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CHEMICAL AND BIOLOGICAL INVESTIGATIONS OF THE ROOTS OF *SONCHUS OLERACEUS* L. GROWING IN EGYPT

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من خلال الدراسه الكيميائيه لجذور نبات السنكس أوليراسيوس التابع للعائله المركبه والذى ينمو فى مصر، تم فصل خمسة مركبات هي: لاليوليد ١ والذى يفصل لأول مره من جنس السنكس بالإضافة الى ١٥-جلوكوبيرانوزيل- بيتا١١،١٣ -ثنائى هيدرو يوروسبرمال أ ٢ ، حمض الأورسوليك ٣ ، لوبيول ٤ ، بيتا سيتوستيرول-٣-جلوكوبيرانوزيد ٥ والتى تفصل لأول مره من نبات السنكس أوليراسيوس. وقد تم التعرف على المركبات المفصولة عن طريق الدراسة الطيفية المختلفه (أحادية وثنائية الأبعاد ومطياف الكتله).

الدراسه الحيويه للمركبات التى تم فصلها أثبتت أن لمركبي اللاليوليد ١ و ٥ جلوكوبيرانوزيل بيتا١١،١٣ -ثنائى هيدرو يوروسبرمال أ ٢ تأثير مثبط للأورام عند استخدام الخلايا من نوع PC33 و L5187Y. كما ثبت ان لهذين المركبين تاثير مضاد للبكتريا من نوع: باسيلاس سبتيليس، استافيلاس ايوريوس، اى كولاى ونيسيريا جونوريا عند تركيز ١٠ و ٥ ميكروجرام.

*Phytochemical study of the roots of Sonchus oleraceus L. (Astraceae) growing in Egypt, afforded loliolide **1** for the first time from the genus Sonchus in addition to 15-O-β-glucopyranosyl-11β,13-dihydrourospermal A **2**, ursolic acid **3**, lupeol **4,*** and β-*sitosterol-3-O-β-glucopyranoside **5** for the first time from the species. The biological evaluation of the isolated compounds showed cytotoxic activity of **1** and **2** against L5187Y cell line, while compound **2** showed activity against PC33 cell line. In addition to antibacterial activity of compounds **1** and **2** against S. aureus, B. subtilis, E. Coli, and N. gonorrhoea. The structures of the isolated compounds were elucidated using 1D (^{1}H and ^{13}C), 2D (H-H COSY, HMQC and HMBC) NMR and MS spectroscopic data.*

INTRODUCTION

The genus *Sonchus* belongs to sub-tribe Crepidinea, tribe Lactuceae and family Astraceae[1] and includes more than 50 species[2]. This genus is represented in Egypt by five species namely: *maritimus*, *oleraceus*, *asper*,

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macrocarpus and *tenerrimus*[3]. Sonchus plants are well-known with their content of sesqueterpene lactones of the eudismanolide[4&5] and guaianolide structures[6]. Other constituents include ionone glycosides[7], phenyl propanoids[8], phenolics [flavonoids and coumarins][9], in addition to sterols and lignans[10].

Sonchus oleraceus L. which is a common annual herb, with erect stem branched near the pale yellow inflorescence[11] and known as smooth sow-thistle[12]. In Upper Egypt it is commonly known as lobbain due to its milky juice secretion. Previous studies of *S. oleraceus* L. reported the isolation of eudes-manolide and guaianolide lactone glycosides from the aerial parts of the plant growing in Japan[13] and the detection of flavone glycosides in the plant growing in Canary Island[2]. This paper describes the phytochemical investigation of the roots of the plant growing in Egypt as well as the biological evaluation of the isolated compounds. Where the monoterpene loliolide **1** was isolated for the first time from the genus *Sonchus*, in addition to 15-*O*-β-glucopyranosyl-11β, 13-dihydrourospermal A **2**, ursolic acid **3**, lupeol **4** and β-sitosterol-3-*O*-glucopyranoside **5** (Fig. 1), which were isolated for the first time from the species. Besides, the crude alcoholic extract, compound **1** and **2** showed antibacterial and antifungal activities against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Neisseria gonorrhoea*, and the fungal strains: *Candida albicans* and *Aspergillus flavus*. Compounds **1** and **2** showed *in-vitro* cytotoxic activity against L5187Y cell line while **2** only showed cytotoxic activity against PC33 cell line.

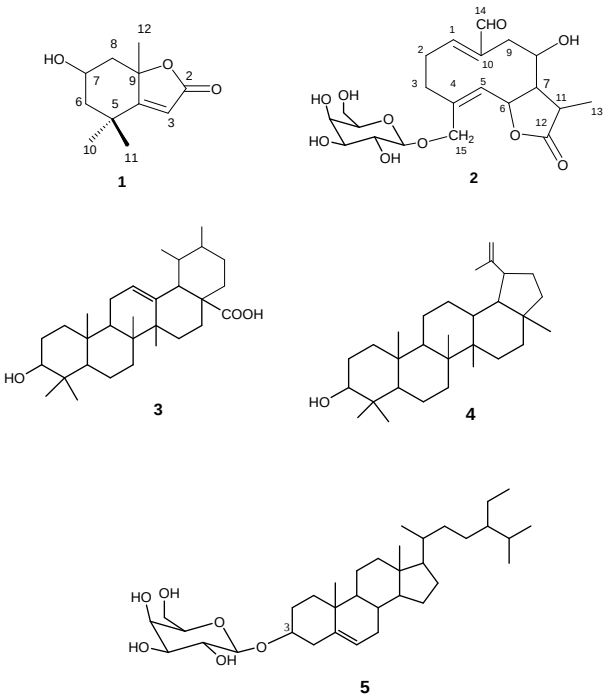


Fig. 1: Structure of compounds **1**-**5**.

EXPERIMENTAL

General experimental procedures

Pre-coated silica gel 60 F_{254} plates (Merck) were used for TLC. Vacuum liquid chromatography (VLC) was carried out using silica gel 60, 0.04-0.063 mm mesh size (Merck). The solvent systems used for TLC analysis were n-hexane-EtoAc (9:1, system I), $CHCl_3$-MeOH (9:1, system II) and $CHCl_3$-MeOH (75:25, system III). The TLC plates were visualized by spraying with *p*-anisaldehyde /H_2SO_4 reagent and heating at 110°C for 1-2 min. ^1H and ^{13}C-NMR spectra were recorded on a JEOL-JNM-EX-400 spectrometer (400 MHz for ^1H and 100 MHz for ^{13}C). EI-MS data were obtained with a JEOL JMS−700T mass spectrometer. The melting point was determined using an Electrothermal 9100 Digital melting point apparatus (Electro-thermal Engineering Ltd, Essex, England). The IR spectrum was carried out using Shimadzu Infrared-400 spectrophotometer (Kyoto, Japan). All solvents were distilled prior to use. NMR grade solvents (Merck) were used for NMR analysis.

Plant material

The fresh roots of *S. oleraceus* L. were collected in March and April 2007 from the wild plants around the campus of Al-Azhar University, Assiut, Egypt. The plant material was kindly identified by Prof. Dr. A. Fayed, Professor of Plant Taxonomy, Faculty of Science, Assiut University, Egypt. A voucher specimen was deposited in the Department of Pharmacognosy herbarium, Faculty of Pharmacy, Al-Azhar University, Assiut (Registration code W. Az-007 So).

Extraction and isolation

The air-dried powdered roots of *S. oleraceus* (0.9 kg) were extracted with 70% MeOH (4x3 L) at room temperature; evaporation of the methanol extract under reduced pressure affords a dark brown oily residue (7.6 g). The residue was subjected to VLC on silica gel using $CHCl_3$: MeOH gradient (starting with 100% $CHCl_3$ to 100% MeOH) and afforded 6 fractions. Fraction I was chromatographed on silica gel column and eluted with $CHCl_3$: MeOH (95:5) afforded compounds **1** (5.2 mg), **3** (13.7 mg) and **4** (11.4mg). Fraction II was subjected to silica gel column and eluted with $CHCl_3$: MeOH (85:15) to afford compound **5** (18 mg). Finally, fraction III was chromatographed on silica gel and eluted with $CHCl_3$: MeOH (8:2), and further purified by silica gel column chromatography using $CHCl_3$: MeOH (8:2) to yield compound **2** (4.3 mg).

Biological study

Cytotoxicity assay

The cytotoxicity was evaluated by the [^3H] thymidine assay[14] against mouse lymphoma (L5178Y) and rat brain cancer (PC33) cell lines. All cells were mycoplasma-free and cultures were propagated under standardised conditions[15].

Antimicrobial assay

The antibacterial and antifungal activities were evaluated using the agar plate diffusion assay[16]. Susceptibility discs (5.5 mm) were impregnated with solution of each of the alcoholic extract, compounds **1** and **2** at concentrations of 5 and 10 µg/ml. The discs were dried and placed on agar plates inoculated with the test bacterial strains: *B. subtilis*, *S. aureus*, *E. coli* and *N. gonorrhoea*, and the fungal strains: *C. albicans* and *A. flavus*. Each plate was inoculated with a single organism and the test was run in duplicates. The plates were incubated at 37°C and checked for inhibition zones after 24 hrs for bacteria and after 48 hrs for fungi. Benzyl-penicillin was used as a positive reference standard.

RESULTS AND DISCUSSION

Compound **1**. Was isolated from choloroform/methanol (95:5) fraction, recrystallized from acetone as white needle crystals with melting point 151-153°C. The EIMS showed molecular ion peak at m/z 197 $[M+H]^+$ calculated for $C_{11}H_{16}O_3$ with significant fragment ions at m/z 181 $[M- CH_3]^+$, 178 $[M- H_2O]^+$, 163 $[M- H_2O- CH_3]^+$. The IR spectrum (KBr) showed absorption bands at 3450, 1735, 1630, and 850 cm^{-1} characteristic for the presence of hydroxyl group, γ-lactone, α,β-unsaturated ketone, and tri-substituted double bond, respectively[4]. They were confirmed by the observed signals in ^1H- and ^{13}C-NMR spectra at δ_H 5.69/ δ_C 113.2 and 182.1 characteristic for the tri-substituted double bond (H-3 /C-3), 4.31/66.9 (hydroxymethine) and at δ_C 172.0 and 87.0 for the lactone moiety. Furthermore, the ^1H-NMR spectrum (Table 1) showed the presence three methyl singlet signals: two of them were geminal methyl at δ_H 1.27 (Me-10) and 1.47 (Me-11) and one at δ_H 1.78 (Me-12) which was bound to a quaternary carbon. These findings were supported by the observed HMBC correlations of the olefenic proton at δ_H 5.69 (H-3) and the carbons at δ_C 182.1 (C-4), 172.0 (C-2) and 87.0 (C-9). The HMBC cross peaks between Me-10 (δ_H 1.27) with C-5, C-6 and C-4, Me-11 (δ_H 1.47) with C-5, C-6 and C-4, H-7 (δ_H 4.31) with C-6 and C-8, in addition to the cross peaks between Me-12 (δ_H 1.78) with C-9, C-8 and C-4. Compound **1** therefore corresponded to loliolide which was previously isolated from *Alchornea glandulosa* (Euphorbiaceae)[17], in addition to several plants including *Eirmocephala megaphylla* (Astraceae)[18], *Digitalis lanata* (Scrophulariaceae)[19] and *Arnica Montana* (Astraceae)[20]. Loliolide was considered as a biosynthetic degradative product of terpenoids[21]. It is the first time of isolation for loliolide from genus *Sonchus*.

Compound **2**. Was isolated as an oily residue from chlororform/ methanol (8:2) fraction. The EIMS showed a molecular ion peak at m/z 443 $[M+H]^+$ calculated for $C_{21}H_{31}O_{10}$ with significant fragment ions at m/z 427 $[M- CH_3]^+$, 424 $[M- H_2O]^+$, 413

[M- CHO]$^+$ and 263 [M - glucose]$^+$. The IR spectrum (KBr) showed similar diagnostic absorption bands to those of **1** at 3445 (hydroxyl group), 1770 (γ-lactone) and 860 cm^{-1} due to tri-substituted double bond. The ^{1}H-NMR spectrum (Table 1) showed signal for aldehydic proton at δ_H 9.60 (s), two olefenic protons at δ_H 6.85 (t, *J* = 8.5 Hz) and 5.11 (d, *J* = 10.3 Hz), in addition to a hydroxymethine at δ_H 4.03 (m) and a secondary methyl at δ_H 1.37 (d, *J* = 6.7 Hz). Furthermore, the spectrum showed an anomeric proton of glucose at δ_H 4.41 with coupling constant 7.6 Hz indicated β-configuration. The ^{13}C-NMR (Table 2) showed the presence of 21 carbons, including six carbons of a glucopyarnosyl moiety. The DEPT and HMQC experiments confirmed the presence of the aldehydic carbon at δ_C 199.8, four olefinic carbons associated with two double bonds at δ_C 129.4, 137.0, 144.6 and 160.0, in addition to oxygenated methine at δ_C 71.1 and an oxygenated methylene at δ_C 67.5. The carbon resonances at δ_C 41.0, 55.9, 76.2 and 180.0, in addition to the methyl at δ_C 15.5 indicated the presence of methyl-γ-lactone moiety[8] as suggested by the IR spectrum. The

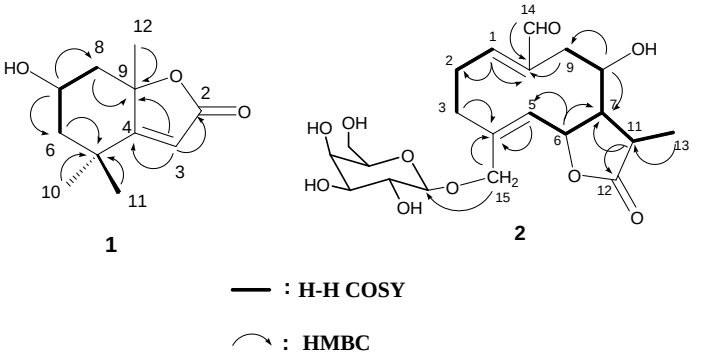
Table 1: ^{1}H-NMR data of compounds **1** ($CDCl_3$) and **2** (DMSO-d_6) at 400 MHz.

Position	**1**	**2**
1	-	6.85 (1H, t, *J* = 8.5 Hz)
2	-	2.51 (2H, m)
3	5.69 (1H, s)	2.07 (1H, m) 2.73 (1H, m)
4	-	-
5	-	5.11 (1H, d, *J* = 10.3 Hz)
6	1.79 (1H,dd, *J* = 9.3, 4.0 Hz) 1.53 (1H, dd, *J* = 14.6, 3.7 Hz)	4.88 (1H, t, *J* = 10.3 Hz)
7	4.31 (1H, m)	1.64 (1H, m)
8	2.47 (1H, dt, *J* = 14.1, 2.6 Hz) 1.98 (1H, dt, *J* = 14.6, 2.7 Hz)	4.03 (1H, m)
9	-	2.35 (1H, d, *J* = 15.7) 2.94 (1H, m)
10	1.27 (3H, s)	-
11	1.47 (3H, s)	2.64 (1H, m)
12	1.78 (3H, s)	-
13	-	1.37 (3H, d, *J* = 6.7 Hz)
14	-	9.60 (1H, s)
15	-	4.56 (1H, d, *J* = 11.5 Hz) 4.28 (1H, d, *J* = 11.5 Hz)
glucose H-1`	-	4.41 (1H, d, *J* = 7.6 Hz)
H-2` to H-6`	-	3.2 -3.9 (10H, m)

Table 2: ^{13}C-NMR data of compounds **1** ($CDCl_3$) and **2** (DMSO-d_6) at 100 MHz.

Position	**1**	**2**
1	-	160.0
2	172.0	27.7
3	113.2	33.3
4	182.1	137.0
5	35.8	129.4
6	47.5	76.2
7	66.9	55.9
8	45.4	71.1
9	87.0	32.7
10	30.4	144.6
11	26.5	41.0
12	27.1	180.0
13	-	15.7
14	-	199.8
15	-	67.5
glucose C-1`	-	102.5
C-2`	-	73.4
C-3`	-	76.8
C -4`	-	69.7
C- 5`	-	76.0
C -6`	-	61.5

presence of the methyl-γ-lactone moiety was further confirmed by the HMBC cross peaks (Fig. 2), between H-6 with C-7, H-7 with C-6 and C-11 and between H-11 with C-12, C-7 and C-13, moreover the cross peaks between Me-13 (δ_H 1.37) with C-11, confirmed the methyl-γ -lactone moiety. The sequence of the aliphatic and olefenic protons was made-up using the H-H COSY experiment (Fig. 2), which afforded the series from H-1 to H_2-3 and from H-5 to H_2-9. The spin system from H-6 to H_3-13 through H-7 and H-11, afforded further evidence for the methyl-γ-lactone moiety. The HMBC cross peaks between H-1 with C-10, H_2-3 with C-4, H_2-9 with C-10 and H-5 with C-6 and C-4, and the cross peaks between H_2-15 with C-4, indicated a costunolide nucleus[22]. The position of the aldehydic group at C-10 was established by the HMBC correlation of H-14 with C-10. The cross peak of H_2-15 with C-1' indicated the attachment of the glucopyarnosyl moiety to C-15. From the abovementioned data **2** was identified as 15-O-β-glucopyranosyl-11β,13-dihydrourospermal A which was previously isolated from the roots of *Sonchus asper*[6], but this is the first isolation from *S. oleraceus.*



1 **2**

: H-H COSY

: HMBC

Fig. 2: Important 2D correlations of compounds **1** and **2**.

Compounds **3-5** were identified as ursolic acid[23], lupeol[24] and β-sitoststerol-3-*O*-β-glucopyranoside[25], respectively on comparing their physical and spectral data with literatures. These compounds were isolated for the first time from *S. oleraceus* L.

The *in-vitro* evaluation of the cytotoxic activity of compounds **1** and **2** using the thymidine assay, showed that 15-O-β-glucopyranosyl-11β,13-dihydrourospermal A **2** was active against L5178Y and PC33 cell lines (ED_{50} 6.2 and 5.2 µg/ml, respectively), meanwhile loliolide **1** was active only against L5178Y (ED_{50} 4.7 µg/ml).

The antimicrobial activity of the alcoholic extract and compounds **1** and **2** (Table 3), revealed antibacterial activity against; *B. Subtilis*, *E coli*, *S. aureus* and *N. gonorrhoea*. The alcoholic extract (10 µg/ml) showed inhibition zones of 10, 9, 9 and 8 against the tested strains, respectively. Compound **2** (10 µg/ml) was the most active as it showed inhibition zones of 16, 16, 15 and 15, while compound **1** (10 µg/ml) was less active as the inhibition zones of 12, 13, 14 and 15. None of the tested compounds or the alcoholic extract showed activity against the fungi *C. albicans* or *A. flavus*.

It is noteworthy to mention that this is the first cytotoxic and antimicrobial evaluation of loliolide **1** and 15-*O*-β-glucopyranosyl-11β,13-dihydrourospermal A **2**, although loliolide was reported to have immunosuppressive activity against T and B-lymphocytes[26].

Table 3: Inhibition zones of the alcoholic extract and compounds **1** and **2**.

Sample		*B. subtilis*	*E. coli*	*S. aureus*	*N. gonorrhoea*
Alc. Ext.	5 µg	8	7	8	7
	10 µg	10	9	9	8
1	5 µg	9.5	10	10.5	12
	10 µg	12	13	14	15
2	5 µg	10.5	11	12	13.5
	10 µg	16	16	15	15

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