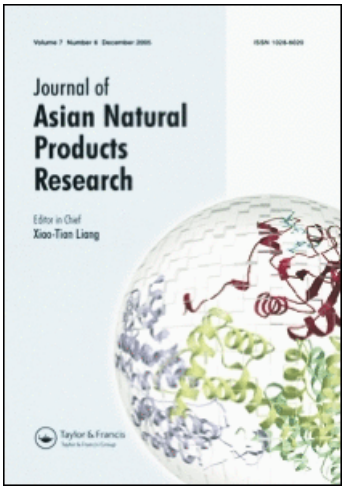


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# Portulene, a new diterpene from *Portulaca oleracea* L.

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# Portulene, a new diterpene from *Portulaca oleracea* L.

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Chromatographic fractionation of the chloroform extract of *Portulaca oleracea* L. growing in Egypt afforded a new clerodene diterpene portulene (**1**), in addition to the known compounds lupeol (**2**), β-sitosterol (**3**), and daucosterol (**4**), which were reported for the first time from the title plant. The structures of the isolated compounds were unambiguously established through 1D, 2D, and mass spectral analyses. Co-treatment of $CCl_4$ hepatic injured rats with 70% alcohol extract of *P. oleracea* significantly restored the hepatic marker enzymes and total bilirubin to near-normal values, which demonstrated hepatoprotective activity. In addition, the *P. oleracea* extract showed antibacterial and antifungal activities.

**Keywords:** portulene; rigla; *Portulaca oleracea*; hepatoprotective activity



## 1. Introduction

*Portulaca oleracea* L. (Portulacaceae) is a cosmopolitan annual herb growing in warm areas of the world [1]. It is commonly known as rigla (Egypt), purslane (USA and Australia), pigweed (England), and pourpier (France) [2]. It is an excellent source of ω-3 fatty acids, antioxidant vitamins [3], β-carotene [4], and iron [5]. Surveying the traditional and folk uses of *P. oleracea* showed that it has been used in scurvy, liver complaints, pulmonary diseases, dysentery, and mouth ulcers. Also, it is used for dry cough, burns, and skin diseases [4,6]. Recent research revealed a wide range of biological activities such as antifungal effect against dermatophytes of the genera Trichophyton [7], antinociceptive [8], antioxidant [9], and wound-healing activity [10]. A bronchodilatory effect in asthmatic patients [11], skeletal muscle relaxant [12], and antifertility effect [13] were also reported for *portulaca*. Previous chemical investigations of *P. oleracea* revealed the presence of flavonoids, coumarines [14], monoterpene glycoside [15], several nitrogenous compounds such as *N-trans*-feruloyltyramine, dopamine, dopa, and a high concentration of norepinephrine, in addition to several alkaloidal compounds [16].

In the present study, investigation of the chloroform extract of *P. oleracea* afforded a new clerodene diterpene named as portulene (**1**), in addition to the three known compounds lupeol (**2**), β-sitosterol (**3**), and daucosterol (**4**), which were isolated for the first time from the plant growing in Egypt.

## 2. Results and discussion

Compound **1** was isolated as white amorphous powder and its molecular formula was assigned as $C_{20}H_{34}O_4$ by HR-ES-IMS. The IR spectrum suggested the presence of hydroxyl and carboxyl groups in addition to substituted olefin bands 3455, 1729, and 2905 $cm^{-1}$. Analysis of the NMR spectral data ($^1H$ and $^{13}C$ aided with HMQC) revealed the presence of a carboxyl group, trisubstituted olefin, two hydroxylmethylenes, three methyls, three

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methines, two $sp^3$ quaternary carbons and seven methylenes. The $^1H-^1H$ COSY experiment identified three spin systems as it showed the assembly of the three methylenes $H_2$-1, $H_2$-2, and $H_2$-3 with H-10 and H-4 with $H_3$-18 as the first spin system; the methylenes $H_2$-6, $H_2$-7, and H-8 with $CH_3$-17 as the second spins system and the assembly of $H_2$-11 with $H_2$-12 as the third spin system.

The HMBC correlations of H-4 with C-5 and C-19 (179.4), H-10 with C-1, C-9, and C-5, in addition to correlations of H-8 with C-9 and C-10 revealed the presence of two cyclohexane rings, to which a carboxyl group was attached at C-5. Moreover, the HMBC correlations of H-14 with C-13, C-12, and C-15 and the correlations of $H_2$-16 with C-12, C-13, and C-14 showed the presence of a 1,4-dihydroxy-2-buten-2-yl-ethyl side chain, characteristic for the terpenoids of the portulaca plant [17–21]. The HMBC correlations of $H_3$-20 with C-9 and C-11 afforded the attachment of the side chain at C-9 (Figure 2). The configurations at C-5, C-8, C-9, and C-10 was determined by comparing the $^1H$ and $^{13}C$ chemical shifts of C-5, C-9, C-17, and C-20 with literatures [17,21], Thus, the structure of **1** was established as shown in Figure 1 and named portulene.

Compounds **2**–**4** were identified as lupeol [22], β-sitosterol [23], and daucosterol [24] by comparing their physical and spectral data with those reported.

Antimicrobial screening of the 70% methyl alcohol extract showed activity against the Gram-positive strains: *Bacillus subtilis*, *Staphylococcus aureus,* and *Streptococcus faecalis* (inhibition zones of 14, 13, and 15 mm, respectively) and the Gram-negative stains: *Escherichia coli*, *Neisseria gonorrhea* and *Pseudomonas aeruginosa* (inhibition zones of 14, 15, and 15 mm, respectively), in addition to antifungal activity against *Candida albicans* with inhibition zone of 12 mm.

Intraperitoneal $CCl_4$ administration caused liver injury in rats that was manifested by significant elevation in the levels of serum hepatic marker enzymes [glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT)] and total bilirubin (TB) as shown in Table 2.

Table 2 shows the activities of GOT and GPT in the serum of control and experimental animal groups. The GOT and GPT activities are expressed as mmol of pyruvate liberated per mg of protein per hour.

Results revealed marked elevation in enzyme activities of group 2 ($CCl_4$-administered rats) when compared with group 1 (control rats). Activities of the enzymes were maintained at near-normal levels in group 4 (rats co-treated with extract of *P. oleracea*). Group 3 (rats treated with *P. oleracea* extract alone) did not show any changes when compared with group 1 (control rats), which confirms the nontoxic effect of the *P. oleracea* extract.

## 3. Experimental

### *3.1 General experimental procedures*

Silica gel 60, 0.04–0.063 mm mesh size (Merck, Darmstadt, Germany), was used for column chromatography. TLC was performed on TLC plates pre-coated with silica gel $F_{254}$ (Merck, Darmstadt, Germany). IR spectra were measured on Shimadzu 470 infrared spectrometer. $^1H$ (1D, 2D COSY) and $^{13}C$ (1D, 2D HMBC) NMR spectra were recorded on Jeol 500 NMR spectrometer. FABMS measured on Finnigan MAT 8430 mass spectrometer and HR-ES-IMS obtained on JEOL JMS-700T mass spectrometer. Solvents were distilled prior to use and spectral grade solvents were used for spectroscopic measurements.

### *3.2 Plant material*

*P. oleracea* was collected from the wild-growing plants around Al-Azhar University, Assiut in April 2006. The plant material was kindly identified by Professor Dr A. Fayed, Professor of Plant Taxonomy, Faculty of Science, Assiut University. A voucher specimen is deposited in herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Al-Azhar University, Assiut, under the registration number Phg.Az.06.

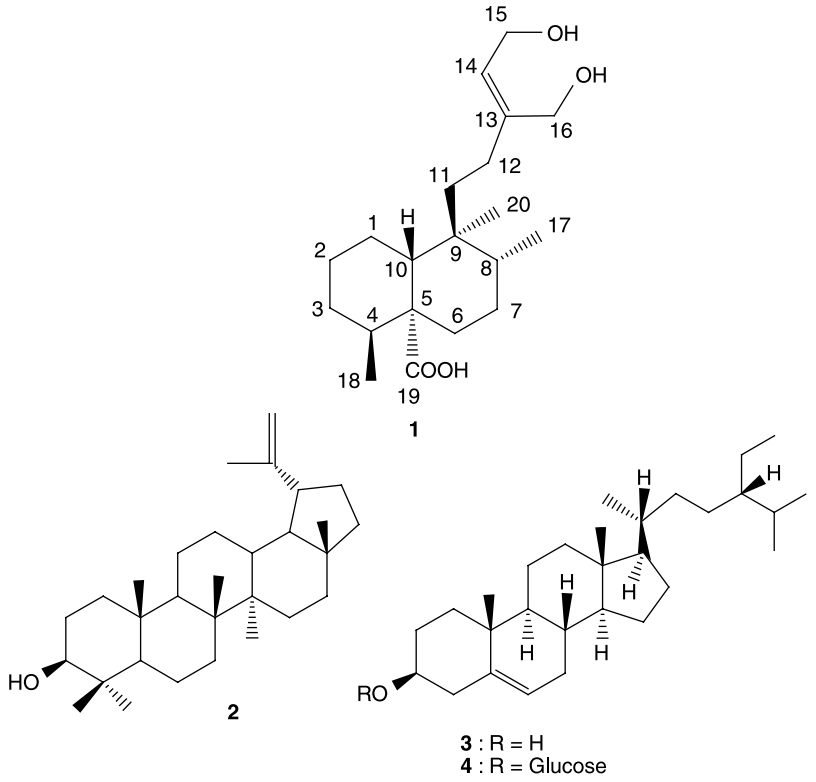


Figure 1. Structures of compounds **1–4**.

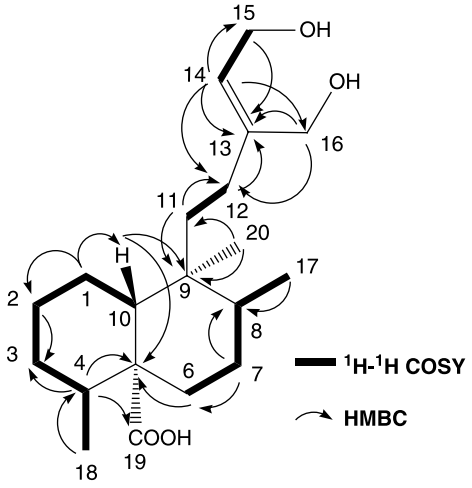


Figure 2. Key HMBC and $^{1}H-^{1}H$ COSY correlations of **1**.

### *3.3 Extraction and isolation*

The air-dried plant (1450 g) was extracted with $CHCl_3$ (31 × 7), and the extract (46.3 g) was subjected to silica gel vacuum liquid chromatography using hexane/ethyl acetate gradient to give 10 fractions. The fraction eluted with hexane/ethyl acetate (9/1) was chromatographed on silica gel and eluted with hexane/diethyl ether (85/15) to afford portulene (**1**), which was subjected to silica gel (hexane/diethyl ether 85/15) column for further purification. The fraction eluted with hexane/ethyl acetate (85/15) was chromatographed on silica gel to afford lupeol (**2**) and β-sitosterol (**3**). The fraction eluted with hexane/ethyl acetate (1/1) was chromatographed on silica gel to afford daucosterol (**4**).

Another sample of the dried plant (100 g) was extracted with 70% MeOH (0.51 × 3) to afford 11.3 g of the total extract for the biological screening.

Table 1. $^{1}H$ and $^{13}C$ NMR spectral data of portulene (**1**) (500 MHz for $^{1}H$ and 125 MHz for $^{13}C$ in $CDCl_3$).

| Position | $^{1}H$ | $^{13}C$ |
|---|---|---|
| 1 | 1.65(1H, m), 2.34 (1H, m) | 23.4 |
| 2 | 1.92 (2H, m) | 26.2 |
| 3 | 1.64 (1H, m), 1.99 (1H, m) | 30.3 |
| 4 | 1.67 (1H, m) | 36.5 |
| 5 | – | 55.3 |
| 6 | 1.27 (1H, m), 2.27 (1H, m) | 34.1 |
| 7 | 1.54 (2H, m) | 28.8 |
| 8 | 1.59 (1H, m) | 37.9 |
| 9 | – | 40.6 |
| 10 | 1.61 (1H, m) | 49.7 |
| 11 | 1.56 (2H, m) | 36.8 |
| 12 | 1.78 (1H, m), 2.11 (1H, m) | 29.1 |
| 13 | – | 144.7 |
| 14 | 5.47 (1H, t, $J = 6.8$ Hz) | 128.1 |
| 15 | 4.21 (2H, m) | 59.1 |
| 16 | 4.1 (2H, s) | 60.8 |
| 17 | 0.85 (3H, d, $J = 5.8$ Hz) | 16.1 |
| 18 | 0.85 (3H, d, $J = 5.8$ Hz) | 16.4 |
| 19 | – | 179.4 |
| 20 | 0.76 (3H, s) | 18.8 |

#### 3.3.1 *Portulene (**1**)*

White amorphous powder; IR $\gamma_{max}$ (KBr): 3455, 2905, 1729 $cm^{-1}$; $^{1}H$ NMR (500 MHz, $CDCl_3$): Table 1; $^{13}C$ NMR (125 MHz, $CDCl_3$): Table 1; HR-ES-IMS *m/z* 337.2381 $[M - H]^-$ (calculated for $C_{20}H_{33}O_4$, 337.2379), FABMS: *m/z* (rel. int. %): 339 $[M + H]^+$(16), 27 (20), 185 (100), 149 (50), 75 (86).

### 3.4 *Antimicrobial assay*

Susceptibility disks (5.5 mm) were impregnated with 1 mg of the 70% alcoholic extract dried and placed on agar plates with the test bacterium: *B. subtilis*, *S. aureus*, *S. faecalis*, *E. coli*, *N. gonorrhea*, *P. aeruginosa*, and *C. albicans*. The plates were incubated at 37°C and checked for inhibition zones after 24 h for bacteria and after 48 h for fungi [25].

### 3.5 *Hepatoprotective activity*

#### 3.5.1 *Experimental animals*

Adult male albino rats of Charles River strain weighing 120–150 g were obtained from Assiut University Animal House.

#### 3.5.2 *$CCl_4$-induced hepatotoxicity*

The animals were divided into four groups of six animals each [26].

*Group I*. Normal control, received distilled water (1 ml/kg) daily for 5 days and received olive oil (1 ml/kg, intraperitoneal) on days 2 and 3.

*Group II*. $CCl_4$ control, received distilled water (1 ml/kg) daily for 5 days and received $CCl_4$ diluted with olive oil (50% v/v) and administered intraperitoneally (200 µl/100 g of body weight) on days 2 and 3.

*Group III*. Treated with *P. oleracea* extract orally through intragastric feeding tube at a dose of 30 mg/kg. The extract dose was fixed after trying out different doses (10, 20, 30, 40, and 50 mg/kg).

*Groups IV*. Treated with *P. oleracea* extract doses of 30 mg/kg, for 5 days and 30 min after administration of extract received $CCl_4$: olive oil (1 ml/kg, intraperitoneal) on days 2 and 3.

Table 2. Effect of *P. oleracea* on the activities of the hepatic function tests in experimental animal groups (mean ± SE).

| Animal groups | GOT | GPT | TB |
|---|---|---|---|
| Control | 21.4 ± 2.4 | 35.7 ± 3.7 | 0.62 ± 0.16 |
| $CCl_4$ treated | 41.2 ± 3.2 | 86.4 ± 3.84 | 1.81 ± 0.31 |
| *P. oleracea* extract | 21.9 ± 1.6 | 36.1 ± 4.1 | 0.63 ± 0.2 |
| *P. oleracea* and $CCl_4$ | 26.6 ± 1.4 | 38.5 ± 4.7 | 0.85 ± 0.23 |

Results are expressed as mean ± SD ($n = 6$). $p < 0.01$.
Comparisons are made between group 1 (control) and group 2 ($CCl_4$, induced injury), and between group 2 and group 4 (*P. oleracea* and $CCl_4$).

### 3.5.3 Biochemical estimations

The rats were killed on the day 6 and blood was collected from orbital sinus in plain tubes. The serum was obtained by centrifugation and serum samples were taken for biochemical assays, namely GOT, GPT [27], and TB [28].

The data were expressed as mean ± SE ($n = 6$). Results were analyzed statistically by one-way ANOVA followed by comparison using Prism software (Graph Pab. Ver. 3.0). The difference was considered significant if $p < 0.01$.



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