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Cytotoxic and Antibacterial Constituents from the Roots of *Sonchus oleraceus* L. Growing in Egypt

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ABSTRACT

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-11*β*,13-dihydrourospermal A 2, ursolic acid 3, lupeol 4 and β-sitosterol-3-O-glucopyranoside 5 for the first time from the plant. The biological evaluation of the isolated compounds showed cytotoxic activity of 1 and 2 against PC33 and L5187Y cell lines, in addition to antibacterial activity against *S.aureus*, *B. subtilis*, *E. Coli* and *N. gohnorea*. The structures of the compounds were elucidated using 1D (^{1}H and ^{13}C), 2D (H-H COSY, HSQC and HMBC) NMR and MS spectroscopic data.

KEYWORDS: *Sonchus oleraceus, Astraceae, Terpenes, Cytotoxicity, Antibacterial activity.*

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*, *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 includes ionone glycosides (7), phenyl propanoids (8), phenolics [flavonoids and coumarines] (9), in addition to sterols and lignans (10).

Sonchus oleraceus L. which is a common annual herb, with erect stem branched near a 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. Previous studies of *S. oleraceus* reported the isolation of eudesmanolide and guaianolide lacone glycosides from the plant growing in Japan (13) and the detection of flavones glycosides in the plant growing in Canary Island (2). This paper describes the phytochemical invetigation 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-11*β*,13-dihydrourospermal A 2, ursolic acid 3, lupeol 4 and *β*-sitosterol-3-O-glucopyranoside 5 which were isolated for the first time from the plant. Besides, the crude alcoholic extract, compound 1 and compound 2 showed antibacterial activites against against *S. aureus*, *B. subtilis*, *E. coli* and *N. gohnorea*. Compounds 1 and 2 showed *in vitro* cytotoxic activity against L5187Y cell line while compound 2 only showed cytotoxic activity against PC33 cell line.

MATERIALS AND METHODS

Plant Material

The fresh roots of *S. oleraceus* L. were collected in March-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).

Pre-coated silica gel 60 F_{254} plates (E. 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 analyses were $CHCl_3$-MeOH (97:3, 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. HPLC was performed on semi-preparative RP-18 column (Cosmosil 5C18 ARII, 250 × 10 mm) with a UV detector at λ_{max} 220 nm and flow rate of 2.5 ml/min. ^{1}H and ^{13}C-NMR spectra were recorded on a JEOL-JNM-EX-400 spectrometer (400 MHz for ^{1}H and 100 MHz for ^{13}C, respectively). EI-MS data were obtained with a JEOL JMS–700T mass spectrometer. All solvents were distilled prior to use. NMR grade solvents (Merck) were used for NMR analysis.

Extraction and Isolation

The air-dried powdered aerial parts of *S. oleraceus* (1.2 kg) were extracted with 70 % MeOH (4 × 3 L) at room temperature; evaporation of the methanol extract under reduced pressure affords a dark brown residue (14.2 g). The residue was subjected to VLC on silica gel using $CHCl_3$: MeOH gradients and afforded 6 fractions. Fraction I was chromatographed on silica gel and eluted with $CHCl_3$: MeOH gradient and afforded compounds 1 (6.2 mg), 3 (13.7 mg) and 4 (11.4mg). Fraction II was chromatographed on silica gel and eluted with $CHCl_3$: MeOH gradient to afford compound 5 (18 mg). Meanwhile, fraction III was chromatographed on silica gel and eluted with $CHCl_3$: MeOH gradient, followed by semi-preparative RP-18 HPLC [MeOH: H_2O (40:60)], to compound afford 2 (4.3 mg).

NMR data.

Loliolide 1: ^{1}H-NMR ($CDCl_3$, 400MHz): 5.69 (1H, s, H-6), 4.33 (1H, ddd, *J* = 3.2,3.6,3.18 Hz, H-2), 2.47 (1H, dt, *J* = 14.1,2.6, H-1a), 1.98 (1H, dt, *J* = 14.6,2.7, H-1b), 1.79 (1H, dd, *J* = 9.3,4 Hz, H-3a), 1.53 (1H, dd, *J* = 14.6,3.7 Hz, H-3b), 1.78 (3H, s, Me-12), 1.47 (3H, s, Me-11), 1.27 (3H, s, Me-10).

^{13}C-NMR ($CDCl_3$, 100MHz): 182.1 (C-5), 172 (C-7), 113.2 (C-6), 87 (C-9), 66.9 (C-2), 47.5 (C-3), 45.4 (C-1), 35.8 (C-4), 30.4 (C-10), 27.1 (C-12), 26.5 (C-11).

15-O-β-glucopyranosyl-11β,13-dihydrourospermal A 2: ^{1}H-NMR (DMSO-d_6, 400MHz): 9.6 (1H, s, H-14), 6.85 (1H, t, *J* = 8.5 Hz, H-1), 5.11 (1H, d, *J* = 10.3 Hz, H-5), 4.88 (1H, t, *J* = 10.3 Hz, H-6), 4.56 (1H, d, J = 11.4 Hz, H-15a), 4.28 (1H, d, J = 11.7 Hz, H-15b), 3.91 (1H, m, H-8), 2.94 (1H, m, H-9a), 2.35 (1H, d, J = 15.7 Hz, H-9b), 2.64 (1H, m, H-11), 2.51 (2H, m, H_2-2), 2.07 (2H, m, H_2-3), 1.64 (1H, m, H-7), 1.37 (3H, d, J = 6.7 Hz, Me-13). The glucose moiety 4.52 (1H, d, J = 7.4 Hz, H-1'), 3.2-3.9 (10H, m, H-2' to H-6').

^{13}C-NMR (DMSO-d_6, 100MHz): 199.1 (C-14), 181.3 (C-12), 160 (C-1), 144.8 (C-10), 136.8 (C-4), 129.7 (C-5), 76.2 (C-6), 71.3 (C-8), 67.5 (C-15), 55.9 (C-7), 41 (C-11), 33.3 (C-3), 33 (C-9), 27.7 (C-2), 16.1 (C-13). The glucose moiety: 101.8 (C-1'), 76.8 (C-3'), 76 (C-5'), 73.2 (C-2'), 69.8 (4'), 61.4 (C-6').

BIOLOGICAL STUDY

Cytotoxicity Assay. The cytotoxicity was evaluated by the [^{3}H] Thymidine assay (14) against mouse lymphoma (L5178Y), rat brain cancer cells (PC33) and human nasopharynx carcinoma cells. 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 (5.5mm) were impregnated with solution of each of the alcoholic extract and 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: *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Neisseria gonorrheae*, and the fungal strains: *Candida albicans* and *Aspergillus 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 h for bacteria and after 48hrs for fungi.

RESULTS AND DISCUSSION

Compound 1. Was isolated as needle crystals, the EIMS showed molecular ion peak at m/z 197 $[M+H]^+$ with a significant fragment at m/z 178 $[M- H_2O]^+$ suggested the presence of an hydroxyl substituent. The ^{1}H-NMR spectrum, showed the presence of an olefenic proton at δ_H 5.69 (s), three methyls at δ_H 1.27 (s), 1.47 (s) and 1.78 (s). In addition to oxymethine signal at δ_H 4.33 (dt, *J* = 3.8, 3.6 Hz). The ^{13}C-NMR spectrum showed the presence of 11 carbons. The carbon resonances suggested an α,β- unsaturated lactone as elicited by the carbons at δ_C 182.1, 172 and 113.2, which was confirmed

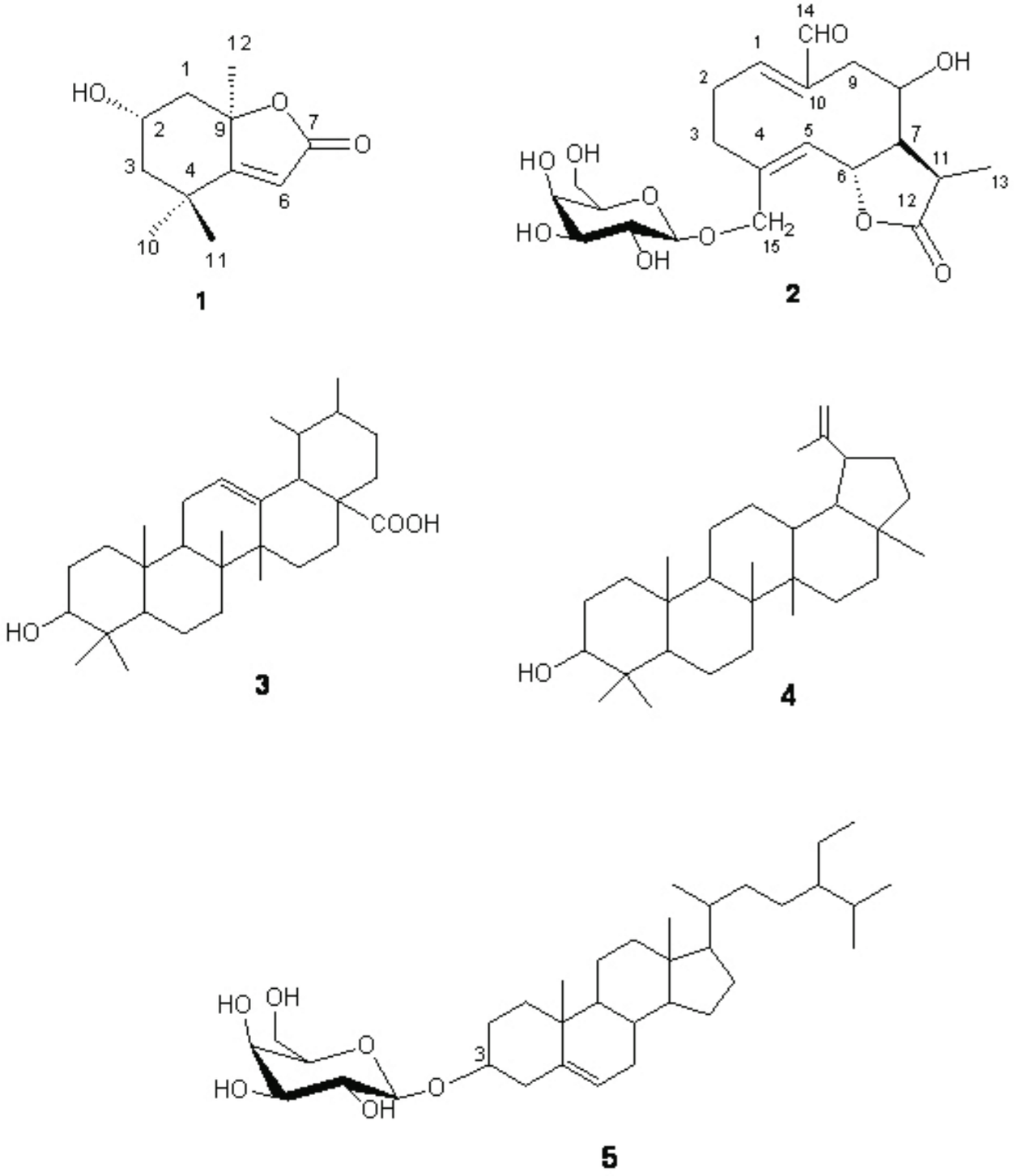

Figure 1. Structure of compounds 1-5.

by the HMBC cross peaks of the olefenic proton at δ_H 5.69 (H-6) with the carbons at δ_C 182.1 (C-5), 172 (C-7) and 87 (C-9). The HMBC cross peaks of Me-10 with C-4, Me-11 with C-4, H-2 with C-1 and C-3, in addition to the cross peaks of Me-12 with C-9 have assigned structure 1. From the previous data, 1 was identified as loliolide (17,18), and this is the first isolation from genus *Sonchus*.

Compound 2. Was isolated as an oily residue, the EIMS showed a molecular ion peak at m/z 465 $[M+Na]^+$. The ^{1}H-NMR spectrum showed signals identified as aldehydic proton at δ_H 9.6 (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 3.91 (m) and a tertiary methyl at δ_H 1.37 (d, J = 6.7 Hz). Furthermore, the spectrum showed an anomeric proton at δ_H 4.52 (d, J = 7.4 Hz). The ^{13}C-NMR showed the presence of 21 carbons, including six carbons of a glucopyarnosyl 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, in addition to the chain from H-6 to H_3-13 through H-7 and H-11. The HMBC experiment (fig. 2) showed the cross peaks of H-1 with C-10, H_2-3 with C-4, H_2-9 with C-10 and H-11 with C-12 and the cross peaks of H_2-15 with C-4, have afforded a costtunolide nucleus (19). The cross peaks of H-14 with C-10 and H_2-15 with C-1' assigned structure 2. From the previous data, 2 was identified as 15-O-β-glucopyranosyl-11β,13-dihydrourospermal A (6), and this is the first isolation from *S. oleraceus*.

Compounds (3–5) were identified as ursolic acid (20), lupeol (21) and β-sitoststerol-3-O-β-glucopyranoside (22), respectively on comparing their physical and spectral data with literatures. These compounds were isolated for the first time from *S. oleraceus*.

The *in vitro* evaluation of the cytotoxic activity of compounds 1 and 2 using the microculture tetrazolium technique (MTT), for the isolated compounds 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) and 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, revealed antibacterial activity against; *B. Subtilis*, *E coli*, *S. aureus* and *N. gonorrhoeae* (Table 1). The alcoholic extract (10μg/ml) showed inhibition zones of 10, 9, 9 and 11 against the tested strains, respectively. Compound 1 (10 μg/ml) was the most active as it showed

Table 1. Zones of inhibition of the Alcoholic extract and compounds 1 and 2.

Sample		*B. subtilis*	*E. coli*	*S. aureus*	*N. gonorrhoeae*
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

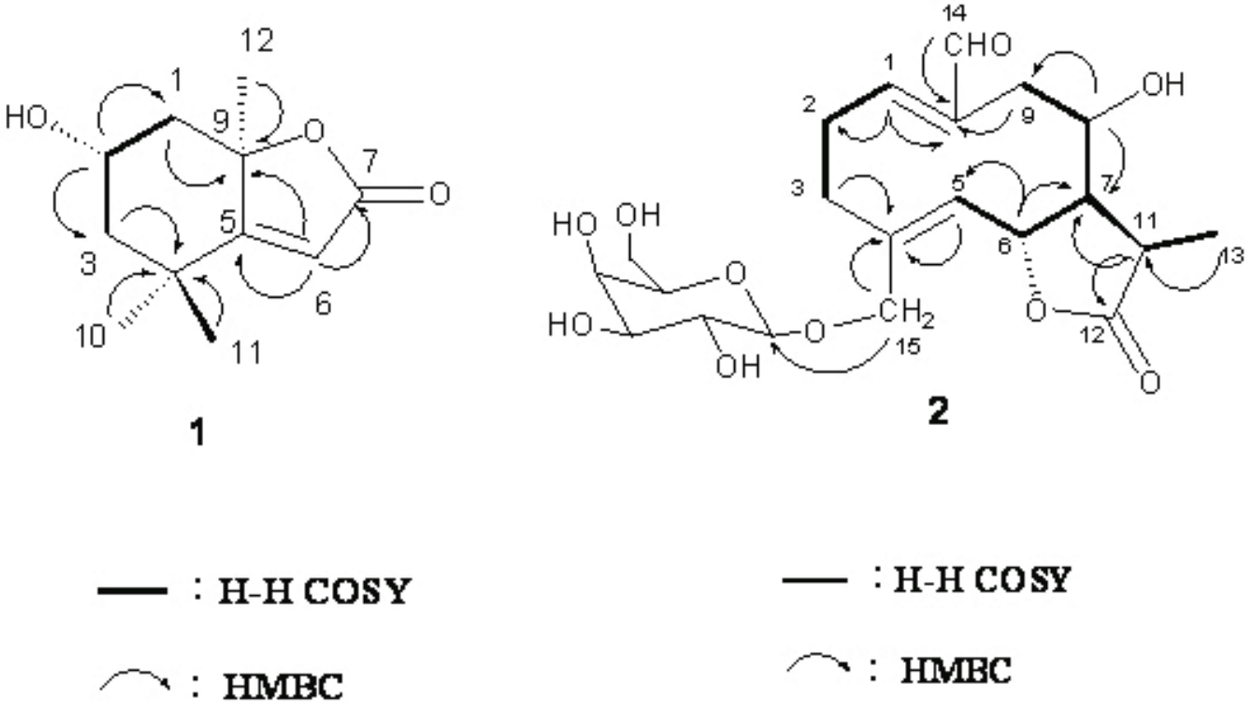

Figure 2. Important 2D correlations of compound 1 and 2.

inhibition zones of 16, 16, 15 and 15, while compound 2 (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 any activity against the fungi *Candida albicans* or *Aspergillus 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 has immunosuppressive activity against T and B-lymphocytes (23).

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