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ISOLATION AND CHARACTERIZATION OF TERPENOID DERIVATIVES FROM MEDICINAL PLANT ROOTS BY THIN LAYER AND FLASH COLUMN CHROMATOGRAPHY (TLC & FCC) TECHNIQUES

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ABSTRACT

The present work includes separation and identification of triterpenoids like sterol, betulinic acid or oleanolic acid extracted from *Alhagi* roots plant by three stages. The first one involved extraction with soxhlet and identification process of the crude extract using thin layer chromatography (TLC) technique that depends on difference in the polarity of solvents of the mobile phase, while the second stage was isolation of the oleanolic derivative by flash column chromatography (FCC). The final stage includes full elucidation of isolated component by spectroscopic analysis as Fourier Transformer Infra Red (FT-IR with KBr disk and ATR mode), ^{1}H-NMR, MS, and Elemental Analysis (C:H:N).

Key words: Alhagi, Medical plants, Sterols, Flash column chromatography.

INTRODUCTION

It's well known that alhagi maurorum has one of the most important medicinal plants. It's common names are Al-akool, and Camel Thorn Plant. Alhagi maurorum or camel thorn is a member of pea family. It is a deep rooted up to 15 feet, while its height is about 1-4 feet. It is used in USSR for camels, sheep and goats[1]. It grows in dry soil because of its deep root. Some researchers have suggested that exhaust food reserves in the roots. Towhidi A. stated that alhagi contains 9% dry matter, 10% crude protein, 43% dry matter digestibility, 38% organic matter which contents minerals and anti parasitic substance[2]. Ghane and Badiei[3] stated that Alhagi maurorum is used in folk medicine as laxative, expectorant and diuretic. Marashdah and Farraj[4], studied the effects of the extract on mice rectal temperature, which

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decreases from 4.3-7.3°C according the dose by method described by Gray et al.[5] They also studied the effect of Ahagi extract on rectus abdominis muscle of frog in dose 10-25 mg/mL according method described by Fliesher et al.[6] Recently, Kazem and Seyyed[7] studied effect of Alhagi extract on gastric ulcer on male mice.

Atta et al.[8], proved that Alhagi extract has diuretic effect for this reason. They studied the effect of methanol extract of Alhagi maurorum. They noted that urine and electrolytes excretion similar to furosemide. Also Srivastava et al.[9] alhagi as a remedy for rheumatic pains, bilharziasis and various types of gastrointestinal discomforts, urinary tract diseases and liver diseases. At the present time, there is an increasing tendency toward the use of herbal medicine specially in Arab land that reflects the trust and confidence in such remedies[4]. According to previous facts the analytical studies of this herb was very necessary. On the other hand, Oleanolic acid and ursolic acid are the main active components in fruit of *Ligustrum lucidum* Ait, and possess anticancer, antimutagenic, anti-inflammatory, antioxidative activities[10]. Hamed et al.[11] isolated successfully new Triterpene glycosides from the Alhagi maurorum. Yeboah et al.[12] approved that oleanolic acid (OA) have anti-fertility effect in several animals. Babalola and Shode[13] used oleanolic acid and ursolic acid as beneficial potentials in nutrition, cosmetics and drugs. Therefore, we became more interested and desirable to investigate of this plant in order to Isolation and characterization new components by different spectroscopic techniques.

EXPERIMENTAL

Material and methods

General procedure for plant preparation

The fresh plant roots of alhagi were collected from Al-Mahaweel area in Babylon government during August 2014 and stored immediately after collection at 8°C. The samples roots were cleaned and cut to small pieces for grinding Fig. 1. Weight of 15 g of powdered roots were extracted with ethanol by using Soxhlet extraction apparatus for about 30 mins. The crude extract was concentrated under vacuum by using lympholizer apparatus (Freeze dryer) to produce about (2.4 g, 16%) of crude extract.

General procedure for plant extraction

The crude was solvated again in ethanol and subjected to the thin layer chromatography (TLC plate with the following description, aluminum silica 60. Size 20 × 20 cm, fluorescent indicator) and eluted with mixture of (ethanol : ethyl acetate), (dichloromethane : hexane), and (methanol: chloroform) (10:90), (20:80), (30:70) ratio,

respectively. More than three components were observed. The best separation was the (dichloromethane : hexane) trial. They viewed under UV light at about 257 nm for development spots (Fig. 2).

General procedure of isolation product

The same crude extract directly packed into flash column chromatography (FCC) with the following descriptions (Column size 30 × 500 mm, packed with stationary phase silica gel 220-400 mesh size. The mobile phase used (30 : 70, dichloromethane : hexane) which was the best selection ratio for elution to obtain a pure component of oleanolic acid evaporation the solvents under vacuum by lypholyzer to afforded solid substance milk color, melting point = 296-298°C decom. While the other fractions were negligible, which contenting other ingredients. This procedure is done according to the method given by Tripathee et al.[14]

Analysis techniques conditions

Electrothermal melting point (Stuart) uncorrected was used for determination the melting point of isolated compound. Freeze Dryer (CHRIST, Alpha 1-4 LD, with Vacum Pump RZ 6, up to $4\text{-}10^{-4}$ mbar). Infra red spectra were recorded on Bruker-Tensor 27 (FT-IR spectroscopy with both KBr disk and ATR Unit). Proton with carbon nuclear magnetic resonance (^{1}H-NMR) bruker spectrometer, model ultra shield spectrometer-300 MHz with tetra methyl silane (TMS) as a standard with dimethyl sulfoxide (DMSO), and deuteriated water (D_2O) as a solvents. Mass spectra were recorded on Shimadzu Qp-2010 plus, ion source Temp. = 150°C, Ionization Mode: SCI, DI Temperature = 350°C with detector Gain : 0.68 kV and elemental analysis was recorded using Euro EA 3000. All the chemicals were used in this research including the solvents were from sigma Aldrich Company.

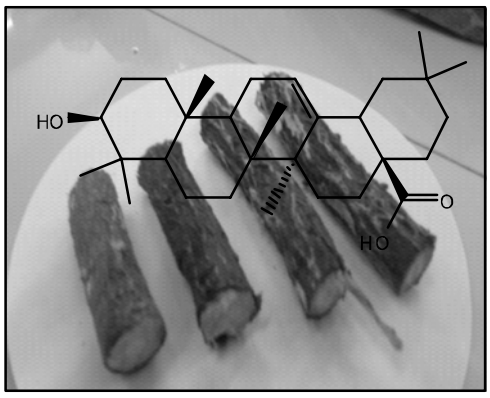


Fig. 1: Section of alhaqi roots after cleaning

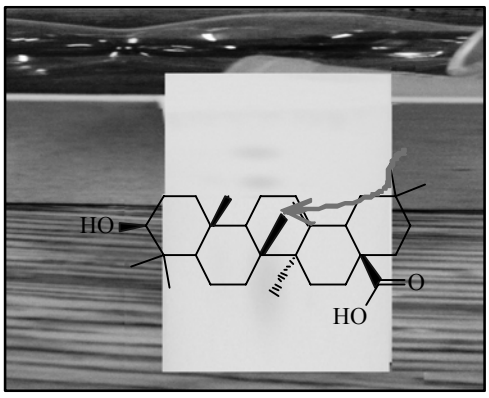
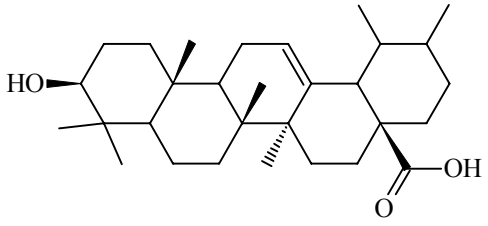


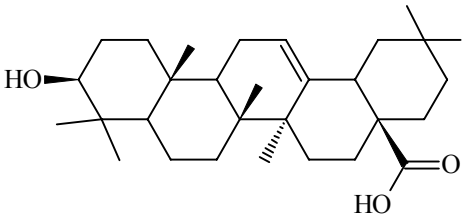
Fig. 2: TLC of crude extract

RESULTS AND DISCUSSION

The experimental data of spectroscopic analysis showed a good evidence to presence of oleanolic acid or it's derivatives type (A, B). The TLC analysis of the alhagi crude gives more than two spots with tailing, which was observed clearly in the (dichloromethane : hexane) solvents Fig. 2. Therefore, the separation of these components became very necessary. We used the same mobile phase in the (TLC) to isolate these spots in (FCC) but with different ratio. The fractions of collection samples from FCC showed there were more than one component. Our interest was to collected only the oleanolic acid as possible. Evaporation of the sample from the solvent under reduces pressure by Freeze Dryer to get on oily sample. When treated with n-pentane a solid component formed with m.p. = 290-292 decom. The Infra red spectroscopy of this sample reveled a clear evidence by presence of hydroxyl groups (OH) at stretching frequency θ = (3303-3350 cm^{-1}) in Fig. (3a). Also the strong absorption band at θ = (1707-1730 cm^{-1}), which refer to the carbonyl group (C=O) of carboxylic acid and ester while the rest of peaks at about θ = (2937, 1445- 1229 cm^{-1}) belong to CH=C-, CH_2 and CH_3, respectively with finger print region[15]. On the other hand, ^{1}H-NMR in DMSO solvent Fig. (3a) showed clearly presence of all the methyl group (CH_3), methylene (CH_2) in addition two of hydroxyl groups (alcoholic and Carboxylic site) as follows: (s, 9H, $3CH_3$, methyl groups) at δ = 1.22 ppm, (s, 12H, $4CH_3$, methyl groups) at δ = 3.34-3.55 ppm, (m, 20H, $10CH_2$, methylene groups) at about δ = 3.62-3.90 ppm, (d, 1H, OH, alcoholic group) at δ = 4.40-4.55 ppm, (s, 1H, OH, carboxylic group) at about δ = 4.82 ppm and (m, 1H, CH = C, alkene group) at δ = 5.1-5.20 ppm. The most important fact was disappearance of both hydroxyl groups (alcoholic and carboxylic) when measured in deuterium water H_2D as shown in Fig. (3b). Mass spectra of isolated compound confirmed presence of Oleanolic acid, which show molecular ion peak, (m/z, %) = 456 [M^+ + 4], with relative intensity 10%. Other fragment were about; (350, 9), (280, 92), (200, 70). The last evidence of isolation (OA) was elemental analysis, which clearly a proved to be identical till 92% with virtual calculation, (calculated/experimental); (C, 78.68/ 79.16; H, 10.47/ 11.09; O, 10.84/11.31). From all the above evidence, we can judge that the isolated product is triterpenoid derivatives like type A or B.



[Type A]



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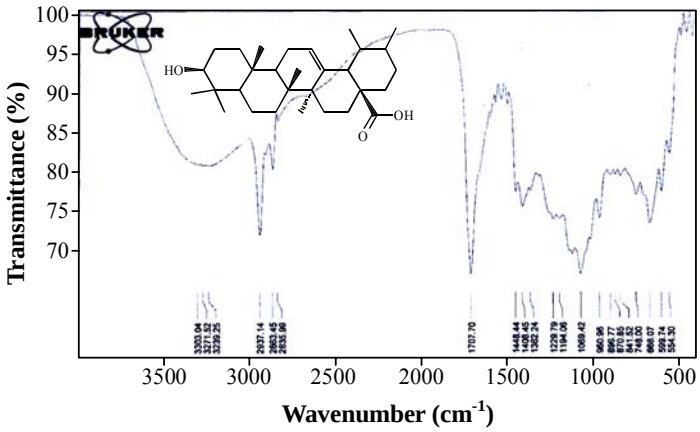

Fig. 3a: Infra red spectra (FT-IR) of oleanolic acid in ATR unit at 22°C

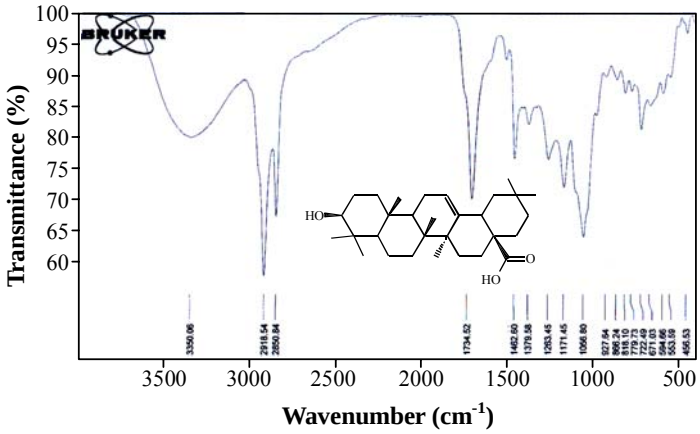

Fig. 3b: Infra red spectra (FT-IR) of oleanolic acid in KBr disk at 22°C

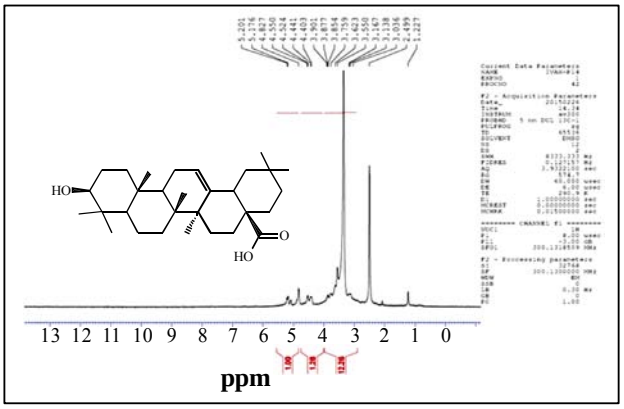

Fig. 4a: ^{1}H-NMR spectra of the isolated product (triterpenoid) in DMSO solvent

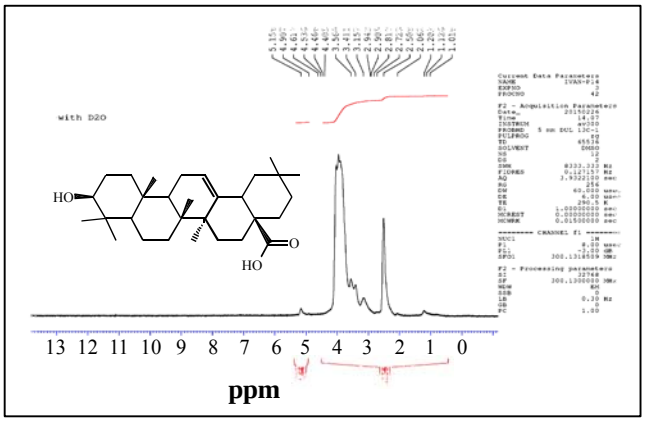

Fig. 4b: ^{1}H-NMR Spectra of isolated product (Triterpenoid) in DMSO + D_2O solvents

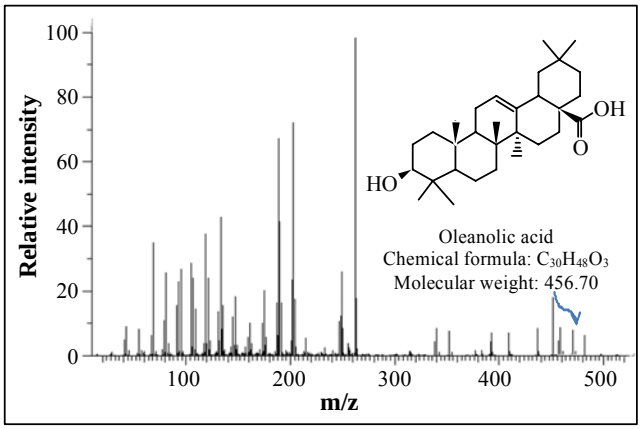

Fig. 5: Mass spectra of oleanolic acid from alhagi roots

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