Isolation and Quantificative Analysis of Oleanolic Acid from *Satureja mutica* Fisch. & C. A. Mey.

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Abstract

Background: ***Satureja mutica*** **(Lamiaceae) is an herbaceous plant which grows in North-West of Iran. Oleanolic acid has been previously isolated from some Lamiaceae genus and showed a broad range of biological activities. Besides *Silphium* spp., *Panax quinquefolium* root and *Calendula officinalis* flower as the interesting source of oleanolic acid used in the herbal industry, here we report the isolation and quantitative evaluation of oleanolic acid, as one of the major constituents in *S. mutica*.**

Methods: **Dried aerial parts of *S. mutica* were successively extracted with diethyl ether. This extract was used for further isolation on silica gel column chromatography to obtain compound 1. Isolated compound was identified based on the spectral data of ^{1}H- NMR, ^{13}C-NMR and Mass spectroscopy. Densitometric analysis of the developed plate of TLC was carried out to quantify the oleanolic acid using TLC scanner.**

Results: **Isolated compound (1) was structural elucidated as oleanolic acid and its NMR data showed good agreement with the reference data mentioned in literature. Spectrodensitometric analysis showed that the band characterized by absorption maximum of oleanolic acid was placed at λmax = 270 nm without derivatization. The contents of oleanolic acid are calculated 17.5 mg in 100 g of *S. mutica* leaves based on dry weight.**

Conclusions: **Oleanolic acid is a ubiquitous triterpenoid in plant kingdom, medicinal herbs, and is integral part of the human diet. Densitometric analysis is the accurate, selective, and precise method which can be used for routine quality control analysis and quantitative determination of oleanolic acid in Lamiaceae plants specially *Satureja* species, as one of the industrial source of this compound.**

Keywords: **Lamiaceae, *Satureja mutica*, Oleanolic acid, TLC scanner**

Introduction

Satureja genus belongs to Lamiaceae family and comprises 13 species in Iran. Some of them like *S. atropatana* and *S. khuzistanica* excursively grow in Iran and others also grow in Iraq, Turk mania, and Turkey [1]. Some of the *Satureja* species (*S. parvifolia, S. odora* and *S. macrantha*) contain potent cytotoxic and antitumor agents [2, 3]. Several traditional usages of *Satureja* (especially for *S. hortansis*) such as antidiarrhea, antispasmodic, antioxidative and pesticide activity have been approved [4, 5].

Satureja mutica Fisch. & C. A. Mey., generally called white or forest savory, is an herbaceous plant which grows in North-West of Iran [7]. Oleanolic acid, a pentacyclic triterpenoid, has been previously isolated from some Lamiaceae genus such as *Satureja, Salvia* and *Dracocephalum* [3, 8, 9]. We found it as a potent trypanocidal and cytotoxic components of the mentioned plants [3, 8, 10]. Also, it was found as the major HIV-1 Reverse Transcriptase-Inhibitory compound in *Salvia officinalis* [9].

Oleanolic acid and its homologue, ursolic acid, are ubiquitous triterpenoids in plant kingdom, medicinal herbs, and are integral part of the human diet. During the last decade over 700 research articles have been published on their research, reflecting tremendous interest and progress in our understanding of these triterpenoids [11]. The literature states that oleanolic and ursolic acids show antibacterial, antifungal, insecticidal, complement inhibitory, diuretic, antidiabetogenic, and gastrointestinal transit modulating activities. Moreover, oleanolic and ursolic acids have protective action to liver, antiinflammatory effects, antitumor activity and immunomodulatory activity. Besides commonly known plants, North American perennials of the *Silphium* genus is an interesting source of oleanolic acid glycosides as compared to materials used in the herbal industry such as *Panax quinquefolium* root and *Calendula officinalis* flower [12]. For the first time, we describe the isolation, identification and quantitative evaluation of the pentacyclic triterpenoid, oleanolic acid, as one of the major constituents in *S. mutica* by the valuable method of spectrodensitometry using TLC scanner.

Material and Methods

Plant material

Satureja mutica was collected, during the full flowering stage, in September 2007 from Gilan province. A voucher specimen was deposited at the Herbarium of the Institute of Forests and Rangelands Researches. Plant specimen was identified by Dr. Vali-allah Mozaffarian from the same institute.

Experimental

^{1}H- and ^{13}C-NMR spectra were measured on a Brucker Avance 500 DRX (500 MHz for ^{1}H and 125 MHz for ^{13}C) spectrometer with tetramethylsilane as an internal standard and chemical shifts are given in δ (ppm). MS data were recorded on Agilent Technology (HP) instrument with 5973 Network Mass Selective Detector (MS model). Silica gel $60F_{254}$ pre-coated plates (Merck) were used for TLC. The spots were detected by spraying anisaldehyde-H_2SO_4 reagent followed by heating. All chemicals and reagents used for TLC were of analytical grade.

Separation process of oleanolic acid

Dried aerial parts of *S. mutica* (400 g) were cut into small pieces and extracted with diethyl ether at room temperature (for 48 hours) using

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percolation method. The solvent was evaporated by a Rotary Evaporator to give diethyl ether (18 g) extract. The diethyl ether extract (8 g) was submitted to silica gel column chromatography (CC) with Hexane: $CHCl_3$ (8:2, 0:1) and MeOH as eluent, to give eight fractions (A-D). Fraction C (700 mg) was separated by silica gel CC with Hexane: EtOAc (7:3) to obtain four fractions (C1-C4). Fraction C3 was submitted to silica gel CC with Hexane: EtOAc (3:1) and then $CHCl_3$: EtOAc (19:1) to give compound **1** (32 mg, R_f = 0.22 in $CHCl_3$: Hexane, 4:1).

Oleanolic acid (**1**). White amorphous powder. m.p.: 271-273° C. ^{1}H-NMR (500 MHz, $CDCl_3$): 0.75, 0.77, 0.90, 0.91, 0.93, 0.98 (each 3H, *s*, CH3 ×6), 1.13 (3H, *s*, H-27), 2.82 (1H, *dd*, *J*= 3.6, 13.2 Hz, H-18), 3.23 (1H, *dd*, *J*= 11.2, 4.4 Hz, H-3), 5.27 (1H, *t*, *J*=3.5 Hz, H-12). ^{13}C-NMR (125 MHz, Pyridine-d_5): δ_C (from C-1 to C-30) 39.0, 28.2, 78.1, 39.4, 55.8, 18.8, 33.3, 39.8, 48.2, 37.4, 23.7, 122.6, 144.8, 42.2, 28.4, 23.8, 46.7, 42.0, 46.5, 31.0, 34.3, 33.2, 28.8, 16.6, 15.6, 17.5, 26.2, 180.2, 33.3, 23.8.

Sample preparation for TLC scanner

Dried and pulverized leaves (about 30 g) were submitted to extraction with 400 ml of petroleum ether (60-80°C) in a Soxhlet apparatus for 24 h. Unsaponifiable matter were separated from saponified petroleum ether extract. The solvent were evaporated by Rotary Evaporator and the extract was dried under a nitrogen gas flow [13]. Dried extract was diluted with chloroform at different concentrations. The standard solutions of oleanolic acid were prepared in chloroform.

Thin - layer chromatography

The plates were pre washed with methanol and dried for 24 h at room temperature. Before use they were activated at 120°C for 30 min. The activated plates were manually spotted with 5 µL aliquots of the solutions. The mobile phase (hexane: AcOEt, 8:2) was used per development. Plates were developed to a distance of 14 cm in chromatographic chamber previously saturated with the mobile phase for 30 min at room temperature. Then the plates were dried in a current of air by means of an air dryer. Densitometer scanning was then performed at λmax = 270 nm. The radiation source was a deuterium lamp emitting a continuous UV spectrum between 200-370 nm. Each analysis was repeated five times, whilst each track scanned three times, and baseline correction (lowest slope) was used. The start wavelength was 200 nm and the end wavelength was 370 nm. The oleanolic acid was quantified by densitometric scanning of the developed plate at 270 nm.

Validation of the Method

A) Linearity of detector response

The linearity of the TLC method was evaluated by analysis of 5 standard solutions of oleanolic acid at concentrations 5, 10, 15, 20 and 25 µg/ml. The solutions were applied on the same plate. The plate was developed using the above-mentioned mobile phase.

B) Specificity

The specificity of the method was ascertained by comparing the R_f values and the spectrum of oleanolic acid standard with the spectrum obtained from a sample of the extract, at three different positions on the bands, i.e. peak start (S), peak apex (M), and peak end (E).

Results and discussion

Dried aerial parts of *S. mutica* were successively extracted with diethyl ether. This extract was used for further isolation on silica gel column chromatography to obtain

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compounds **1**. Compound **1** gave a positive Liebermann-Burchardt and anisaldehyde test and the mass spectrum of it showed a molecular ion at *m*/*z* 456 corresponding to $C_{30}H_{48}O_3$. The ^{1}H-NMR spectrum of compound **1** showed seven tertiary methyl groups at δ 0.75, 0.77, 0.90, 0.91, 0.93, 0.98 and 1.13 on an oleanane skeleton. A doublet-doublet of one proton at δ 2.82 and a triplet of one vinyl proton at δ 5.27 were assigned to H-18 and H-12, respectively, suggesting an olea-12-ene skeleton. One methine proton at δ 3.23 (*dd*, *J*= 11.2 and 4.4 Hz) showed that **1** has at least one hydroxyl group. In ^{13}C-NMR spectrum, the signal corresponding to the carboxyl C-28 appeared at δ 183.8. The spectral data were similar to the ones reported for oleanolic acid (Fig. 1) [14].

Densitometric analysis of the developed plate was carried out to quantify the oleanolic acid. Spectrodensitometric analysis showed that the band characterized by absorption maximum of oleanolic acid was placed at λmax = 270 nm without derivatization, while it was evaluated after derivatization with anisaldehyde reagent at 560 nm. The chloroform extract revealed a pronounced band (R_f = 0.75), corresponding to oleanolic acid. The contents of oleanolic acid are calculated 17.5 mg in 100 g of *S. mutica* leaves (based on dry weight) coming from the collections in the year of 2007. The presented method is accurate, selective, and precise, and can be used for routine quality control analysis and quantitative determination of oleanolic acid in *Satureja* species.

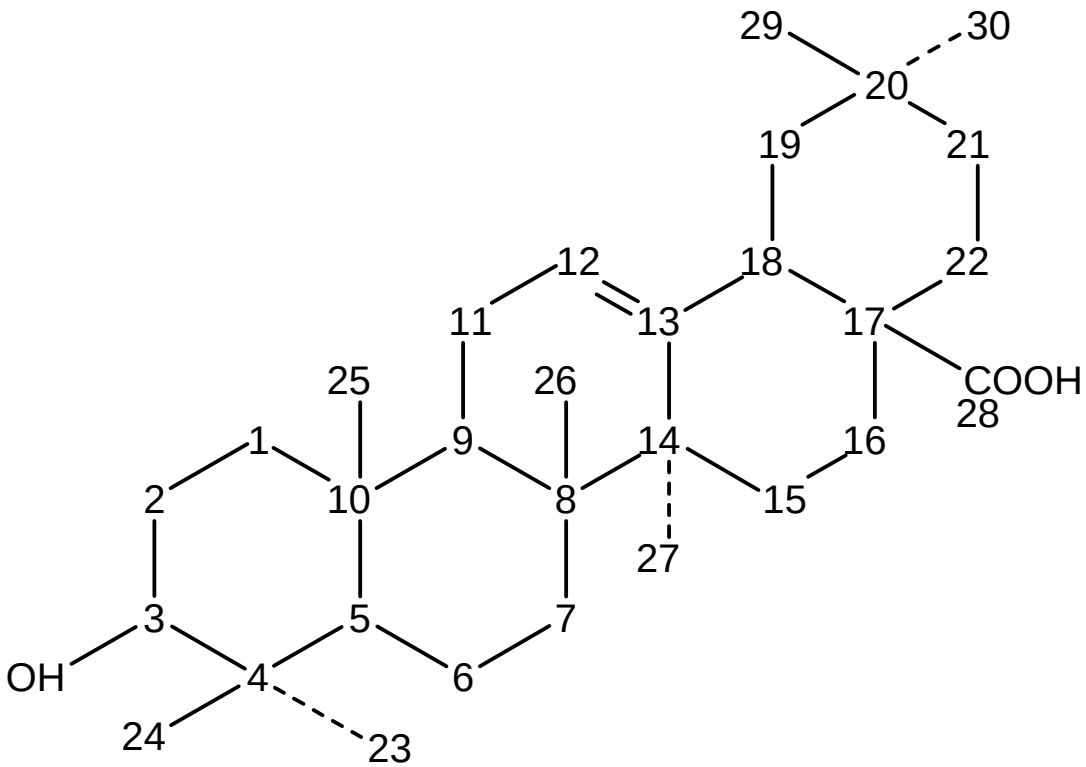

Fig. 1- Structure of the compound 1, oleanolic acid

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