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RESEARCH PAPER

Chemical Constituents, Cytotoxic, Antioxidant and Cholinesterases Inhibitory Activities of *Silene Compacta* (Fischer) Extracts

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ABSTRACT

In Turkey, *Silene* species have been used as infusion in urinary bladder and biliary tract diseases in traditional medicine. *Silene* species have been also consumed as food in Anatolia and Europe. In this study, the phenolic, fatty acid and essential oil profiles and the cytotoxic, antioxidant and cholinesterases inhibitory activities, and total phenolic-flavonoid content of *Silene compacta* Fischer (SC) were studied. The essential oil and fatty acid compositions of *S. compacta* were determined by using GC/MS in the current study. The chemical composition of the methanol extract was determined using LC-MS/MS for quantitative and qualitative purposes. The major components of the essential oil and fatty acid were identified as α-selinene (12.4%) and palmitic acid (26.3%), respectively. The methanol extract (SCM: *S. compacta* methanol extract) which possessed the best activity in four tested antioxidant methods among the tested extracts exhibited very strong cholinesterases inhibitory activities. Additionaly, this extract indicated the highest cytotoxic effect against A549 cells. In the SCM extract, hesperidin and rutin, quinic and malic acids were quantified by LC-MS/MS as major contituents.

Keywords: *Silene compacta*, LC-MS/MS, fatty acid, essential oil, anticholinesterase, antioxidant, cytotoxicity.

INTRODUCTION

The genus *Silene* L. (Caryophyllaceae) is represented by 150 taxa in Turkey, 67 of them are endemic [1]. *Silene* species are known in Anatolia as "Nakıl çiçeği", "Gıvışganotu", "Gıcı gıcı", "Salkım çiçeği" [2]. Their roots and aerial parts have been used as infusion in urinary bladder and biliary tract diseases. *Silene vulgaris* (Moench) have been also consumed as food in Anatolia and Europe [2, 3].

Silene species possess mainly triterpene saponins and ecdysteroids [4, 5]. Ecdysteroids have been found in more than 120 species and subspecies of *Silene* from 155 species so far tested [6]. The steroid-containing fractions of some *Silene* species are immunosuppressive, potentially anti-inflammatory [6], inhibit the proliferation of cancer cells by inducing apoptosis [5], and show *in vivo* anticancer activity in mice [7]. On the other hand, several studies are devoted to the fatty acid and essential oil composition of various *Silene* species [8-10].

Depending on the life expectancy and the aging of the world's population, various health problems gain importance both socially and economically. Nowadays, the most beneficial results in the treatment of Alzheimer's disease, taken as a

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single drug group is indicated by acetylcholinesterase (AChE) inhibitors. However, only this group of drugs is used in the mild treatment to moderate Alzheimer's disease and side effects required make identify new anti-Alzheimer's drugs. With the prolonging human life, the increase of chronic diseases (cancer, cardiovascular disease, Alzheimer's, and so on) has attracted the interest to the antioxidants nowadays [11, 12]. On the other hand, synthetic antioxidants are used to extend the shelf life of foods and prevent their degradation. Synthetic antioxidants, BHA (butylated hydroxyanisole), BHT (butylated hydroxytoluene), PG (propyl gallate) and TBHQ (t-butyryllhydroquinone), and by-products formed from them can lead to various diseases [13]. For this reason, finding new antioxidant substances in replace of synthetic ones have gained importance in this field. The antioxidants may be also relevant in slowing the progression of Alzheimer's disease which is frequently seen among elderly people all around the world. Consequently, consumers have been more interested in natural antioxidants to protect their health [14]. Additionaly, recent several scientific studies have been focused on the phenolic compounds of the plants having a number of pharmacological effects and the biological activities [15].

A literature survey showed that there are no reports on the fatty acid, essential oil, phenolic profiles, cytotoxic, antioxidant and anticholinesterase activities of *S. compacta*. At the beginning, the essential oil and fatty acid compositions of *S. compacta* were determined by using GC/MS in the current study. In the next step, related cytotoxic, antioxidant and cholinesterases inhibitory activities; total phenolic-flavonoid contents were examined. Finally, the chemical composition of the methanol extract was determined using LC-MS/MS for quantitative and qualitative purposes.

MATERIALS AND METHODS

Plant material

The aerial parts of *Silene compacta* FISCHER were collected from northwestern Turkey (Şile-Istanbul) in August 2012 by Dr. A. Ertaş, and identified by Dr. Yeter Yeşil. A voucher specimen was deposited in the Herbarium of Istanbul University (ISTE 98051).

Extraction of essential oil and GC/MS conditions steam distillation

Essential oils were obtained using a Clevenger apparatus from the aerial parts of plant, which were crumbled into small pieces and soaked in distilled water for 3 h. The obtained essential oils were dried over anhydrous Na_2SO_4 and stored at +4 °C for a sufficient period of time. The essential oil were diluted using CH_2Cl_2 (1:3 v/v) prior to GC/FID and GC/MS analysis. The GC/MS and GC/FID procedure described by Ertas *et al.* [15] were applied. GC/FID performed using Thermo Electron Trace GC FID detector and GC/MS performed using same GC and Thermo Electron DSQ quadrupole for MS.

Preparation of extracts for biological activities and GC-MS

Powdered the aerial parts of *S. compacta* were weighed (100 g) and sequentially macerated three times with petroleum ether (250 mL), acetone (250 mL), methanol (250 mL) and water (250 mL) at 25°C for 24 h. After filtration, the solvent was evaporated to get the crude extracts. This yielded 0.89% petroleum ether extract, 1.1% acetone extract, 5.8% methanol extract, and 3.4% water extract (w/w). The petroleum ether extract was analyzed to determine its fatty acid composition by GC/MS.

GC/MS conditions and esterification of fatty acid

Esterification of the petroleum ether extract and GC/MS procedure described by Ertas *et al.* [16] were applied. Thermo Scientific Polaris Q GC-MS/MS was used.

Preparation of methanol extract for LC-MS/MS

The air-dried and powdered plant materials (10 g) were extracted three times with 100 mL of methanol for 24 h at room temperature. The solvent was removed from the filtered extract under vacuum at 30°C in a rotary evaporator. Methanol extract was diluted to 250 mg/L and fitrated with 0.2 µm microfiber filter prior to LC MS/MS analysis.

Method validation parameters for LC-MS/MS

In this study, twenty-four phenolic compounds (flavonoids, flavonoid glycosides, phenolic acids, phenolic aldehyde, coumarin) and three non-phenolic organic acids were qualified and quantified in *S. compacta*. Rectilinear regression equations and the linearity ranges of the studied standard compounds were given in Table 1. Correlation coefficients were found to be higher than 0.99. The limit of detection (LOD) and limit of quantitation (LOQ) of the reported analytical method were shown in Table 1. For the

studied compounds, LOD ranged from 0.05 to 25.8 µg/L and LOQ ranged from 0.17 to 85.9 µ/L (Table 1). Moreover, the recoveries of the phenolic compounds ranged from 96.9 % to 106.2 % [12, 16].

Determination of total phenolic and flavonoid contents

Total phenolic and flavonoid contents which were expressed as pyrocatechol and quercetin equivalents, respectively, were determined as reported in the literature [17, 18]. The following equations were used to calculate total phenolic and flavonoid contents of the extracts:

Absorbance = 0.0128 pyrocatechol (µg) + 0.0324 (R^2 = 0.9924)

Absorbance = 0.1701 quercetin (µg) - 0.0778 (R^2 = 0.9939)

Antioxidant activity of the extracts

β-Carotene-linoleic acid test system [19], DPPH free radical scavenging activity [20], ABTS cation radical decolorisation [21] and cupric reducing antioxidant capacity (CUPRAC) [22] assays were carried out to determine the antioxidant activity.

Cholinesterases inhibitory activities of the extracts

A spectrophotometric method developed by Ellman *et al.* [23] was used to indicate the acetyl- and butyryl-cholinesterase inhibitory activities.

Cytotoxic activity

Cell culture

A549 and L929 fibroblast cells which were stored in liquid nitrogen tank were centrifuged after dissolution. Later, these cells were placed on a 96-well plate after addition of 3 mL (DMEM 10% + fetalbov serum 100%+ containing 1% antibiotics), and incubated under 5% CO_2 and at 37°C. When the cells reached sufficient growth, they were discharged with trypsin-EDTA solution, and passaging process was continued.

Determination of cytotoxicity by MTT assay

The MTT assay was performed in accordance with ISO 10993-5 standards. This method is sensitive for cell proliferation measurement that 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) tetrazolium salt is used. MTT is reduced to insoluble formazan dye in water by mitochondrial enzymes associated with metabolic activity. MTT reduction is primarily associated with the glycolytic activity in the cells and depends on the presence of NADH (nicotin amide adenine dinucleotide) and NADPH (nicotinamide adenine dinucleotide phosphate). In the reactions of mitochondria of healty cells or early stages of apoptotic cells, colored formazan crystals constitute with the degredation of the tetrazolium ring which is found in MTT solution by dehydrogenase enzymes in cell mitochondria. The color change which observed in living cells gives the absorbance values in Elisa reader.

L929 fibroblast and A549 cells was cultivated on 96-well plate ($10x10^3$ cells/well). Cells were incubated for 24 h. Later, previously prepared plant extracts at different concentrations (0-12.5 µg/mL – 25 µg/mL – 50 µg/mL – 100 µg/mL) was applied onto the cells and then incubated for 24 h. The samples were studied 5 times. As a positive control, the medium was only applied onto cells. After 24 h, waste in each well were discarded and 100 µL of medium and 20 µL of MTT solution were added. After 3.5 h incubation at 37°C, 150 µL of MTT solvent was added to wells, and cells were incubated for extra 15 minutes. In order to determine cell viability, absorbance values of plates were recorded by ELISA reader at 570 nm. According to the absorbance values of control group, percentage cell viability was calculated.

Statistical analysis

The total phenolic-flavonoid contents, antioxidant and anticholinesterase activity assays results were shown as means ± standard deviation. The results were evaluated using an unpaired *t*-test and one way analysis of variance ANOVA. The differences were regarded as statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

Quantitative analysis of phenolic compounds by LC-MS/MS

Several studies are present in literature reporting the use of liquid chromatography electrospray ionization tandem mass spectrometry to perform quantitative analyses of phenolic compounds [12, 14, 24]. Thus, for quantitative purpose, an accurate method on a mass spectrometer equipped with a triple quadrupole analyzer was developed for the analyses of twenty-four phenolic compounds (flavonoids, flavonoid glycosides, phenolic acids, phenolic aldehyde, coumarin)

and three non-phenolic organic acids in the methanol extract of *S. compacta* methanol extract (SCM). When considered the results of LC-MS/MS in general, rutin (2147.97 ± 107.35 µg/g extract) and hesperidin (1941.64 ± 95.06 µg/g extract) were found to be the most abundant compounds in the SCM extract (Table 1 and Figure 1).

Furthermore, vanillin (95.59 ± 4.65 µg/g extract), hyperoside (439.40 ± 21.51 µg/g extract), luteolin (72.46 ± 4.12 µg/g extract), kaempferol (34.63 ± 2.11 µg/g extract), apigenin (50.43 ± 2.65 µg/g extract) and chrysin (16.74 ± 0.85 µg/g extract) were also detected in the SCM extract as flavonoid (Table 1 and Figure 1). Additionally, gallic (22.59 ± 1.13 µg/g extract), chlorogenic (151.79 ± 7.40 µg/g extract), protocatechuic (178.10 ± 9.07 µg/g extract), tr-caffeic (only detect), p-coumaric (134.68 ± 6.83 µg/g extract), rosmarinic (152.53 ± 7.44 µg/g extract), 4-OH benzoic (50.68 ± 2.60 µg/g extract) and salicylic acids (41.16 ± 2.05 µg/g extract) were determined in SCM extract as phenolic acids. Also, quinic (1744.71 ± 83.71 µg/g extract), malic (1625.02 ± 86.12 µg/g extract) and tr-aconitic acids (462.69 ± 22.84 µg/g extract) were detected and quantified in SCM extract as non-phenolic acids. According to the results of LC-MS/MS, the existence of the most of these compounds was not reported in *Silene* species before. Additionaly, the current study is the first report on the phenolic composition of *S. compacta*. In literature, there are few studies about chemical profile of *Silene* species with HPLC [25].

Table 1: Analytical parameters of LC- MS/MS method; Identification and quantification of compounds in methanol extract of *S. compacta* (SCM) by LC-MS/MS.

Analyte no	Analytes	RT[a]	Parent ion (m/z)[b]	R^{2c}	RSD%[d]	Linearity Range (mg/L)	LOD/LOQ (µg/L)[e]	Recovery (%)	Quantification (µg analyte/g extract)[f]
1	Quinic acid	3.36	190.95	0.9927	0.0388	250-10000	22.3 / 74.5	103.3	1744.71±83.71
2	Malic acid	3.60	133.05	0.9975	0.1214	250-10000	19.2 / 64.1	101.4	1625.02±86.12
3	tr-Aconitic acid	4.13	172.85	0.9933	0.3908	250-10000	15.6 / 51.9	102.8	462.69±22.84
4	Gallic acid	4.25	169.05	0.9901	0.4734	25-1000	4.8 / 15.9	102.3	22.59±1.13
5	Chlorogenic acid	5.29	353	0.9932	0.1882	250-10000	7.3 / 24.3	99.7	151.79±7.40
6	Protocatechuic acid	5.51	152.95	0.9991	0.5958	100-4000	25.8 / 85.9	100.2	178.10±9.07
7	Tannic acid	6.30	182.95	0.9955	0.9075	100-4000	10.2 / 34.2	97.8	39.92±1.99
8	tr- caffeic acid	7.11	178.95	0.9942	1.0080	25-1000	4.4 / 14.7	98.6	D.[g]
9	Vanillin	8.57	151.05	0.9995	0.4094	250-10000	10.1 / 33.7	99.2	95.59±4.65
10	p-Coumaric acid	9.17	162.95	0.9909	1.1358	100-4000	15.2 / 50.8	98.4	134.68±6.83
11	Rosmarinic acid	9.19	358.9	0.9992	0.5220	250-10000	10.4 / 34.8	101.7	152.53±7.44
12	Rutin	9.67	609.1	0.9971	0.8146	250-10000	17.0 / 56.6	102.2	2147.97±107.35
13	Hesperidin	9.69	611.1	0.9973	0.1363	250-10000	21.6 / 71.9	100.2	1941.64±95.06
14	Hyperoside	9.96	463.1	0.9549	0.2135	100-4000	12.4 / 41.4	98.5	439.40±21.51
15	4-OH Benzoic acid	11.38	136.95	0.9925	1.4013	25-1000	3.0 / 10.0	106.2	50.68±2.60
16	Salicylic acid	11.39	136.95	0.9904	0.6619	25-1000	4 / 13.3	106.2	41.16±2.05
17	Myricetin	11.42	317	0.9991	2.8247	100-4000	9.9 / 32.9	106.0	N.D.[h]
18	Fisetin	12.10	284.95	0.9988	2.4262	100-4000	10.7 / 35.6	96.9	N.D.
19	Coumarin	12.18	146.95	0.9924	0.4203	100-4000	9.1 / 30.4	104.4	N.D.
20	Quercetin	13.93	300.9	0.9995	4.3149	25-1000	2.0 / 6.8	98.9	N.D.
21	Naringenin	14.15	270.95	0.9956	2.0200	25-1000	2.6 / 8.8	97.0	N.D.
22	Hesperetin	14.80	300.95	0.9961	1.0164	25-1000	3.3/ 11.0	102.4	N.D.
23	Luteolin	14.84	284.95	0.9992	3.9487	25-1000	5.8 / 19.4	105.4	72.46±4.12
24	Kaempferol	14.85	284.95	0.9917	0.5885	25-1000	2.0 / 6.6	99.1	34.63±2.11
25	Apigenin	16.73	268.95	0.9954	0.6782	25-1000	0.1 / 0.3	98.9	50.43±2.65
26	Rhamnetin	18.41	314.95	0.9994	2.5678	25-1000	0.2 / 0.7	100.8	N.D.
27	Chrysin	20.60	253	0.9965	1.5530	25-1000	0.05 / 0.17	102.2	16.74±0.85

[a]RT: Retention time, [b]Parent ion *(m/z):* Molecular ions of the standard compounds (mass to charge ratio), [c]R^2: coefficient of determination, [d]RSD: relative standard deviation

[e]LOD/LOQ (µg/L): Limit of deteection/Limit of quantification, [f]Values in µg/g (w/w) of plant methanol extract, [g]D: peak observed, concentration is lower than the LOQ but higher than the LOD, [h]N.D: not detected.

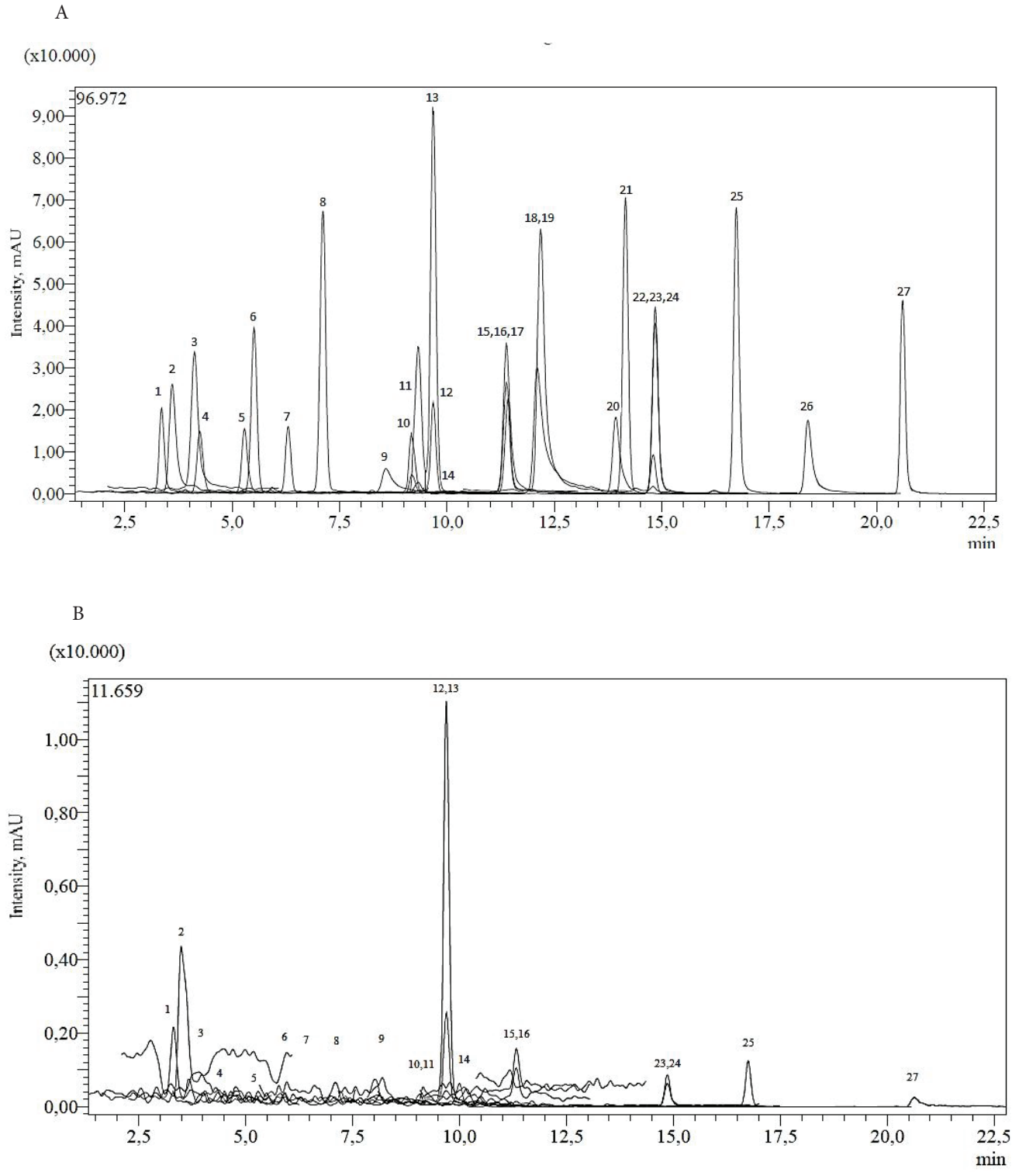

Figure 1. LC-MS/MS chromatograms of A: 250 ppb standard mix, B: *S. compacta* methanol extract. 1: Quinic acid, 2: Malic acid, 3: tr-Aconitic acid, 4: Gallic acid, 5: Chlorogenic acid, 6: Protocatechuic acid, 7: Tannic acid, 8: tr- caffeic acid, 9: Vanillin, 10: p-Coumaric acid, 11: Rosmarinic acid, 12: Rutin, 13: Hesperidin, 14: Hyperoside, 15: 4-OH Benzoic acid, 16: Salicylic acid, 17: Myricetin, 18: Fisetin,19: Coumarin, 20: Quercetin, 21: Naringenin, 22: Hesperetin, 23: Luteolin, 24: Kaempferol, 25: Apigenin, 26: Rhamnetin, 27: Chrysin.

Essential oil and fatty acid compositions by GC-MS

The essential oil of *S. compacta* was determined by GC/MS analysis. 25 components were identified, constituting 96.1% of the total oil. The identified compounds are listed in Table 2. According to the results the main constituents of the essential oil were α-selinene (12.4%), *Z*-8-octadecen-1-ol acetate (10.3%) and terpinolen (7.7%).

As far as our literature survey could ascertain, no report is available for the essential oil composition of *S. compacta*. Therefore, this study could be considered the first report on this topic. There are very few studies on essential oil composition of *Silene* species in the literature. According to a report Vivek *et al.* the main constituents of the essential oil of *S. armeria* were 1-butene (39.20%), methylcyclopropane (21.48%), 2-butene (17.97%) and caryophyllene oxide (7.20%) [10].

The petroleum ether extract of *S. compacta* were determined by GC/MS analysis for fatty acid composition. As shown in Table 3, 14 components were identified, consitituting 99.30 % of the petroleum ether extract. According to the results, a large rate in the amounts of palmitic acid (26.3 %), linoleic acid (17.7 %) and oleic acid (17.1 %) were identified in the extract.

As far as our literature survey could ascertain, no report is available for the fatty acid composition of *S. compacta*. There are few studies on fatty acid composition of *Silene* species in the literature. According to the report of Kucukboyacı *et al.* the main constituents of the fatty acid of *S. vulgaris* and *S. cserei* subsp. *aeoniopsis* were linoleic acid (65.40 %), oleic acid (17.80%) and palmitic acid (8.80%), respectively [9]. According to the report of Mamadalieva *et al. S. barchucia*, *S. viridiflora* and *S. wallichiana* were determined by GC/

Table 2: Chemical composition of the essential oil from *S. compacta*.

Rt (min)[a]	Constituents[b]	Composition %	RI[c]
18.98	Terpinolen	7.7	1086
24.05	1,3-Di-tertbutyl benzene	3.0	1249
29.38	Geranyl acetone	2.4	1431
29.47	β-Farnesene	1.5	1447
30.48	Valencene	3.3	1484
30.56	Germacrene-D	2.2	1485
30.87	α-Selinene	12.4	1498
31.01	β-Himachalene	1.9	1505
31.42	β-Cadinene	3.0	1523
31.83	α-Amorphene	1.9	1528
35.52	2-Methyl heptadecane	3.0	1746
36.14	Pentadecanol	1.8	1778
36.45	Octadecane	2.1	1800
36.74	2-Methyl-1-hexadecanol	1.5	1890
36.87	1-Nonadecanol	4.2	2156
38.22	Z-14-Octadecen-1-ol acetate	1.5	2185
38.34	Z-8-Octadecen-1-ol acetate	10.3	2185
39.96	Heneicosane	4.5	2109
40.13	2,5-Di-tert octyl-p-benzoquinone	7.5	2259
40.61	Arachidic acid	5.8	2366
40.87	Tetracosane	4.7	2407
43.30	Heptacosane	1.8	2700
43.63	1-Hexacosanol	4.0	2852
44.10	Nonacosane	2.4	2900
44.41	Ethyl iso-allocholate	1.7	3094
	Total	96.1	

[a]Retention time (as minutes), [b]Compounds listed in order of elution from a HP-5 MS column. A nonpolar Phenomenex DB-5 fused silica column, [c]RI Retention indices (DB-5 column).

Table 3: GC-MS Analysis of the petroleum ether extract of *S. compacta*.

Rt (min)[a]	Constituents[b]	Composition %
12.00	Lauric acid, methyl ester	1.8
18.60	Myristic acid, methyl ester	4.5
24.94	Palmitoleic acid, methyl ester	0.2
25.27	Palmitic acid, methyl ester	26.3
28.47	Margaric acid, methyl ester	1.0
29.75	Phytol	1.2
30.64	Linoleic acid, methyl ester	17.7
30.77	Oleic acid, methyl ester	17.1
30.86	Linolenic acid, methyl ester	10.8
31.54	Stearic acid, methyl ester	5.1
36.23	Nonacosanol	6.1
37.38	Arachidic acid, methyl ester	3.2
39.36	Docosane	0.7
43.82	Behenic acid, methyl ester	3.6
	Total	99.3

[a]Retention time (as minutes), [b]Compounds listed in order of elution from a HP-5 MS column. A nonpolar Phenomenex DB-5 fused silica column.

MS analysis for fatty acid composition [8]. According to this report, the main components were determined as linolenic (25.00, 32.80, 15.50%), palmitic acid (18.50, 24.70, 26.60%) and linoleic acid (11.00, 18.80, 22.50%), respectively. Consequently, when the results of current study and previous studies in literature were examined together, it could be concluded that the unsaturated fatty acid content of *Silene* species were higher than their saturated fatty acid content.

Total phenolic-flavonoid contents and antioxidant activity

Total phenolic and flavonoid contents of the extracts were also determined as pyrocatechol (PEs) and quercetin (QEs) equivalents, respectively (Table 4). The phenolic and flavonoid contents of the methanol (SCM) extract are higher than those of the petroleum ether (SCP), acetone (SCA) and water (SCW) extracts. The total phenolic and flavonoid content of the SCM extract was characterized as the richest among all (108.94 ± 2.68 µg/Pes mg extract and 55.88 ± 0.15 µg/Qes mg extract, respectively).

The petroleum ether, acetone, methanol and water extracts prepared from the aerial parts of *S. compacta* were screened for their possible antioxidant activity by using four complementary methods, namely the amount of β-carotene bleaching, DPPH free radical scavenging, ABTS cation radical decolorisation assays and Cupric reducing antioxidant capacity assays.

As seen in Table 4, while the SCM extract showed good lipid peroxidation activity (IC_{50}: 26.37 ± 0.88 µg/mL) in β-carotene bleaching method, the SCP, SCA and SCW extracts exhibited moderate lipid peroxidation activity (IC_{50}: 52.45 ± 1.21, 42.91 ± 0.72 and 41.94 ± 1.12 µg/mL, respectively). As it can be observed in Table 4, the SCM extract showed good activity (IC_{50}: 56.78 ± 2.12 µg/mL) in DPPH free radical scavenging method. Besides, the SCP, SCA and SCW extracts showed weak activity in DPPH free radical scavenging method.

As shown in Table 4, the SCP, SCA, SCM and SCW extracts showed the following IC_{50} values in ABTS cation radical scavenging assay, 118.18 ± 2.19, 57.78 ± 1.19, 16.91 ± 0.66 and 55.45 ± 2.18 µg/mL, respectively. Particularly, the SCM extract indicate strong activity in ABTS cation radical scavenging assay. The SCM extract and α-tocopherol exhibited 1.71 and 1.60 absorbance values in CUPRAC at 100 µg/mL, respectively. The other tested extracts showed weak activity in CUPRAC (Data not shown).

Generally speaking, after examining the antioxidant properties of the four extracts, the SCM extract showed the highest activity among the studied methods. The high antioxidant activity of SCM extract might be stemmed from high total phenolic content or high rutin amount that is known with its high antioxidant capacity.

Table 4: Antioxidant activity*, total phenolic-flavonoid contents* and anticholinesterase activity (200 µg/mL) * of *S. compacta* extracts, BHT, α-TOC and galantamine.

Samples	Inhibition % against AChE	Inhibition % against BChE	Phenolic content (µg PEs/mg extract)$^{\alpha}$	Flavonoid content (µg QEs/mg extract) $^{\beta}$	Lipid Peroxidation	IC_{50} (µg/mL) DPPH Free Radical	ABTS Cation Radical
SCP	86.03±2.10^{a}	28.65± 1.2^{a}	59.61± 3.74^{a}	27.61 ± 0.41^{a}	52.45±1.21^{a}	141.45±2.19^{a}	118.18±2.19^{a}
SCA	75.21±0.73^{b}	37.65± 3.2^{b}	84.14 ± 2.22^{b}	47.23 ± 0.14^{b}	42.91±0.72^{b}	155.83±3.69^{b}	57.78±1.19^{b}
SCM	93.75±1.^{2}3c	84.43± 1·52c	108.94 ± 2.68^{c}	55.88 ± 0.^{1}5c	26.37±0.88^{c}	56.78±2.12^{c}	16.91±0.66^{c}
SCW	65.47±0.57^{d}	55.12± 2.44^{d}	72.56 ± 0.06^{d}	47.16 ± 0.09^{b}	41.94±1.12^{b}	128.98±1.91^{d}	55.45±2.18^{b}
Galantamine†	85.55±0.55^{a}	79.22±1.22^{e}	-	-	-	-	-
α-TOC†	-	-	-	-	11.45±0.45^{d}	19.09±0.11^{e}	9.92±0.17^{d}
BHT†	-	-	-	-	8.41±0.31^{e}	48.64±0.06^{f}	10.90±0.16^{e}

* Values expressed are means ± SEM of three parallel measurements (p<0.05), †Standard drug, $^{\alpha}$ PEs, pyrocatechol equivalents (y=0.0128x + 0.0324 R^2=0.9924), $^{\beta}$QEs, quercetin equivalents (y=0.1701x – 0.0778 R^2=0.9939).

Cholinesterases inhibitory activities

As shown in Table 4, the SCM extract exhibited strong cholinesterases inhibitory activities, and this extract indicated higher inhibitory effect against acetyl- and butyryl-cholinesterase enzymes (93.75±1.23 and 84.43±1.52% inhibition, respectively) than the reference compound, galantamine, at 200 µg/mL.

To the best of our knowledge, there is not any reports on the cholinesterases inhibitory activities of *Silene* species. Taking into account that our results were higher than galanthamine and there has been no such study on *Silene* species, the cholinesterases inhibitory activities results of *S. compacta* will be important data in this field.

Cytotoxicity activity

MTT assay was used to determine cell viability. Only the medium was applied for control group. Figure 2 shows viability of L929 fibroblast cells and Figure 3 cell viability of A549. In the control group there were no toxicity. Generally

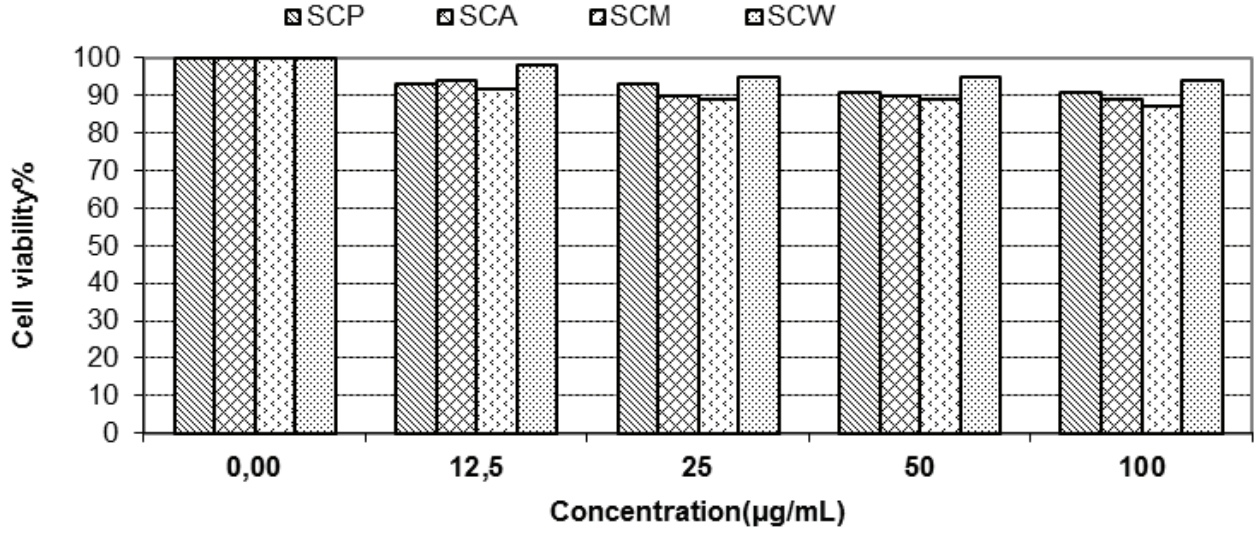

Figure 2. L929 Cytotoxic activity of *S. compacta* extracts.

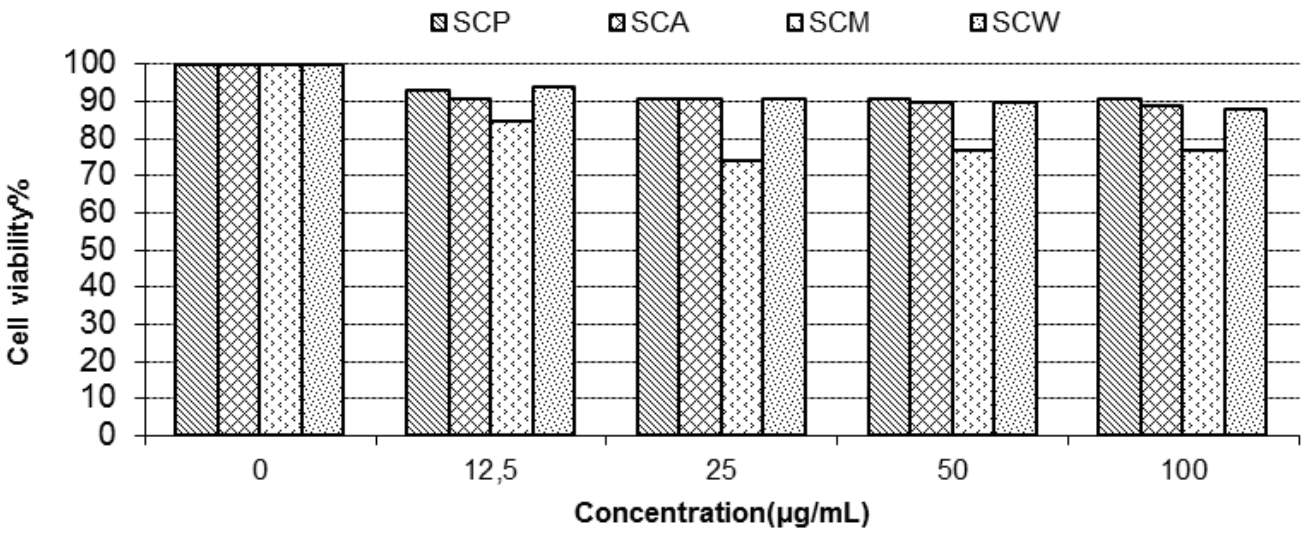

Figure 3. A549 Cytotoxic activity of *S. compacta* extracts.

at all concentration, the highest cytotoxic effect was found in SCM extract applied to A549 cells. The highest effect has shown by SCM extract at 25 µg/mL concentration (74% viability). In addition, when SCM extract applied to L959 fibroblast cells in the same concentration (25 µg/mL), SCM extract has shown high nontoxic effect (89% viability).

It is concluded that the report represents the first study on chemical composition and biological activities of *S. compacta*. *Silene* species possess mainly triterpene saponins and ecdysteroids [4, 5]. Although there are a lot of studies in this area, there are few studies on their phenolic contents. Thus, the phenolic content of *Silene compacta* might be a valuable data in this field. While the methanol extract displayed a good level of antioxidant properties in four tested methods, the present study concluded that this extract showed very strong cholinesterases inhibitory activities. Further phytochemical and biological studies are needed to characterize the active constituents of *S. compacta*.

***Silene compacta* (Fischer) Ekstrelerinin Kimyasal İçerikleri, Sitotoksik, Antioksidan ve Kolinesteraz İnhibitor Aktiviteleri**

ÖZ

Silene türleri Türkiye'de halk arasında mesane ve safra yolları infüzyonunda kullanılmaktadır.

Silene türleri Avrupa ve Anadolu'da yiyecek olarak tüketilmektedir. Bu çalışmada *Silene compacta* Fischer (SC) bitkisinin fenolik, yağ asidi ve uçucu yağ kompozisyonları ile sitotoksik, antioksidan ve antikolinesteraz aktiviteleri, toplam fenolik ve flavonoid içerikleri çalışılmıştır. Uçucu yağ ve yağ asidi bileşenleri GC-MS cihazı ile belirlendi. Metanol ekstresinin kimyasal bileşimi kalitatif ve kantitatif olarak LC-MS/MS cihazı ile belirlendi. Uçucu yağ ve yağ asidinin ana bileşenleri sırasıyla α-selinen (%12.4) ve palmitik asit (%26.3) olarak belirlendi. Çalışılan dört antioksidan test yöntemlerinde aktif olan methanol ekstresi çok güçlü antikolinesteraz enzim inhibisyon aktivitesi göstermiştir. Aynı zamanda methanol ekstresinin A549 hücre serilerine karşı en yüksek sitotoksik etkiyi gösterdiği belirlenmiştir. Metanol ekstresinin LC-MS/MS analizi sonucuna göre ana bileşenleri hesperidin, rutin, kuinik asit ve malik asit olarak belirlenmiştir.

Anahtar kelimeler: *Silene compacta*, LC-MS/MS, Yağ asidi, uçucu yağ, antikolinesteraz, antioksidan, sitotoksisite.

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