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Retusasterol , a Novel Sterol and Glycoside Sitosterol Isolated from Nitraria Retusa. L.

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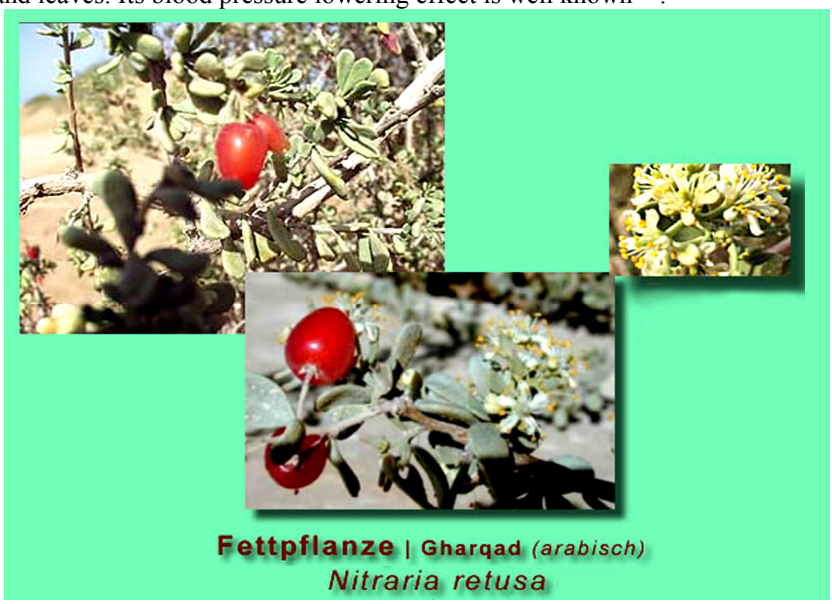
Abstract
Phytochemical analysis of the $CHCl_3$ and methanol fractions of *Nitraria Retusa* L. has led to the isolation and identification of a new sterol; Retusasterol and another known compound; β- sitosterol glucoside. The structure elucidation of both compounds was based on spectroscopy data IR, MS, ^{1}H and ^{13}C – NMR, DEPT (90,135), HMQS, COSY and HMBC.
Keywords: *Nitraria Retusa.* L. , Retusasterol , β-Sitosterol , Sterol.

1.Introduction

The genus Nitraria which belongs to the family Zygophyllaceae, includes seven species, distributed throughout the world, in particular around the dead sea in Palestine, in Jayroud saline area in Syria, Sinai in Egypt, Kuwait, Turkey, Russia and in Siberia[1] .

Nitraria Retusa (Forssk.) is one of the perennial species. A thorny shrub with fleshy grayish heart-shaped leaves[2], and red fleshy tasty fruits, that grows along the shallow sand hummocks on saline ground near the coastal areas. It is salt-tolerant and drought-resistant plant which propagates by seeds. Many wildlife forms feed on its fruits and leaves. Its blood pressure lowering effect is well known [3].



***Nitraria Retusa* L. Figure 1**

2.Materials and Methods

2.1.Instrumentations

Melting points were measured on an Electrothermal Entineering melting point apparatus / LTD / and are uncorrected.

MS, 1H-NMR,13 C-NMR, and IR spectra were recorded on GC-MS-QP 2010 Shimadzu Bruker Ultra Shield 400MHz and Jasco FT-IR 410 respectively.

Rotational evaporator / Buchii /, analyzing preparative plates /TLC/ made of glass and aluminum, painted with Silica gel / Merck /, and solvents / Merck/.

2.2.Plant collection and extraction procedure

Green parts of Nitraria Retusa L. were collected from Jayroud saline area in Syria, in 2009, air-dried (600 g) and extracted twice with CHCl3. Obtained extracts were combined and concentrated under low pressure, yielding 19.50 g of extract I. The residue from extract I was further extracted with CH3OH and the obtained extract was then concentrated under vacuum resulting in 7.30 g of extract II.

Fraction I (5.0 g) was adsorbed onto silica gel (230 – 400 mesh, ASTM) and subjected to column

chromatography (2×120 cm). The column was eluted successively with: n- hexane: benzene (70:30, 600 ml), benzene (400 ml), benzene:chloroform (50:50, 500 ml), and chloroform (600 ml).

5 g from extract II was loaded on a similar column and eluted with: chloroform (500 ml), chloroform:methanol (90:10, 600 ml) chloroform: methanol (80: 20, 600 ml).

2.3. Retusasterol 1

A white solid was obtained from fraction (5gr) of extract **I** and purified on preparative TLC by using of $CHCl_3$: MeOH (99.5 : 0.5, R_f =0.31) mixture and recrystallized from hexane : chloroform mixture to give Retusasterol 1 (39 mg). The compound is soluble in cold $CHCl_3$ and in hot n- Hexane and Benzene,
m.p = 174-177 °C.
IR (KBr) cm-1 : 2431 , 2934 , 2846 , 1638 , 1465 , 1380 , 1056
Mass: m/z (%) : M+ 440 (89 %), 412(100), 394(40), 379(20), 351(37), 300(46), 281(18), 272(62), 255(87), 213(39),185(15), 159(57), 133(56), 105(46), 83(63), 55(77) .
^{1}H-NMR and ^{13}C-NMR ($CDCl_3$) δ (ppm) see Table 1.

2.4. β- Sitosterol glucoside 2

A white solid was obtained from fraction (5gr) of extract II and purified on preparative TLC by using of CHCl3: MeOH (90.5 : 10.5 , R_f = 0.23) mixture and recrystallized from methanol : chloroform mixture to give β- Sitosterol glucoside (87 mg). The compound is soluble in cold water, and in hot MeOH .
m.p = 266-268 °C.
IR (KBr) cm^{-1}: 3398 , 2934 , 2845 , 1638 , 1465 , 1379 , 1025 .
Mass : m/z(%) : M^+ 576 (18%)
530(5), 498(10), 468(14), 442(21), 413(47), 369(9), 342(24), 314 (6), 281(48), 256(17), 227(6), 205(100), 179(46), 135(74), 95(92), 75(55) .
^{1}H-NMR and ^{13}C-NMR (DMSO-d_6) δ (ppm) see Table 2.

3.Results and discussion

3.1.Elucidation of structure of Retusasterol:

Retusasterol, compound **1**,was isolated from the concentrated chloroform extract of the air – dried leaves and flowers of the plant using silica gel column chromatography. Similarly, β- Stigmasterol.
Compound **2**, was isolated by column chromatography from the concentrated methanolic extract of the residue left after the chloroform extraction[4-5].

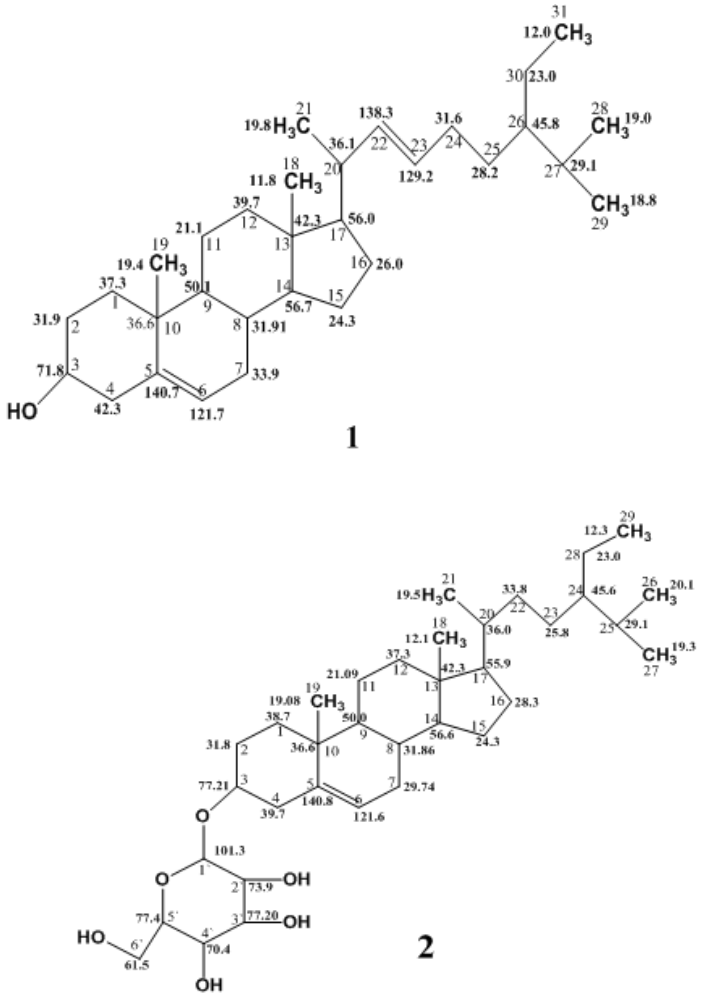


Figure 2: isolate compounds from *Nitraria Retusa* L.

The determination of the structure of Retusasterol **1**, was based on the usual spectral methods. Thus, the IR spectrum of **1** shows a broad band at 3431 cm^{-1} (O-H stretching), strong absorption band at 2934-2846 cm^{-1} (C-H stretching), a weak band at 1638 cm^{-1} (C=C stretching), and two medium bands at 1465 cm^{-1} and 1380 cm^{-1} (CH bending and CH_3 groups)

Moreover, the ^{13}C-NMR of **1**, exhibits 31 signals indicating the presence of at least 31 carbon atoms in the molecule. (Table 1, Figure 3)

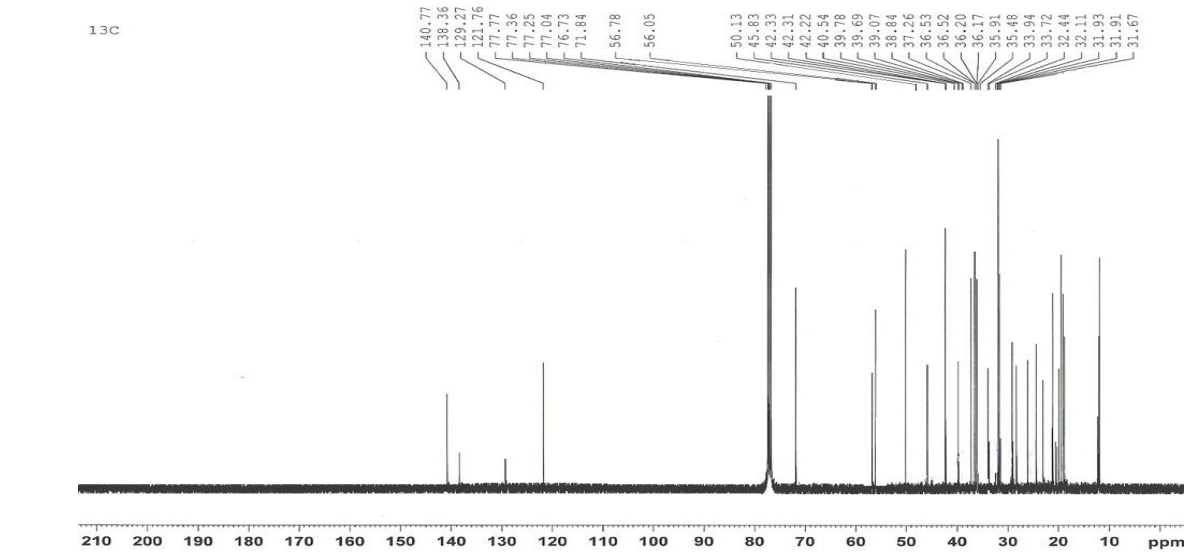


Figure 3: ^{13}C-NMR of Retusasterol in $CDCl_3$

DEPT - 135, and DEPT- 90, however, show that these include 11 secondary, 11 tertiary, 3 quaternary and six primary carbons. (Figure 4,5).

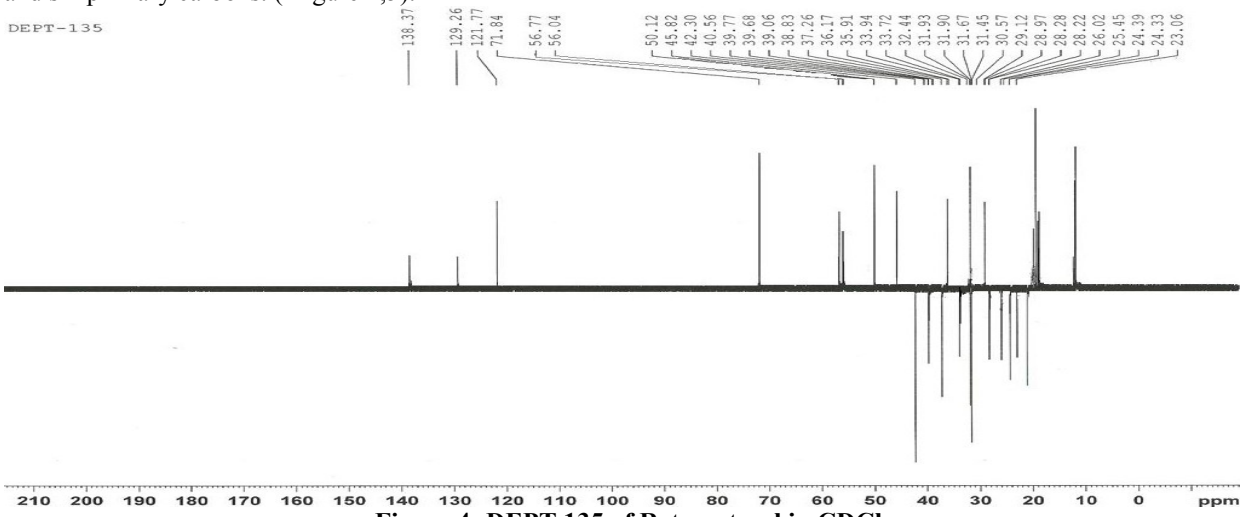


Figure 4: DEPT 135 of Retusasterol in $CDCl_3$

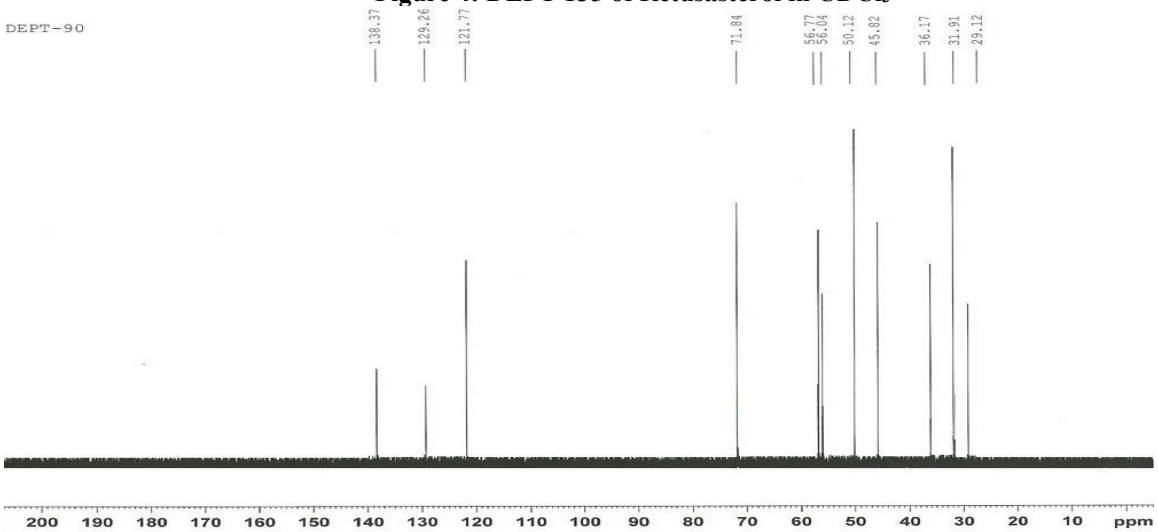


Figure 5: DEPT 90 of Retusasterol in $CDCl_3$

On the other hand, the mass spectrum of compound 1 shows the molecular ion peak at m/z 440.4 corresponding to the correct molecular formula $C_{31}H_{52}O$ (Figure 6).

The latter with 6 degrees of unsaturation, pointed to the two (C=C) bonds disclosed by ^{13}C-NMR (δ_C :

121.76, 129.27, 138.36, 140.77) and four cycles. Together with hydroxyl group, the formula must be that of a sterol. Moreover, the comparison of the MS spectrums of **1** with those available in the database of the instrument, showed good similarity with β- Stigmasterol, (Figure 7), despite the fact that sterol **1** had two extra secondary carbons. The close similarity of ^{13}C-NMR of **1** and β- Stigmasterol Supports this conclusion [4-7].

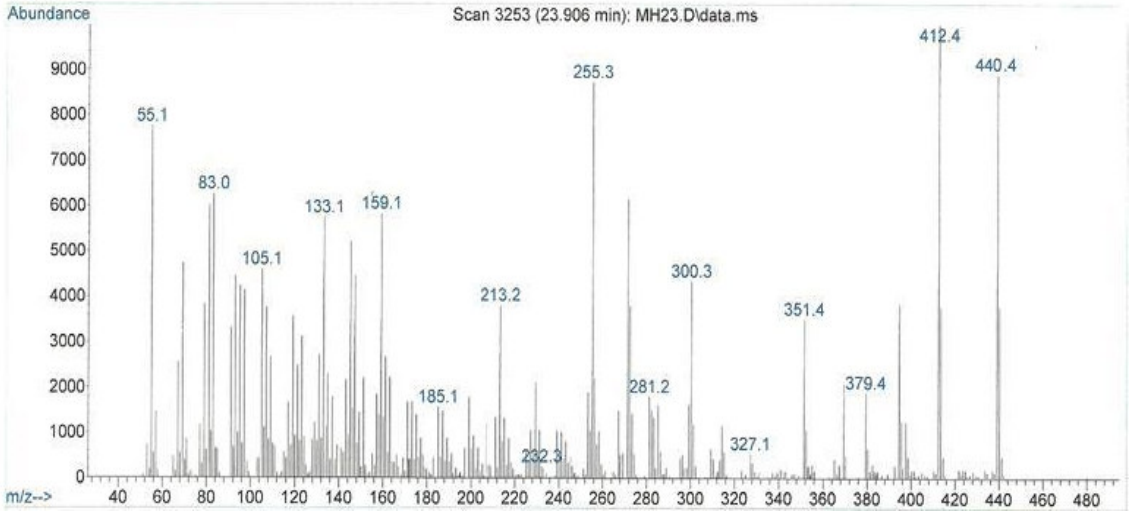


Figure 6: Mass spectrum of Retusasterol

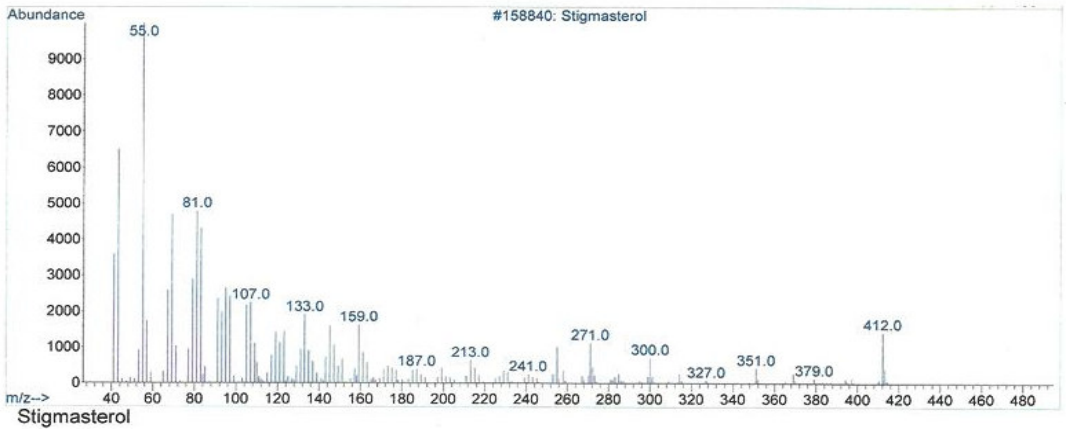


Figure 7: Mass spectrum of β- Stigmasterol

Table 1, ^{1}H-^{1}H COSY and ^{1}H-NMR spectrums also display the spin – spin coupling between different protons (Figure 8,9).

Table 1and HMBC spectrum show the correlations between hydrogen and carbon atoms adjacent to them in Retusasterol 1 (Figure 10). these correlations are shown in (Figure 11).

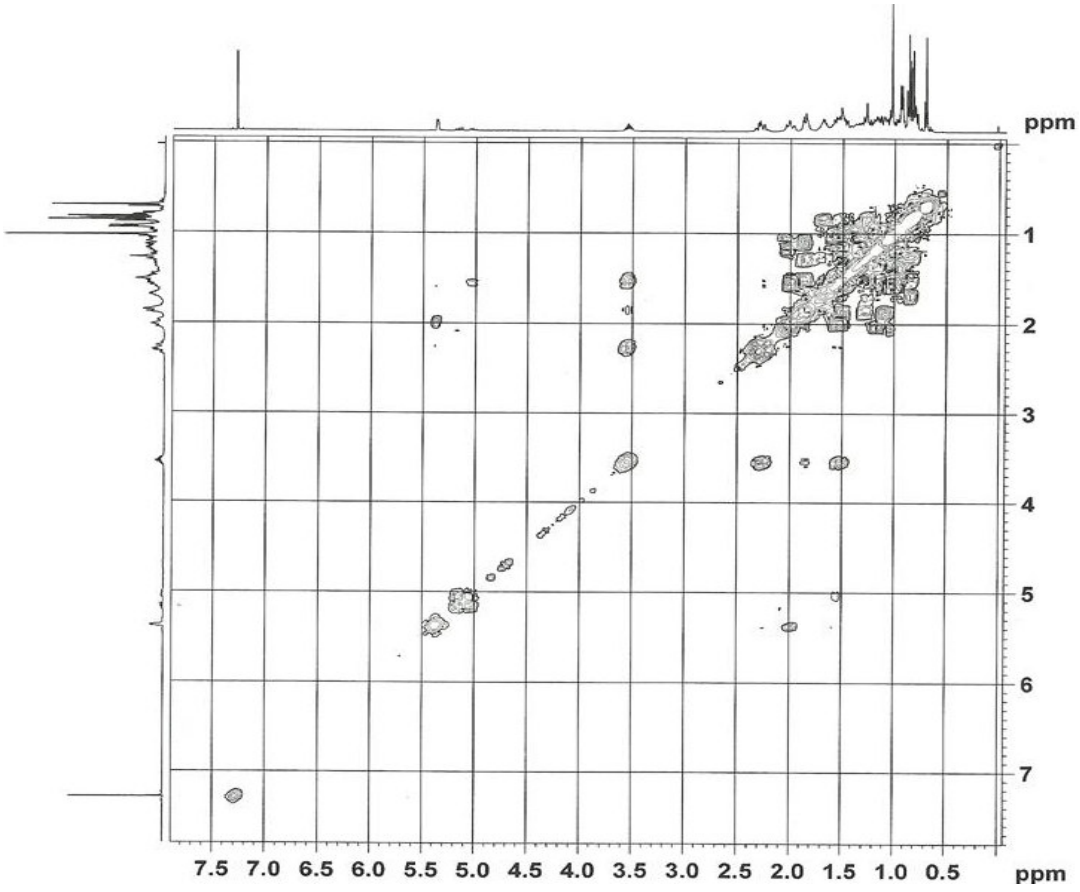


Figure 8: ^{1}H-^{1}H COSY of Retusasterol in $CDCl_3$

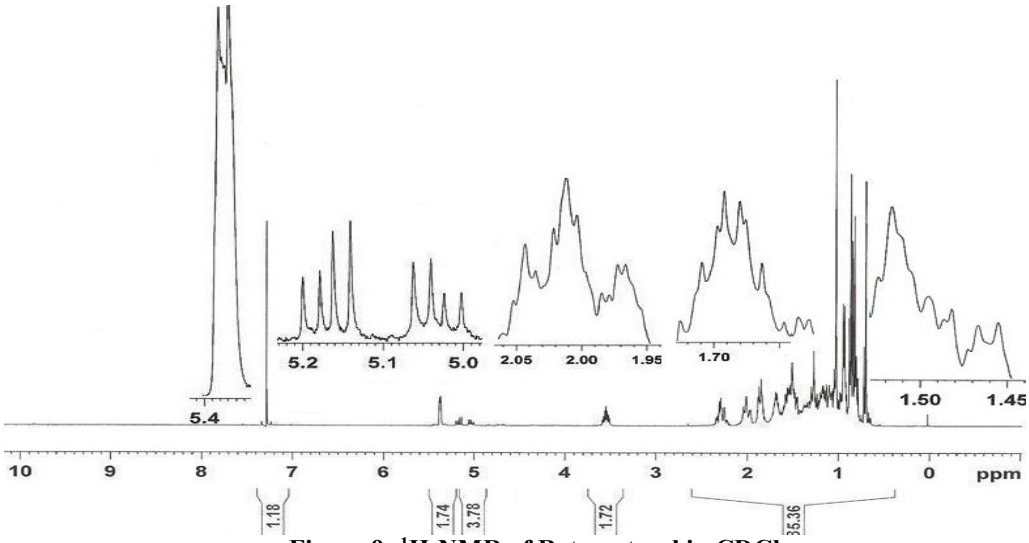


Figure 9: ^{1}H-NMR of Retusasterol in $CDCl_3$

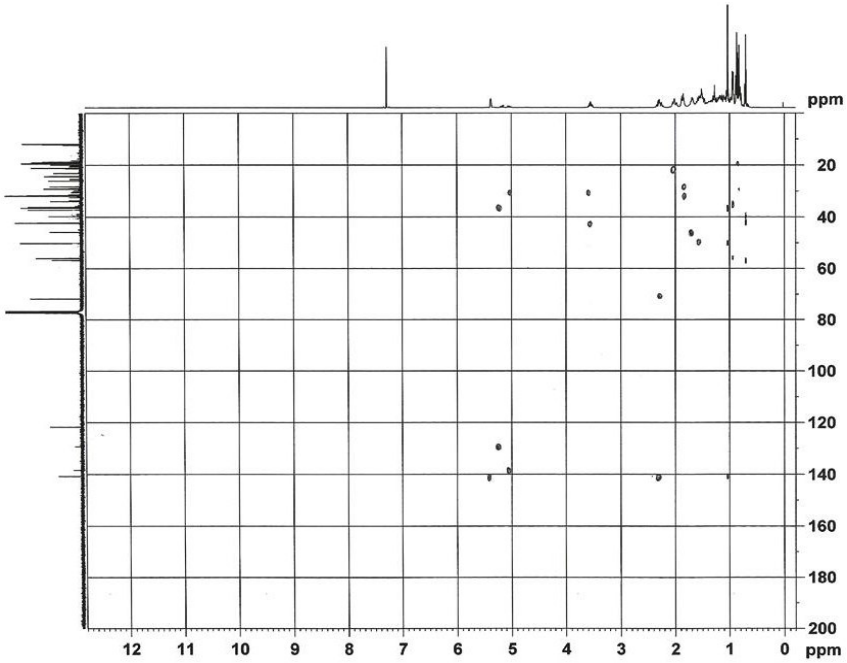


Figure 10: HMBC of Retusasterol in $CDCl_3$

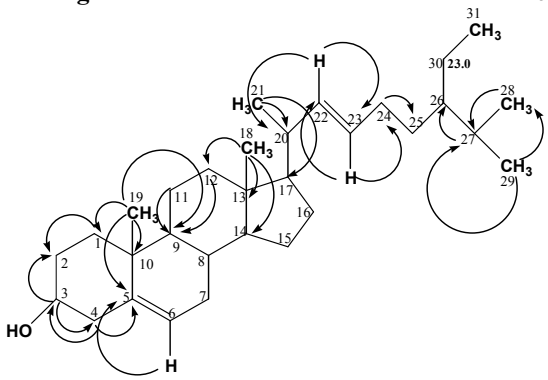


Figure 11: HMBC spectroscopy for the compound.

Table 1: ^{1}H-NMR, ^{13}C-NMR, DEPT, HMQC, COSY and HMBC data of Retusasterol

C	^{13}C δ_C (ppm)	DEPT (135-90)	HMQC δ_H (j =Hz)	^{1}H-^{1}HCOSY δ_H (ppm)	HMBC
1	37.26	CH2	1.84 -1.1 t j=6.3	3.55	C2
2	31.93	CH2	1.49 d j=6.1	3.55/1.84	
3	71.84	CH	3.55 m		C-4, C2
4	42.33	CH2	2.3 t j=6.2	3.55	C-3, C-5
5	140.77	C	----------	----------	
6	121.76	CH	5.37 d j=5.4		C-5
7	33.94	CH2	1.98 d j=4.3	5.37	
8	31.91	CH	1.96 d j=5.1	5.37	
9	50.13	CH	0.95 d j=6	1.55	
10	36.52	C	----------	----------	
11	21.10	CH2	1.55 m	2.05	C9
12	39.78	CH2	2.05-1.16 t j=4.4	1.55	C11
13	42.31	C	----------	----------	
14	56.78	CH	1.04 m	1.58	
15	24.33	CH2	1.58 m	1.96	
16	26.04	CH2	1.19 m	1.58/2.05	
17	56.05	CH	1.14 m	2.05	
18	11.89	CH3	0.7 s	----------	C-12, C-13, C14
19	19.43	CH3	1.03 s	----------	C-1, C-10, C-9, C5
20	36.17	CH	1.38 m		
21	19.86	CH3	0.94 d j=6.6	1.38	C-17, C-20
22	138.36	CH	5.17 dd j=16-5		C23, C20
23	129.27	CH	5.04 dd j=15-4.6	5.17	C-22, C24
24	31.67	CH2	1.88-1.48 d j=4	5.04	C-25
25	28.28	CH2	1.68 m		
26	45.83	CH	0.97 d j=5.3	1.28/1.48	
27	29.13	CH	1.69 qd j=7-2		C26
28	19.05	CH3	0.86 d j=6.1		C27
29	18.80	CH3	0.84 d j=4.5	1.28/1.69	C-27, C28
30	23.07	CH2	1.28 m	1.88	
31	12.01	CH3	0.82 t j=4.6		

3.2.Identification of β- Sitosterol glucoside 2

This glycoside was isolated before from other plants. However, this is the first time in which it has been isolated from *Nitraria Retusa* L. A similar strategy for identification of 2 was followed as in 1 Again, spectral methods were used, in particular, IR, MS, ^{1}H and ^{13}C – NMR, DEPT (90,135), HMQS, COSY and HMBC.

The IR spectrum showed broad strong stretching of O-H at 3398 cm^{-1}, the C-H absorption at 2934 cm^{-1}, C=C band at 1638 cm^{-1}, C-H bending and C-O stretching [6-8].

^{13}C-NMR displayed 35 signals indicating the presence of 35 carbon atoms at least. This was confirmed by DEPT 135 and DEPT 90 which showed 12 secondary, 14 tertiary, 3 quaternary, and 6 primary carbons,

the chemical shifts of which are listed in table 2 Moreover, the molecular mass, 576.6, obtained from the mass spectrum, together with the aforementioned findings, indicated that the molecular formula was **$C_{35}H_{60}O_6$** Matching the MS spectrum of **2** with those in the database showed perfect match with β- Sitosterol glucoside, and further investigation in the literature left no doubt about the structure of compound **2**

The authors have included in table 2, the ^{1}H and ^{13}C – NMR, IR , DEPT 135, DEPT 90, HMQC,^{1}H-^{1}HCOSY , and HMBC for the sake of presenting complete set of data [9] **.**

In this respect, it is worth mentioning the correlation of the anomeric proton 1` with C-3 on ring A and C-2` on the glucose moiety as shown in Figure 12[10-11].

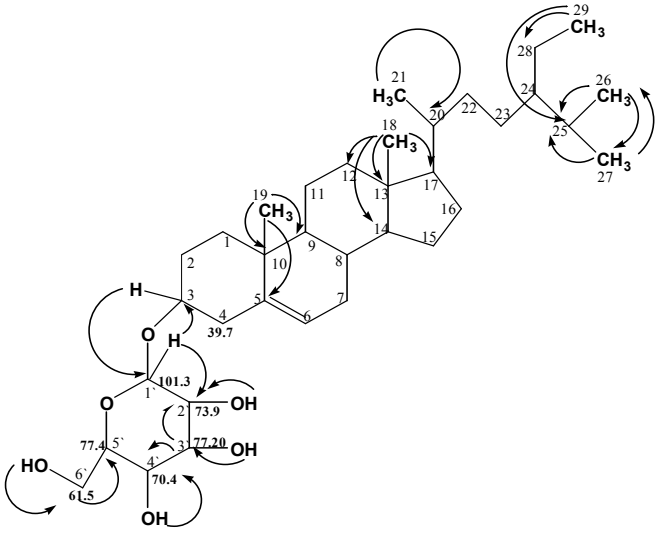


figure 12: HMBC spectroscopy for the compound.

Table 2: ^{1}H-NMR, ^{13}C-NMR, DEPT, HMQC, COSYand HMBC data of β- Sitosterol glucoside:

C	^{13}C δ_C (ppm)	DEPT (135-90)	HMQC δ_H (j =Hz)	^{1}H-^{1}HCOSY δ_H (ppm)	HMBC
1	38.78	CH_2	2.35 t j=4.9	3.44	
2	31.87	*CH_2*	1.46 d j=8.4	3.44/2	
3	77.21	*CH*	3.44 m		
4	39.73	CH_2	2.07-2 t j=5.8	3.45/5.35	
5	140.89	*C*	----------		
6	121.65	CH	5.35 t j=6.7		C-5, C-7
7	29.74	CH_2	1.82 d j=6.2	5.35	
8	31.88	*CH*	1.9 d j=5.6	5.35	
9	50.07	CH	0.9 d j=6.1	1.52	
10	36.67	C	----------		
11	21.09	CH_2	1.52 m		
12	37.32	CH_2	1.8 t j=6		
13	42.32	*C*	----------		
14	56.66	CH	1.01d j=6.7	1.8 / 1.9	C-20
15	24.35	CH_2	1.50 d j=5.1	1.9	
16	28.30	CH_2	1.78 m		
17	55.92	CH	1.06 m	1.35	
18	12.12	CH_3	0.65 s		C-12, C-13, C14, C17
19	19.08	CH_3	0.96 s		C-1, C5, C-10
20	36.00	CH	1.35 d j=6.9		
21	19.56	CH_3	1.02 d j=7.2		
22	33.82	CH_2	1.33 m	1.78	
23	25.88	CH_2	1.16 m	1.33	
24	45.61	CH	0.83 t j=6.6	1.3/1.33	
25	29.15	CH	1.75 m		
26	20.17	CH_3	0.81 d j=6.5		C-25, C-27
27	19.39	CH_3	0.8 d j=6		C-25, C26
28	23.07	CH_2	1.1-1.3 m		
29	12.35	CH_3	0.78 d j=5.2		C-28
1`	101.30	CH	4.2 d J=8.7		C-3, C2`
2`	73.90	CH	2.91 m	3.06/4.2/OH	C-1`, C-3`
3`	77.20	CH	3.06 m	OH	C-2`, C4`
4`	70.47	CH	3.02 m	3. 2/OH	
5`	77.43	CH	3.15 dd j=7-4	3.64/OH	
6`	61.50	CH_2	3.64 dd j=8-4	OH	C1`

4.Acknowledgment
The authors would like to thank Prof . Dr. Anwar Al- Khatib for his help in classifying the studied plant.

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