086 74X with respective to height and Y=186.1+2.004X with respect to area, where Y is the peak height/area and X is concentration of oleanolic acid, the amount in methanol extract was 218.30 ng/mL. System precision was performed by spotting four samples each from the standard stock solutions, the results of areas were given. A triterpene aglycone, ursolic acid used as reference substance R_f 0.49 have been identified in these TLC condition, after spraying with anisaldehyde, glacial acetic acid, sulphuric acid and ethanol (Figure 1).

The detection of ursolic acid was observed in the linear calibration curves of standard compounds were found to be linear over the range 100-250 ng/mL (Figure 2). When the content of the ursolic acid in the methanol extract were analyzed by linear regression by HPTLC method was observed height and area is r=0.952 99, 0.950 42, the amount of ursolic acid was found to be 509.99 ng/mL. Using the techniques of the HPTLC and UV Vis spectra the amount of standards in the methanol extract were found to 218.30 and 509.99 ng/mL after compare nearest matching peaks of standards (Table 1).

This indicates that the HPTLC method is reliable for good estimation of the unknown compounds in methanol extract. Therefore the method could be used for initial screening or semiquantitative analyses.

4. Discussion

In conclusion, the method used in this work resulted in good peak shape and enabled good resolution of terpenoids from other constituents of the methanol extract. Using HPTLC analytical method, oleanolic acid and ursolic acid could be determined simultaneously, and the validity of the method was also verified.

Conflict of interest statement

We declare that we have no conflict of interest.

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