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Original article

DIHYDROISOCOUMARIN DERIVATIVES ISOLATED FROM THE ROOTS OF *SCORZONERA LATIFOLIA*

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Abstract

Scorzonera latifolia (Fisch. & Mey.) DC. is a perennial herb that grows in central and eastern region of Anatolia, Turkey. A traditional mastic named yakı sakızı has been prepared from the roots of this plant and used as an analgesic in Turkish folk medicine. In this study, the methanolic extract obtained from the roots of S. latifolia were investigated for the chemical composition. Two dihydroisocoumarin derivatives were isolated from the roots. The compounds were identified as (±) hydrangenol and (-)-scorzotomentosin 4'-O-β-glucopyranoside. The structures were determined using spectroscopic methods (^{1}H-NMR, ^{13}C-NMR, HMBC, HSQC, COSY, TOCSY, NOESY, DEPT) and chemical correlations with known compounds that have been described in the literature.

Key words: *Scorzonera latifolia, Compositae, (±) Hydrangenol, (-)-Scorzotomentosin 4'-O-β-glucopyranoside*

Scorzonera latifolia Köklerinden İzole Edilen Dihidroizokumarin Türevi Bileşikler

Scorzonera latifolia (Fisch. & Mey.) DC. orta ve doğu Anadolu'da yetişen çok yıllık bir bitkidir.Bu bitkinin köklerinden Türk halk tıbbında analjezik olarak kullanılan, yakı sakızı olarak isimlendirilen geleneksel bir sakız elde edilmektedir. Bu çalışmada, S. latifolia köklerinden elde edilen metanollü ekstre kimyasal bileşimi açısından araştırılmıştır. İzole edilen bileşikler (±) hidrangenol ve (-)-skorzotomentozin 4'-O-β-glukopiranozit olarak tanımlanmıştır. Bileşiklerin yapıları spektroskopik metotlar (^{1}H-NMR, ^{13}C-NMR, HMBC, HSQC, COSY, TOCSY, NOESY, DEPT) kullanılarak aydınlatılmış ve literatür bilgileriyle doğrulanmıştır.

Anahtar kelimeler: *Scorzonera latifolia, Compositae, (±) Hidrangenol, (-)-Skorzotomentozin 4'-O-β-glukopiranozit*

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INTRODUCTION

The genus *Scorzonera* L. is one of the member of Compositae which is the largest family of flowering plants (1) includes more than 175 species in the world from Central Europe to Central Asia (2). In Europe, there are 28 species distributed all over the continent, from Northern Russia to Spain and Crete (3, 4). In Europe some species of the *Scorzonera* have been widely used as a food as well as medicinal plants (5-7). *S. hispanica* L. (black salsify) which is the most popular member of this genus known as vipergrass, used as a vegetable and herbal remedy for some medicinal properties, such as mucolytic, diuretic, antipyretic, stomachic and in a treatment of some pulmonary diseases (6, 8). Another species, *S. humilis* L. is also used in Central Europe to treat wounds and gastro-intestinal disorders (9, 10). *S. cretica* Willd, which has been also recorded in the Flora of Turkey, is known as an appetitive agent in Greek cuisine, (3, 4). *S. mongolica* Maxim. and *S. austriaca* Willd. are some of the species that have medicinal usage in Chinese and Tibetan folk medicine as an antipyretic and antiinflammatory agent in breast inflammation and against the abscess (11). Moreover in Mongolia most of the members of *Scorzonera* genus have medicinal usage in traditional Mongolian medicine, such as; *S. divaricata* Turcz. has antipyretic and antidote activity and is used as an therapeutic agent in poisonous ulcer and malignant stomach neoplasia, *S. pseudodivaricata* Lipsch is used for treatment of diarrhea, lung oedema and parasitic diseases and possess diuretic and antipyretic activity (6). *S. radiata* is one of another species that used in traditional Mongolian medicine for its diuretic and galactagogue activities as well as medical activities in treatment of poisonous ulcer and in treatment of fever accompanying bacterial and viral infections (7).

In the Flora of Turkey, which is estimated to contain more than 11000 species of higher plants (12), the *Scorzonera* genus is represented by 39 species (4). With the addition of new species, the number is now increased to 49 (4, 13-15).

Especially, in East Anatolia, some *Scorzonera* species are known with the traditional name as *yemlik* and their roots as well as green buds are edible freshly or after cooked. *S. mollis* Bieb. and *S. suberosa* C. Koch are some examples of the edible species in north and east Anatolia and they are known as a *goftigoda* and *wild carrot,* respectively (16). Leaves of *S. cana* which is another edible species known as *karakök* or *teke sakalı* especially used in salads (17).

Scorzonera species have also some medicinal usage in Turkish folk medicine for the treatment of arteriosclerosis, kidney diseases, hypertension, diabetes mellitus and rheumatism (16).The roots of *S. tomentosa* L. contain latex, and both latex and roots are used for their wound healing activity (18). *Scorzonera latifolia* (Fisch. & Mey.) DC, is another species that has traditional usage in Turkish folk medicine. A mastic named *yakı sakızı* has been prepared from the latex of this plant and used as analgesic externally and against to infertility and internally used for its anthelmintic activity (16, 17).

The constituents of the plant are dihydroisocoumarines (3), bibenzyl derivatives (9, 10, 19), flavonoids (20, 21), lignans (10, 22, 23), phenolics (7, 24), sesquiterpenes (8, 11) and triterpenes (25).

There are few biological activity researches have been performed on *Scorzonera* species previously. Tyrolobibenzyls which were isolated from *S.humilis* showed no cytotoxic activity against P-388 cells and neither antibacterial activity against *Bacillus subtilis* nor antifungal activity against *Candida albicans* and *Trichophyton mentagrophytes* (9, 19).

Moreover, some phenolic compounds were isolated which were found to be responsible for antioxidant activity from medicinal Mongolian plants *S. divaricata*, *S. pseudodivaricata* and *S. radiata* (6, 7).

Khobrakova et al. (23) reported that syringaresinol glucoside I isolated from *S. hispanica* possesses pronounced immunomodulating properties with respect to both cellular

and humoral immunity response on the experimental model of azathioprine-induced immunosuppression.

It has been recently reported that acetylated derivative of biguaiascorzolide A which was isolated from *S. austriaca* exhibits a moderate activity against K562/ADM cell line (IC_{50} 39.8 μM) and is inactive towards MGC-803 cell line (11).

In our previous study, four *Scorzonera* species; *Scorzonera latifolia* (Fisch. & Mey.) DC, *S. tomentosa* L., *S. suberosa* C. Koch ssp. *suberosa* , *S. mollis* Bieb. ssp. *szowitzii* and *yakı sakızı* which is prepared from the root of *S. latifolia* were investigated for their antinociceptive activities. All of the methanolic extracts showed the antinociceptive activity but *S. latifolia* was established the most active species. Three triterpen derivatives were also isolated with bioactivity guided fractionation as an active constituents from n-hexane fraction of *S. latifolia* methanolic extract (26).

Herein, we report the isolation of the chemical constituents from the chloroform and ethyl acetate extract of the roots of *Scorzonera latifolia*. To the best of our knowledge, this is the first report of (±) hydrangenol **(1)** and (-)-scorzotomentosin 4'-O-β-glucopyranoside **(2)** from the *S. latifolia.*

EXPERIMENTAL

General experimental procedures

Mass spectra were measured using a Waters 2695 Alliance Micromass ZQ, LC/MS. Optical rotation power were measured on a Jasco P2000 digital polarimeter. Varian Mercury 400, 400 MHz High Performance Digital FT-NMR Spectrometer was used for ^{1}H NMR, ^{13}C NMR and 2D NMR (HMBC, HSQC, COSY, TOCSY, NOESY, DEPT) (in $CDCl_3$ and CD_3OD).

Plant material

S. latifolia (Fisch. & Mey.) DC was collected from Kars, Arpaçay in August 2005 during the flowering season. The taxonomic identification of the plant was confirmed by H. Duman, a plant taxonomist in the Department of Biological Sciences, Faculty of Art and Sciences, Gazi University. Voucher specimen was kept in the herbarium of Ankara University, Faculty of Pharmacy (AEF 23830).

Extraction method

The air-dried and powdered roots of *S. latifolia* (700 g) were extracted with methanol seven times at room temperature, each extraction lasted eight hours. The extract was filtered and concentrated under reduced pressure to yield crude extract of methanol (182.46 g).

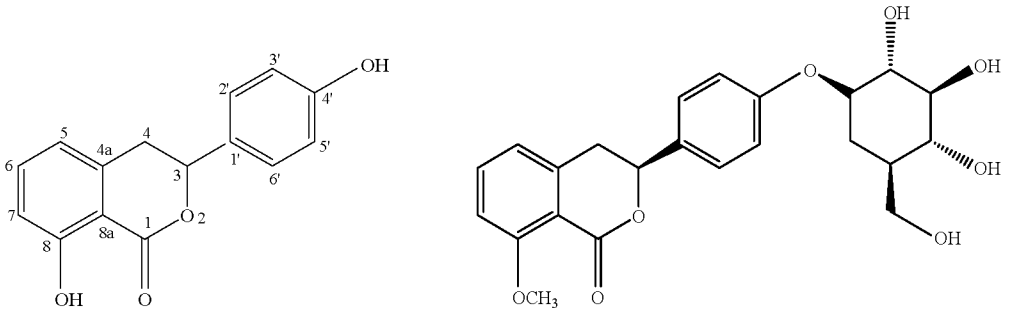
The methanolic extract was suspended in 500 ml distilled water and fractionated through successive extractions with n-hexane, chloroform, ethyl acetate, n-buthanol/saturated with water. Each fraction was concentrated to dryness under reduced pressure and below 40-50 °C on a rotary evaporator to give n-hexane fraction (n-hexane-Fr, 22.64 g), $CHCl_3$ fraction ($CHCl_3$-Fr, 7.93 g), EtOAc fraction (EtOAc-Fr, 11.02 g), n-BuOH fraction (n-BuOH-Fr, 30.39 g) and the remaining aqueous fraction (R-H_2O-Fr, 50.39 g), respectively.

In order to isolate the compounds, $CHCl_3$-Fr was applied to silicagel 60 (70-230 mesh, 0.063-0.2 mm) (Merck 1.07734.1000 ASTM) column chromatography and eluted with CH_2Cl_2:MeOH (100:0), (98:2), (95:5), (90:10), (85:15), (80:20), (75:25), (50:50) and (0:100), successively. Eluents were combined into subfractions; according to TLC behaviour using CH_2Cl_2:MeOH (95:5) solvent system. (Fr. 17-18) was subjected to second separation on Kieselgel 60. (Fr. 26-28) were purified with the preparative TLC on precoated silica gel 60 F_{254} TLC sheets (Merck 1.05744, 20x20, 0.5 mm). The precipitate would occured when dissolved

in MeOH and kept in refrigerator was removed through filtration. On TLC analysis (using CH_2Cl_2:MeOH (95:5) solvent system) of (Fr. 26-28), was shown to possess a single compound **1** (5 mg).

The EtOAc-Fr was also applied to Kieselgel 60 (0.2-0.5 mm) (Merck, Darmstadt Art. No. 7733) column chromatography and eluted with EtOAc : MeOH : H_2O (100:13.5:10) to obtain 47 fraction. Fractions were combined according to TLC results. (Fr. 18-27) was re-chromatographed on Kieselgel 60 (0.08 mm, Merck, Darmstadt Art. No. 7729). EtOAc:MeOH mixture in increasing polarity were used for elution and 153 fractions were obtained. Compound **2** (130 mg) were crystallized from (Fr. 57-60).

Spectroscopic techniques such as ^{1}H-NMR, ^{13}C-NMR and 2D NMR (HMBC, HMQC, COSY, TOCSY, NOESY, DEPT) were used for the structure elucidation of the isolated compounds. Their structures were determined as (±) hydrangenol **(1)** and **(-)**-scorzotomentosin 4'-*O*-β-glucopyranoside **(2)** (Figure 1) and results were confirmed with the known compounds that have been described in the literature (24, 27).



(±)-hydrangenol **(1)** (-)-scorzotomentosin-4'-*O*-β-glucoside **(2)**

Figure 1. Compounds isolated from *Scorzonera latifolia.*

(±) Hydrangenol (1) White crystals; $(\alpha)_D = 0$ (*c* = 2,5 $CHCl_3$); EIMS: $C_{15}H_{12}O_4Na$, $(M+ Na)^+$, *m/z* 279.58. **^{1}H NMR** ($CDCl_3$, 400 MHz, δ, ppm, J/Hz): 10.98 (1H, s, 8-OH), 7.45 (1H, t, (*J*=8.0), H-6), 7.32 (2H, d,(*J*=8.0), H-2', H-6'), 6.94 (1H, br d, (*J*=8.4), H-5), 6.88 (2H, d, (*J*=8.0), H-3', H-5'), 6.75 (1H, br d, (*J*=7.2), H-7), 5.53 (1H, dd, (*J*=3.2, 12), H-3), 5.19 (1H, br s, 4'-OH) 3.33-3.09 ((1H, dd, *J*=12.4, 16.4) - (1H, dd, *J*=3.2, 16.6) 4-H); **^{13}C NMR** ($CDCl_3$): 170.0 (C-1), 80.79 (C-3), 35.0 (C-4), 139.4 (C-4a), 116.4 (C-5), 136.6 (C-6), 117.9 (C-7), 162.2 (C-8), 108.3 (C-8a), 130.0 (C-1'), 127.9 (C-2', C-6'), 115.6 (C-3', C-5'), 156.1 (C-4').

(-)-Scorzotomentosin 4'-*O*-β-glucopyranoside (2) White crystals; $(\alpha)_D$ = -140 (*c* = 2,5 MeOH); EIMS: $C_{22}H_{25}O_9Na$, $(M+ Na)^+$, *m/z* 455.65 **^{1}H NMR** (CD_3OD, 400 MHz, δ, ppm, J/Hz): 7.59 (1H, t, (*J*=8.0), H-6), 7.42 (2H, d,(*J*=8.8), H-2', H-6'), 7.14 (2H, d, (*J*=8.8), H-3', H-5'), 7.07 (1H, br d, (*J*=8.0), H-7), 6.94 (1H, br d, (*J*=8.8), H-5), 5.45 (1H, dd, (*J*=3.2), H-3), 3.29 – 3.11 ((1H, dd, *J*=2.8, 16.4) - (1H, dd, *J*=2.8, 16.4) 4-H); **^{13}C NMR** (CD_3OD): 161.4 (C-1), 79.5 (C-3), 35.9 (C-4), 142.5 (C-4a), 119.5 (C-5), 135.3 (C-6), 111.6 (C-7), 164.1 (C-8), 112.9 (C-8a), 132.7 (C-1'), 127.5 (C-2', C-6'), 116.6 (C-3', C-5'), 158.4 (C-4'), 101.6 (C-1"), 73.7 (C-2"), 76.9 (C-3"), 70.1 (C-4"), 76.8 (C-5"), 61.3 (C-6"), 55.3 (OCH_3).

RESULTS AND DISCUSSION

The genus *Scorzonera* is very rich source of phenolic compounds, such as dihydrocoumarins, flavonoids, bibenzyl derivatives, lignans as well as some of terpenic compounds, which some of them are known for their medicinal properties. In our previous study, three triterpenes were isolated from the n-hexane fraction of the roots of *S. latifolia* which they were established as active compounds responsible for the analgesic activity of this plant (26).

In the current study, two dihydroisocoumarin derivatives (**1** and **2**) which reported for the first time in *Scorzonera latifolia* were isolated from the $CHCl_3$ and EtOAc fractions using open column chromatography. Identification of the compounds was established using NMR spectrometry.

Compound **1** was isolated as white powder and LC-MS analysis result revealed that the molecular weight was measured as *m/z* 279.58 $(M+ Na)^+$. ^1H NMR spectra of **1** showed the presence of disubstituted benzene ring (δ 7.32 (d, *J*=8.0, 2', 6'-H); 6.88 (d, *J*=8.0, 3', 5'-H); 5.19 (br s, 4'-OH)), a trisubstituted benzene ring (δ 7.45 (t, *J*=8.0, 6-H); 6.94 (br d, *J*=8.4, 5-H); 6.75 (br d, *J*=7.2, 7-H), 11.0 (br s, 8-OH)) and δ lactone ring (δ 5.53 (dd, *J*=3.2, 12, 3-H); 3.33 (dd, *J*=12.4, 16.4, 4-H) and 3.09 (dd, *J*=3.2, 16.6, 4-H)). The signals observed in the ^{13}C NMR spectra displayed thirteen C atoms. HSQC spectrum of the compound **1** exhibited correlation between δ 3.33- δ 3.09 and C(4) (δ 34.9); δ 7.32 and C(2', 6') (δ 127.9); δ 7.45 and C(6) (δ 136.6); δ 6.88 and C(3', 5') (δ 115.6); δ 6.94 and C(5) (δ 116.4); δ 6.75 and C(7) (δ 117.9); δ 5.54 and C(3) (δ 80.79). In the HMBC spectrum H-C(4) and H-C(2', 6') showed correlation with C(3) at δ 80.79; H-C(3) was correlated with C(1') at δ 130.04 and H atoms of –OH group at δ 11.0 was correlated with C(8) at δ 162.2, C(7) at δ 117.9 and C(8a) at δ 108.3. These data led us to confirm a structure of compound **1** as (±)-hydrangenol.

Compound **2** was isolated as white powder and molecular weight was determined as *m/z* 455.65 $(M+ Na)^+$. ^1H NMR and ^{13}C NMR spectra were found to be very similar to compound **1** except glucoside residue. ^1H NMR spectrum of **2** revealed the presence of disubstituted benzene ring (δ 7.42 (d, *J*=8.8, 2', 6'-H); 7.14 (d, *J*=8.8, 3', 5'-H)), a trisubstituted benzene ring (δ 7.56 (t, *J*=8.0, 6-H); 7.08 (d, *J*=8.8, 7-H); 6.94 (d, *J*=7.6, 5-H), 3.91 (s, 8-H_3CO-)) and δ lactone ring (δ 5.45 (dd, *J*=2.8, 11.6, 3-H); 3.29 (dd, *J*=2.8, 16.4, 4-H) and 3.11 (dd, *J*=2.8, 16.4, 4-H)). Comparison of the ^1H NMR and ^{13}C NMR data with compound **1** and the literature (24) exhibited compound **2** contains glucoside residue. Glucoside linkage position was determined from the correlation between the H-C(1'') and the C-4' at δ 158.4 in the HMBC spectrum. HMBC spectrum also showed the correlation between H atoms of CH_3O- group (δ 3.91) with C-8 at δ 164.1. NOESY spectrum was indicated that the presence of β-glucopyranose moiety and these data showed us compound **2** was (-)-scorzotomentosin 4'-*O*-β-glucopyranoside.

Hydrangenol has been previously isolated from *Hydrangea macrophylla* Seringe var. *thunbergii* Makino (28), *H. macrophylla* subsp. *serrata* (Thunb.) Makino (29) and *Scorzonera tomentosa* (24).

Up to know, naturally occuring coumarins have been isolated from over 800 species. Significant pharmacological activities have been described for some of these compounds such as antihistaminic, antioxidant, anticancer, antibacterial (30). Moreover, the coumarin derivatives which were reported in present study have also some important biological activities.

(±)-Hydrangenol has been showed suppressing activity on T lymphocyte proliferation induced by concanavalin A (31) and this compound was considered to be the principal antiallergenic compound of *H. macrophylla* var. *thunbergii* (32). Moreover, hydrangenol lowered blood glucose and free fatty acid levels 2 weeks after treatment with 200 mg/kg dose in mice (33).

(-)-Scorzotomentosin 4'-*O*-β-glucopyranoside, was previously isolated from *S. tomentosa* (24). In our study this compound was isolated from another *Scorzonera* species.

$CHCl_3$ fraction in which we isolated the (±)-hydrangenol, showed potent antinociceptive activity in acetic acid-induced writhing and tail-flick test and hydrangenol was determined as one of the major component of this fraction. EtOAc fraction also was determined as an active fraction in the same study (31). The antinociceptive activities of $CHCl_3$ and EtOAc fractions may be attributed to the isolated compounds. Further studies with the isolated compounds will follow.

Both compounds which determined in present study were also isolated from *S. tomentosa*. (±)-Hydrangenol was found in other species such as *Hydrangea* whereas **(-)**-scorzotomentosin 4'-*O*-β-glucopyranoside was isolated only from *Scorzonera* species up to now. Moreover *S. latifolia* roots were established as a rich source of this compound, therefore it may be considered that (-)-scorzotomentosin 4'-*O*-β-glucopyranoside has chemotaxonomic significance for *Scorzonera* species.

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