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Original Article

Triterpenoids from *Scorzonera veratrifolia* Fenzl

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ABSTRACT

The genus *Scorzonera* (Asteraceae) has several species which are used as a vegetable and in folk medicine all around the world. A phytochemical study was carried out on petroleum ether fraction of methanol extract obtained from the roots of *Scorzonera veratrifolia* Fenzl (Bitlis / Turkey) to investigate the terpenoid composition of the plant. Column chromatography, preparative thin layer chromatography and gas chromatography (GC) were used for separation of the compounds. Their structures were determined by using GC-MS and NMR techniques. Seventeen triterpenoids (ß-amyrin, ß-amyrin acetate, ß-amyrinone, germanicol, germanicol acetate, germanicone, α-amyrin, α-amyrin acetate, α-amyrinone, ψ – taraxasterol, ψ – taraxasterol acetate, taraxasterol, taraxasterol acetate, lupeol, lupeol acetate, lupenone, fern-7-en-3-one) and one sterol (ß-sitosterol) were determined. The presence of these compounds has been shown for the first time in S. veratrifolia. α-amyrinone, ß-amyrin, ß-amyrinone, ψ – taraxasterol and ψ – taraxasterol acetate are new for the genus *Scorzonera*.

Keywords: *Scorzonera veratrifolia*, triterpenoids, sterol, GC-MS

INTRODUCTION

The ancient Mediterranean genus *Scorzonera* L. is a member of the family Asteraceae, subfamily Liguliflorae, tribe Cichorieae. About 160 species of the genus are widely distributed in Eurasia, Central Asia and Africa. Turkey is considered as a diversity centre for the genus with its 52 species, 31 of which are endemic (Altınordu et al. 2015; Coşkunçelebi et al. 2015). Some *Scorzonera* species have been used as a vegetable (raw or cooked). *S. hispanica*, *S. cretica*, *S. austriaca*, *S. mollis*, *S. suberosa*, *S. cana*, *S. semicana* and *S. papposa* are some of the species that are used in the traditional cuisine of various countries (Baytop 1999; Paraschos et al. 2001; Turan et al. 2003; Granica et al. 2015; Mükemre et al. 2016; Xie et al. 2016). Several species of the genus have been utilised as folk remedies. Treatment for pain, fever, rheumatism, wounds, gastrointestinal disorders, snake-bites, carbuncle, mastitis, hepatitis B, malignant stomach neoplasia, dysentery, pulmonary diseases, colds, hypertension, infertility and gout are some of the traditional uses of the genus *Scorzonera* in several countries including Turkey, Mongolia, China and some European countries (Baytop 1999; Zidorn et al. 2000; Tsevegsuren et al. 2007; Granica et al. 2015; Xie et al. 2016; Yang et al. 2016). Previous phytochemical studies of this genus yielded; dihydroisocoumarins, benzyl phthalides, flavonoids, lignans, neolignans, bibenzyl derivatives, phenolic acid derivatives, kavalactones, sesquiterpenes and triterpenes (Sarı 2012; Granica et al. 2015).

Scorzonera veratrifolia Fenzl is native to East Anatolia and grows on dry rocky hillsides at an altitude of 1600 – 2500 m (Chamberlain 1975). Previously, ethyl acetate fraction obtained from the methanol extract of the plant's roots was studied and two new benzyl phthalides and five phenolic acid derivates were reported (Sarı 2010). Furthermore, it has been reported that the antimicrobial activities of the ethanolic extract, petroleum ether, ethyl acetate and n-butanol fractions of the plant can be used against *Staphylococcus aureus, S. epidermidis, Salmonella typhi, Shigella flexneri, Candida albicans* (Sarı et al. 2009). This study aimed to investigate the petroleum ether fraction of the methanol extract obtained from the plant's roots. There are no previous records about the chemical composition of the fraction.

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MATERIALS AND METHODS

Plant material

Scorzonera veratrifolia was collected from Bitlis, Turkey, at an altitude of 2500 m in August 2004. A voucher specimen (F 12 446) was deposited at the Herbarium of the Faculty of Sciences and Letters, Van Yüzüncü Yıl University.

Extraction and fractionation

The air-dried, ground roots of *S. veratrifolia* (600 g) were macerated with MeOH and concentrated under reduced pressure at 45°C using a rotary evaporator (Buchi R-200). The methanol extract was dissolved in MeOH : H_2O (1 : 2) and successively extracted with petroleum ether (PE), ethyl acetate (EtOAc) and *n*-butanol respectively. The PE fraction (20g) was subjected to column chromatography (CC) using silica gel (Merck 60, 0.063-0.200) as an adsorbent. The gradient elution was started with PE, continued with the increasing rate of EtOAc and ended with 100% EtOAc. 82 fractions were provided and grouped based on their Thin Layer Chromatography (TLC, Silica gel, Merck 60 F_{254}) findings. Fr 19-21 (7.3 g) was subjected to CC (Silica gel, PE/$CHCl_3$, 80 : 20, 70 : 30, 50 : 50, 0 : 100) to afford mixture BCV3 (20 mg, α-amyrin acetate + ß-amyrin acetate + germanicol acetate + lupeol acetate + taraxasterol acetate + ψ – taraxasterol acetate) and mixture BCV6 (20 mg, α-amyrinone + ß-amyrinone + germanicone + lupenone +Fern-7-en-3-one). Fr 36-45 (1.3 g) was further separated by CC (silica gel, $CHCl_3$/MeOH, 1 : 1) to provide mixture BCV5 (30 mg, α-amyrin + ß-amyrin + germanicol + lupeol + taraxasterol + ψ – taraxasterol). Fr 52-55 (927 mg) was subjected to CC (silica gel, PE/AcOEt, 1 : 1) and then to prep. TLC (silica gel, PE/AcOEt, 85: 15) to yield pure BCV7 (32.5 mg, ß-sitosterol).

GC-MS and NMR

Thermo Finnigan Trace GC Ultra (Thermo Electron Corporation) with AS 3000 Autosampler for gas chromatography and Thermo Finnigan Trace DSQ (Thermo Electron Corporation) for mass spectrometry were employed. Details of the method were as follows: Column: ZB 1 MS 0.25 µm (30 m x 0.25 mm ID), carrier gas: He, flow rate: 1 mL/min, injection temperature: 300 °C, column temperature: 65 °C for 2 minutes, 300 °C for 20 minutes (increase rate 6 °C), injection volume: 2 µL and ion source temperature: 200 °C. Full-scan mass spectra were acquired from 1 to 1050 *m/z* at a scan interval of 0.2 in EI mode. NMR spectrums were acquired on UNITY INOVA 500 MHz (Varian), in $CDCl_3$.

RESULTS AND DISCUSSION

The PE fraction of methanol extract obtained from *S. veratrifolia* roots was investigated and six oleanane-type (ß-amyrin, ß-amyrin acetate, ß-amyrinone, germanicol, germanicol acetate, germanicone), seven ursane-type (α-amyrin, α-amyrin acetate, α-amyrinone, ψ – taraxasterol, ψ – taraxasterol acetate, taraxasterol, taraxasterol acetate), three lupane-type (lupeol, lupeol acetate, lupenone), one fernane-type (Fern-7-en-3-one) triterpenes along with one sterol (ß-sitosterol) were determined (Table 1).

Table 1: Triterpenes and ß-sitosterol from the PE fraction of the methanol extract obtained from *S. veratrifolia* roots determined by GC-MS

Peak Numbers	Compounds	Retention Time (min)	Molecular Weight	Molecular Formula
Mixture BCV3*				
1	ß-Amyrin acetate	45.16	468	C32H52O2
2	Germanicol acetate	45.34	468	C32H52O2
3	Lupeol acetate + α-Amyrin acetate	46.28	468 + 468	C32H52O2
C32H52O2				
4	ψ – Taraxasterol acetate	47.99	468	C32H52O2
5	Taraxasterol acetate	48.18	468	C32H52O2
Mixture BCV5*				
1	ß-Amyrin	43.76	426	C30H50O
2	Germanicol	43.90	426	C30H50O
3	Lupeol + α-Amyrin	44.48	426 + 426	C30H50O
C30H50O				
4	ψ – Taraxasterol	45.92	426	C30H50O
5	Taraxasterol	46.15	426	C30H50O
Mixture BCV6*				
1	ß-Amyrinone	43.50	424	C30H48O
2	Germanicone	43.63	424	C30H48O
3	α-Amyrinone	44.17	424	C30H48O
4	Fern-7-en-3-one	44.93	424	C30H48O
5	Lupenone	45.52	424	C30H48O
BCV7*	ß-sitosterol	43.27	414	C29H50O

*BCV3, BCV5, BCV6 are the mixtures and BCV7 is the pure compound obtained from the PE fraction of methanol extract of Scorzonera veratrifolia roots

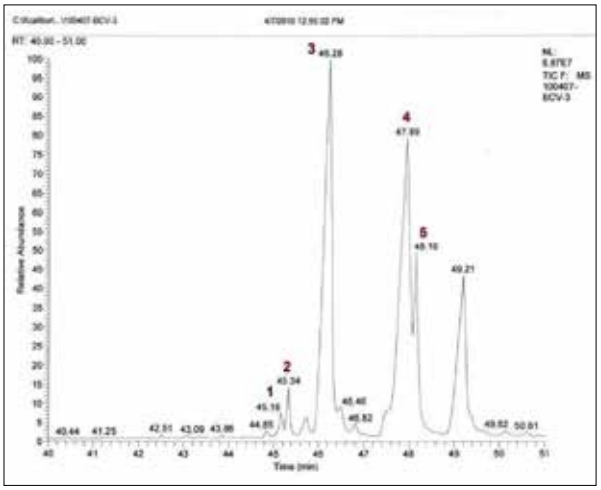


Figure 1. GC chromatogram of mixture BCV3

CC, prep. TLC and GC techniques were used for the separation of the compounds (Figure 1-4). Determination of the compounds was achieved by using GC-MS (Wiley/NIST database) and comparing findings with the literature data (Budzikiewicz et al. 1963; Hooper et al. 1982; Ahmad and Atta ur 1994; Shiojima et al. 1995; Oliveira et al. 2006; Gawrońska-Grzywacz and Krzaczek 2007). Additionally, ^{1}H NMR and ^{13}C NMR techniques were used in the structure elucidation of pure BCV7 (ß-sitosterol) (Table 2). The NMR data of the compound was compared with the literature (Pateh et al. 2009).

All compounds were determined for the first time in *S. veratrifolia*. To the best of our knowledge, α-amyrinone, ß-amyrin, ß-amyrinone, ψ – taraxasterol and ψ – taraxasterol acetate are new for the genus *Scorzonera*. Other triterpenoids were found in several *Scorzonera* species (Table 3). Particularly, *S. veratrifolia* showed a similar triterpenoid composition as *S. cretica*. Also, ß-sitosterol has been reported from several *Scorzonera* species as

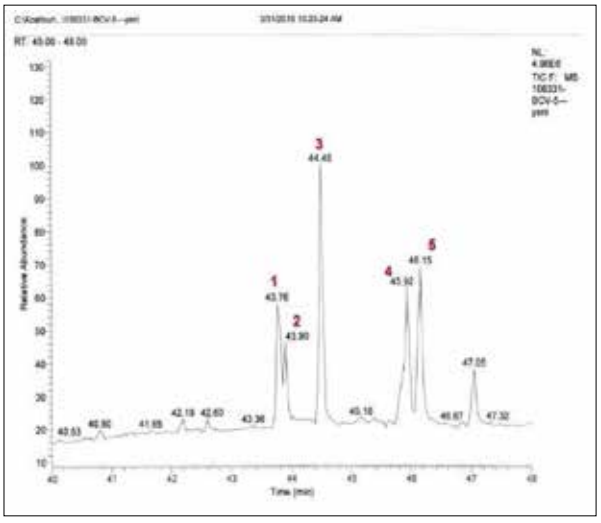


Figure 2. GC chromatogram of mixture BCV5

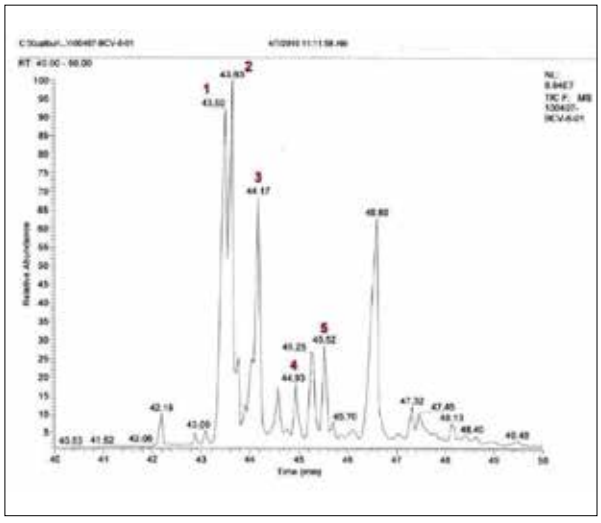
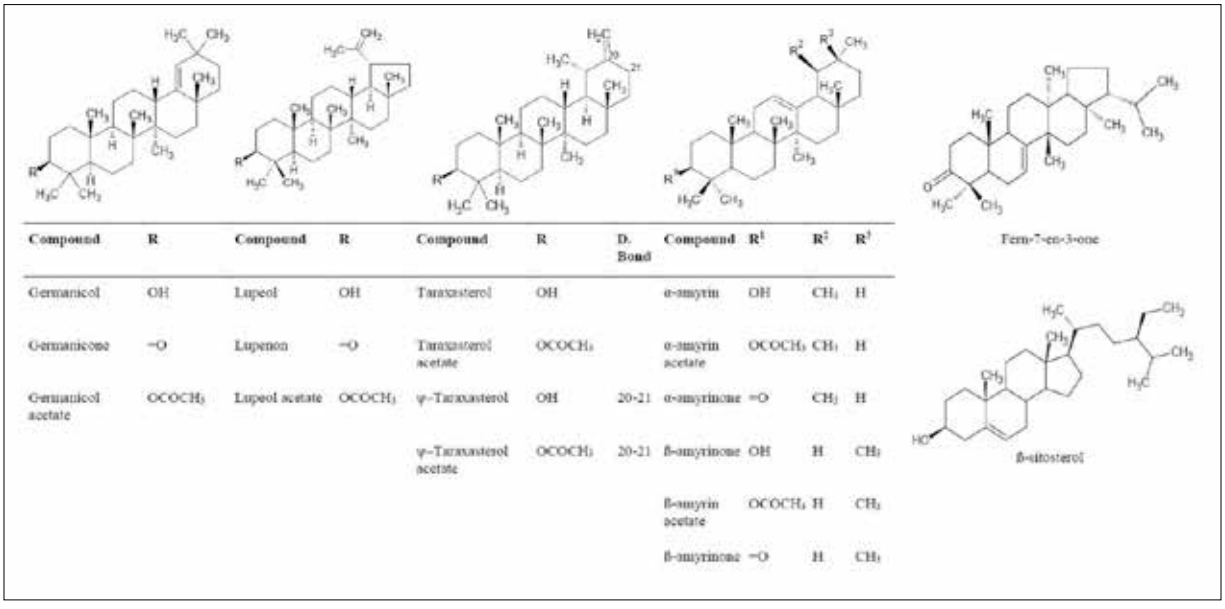


Figure 3. GC chromatogram of mixture BCV6



Compound	R	Compound	R	Compound	R	D. Bond	Compound	R^1	R^2	R^3
Germanicol	OH	Lupeol	OH	Taraxasterol	OH		α-amyrin	OH	CH_3	H
Germanicone	=O	Lupenon	=O	Taraxasterol acetate	$OCOCH_3$		α-amyrin acetate	$OCOCH_3$	CH_3	H
Germanicol acetate	$OCOCH_3$	Lupeol acetate	$OCOCH_3$	ψ-Taraxasterol	OH	20-21	α-amyrinone	=O	CH_3	H
				ψ-Taraxasterol acetate	$OCOCH_3$	20-21	ß-amyrinone	OH	H	CH_3
							ß-amyrin acetate	$OCOCH_3$	H	CH_3
							ß-amyrinone	=O	H	CH_3

Fern-7-en-3-one

ß-sitosterol

Figure 4. Chemical structures of the compounds determined in the PE fraction of the methanol extract obtained from the roots of *Scorzonera veratrifolia*

Table 2: 1H NMR and 13C NMR data of BCV7 (ß-sitosterol)

Position	δH (J, Hz)	δC	Position	δH (J, Hz)	δC	Position	δH (J, Hz)	δC
1		36.2	11		20.0	21	0.85 d (6.3)	17.7
2		28.7	12		38.8	22		32.9
3	3.45 m	72.0	13		41.3	23		25.1
4		41.2	14		55.8	24		44.8
5		139.7	15		23.3	25		27.8
6	5.27 brs	120.7	16		27.2	26	0.74 d (6.8)	18.8
7		30.6	17		54.9	27	0.77 d (6.8)	18.0
8		30.8	18	0.61 s	10.8	28	0.77 t (7.8)	22.0
9		50.2	19	0.94 s	18.3	29	0.74 d (6.8)	10.9
10		35.5	20		35.1			

Note: 1H NMR at 500 MHz in CD3OD and 13C NMR at 125 MHz in CD3OD

Table 3: Triterpenoids determined in the genus *Scorzonera*

Species	Triterpenoids
S. cretica (Paraschos et al. 2001)	germanicol, germanicone, germanicol acetate, lupeol, lupenone, lupeol acetate, taraxasterol, taraxasterol acetate, oleanolic acid, oleanol acetate
S. tomentosa (Öksüz et al. 1990)	lupeol, lupeol acetate, α-amyrin
S. aristata (Jehle et al. 2010)	lupeol, magnificol, 3-α-hidroxyolean-5-ene
S. austriaca (Wu et al. 2011)	lupeol, taraxasterol, ψ-taraxasteryl-3 (3′-methyl-butanonate), 3β-acetyl-11α,12α-oxidotaraxerol, D-friedours-14-en-3β-acetyl-11α,12α-epoxy, α-amyrin-3-acetyl, α-amyrin-3-acetyl-11-oxo, β-amyrin acetate, β-amyrin-3(3′-methylbutanonate), glutinol, 3ß-acetoxyglutin-5(10)-ene-6-oxo, (23Z)-cycloart-23-ene-3β, 25-dihydroxy, 9β,19 cyclolanostane- 24-en-3-oxo
S. columnae (Menichini et al. 1994)	lupeol
S. latifolia (Bahadır et al. 2010; Acıkara et al. 2012; Acıkara et al. 2014)	fern-7-en-3-one, 3ß-hydroxy-fern-7-ene-6-one-acetate, 3-ß-hydroxy-fern-8-ene-7-one-acetate, fern-7-ene-3-ol, taraxasterol acetate, taraxasterol myristate, olean-12-ene-11-one-3-acetyl, urs-12-ene-11-one-3-acetyl
S. undulata ssp. *delicosa* (Harkati et al. 2010)	β-amyrin acetate, methyl oleanate, methyl ursolate
S. undulata ssp. *alexandrina* (Benabdelaziz et al. 2014)	lupeol, 24-methylenecycloartanol
S. mongolica (Wang et al. 2007; Wang et al. 2009)	3ß-tetradecanoyl moradiol, 3-ß-dodecanoyl moradiol, 3ß-tetradecanoyl erythrodiol, 3-ß-dodecanoyl erythrodiol
S. divaricata (Yang et al. 2016)	oleanolic acid, scorzodivaricin B, C, D, 23(Z)-3ß-acetoxy-25-hydroxy-tirucalla-7,23-diene, 23(Z)-3ß, 25-dihydroxy-tirucalla-7,23-diene, 20(R)-3ß, 21-dihydroxy-24(31)-methylene-dammarane, 20(R)-3ß-acetoxy-21-hydroxy-24(31)-methylene-dammarane
S. hispanica tissue culture (Tolstikhina et al. 1988)	oleanolic acid

Note: Bold written compounds are in common with *S. veratrifolia*

well (*S. tomentosa*, *S. austriaca*, *S. columnae*, *S. latifolia*, *S. undulata*, *S. hispanica* tissue culture, *S. suberosa and S. laciniata*) (Tolstikhina et al. 1988; Öksüz et al. 1990; Harkati et al. 2010; Wu et al. 2011; Erden et al. 2013; Acıkara et al. 2014; Benabdelaziz et al. 2014).

GC-MS data of the plants are valuable for setting up chemotaxonomic profiles. However, such studies on the genus *Scorzonera* are scarce. Moreover, terpenoids are considered to be potential anti-cancer, anti-inflammatory, hepatoprotective, anti-viral agents (Dudhgaonkar et al. 2009; Laszczyk 2009; Thyagarajan et al. 2010; Ding et al. 2011; Gao et al. 2011; Narayan et al. 2011; Dakeng et al. 2012; Ezzat et al. 2012). Thus, further investigations on the terpenoids of the genus *Scorzonera* are recommended.

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