



**HIGH CONTENTS OF
OLEANOLIC ACID
IN HIGHLAND
BOLIVIAN PLANTS**

**ALTO CONTENIDO DE
ACIDO OLEANOLICO EN
PLANTAS BOLIVIANAS
DE ALTITUD**

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Full original article

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ABSTRACT

Oleanolic acid is a pentacyclic triterpenoid widely distributed in the plant kingdom, it has several pharmacological properties and it has been marketed in China as hepatoprotective drug since decades. The plants are the main source of this interesting compound, in this sense; during this study we quantified this metabolite in several Bolivian highland plants (*Tetraglochin cristatum*, *Lampaya castellani*, *Junellia seriphioides*, *Baccharis tola*, *Polylepis tomentella*, *P. hieronymi*, *P. besseri* and *Satureja boliviana*) by a high-performance liquid chromatography (HPLC) method. The amount determined (2,87 to 22,69 mg/g) was higher than the concentration reported for the olive fruit (0,17-0,56 mg/g) and similar than the reported for olive leaf (31,0 mg/g) which is the main source of commercial oleanolic acid. So native species, such as *Junellia seriphioides* (22,69 mg/g), *Lampaya castellani* (13,87 mg/g), *Baccharis tola* (11,93 mg/g) and *Polylepis besseri* bark (11,06 mg/g) are an interesting potential natural source of oleanolic acid in the region.

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RESUMEN

Spanish title: *Alto contenido de ácido oleanólico en plantas bolivianas de altitud.* El ácido oleanólico es un triterpeno pentacíclico ampliamente distribuido en el reino vegetal, posee diversas propiedades farmacológicas y ha sido comercializado en China como droga hepatoprotectora desde hace décadas. Las plantas son la principal fuente de este interesante compuesto, en este sentido, durante este estudio cuantificamos este metabolito en varias plantas bolivianas de altura (*Tetraglochin cristatum*, *Lampaya castellani*, *Junellia seriphioides*, *Baccharis tola*, *Polylepis tomentella*, *P. hieronymi*, *P. besseri* y *Satureja boliviana*) por un método de cromatografía líquida de alta resolución (HPLC). La cantidad determinada (2,87 a 22,69 mg/g) fue mayor que la concentración reportada en frutos de oliva (0,17-0,56 mg/g) y similar a la reportada para hojas de oliva (31,00 mg/g) la fuente más importante de ácido oleanólico comercial. Por lo que especies nativas, como *Junellia seriphioides* (22,69 mg/g), *Lampaya castellani* (13,87 mg/g), *Baccharis tola* (11,93 mg/g) y *Polylepis besseri* corteza (11,06 mg/g) son una interesante potencial fuente natural de ácido oleanólico en la región.



INTRODUCTION

The natural product oleanolic acid (3-hydroxyolean-12-en-28-oic acid) (Figure N° 1) is a bioactive pentacyclic triterpenoid compound that has been isolated from more than 1620 plant species, including many food and medicinal plants where it exists as free acid or as an aglycone of saponins [1, 2]

This metabolite is relatively non-toxic and possesses several promising pharmacological activities. Among them, the hepatoprotective effects were widely studied [1] and are the bases of a drug against liver disorders commercialized in China [3]. On the other hand, the antioxidant, anti-inflammatory, anti-HIV, and anticancer activities, among others, motivated a large number of studies, reviews and publications of this compound and its derivatives [1,2,3,4,5], giving as one of the main results, the possible commercialization of the first oleanolic acid-derived drug [5].

The compound is especially prevalent in the Oleaceae family, among which olive (*Olea europaea*) still serves as the main source of commercial oleanolic acid where it is found in a concentration from 0,17 (in fruits) to 31,0 mg/g (in leaves) [6,7,8]. Nevertheless, in our previous studies, we found oleanolic acid as one of the major secondary metabolites in several studied plants of Bolivian highlands (3500 to 4200 m.a.s.l.) [9]. Even plants that biosynthesize few secondary metabolites use part of their energy for the production of this compound, because it seems to play an important role during the periods of frost [9]. However, in our previous report, the measure amount of this metabolite was done by semi-quantitative classic methods, as thin layer chromatography (TLC), isolation by chromatography and recrystallization. In this study, we developed a protocol to quantify this important metabolite in plants by HPLC, because this technique is fast, reproducible, and reliable. Also, it only requires small amounts of sample, compared to classical techniques [10].

The plants are the main source of this metabolite [5,8,11]. So, for this study we selected eight plants from Bolivian highlands: *Tetraglochin cristatum*, *Lampaya castellani*, *Junellia seriphioides*, *Baccharis tola*, *Polylepis tomentella*, *P. hieronymi*, *P. besseri* and *Satureja boliviana* based on the analysis done by TLC in our previous report [9], where all of them showed a majority presence of this metabolite in the EtOH extract, in order to determine new potential natural sources of this interesting metabolite.

RESULTS AND DISCUSSION

In agreement of our previous studies [8], eight plants from Bolivian highlands (*Tetraglochin cristatum*, *Lampaya castellani*, *Junellia seriphioides*, *Baccharis tola*, *Polylepis tomentella*, *P. hieronymi*, *P. besseri* and *Satureja boliviana*) were considered for this study. The plant material was submitted to an EtOH extraction by maceration for 48 h and the content of oleanolic acid in the extracts was quantified by triplicate using the HPLC-DAD developed method.

The HPLC developed method was based in three previously reported methods for triterpenic acids [12, 13, 14] and the calibration curve was done using the oleanolic acid isolated from *Chenopodium quinoa* residues after an acid hydrolysis of its saponins [13,15]. Figure N°1 shows the HPLC-DAD chromatogram of oleanolic acid using a mixture of Acetonitrile/ 0,15 % Formic acid (93:7) as a mobile phase, an EclipsePlus C-18 column at a flow rate 1.0 ml/min, and detecting the analyte at 210 nm where the oleanolic acid appears at retention time $t_R= 8,2$ min. Figure N°2 shows the calibration curve, which was obtained using the peak areas of the standard with different concentration. The area for every point was the average of three runs. The calibration curve was constructed in the range of 400-2500 ug/ml. The linear correlation equations and coefficient (r) was: $12,957x + 735,69$ ($r=0,9983$.) showing a good linearity.

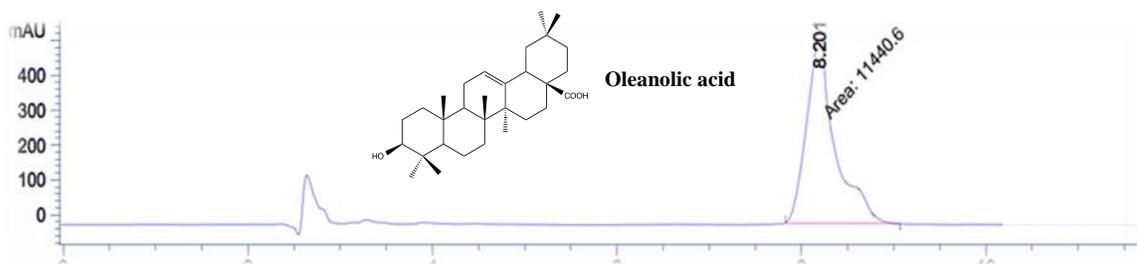


Figure N°1. HPLC chromatogram of oleanolic acid ($t_R= 8,2$); EclipsePlus C-18, wavelength 210 nm and flow rate 1.0ml/min. The limit of detection (LOD) was obtained as the sample concentration that caused a peak with a height three times the baseline noise level and the limit of quantification (LOQ) was calculated as 10 times the base noise level. Thus, the LOD and LOQ calculated were 138.96 $\mu\text{g/ml}$ and 421,11 $\mu\text{g/ml}$ respectively.

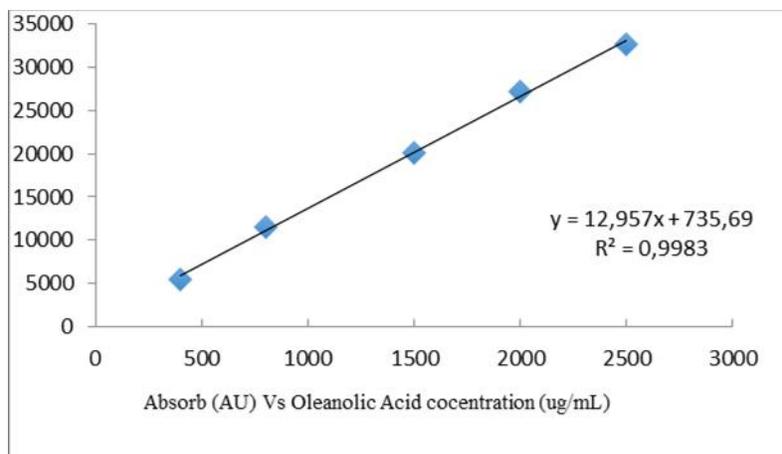


Figure N°2. Calibration curve of oleanolic acid

Then, the concentration of oleanolic acid was measured (Table N°1), showing that the species with high content of oleanolic acid are *Junellia seriphioides*, *Lampaya castellani*, *Baccharis tola* and *Polylepis besseri* bark. Nevertheless, it is important to note that all the plants evaluated showed a higher content of oleanolic acid than those found in the olive fruit (0,17-0,56 mg/g) and similar than the reported for olive leaf (31,0 mg/g) which is still used as main source of commercial oleanolic acid [7, 1]. In addition, one of these plants, *Baccharis tola* commonly known as “tola”, is widely distributed in the Bolivian Highlands, so it could be used as new natural source of this compound. It is also interesting to note that the bark of *Polylepis besseri* has a good content of oleanolic acid, because the shredding bark of *Polylepis* is constantly peeling. Table N° 1 and Figure N° 3 show the high amounts of oleanolic acid in all the collected plants.

Table N°1. Content of oleanolic acid (mg/g) in highland Bolivian plants (n=3)

Code	Plants	(Mean ± SD) (mg/g)	CV (%)
JUNE	<i>Junellia seriphioide</i>	22,69 ±0,41	1,8
TETRA	<i>Tetraglochin cristatum</i>	4,19 ±0,04	1,2
LAMP	<i>Lampaya castellani</i>	13,87 ±0,08	0,6
BTHO	<i>Baccharis tola</i>	11,93 ±0,32	2,7
PTOM **	<i>Polylepis tomentella leaves</i>	4,49 ±0,15	3,5
SATB	<i>Satureja boliviana</i>	8,73 ±0,08	1,0
PTOM *	<i>Polylepis tomentella bark</i>	2,87 ±0,04	1,4
PHIE	<i>Polylepis hieronymi</i>	3,62 ±0,04	1,1
PBESS *	<i>Polylepis besseri bark</i>	11,06 ±0,03	0,3
PBESS **	<i>Polylepis besseri leaves</i>	2,88 ±0,16	5,8

*Bark; **Leaves

In conclusion, we developed a new HPLC-DAD protocol for the quantification of oleanolic acid in plant extracts. The method was used to quantify this compound in selected Bolivian highland plants determining high amounts in all of them, in particular in *Junellia seriphioides*, *Lampaya castellani*, *Baccharis tola* and *Polylepis besseri* bark which could be used as new natural source of this important metabolite.

EXPERIMENTAL

Plant material



Tetraglochin cristatum, *Baccharis tola* and *Lampaya castellani* were collected in Orinoca at Oruro department, Bolivia (3768 m.a.s.l); *Junellia seriphioides* was collected near to the Sajama mountain (3940 m.a.s.l) and finally *Satureja boliviana* and *Polylepis* species were collected at the Botanical Garden of the University of San Andres (UMSA), located in Cota Cota town of La Paz City (3600 m.a.s.l.). Voucher specimens of all plants were deposited in the Bolivian National Herbarium where were identified by the staff.

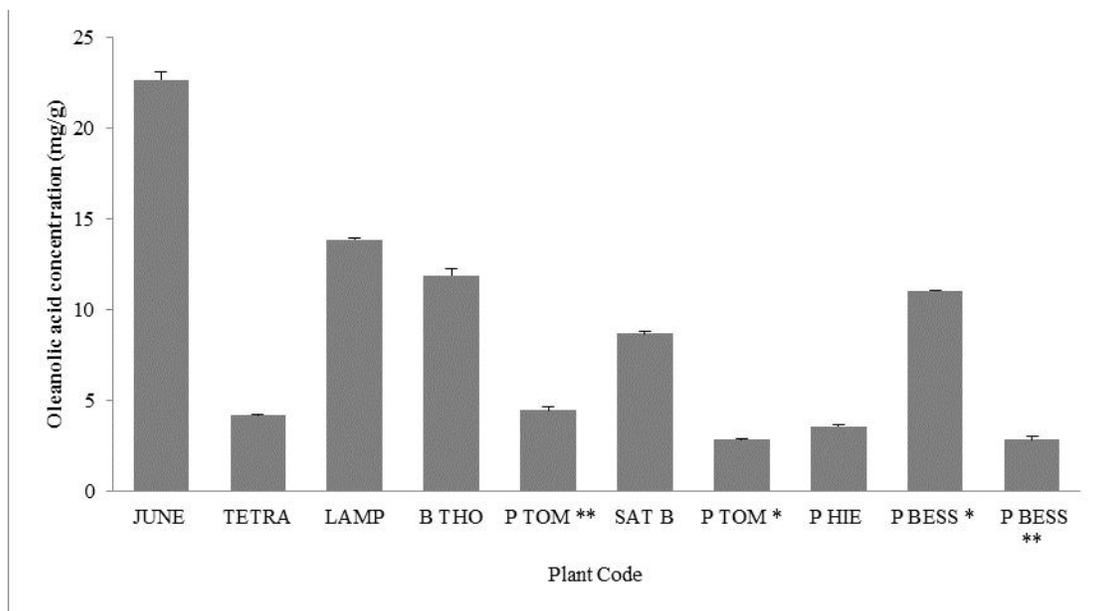


Figure N° 3 Oleanolic acid contents in plants of Bolivian highland.

Oleanolic acid

The oleanolic acid, used as standard, was obtained of the industrial residue of *Chenopodium quinoa* Willd called “mojuelo” rich in saponins [15, 13]. That residue was extracted with EtOH/H₂O (75/25) for 72 h at room temperature. The concentrated EtOH/H₂O extract was submitted to an acid hydrolysis adding HCl 2N under reflux for 3 h, giving the sapogenin extract. The oleanolic acid was isolated out of that extract after a VLC chromatography on Silicagel G-60 and recrystallization, comparing the TLC and NMR data with those of the previous oleanolic acid isolated in our lab [13, 15].

Sample preparation

Aerial parts of *Tetraglochin cristatum*, *Junellia seriphioides* and *Satureja boliviana*; leaves of *Lampaya castellani* and *Baccharis tola*; bark and leaves of *Polylepis tomentella*, *P. hieronymi*, and *P. besseri* were dried in the bioorganic laboratory at room temperature for 2 or 3 days. 500 mg of the dried and ground plant material were extracted in 25 ml of EtOH 96 GL for 48 h. The extract was filtered through filter paper Whatman #4 and the residue was washed with 5 ml of EtOH, then the liquid extract was concentrated *in vacuo*. Before the analysis by HPLC the samples were dissolved and set, with MeOH (HPLC grade), to 5 ml volumetric flasks.

HPLC analysis

The HPLC data were recorded on Agilent Technologies HPLC 1100 series instrument consisting of a quaternary pump, continuous vacuum degasser, manual injection and column compartment coupled to a variable wavelength diode-array detector (DAD). The column was an Agilent Technologies EclipsePlus RP-C18 (4.6×250mm×5µm) with a 0.5 µm pre-column filter (Agilent Technologies), and the column compartment was heated at 35 °C. The acetonitrile (HPLC grade, Sigma-Aldrich), formic acid >98% (Merck) and Ultrapure water at 18.3 M resistance were used for



HPLC mobile phase. Water solvent was filtered through a 0.45 µm filtration system (Sartorius Stedim Biotech) before use.

Calibration curve

A series of oleanolic acid solutions were prepared in MeOH HPLC at five different concentrations (400, 800, 1500, 2000 and 2500 µg/ml). The analyte was detected at 210 nm (DAD) and the area in the chromatogram at retention time 8,2 was recorder by triplicate. The analyte showed a linear relationship between 400 and 2500 µg/ml yielded the following calibration curve $y=12,957x+735,69$ ($R^2= 0,9983$).

Sample quantification

The samples were injected manually; the volume of standards and samples was 25 µl. Chromatographic separation was done with an isocratic solvent of 93 % ACN as solvent D and 7% of 0.15 % (v) formic acid in water as a solvent C, at a flow rate of 1.0 ml/min. The solvent C was filtered with a Whatman 0.45 µm PTFE/cellulose filter prior to use. Then, the area of the compound at retention time $T_{R-8,2}$ min. was recorded by triplicate and the average was used for the calculation of the amount of oleanolic acid in the EtOH extract of every plant.

Statistics

The limit of quantification (LOQ) and the limit of detection (LOD) were calculated on the base of standard deviation of the response and the slope obtained from linear equation following equations: $LOD = 3.3 \times (SD/slope \text{ "b" })$ and $LOQ = 10 \times (SD/ slope \text{ "b" })$; where SD is the standard deviation of the y-intercept.

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