

Secondary Metabolites from *Scorzonera undulata* ssp. *deliciosa* (Guss.) Maire (Asteraceae) and Their Antioxidant Activities

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Abstract: From the shade-dried and powdered roots of *S. undulata* ssp. *deliciosa* eight known compounds; β -Amyrin acetate, methyl Oleanate, methyl Ursolate, Stigmasterol, β -Sitosterol, Galangustin, Coumarin-*O*- β -glycoside and Acteoside were isolated. Their structures were elucidated on the basis of extensive spectroscopic analysis, including 1D and 2D NMR, chemical transformation and comparison with the related known compounds. This is the first report of occurrence of these compounds in *S. undulate* ssp. *deliciosa*. The methanol extract of the roots of *S. undulata* ssp. *deliciosa* was examined for in vitro antioxidant properties using DPPH test (radical scavenging).

Keywords: *Scorzonera undulata* ssp. *deliciosa*; secondary metabolites; antioxidant activities.

1. Plant Source

The *Scorzonera* is a genus belonging to the family of sunflower (Asteraceae). It grows mainly in dry areas of Europe and Asia. It involves about 90 species distributed throughout

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Europe, Asia and Africa. In Algeria, it consists of 8 species: *S. caespitosa* Pomel, *S. coronopifolia* Desf., *S. fasciata* Pomel, *S. laciniata* L., *S. pygmaeae* S., *S. undulata* Vahl, ssp. *alexandrina* (Boiss.) M. and ssp. *deliciosa* (Guss.) Maire [1].

They grow in arid regions, high plateau and the northern desert. Some species of *Scorzonera* were used as cooking vegetables and in traditional medicine both in Europe and Asia [2]. In Algeria *S. undulata* ssp. *alexandrina* is used in traditional medicine mainly against the snake bites [3]. The roots of *S. undulata* are collected in El-aouinet, (Eastern Algeria), during April 2004 and identified by Dr. H. Laouer (Department of Biology, University Ferhat Abbas, Setif, Algeria).

2. Previous Studies

No reports on the isolation of any secondary metabolites from *S. undulata* ssp. *deliciosa* (Guss.) Maire is available to date.

3. Present study

The shade-dried and powdered roots (970 g) of *S. undulata* were extracted with CH_2Cl_2 three times for 24 h at room temperature. The extracts were concentrated under reduced pressure and filtered. The filtrate was then evaporated to yield 23 g of the extract. This residue was extracted with MeOH three times for 24 h at room temperature. The solvent is evaporated under reduced pressure to yield 54 g of the extract. The CH_2Cl_2 soluble extract (11.5 g) was subjected to a gradient elution VLC on silica gel using the solvents Hexane: CH_2Cl_2 (100:10 – 0:100) and finally washed with MeOH to give four fractions (F1– F4). Fraction F2 (3.51g) was subjected to Si gel CC and eluted with Hexane: CH_2Cl_2 (100:0 – 0:100), to give ten fractions F2a – F2j. Fraction F2e (276.6 mg) was subjected to Si gel CC and eluted with cyclohexane: toluene (100:0 – 0:100), to give four fractions F3a – F3d. F 3b purified by column chromatography on Sephadex LH-20 eluting with MeOH to obtain β -amyryn acetate (**1**, 9.8 mg) [4]. F2g was subjected to Si gel CC and eluted with cyclohexane: toluene (90:10 – 0:100), to give four fractions F2g1 – F2g4. F2g3 purified by column chromatography on Sephadex LH-20 eluting with MeOH to obtain mixture of two products (13.7 mg) methyl oleanate [5] as compound **2** and methyl ursolate [6] as compound **3**. F4 (5.2 g) was subjected to Si gel CC and eluted with Hexane: CH_2Cl_2 (100:0 – 0:100), to give eight fractions F4a – F4h. Fraction F4c (194 mg) is spread over silica gel preparation plates and eluted with Hexane: CH_2Cl_2 (7:3) is then separated from the major product which is a mixture of two products (12 mg) stigmasterol as compound (**4**) and β -sitosterol as compound (**5**) [7, 8]. The MeOH soluble extract (54 g) was subjected to column chromatography on Sephadex LH-20 eluting with MeOH, yielding, to give seven fractions (A1– A7). Fraction A2 (3.5 g) were further separated by silica gel column chromatography eluting with Hexane: EtOAc (100:10 – 0:100) and EtOAc: MeOH (100:10 – 0:100) to give seventeen fractions (A2a – A2q). A2i purified by column chromatography on Sephadex LH-20 eluting with MeOH to obtain yellow cotton needles of galangustin (**6**, 15 mg) [8, 9]. A2n(183.3mg) was subjected to chromatography MPLC on polyamide SC6 and eluted with Toluene: MeOH (80:20–0:100), to obtain cichoriin (**7**, 12.6 mg) [10]. A3 (1.23 g) was subjected to MPLC on RP18 and eluted with H_2O : MeOH (90:10–0:100) to obtain acteoside (**8**, 70.0 mg) [11]. The structures (Fig.1) of the eight isolated compounds were identified by a combination of spectroscopic methods (MS, ^1H and ^{13}C NMR, including COSY, HMQC and HMBC) and by comparison with the previously published data.

The antioxidant activity was investigated with the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method using Blois method modified by Brand-Williams et al. [12] and Molyneux [13]. It was found that the methanol extract showed significant antioxidant activity compared to the reference antioxidant trolox in a dose dependent manner. IC50 value of the extract was found to be 0.60 $\mu\text{g}/\text{ml}$ while the IC50 value of the positive control (trolox) was found 0.87 $\mu\text{g}/\text{ml}$.

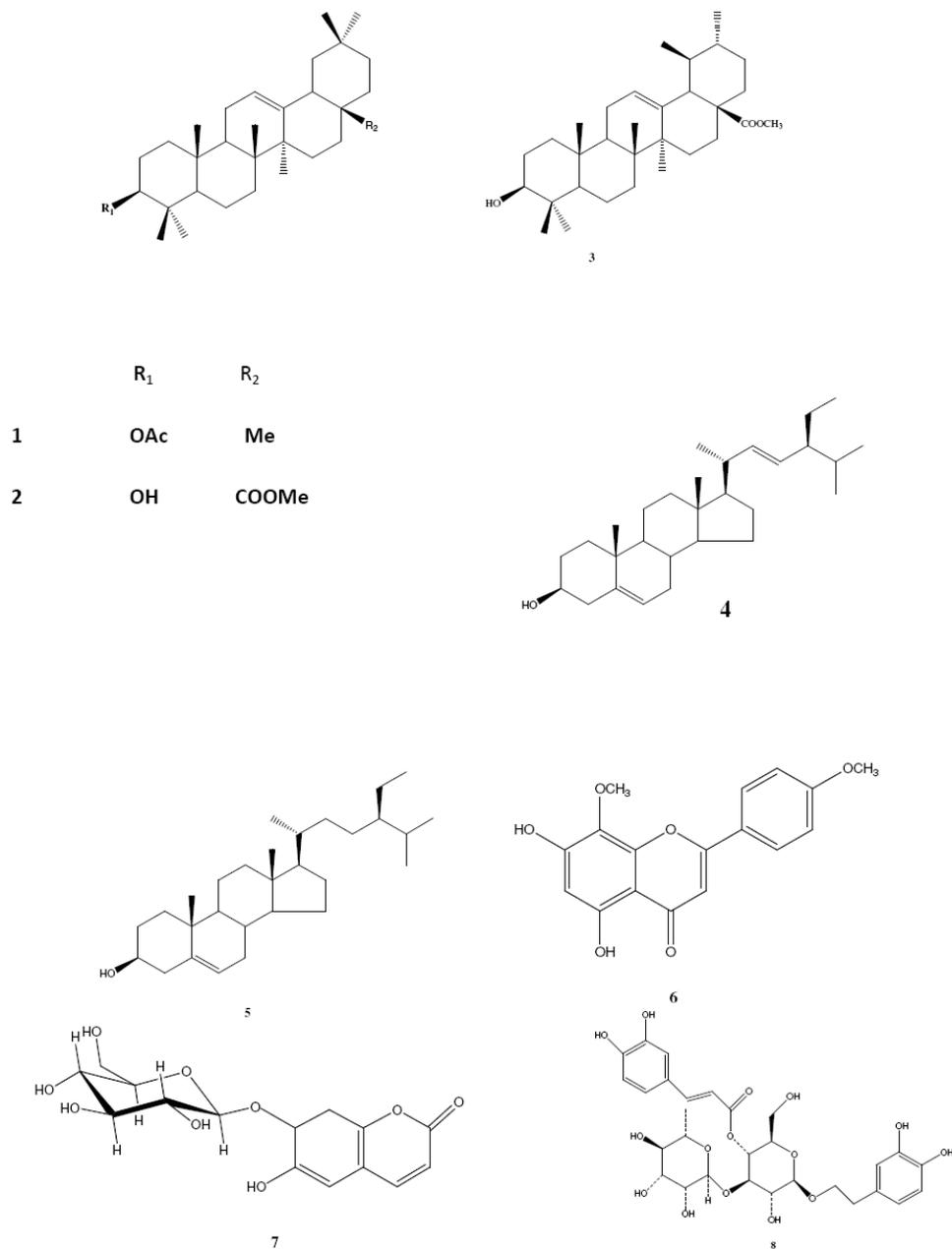


Figure 1. Chemical structures of compounds 1–8.

4. Chemotaxonomic significance

The genus *Scorzonera* is known for the presence of variety of compounds. Previous investigations led to the isolation of triterpenoids [14, 15], coumarins [16], sesquiterpene lactones and sesquiterpene glucoside [17, 18, 19], flavonoide glucoside [20, 21], dimeric guaianolides [22] and phenolic compounds [23, 24].

The present study reported for the first time β -amyrin acetate (1), methyl oleanate (2), methyl ursolate (3), stigmasterol (4), β -sitosterol (5), galangustin (6), coumarin-*O*- β -glycoside (7) and acteoside (8) in the roots of *S. undulata*. The previous compounds stress the fact that flavonoids and terpenes are common in most of the Asteraceae plants and have been encountered in several members of *Scorzonera* species. From the eight compounds characterized in this paper, the galangustin (6) and acteoside (8) were isolated from the genus *Scorzonera* for the first time. Our results show that biosynthesis of these two compounds (6 and 8) are might be a useful contribution to chemotaxonomic studies of *S. undulata*.

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