Isolation and identification of Lupeol from Syrian Euphorbia Helioscopia

Shaza Sitrallah, Joumaa Merza

Abstract— A phytochemical study of Euphorbia Helioscopia from Euphorbiaceae family collected in south west of Hama in Syria, led to isolate and identified the Lupeol from chloroformic extract. The purification, the isolation and the structural identification of this compound was achieved by means of the chromatographic (CC and TLC) analysis and the spectroscopic: nuclear magnetic resonance with different applications (^{1}H-NMR, ^{13}CNMR, COSY, HMQC, HMBC) and FT-IR spectroscopy.

Index Terms— Euphorbia Helioscopia, Euphorbiaceae, Lupeol, ^{1}H-NMR, ^{13}CNMR, COSY, HMQC, HMBC.

1 Introduction

THE Euphorbiaceae is the largest family among the Anthophyta, with 300 genera and 5000 species. The genuses sub cosmopolitan but with strong representation in the humid tropics and subtropics of both hemispheres [1]. The genus Euphorbia is the largest genus in the Euphorbiaceae family with over 2000 species ranging from annuals to trees and is subdivided into many subgenera and sections. All contain latex and have unique flower structures [2]. Euphorbia species are used for the treatment of various ailments such as skin disease, gonorrhea, migraines, intestinal parasites and IJP warts cures. The plant lattices have been used in fish poisons, and insecticide [3]. Based on traditional information, the leaves and the lattices of this genus are used in the Ayurveda system of medicine for bronchitis and rheumatism.
Furthermore, it is stated to possess inflammatory, ant arthritic, ant amoebic, spasmolytic, antiviral, hepatoprotective and antitumor activities. For hundreds of years with traditional Chinese medicine Euphorbia have been used for the treatment of cancers, tumors and warts, and is well known that this pieces contains irritant and tumor-promoting constituents [4]. Quite a number of species are used in folk medicine as drugs and raw materials for pharmaceutical preparations. In Turkish folk medicine, Euphorbia species have been used for rheumatism, swelling and especially as a wart remover [5].
The genus Euphorbia (Euphorbiaceae) mainly contains diterpenoid and triterpenoid compounds that are responsible for the skin irritating, tumor-promoting, and cytotoxic activities [6]. Euphorbia helioscopia L. has been used to treat malaria, bacillary dysentery, osteomyelitis, and tumor in Chinese folk medicine [7].

2 Taxonomic descriptions of Euphorbia Helioscopia

E. helioscopia is a smooth annual plant with an erect, stout stem from eight to twelve inches high, often branched from the base [8]. The branches, as well as the main stem, end in a more or less compound umbel which is subtended by a circle of leaflets. The leaves are scattered along the stem; they are somewhat oblong or wedge-shaped, sometimes nearly round, from one-half to four inches long, finely saw-edged, and narrowed to a short stalk. The rather inconspicuous flowers are of two kinds, the staminate and pistillate on the same plant, both included in a cup-shaped involucre resembling a calyx or corolla. The staminate flowers are numerous, lining the inside of the cup, each consisting of one single stamen in the axil of a very little bract. The pistillate flower is solitary in the centre of the cup and consists of a three-lobed, three-celled ovary which soon protrudes on a long stalk and hangs over the brim of the cup-like involucre. The seeds are reddish-brown, strongly honeycombed. The plant is in bloom from June till October [9].

Fig. 1. photo of Euphorbia Helioscopia

3 Experimental Procedure

3.1 Plant Material

Aerial parts of Euphorbia Helioscopia, were collected and dried in June 2017, from South-Est of Hama, Syria. The plant was authenticated by the Atomic Agent in Syria. A voucher specimen of plant was deposited in the laboratory of chemistry of natural products, Department of chemistry, Faculty of sciences, AL Baath University, Homs, Syria.

3.2 Plant collection and Extraction method of the compound

The green parts of Euphorbia Helioscopia were collected, and air-dried (500 g) were extracted with MeOH in soxhlet. Then

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the methanoic extracts were extracted once with C_6H_{14} Then extracted with $CHCl_3$ and concentrated under vacuum to give 10.5 g. about 4gr were loaded on chromatographic column (2 cm diameter, 120 cm. long) over silica gel (230 – 400 mesh, ASTM). The column has been eluted successively with: n-hexane (200 ml.), and: and n-hexane: ethyl acetate (10: 90, 200 ml.). Lupeol: was obtained from the latter fraction n-hexane: ethyl acetate (10: 90, 200 ml.), purified on preparative TLC by using mixture of n-hexane/ EtOAc (30: 70), to give (50 mg, the Rf = 0.45), it is a white powder solid, its melting point 212-213 ^oC.

4 Results and discussion

4.1 structure identification of Lupeol

The structural determination of the of Lupeol based on the usual spectral methods. The IR (KBr) cm-1: 3452, 1638,2944, 1454, 1384. Figure 2

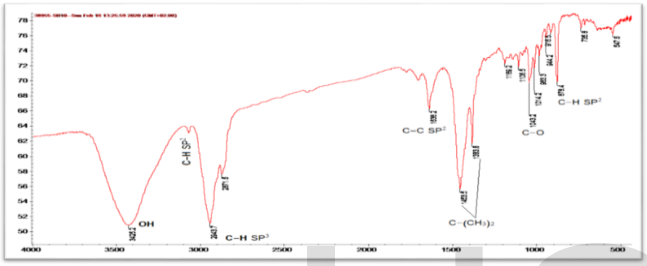


Fig. 2. IR of Lupeol in KBr

The ^{13}C-NMR exhibits 30 absorption signals indicating the presence of 30 carbon atoms in the molecule, these absorptions indicate the compound 1(Table 1, Figure 3).

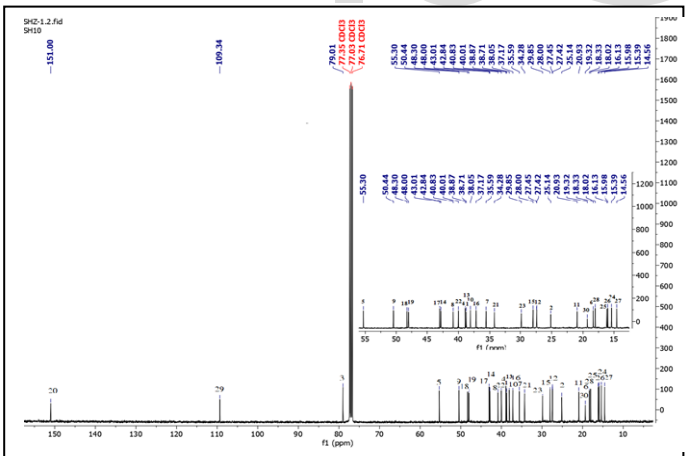


Fig. 3. 13C-NMR Spectrum Lupeol (400 MHz, $CDCL_3$, δ_{TMS} = 0 ppm)

The analysis of the DEPT-135 spectrum, indicate the presence of seven primary carbons at (δ_C=14.56, C_{27}), (δ_C =15.39, C_{24}), (δ_C =15.98, C_{26}), (δ_C =16.13, C_{25}), (δ_C =18.02, C_{28}), (δ_C =19.32, C_{30}), (δC =28.00, C_{23}), 6 tertiary carbons, 11 secondary carbons and 6 quaternary carbons (Figure 4).

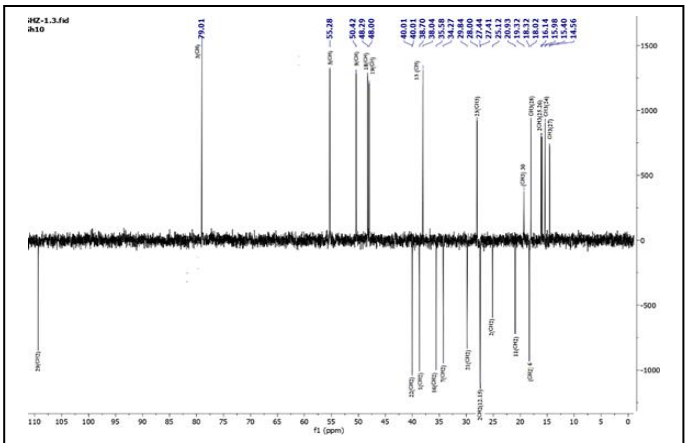


Fig. 4. DEPT- 135 Specter of Lupeol (400 MHz $CDCL_3$, δ_{TMS} = 0 ppm)

The1H-NMR spectrums shows from calculating integrals indicating the presence about 50 hydrogen atoms in the molecule 1: they isolated seven absorption of seven methyl groups at (δ_H=0.96ppm,H_{27}), (δ_H=0.75ppm,H_{24},S) , (δ_H=1.02ppm,H_{26},S) (δ_H=0.82ppm,H_{25},S) ,(δ_H=0.78ppm,H_{28},S), (δ_H=1.67ppm,H_{30},S) (δ_H=0.94ppm,H_{23},S) and two protons of olefin bonds at (δ_H=4.56ppm,H_{29a},dd, J^2=2.4, j^4=1.3Hz) and at (δ_H=4.68ppm,H_{29b},d, J^2=2.2Hz), and quartet signal (doublet of doublet) at: (δ_H =3.18 ppm, dd, J^3=11.3, j^3=4.9 Hz) that due to the proton near the hydroxyl, see (Table 1, Figure 5).

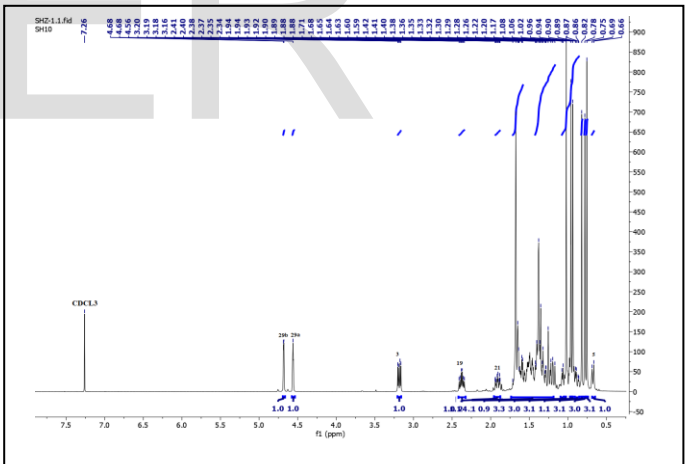


Fig. 5. 1H-NMR Spectrum of Lupeol (400 MHz, $CDCL_3$, δ_{TMS} = 0 ppm)

we determinate from the HMQC spectrum, the heteroatom correlations between hydrogen systems and the carbon atoms carrying these protons (Figure 6).

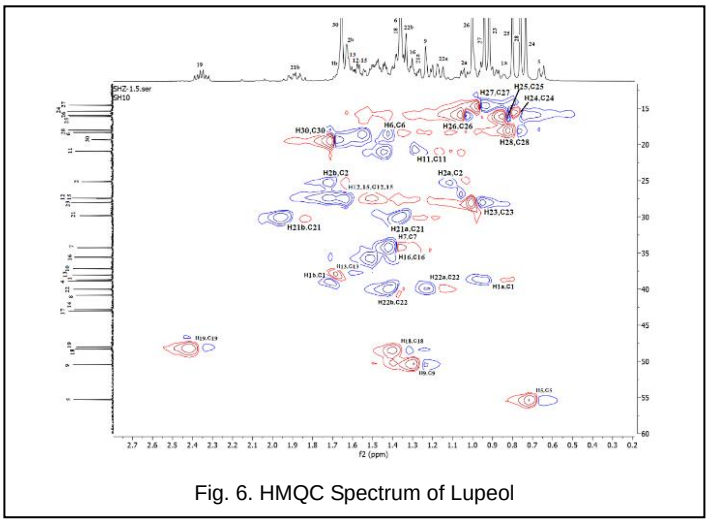


Fig. 6. HMQC Spectrum of Lupeol

we can indicate from the COSY spectrum (Figure 7), the presence of one (spin – spin) scaling coupling system.

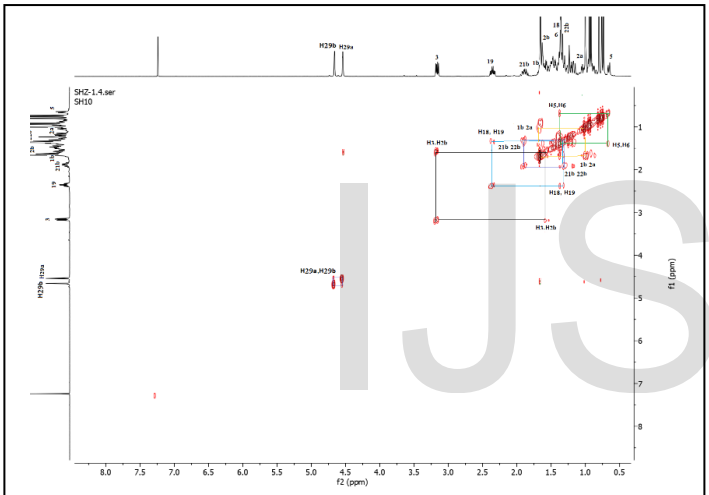


Fig. 7. COSY Spectrum of Lupeol (400 MHz, $CDCL_3$, δ_{TMS} = 0 ppm)

To determaine the hetero-atomes correlation (J^2, J^3, J^4) for obtaining the detail of the skelet of compound 1, we analysed the HMBC spectrum (figure8)

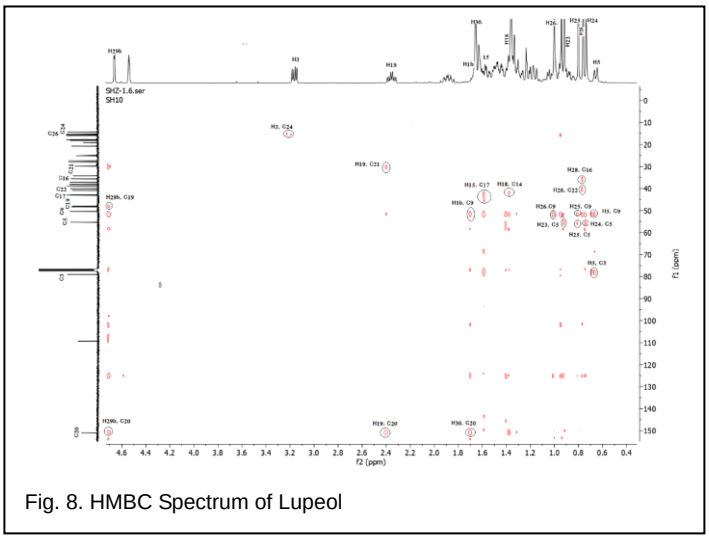
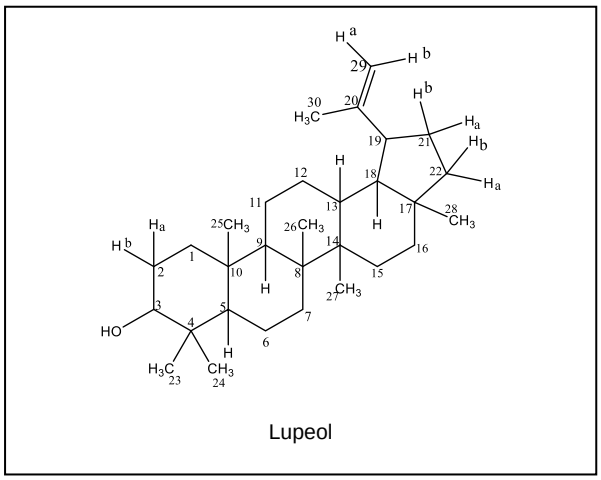


Fig. 8. HMBC Spectrum of Lupeol

TABLE 1
DEPT, COSY AND HMBC DATA OF COMPOUND LUPEOL

C	^{13}C δ_C (ppm)	DEPT (135-90)	HMQC δ_H (j =Hz)	^1H-^1HCOSY δ_H (ppm)	HMBC
27	14.56	CH_3	0.96 (s)	--	--
24	15.39	CH_3	0.75 (s)	--	C_5
26	15.98	CH_3	1.02 (s)	--	C_9
25	16.13	CH_3	0.82 (s)	--	C_9, C_5
28	18.02	CH_3	0.78 (s)	--	C_{16}, C_{22}
6	18.33	CH_2	1.38 (m)	H_5=0.66	--
30	19.32	CH_3	1.67 (s)	--	C_{20}
11	20.93	CH_2	1.21 (m)	--	--
2	25.14	CH_2	H_a=1.07 (m) H_b=1.65 (m)	H_{1b}=1.71 H_3 =3.18	--
12	27.42	CH_2	1.59 (m)	--	--
15	27.52	CH_2	1.59 (m)		C_{17}
23	28.00	CH_3	0.94 (s)		C_5
21	29.88	CH_2	H_a=1.28 (m) H_b=1.92 (m)	H_{22b}= 1.35	
7	34.28	CH_2	1.37 (m)		
16	35.59	CH_2	1.32(m)		
10	37.17	C	--		
13	38.05	CH	1.60 (m)		
1	38.71	CH_2	H_a=0.89 (m) H_b=1.71 (m)	H_{2a}=1.65	C_9
4	38.87	C	--		
22	40.01	CH_2	H_a=1.17 (m) H_b=1.35 (m)	H_{21b}=1.92	
8	40.83	C	--		
14	42.48	C	--		
17	43.01	C	--		
19	48.00	CH	3.36 (ddd, J^3=11.03, 10.98, 5.84)	H_{18} =1.37	C_{21}, C_{20}
18	48.30	CH	1.37 (m)	H_{19}=3.36	C_{14}
9	50.44	CH	1.26 (m)		
5	55.30	CH	0.66 (d, J^3=9.14)	H_6=1.38	
3	79.01	CH	3.18 (dd, J^3 = 11.3, 4.9 Hz)	H_{2b}=1.65	C_{24}
29	109.34	CH_2	H_a=4.56 (dd, J^2=2.4,J^4=1.3) H_b=4.68 (d, J^2=2.2)	H_{29b} = 4.68 H_{29a}= 4.56	C_{19}, C_{20}
20	151.00	C	--		

So we can sugest the structure of compound 1 ($C_{30}H_{50}O$) as: compound Lupeol



4.2 Conclusion

In summary, we demonstrated in this article compound identity Lupeol which is a new compound. The compound seems a white powder solid, fully dissolved in chloroform, and R_f account for this compound in a sentence (n-C_6H_{14}: EtOAc) (30:70) was R_f=0.45.

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