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Research Article

ISOLATION AND CHARACTERIZATION OF OLEANOLIC ACID FROM ROOTS OF *LANTANA CAMARA*

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ABSTRACT

Objective: The aim of the present study is to isolate oleanolic acid from the roots *Lantana camara* roots and its chemical characterization by TLC, HPLC and Infra-red spectroscopy methods.

Methods: The chemical characterization of isolated compound was done by Thin Layer Chromatography (TLC), High Performance Liquid chromatography (HPLC) and Infrared spectroscopy.

Results: The results of spectrophotometric analysis showed that the oleanolic acid isolated from Lantana camara roots gives similar spectral results as shown by standard oleanolic acid.

Conclusion: on the basis obtained results of TLC, HPLC and IR spectra and its interpretation, it can be concluded that oleanolic acid isolated from roots of *Lantana camara* give identical, characteristic signals and absorbance similar to earlier reported reference standards.

Keywords: Oleanolic acid, Lantana camara, HPLC, FTIR, TLC

INTRODUCTION

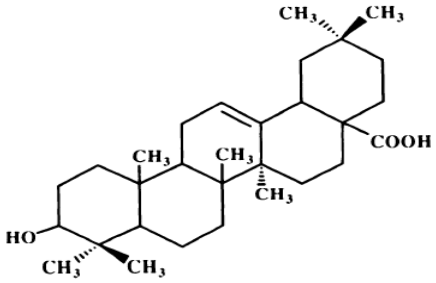

Fig.1 : Structure of Oleanolic acid

Oleanolic acid (3β-hydroxy-olea-12-en-28-oic acid) and its isomer, ursolic acid (3 β -hydroxy-urs-12-en-28-oic acid) are pentacyclic triterpenoids compound with 30 carbon atoms, biosynthetically derived from the cyclization of Squalene, they exist widely in plants in the form of free acid or aglycones of triterpenoid saponins. [1] Saponins can be chemically categorized as comprising an aglycone linked to one or more sugar chains. There are two groups of saponins, one contains a steroidal aglycone, and the other contains a triterpenoid aglycone. Squalene is considered as the common precursor for biosynthesis of both steroid and triterpenoid systems. Like steroids, triterpenoids have many biological effects, and interest in triterpenoids is growing. [2, 3, 4] Traditionally used in Asian medicine, oleanolic acid has long been known to have anti-inflammatory, anti-hyperlipidemic, and hepatoprotective in vivo effects. It has also been found to have antiviral and anti-tumor actions. [5, 6]

MATERIALS AND METHODS

Plant Material

Root of the plant *Lantana camara* Linn. were collected from hill areas of Bhopal, Madhya Pradesh, India. Plant material was collected in the morning hours. The roots were then allowed to dry in air and crushed in small pieces. A coarse powder was obtained.

Authentication of Plant

The plant was authenticated by Department of Botany, Safia College Bhopal and herbarium was submitted. (Authentication No. 280/bot/saf/11).

Process of isolation of Oleanolic acid (OA)

The powdered crude drug (500gm) was taken and defatted thrice overnight with petroleum ether and then extracted exhaustively with ethanol four times over night at room temperature by maceration. The solvent was removed under vacuum and the crude extract was dissolved in CHCl3 and left over night for precipitation. The precipitate so obtained was crystallized with methanol. Precipitation and crystallization process were repeated 4 times, which gave oleanolic acid crystals. [7]

Thin Layer Chromatography

A preliminary TLC pattern of oleanolic acid was performed for the identification by using pre-coated plates of silica gel 60 F254. The solvent used for mobile phase was chloroform: methanol (95:5). [8] An anisaldehyde sulphuric acid reagent was used as spraying agent. [9]

Rf value was calculated by the formula:

$$Rf = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

HPLC analysis

The OA isolated from Lantana camara roots was analyzed using Shimadzu LC 20ATVP HPLC system. Chromatographic separation was performed on Exsil ODS column (250cm x 4mm, 5µ particle size) in isocratic mode with acetonitrile: water (85:15 v/v) as mobile phase. The temperature of the column was kept constant at 30°C and the mobile phase was delivered at a flow rate of 1.0 ml/min and the elution was monitored by photodiode array detector set at 215 nm. [10, 11]

Infrared spectroscopy

IR spectra of isolated oleanolic acid were recorded by using Bruker (Germany) α-ATR, in infrared zone of wave length of 4000 to 400 cm-1. Standard procedure was followed to obtain the IR spectra of the test compound. Results we obtained were illustrated at spectra showed in figure 4. [12]

RESULT & DISCUSSION

Oleanolic acid isolated by above method from Lantana camara roots give a yield of 0.9 % w/w of the dried powder. (Table 1)

Table 1: Yield of oleanolic acid

S. No.	Weight of crude drug taken (gm)	Weight of isolated crystals (gm)	Yield (% w/w)
1.	500	4.5	0.9

Thin Layer Chromatography

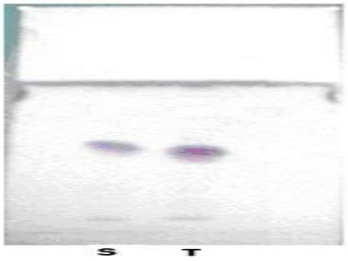

Fig.2: TLC of standard Oleanolic acid (S) & extracted test sample of Oleanolic acid (T)

Preliminary TLC studies revealed well resolved violet spots with retention factor (Rf) 0.50 for the standard oleanolic acid as well as test samples. Developed chromatographic plates presented in figure 2 implicates at good separation both of standards and test in the system of Chloroform: methanol-95:5.

HPLC analysis

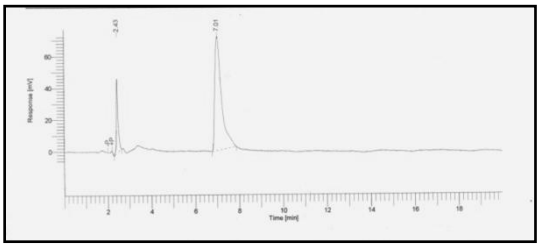

Fig.3: HPLC spectra of standard sample of Oleanolic acid

Standard oleanolic acid HPLC spectra showed two peaks at 2.43 minutes and 7.01 minutes. (figure 3) whereas test oleanolic acid HPLC spectra showed three peaks at 1.73 minutes, 2.44 minutes and 6.94 minutes. (figure 4) From the above observations we can say that isolated sample of oleanolic acid shows resembling HPLC spectra as compared to marker oleanolic acid HPLC spectra with some minor impurities.

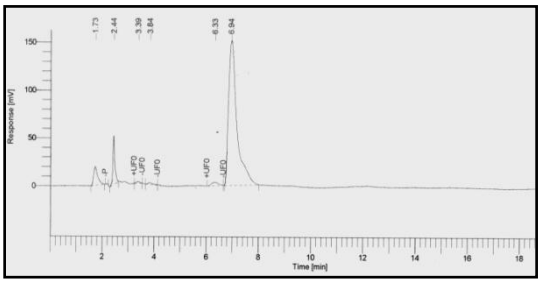

Fig.4: HPLC spectra of Oleanolic acid isolated from *Lantana camara*

Infrared spectroscopy

Oleanolic acid- (3β)-3-Hidroacid-12-en-28-oic acid IR (ν, cm^{-1}): The obtained IR spectra showed different groups in the following spectral regions.

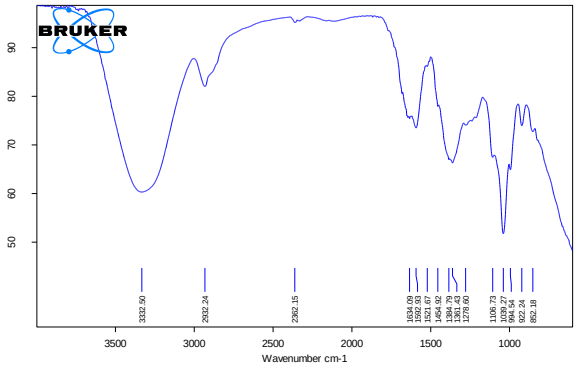

Fig.5: IR spectra of Oleanolic acid isolated from *Lantana camara*

3333 (OH); 2932 (CH_2); 1634 (C=O); 1455 (OH); 1361 (CH_3); 1107 (C-O).

At IR spectra of oleanolic acid appears a very intensive adsorption ribbon, which derivates from OH group in the area of 3333 cm^{-1}. A very intensive absorption ribbon in the area of 2932 cm^{-1} derives from symmetric vibrations of CH_2 cm^{-1} group. In the area of 1634 cm^{-1} appears a characteristic ribbon of carbonyl group (C=O). At 1455 cm^{-1} appears absorption ribbon from OH vibrations of planar distortion. In the area of 1361 cm^{-1} appears a characteristic ribbon, which derives from CH_3 group and at 1107 cm^{-1} stretching vibrations of C-O group of carbonic acid. (figure 5)

In this study the obtained IR spectrums and interpretation of spectrum, on the basis of which it can be concluded that oleanolic acid isolated from roots of *Lantana camara* give identical, characteristic signals and absorbance similar to earlier reported reference standards. (Elvira *et. al*., 2009)

CONCLUSION

In this study on the basis obtained results of TLC, HPLC and IR spectra and its interpretation, it can be concluded that oleanolic acid isolated from roots of *Lantana camara* give identical, characteristic signals and absorbance similar to earlier reported reference standards.

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