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PHYTOCHEMICAL CHARACTERIZATION OF *ACHILLEA GRANDIFOLIA* FRIV. ESSENTIAL OIL AND ITS POTENT AGAINST OXIDATIVE DAMAGE, ACETYLCHOLINESTERASE AND α-AMYLASE

Gülmira ÖZEK*

Department of Pharmacognosy, Faculty of Pharmacy, Anadolu University, 26470, Eskişehir, Turkey

ABSTRACT

The essential oil of *Achillea grandifolia* Friv. was obtained by hydrodistillation of aerial parts of the plant and analyzed with GC-FID and GC/MS techniques. The oil comprised mostly by the oxygenated monoterpenes (76.2 %) with α-terpinyl acetate (54.1 %) as main constituent. The monoterpene hydrocarbons were the second major compound group with *p*-cymene (17.7 %) as the main representative. The essential oil showed free radical scavenging activity against DPPH$^{\bullet}$ radicals (Inh. 52 %) and $ABTS^{+\bullet}$ cation radicals (TEAC 0.62 mM). The oil demonstrated hypoglycemic activity *via* inhibition of α-amylase (IC_{50} 0.15 mg/mL). The effect of the oil on neurodegenerative disease was expressed *via* inhibition of acetylcholinesterase (AChE) and found to be > 82 % of inhibition (an IC_{50} of 1.77 mg/mL).

Keywords: *Achillea grandifolia*, Essential oil, GC/MS, GC-FID, Antioxidant, α-Amylase, Acetylcholinesterase

1. INTRODUCTION

The genus *Achillea* is one of large genera of Compositae family and encompass about 115 taxa. According to the latest records, the genus *Achillea* comprises of six sections and 58 taxa, and 53% of these are endemic to Turkey [1-4]. It has a rather wide geographic distribution spanning Europe, Asia and North Africa, however its center of diversity is located in SE Europe and SW Asia [5].

Wild range of biological activity results were reported for essential oils, extracts, as well as fractions and pure compounds isolated from *Achillea* species: antibacterial [6, 7], anticancer [8-11], cytotoxic, antioxidant [12], antidiabetic [13, 14], anti-cholinesterase [15, 16], analgesic, antiinflammatory [17], hepatoprotective, antispasmodic, calcium antagonist [18] activities. Several aspects on pharmacological potent of the genus *Achillea* have recently been discussed [19-21]. Some *Achillea* species are traditionally used to treat various diseases, *A. biebersteinii* Afan (pireotu) is used against hemorrhoid [22], *A. millefolium* L. subsp. *millefolium* (civanperçemi) as diuretic, appetizer, antiseptic, degasify, and wound healing remedy [23] and for stomach ache [19], *A. millefolium* L. subsp. *pannonica* (Scheele) Hayek as diuretic and emmenagog [24, 25], *A. schischkinii* Sosn. against flatulence [26], *A. nobilis* L. subsp. *neilrechii* (Kerner) Formănek and *A. nobilis* L. subsp. *sipylea* (O. Schwarz) Bässler for abdominal pains and *A. setacea* Waldst.&Kit. against diarrhea and chilblain [27].

A. grandifolia is known under the local name “akyavşan” [28]. Earlier, infusion of *A. grandifolia* was reported for antioxidant, total phenol and total flavonoid contents [29]. The methanol extract from *A. grandifolia* collected from Balkan peninsula was reported to have antibacterial and antioxidant properties [30]. The oil of *A. grandifolia* collected from Serbia was characterized with high percentage of oxygenated monoterpenes with 1,8-cineole (28.8 %), camphor (22.8 %) and borneol (4.9 %) as the major compounds [31]. The oil of *A. grandifolia* growing in Greece was distinguished with high content of *cis*- and *trans*-thujone (20 %) [31, 32]. Two samples of *A. grandifolia* collected from Izmir and Aydin provinces of Turkey have earlier been reported for chemical composition of the essential oils [33].

*Corresponding Author: gulyaozek@gmail.com
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Taking into consideration the previous reports about chemical and biological potential of *Achillea* species as well as information about chemical diversity of *A. grandifolia* from different localities, we aimed to investigate chemical composition and biological potential of the oil from this species. Therefore, the oil of *A. grandifolia* was tested for antioxidant activity using *in vitro* methods like 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and Trolox equivalent antioxidant capacity tests. In addition, the oil was tested *in vitro* for its anti-acetylcholinesterase and anti-α-amylase activities.

Very scarce information is available concerning the anti-enzymatic activity of *A. grandifolia* plant. To the best of our knowledge, the inhibitory activity of the oil of *A. grandifolia* on AChE, which has important role in Alzheimer's disease (AD), has never been studied. Also, no information is available about the antidiabetic activity of the selected plant. Nowadays, there is increasing demand for naturally derived inhibitors of oxidative damage, neurodegenerative processes and carbohydrate digestion disorders. There are number of papers about side effects of synthetic products used for treatments of such disorders. Actually, at present there are no disease-modifying drug available. Nowadays, there is alarming demand for AChE inhibitors which used for symptomatic treatment of AD. Currently available inhibitors are donepezil, galantamine and rivastigmine. Apart from synthetic inhibitors of AChE several phytochemicals have been isolated from *Ginkgo biloba* [34], *Acorus calamus* [35], *Buxus papillosa* [36]. All these reports encouraged us to investigate *A. grandifolia* essential oil for antioxidant, antineurodegenerative and antidiabetic activities. In scope of the present work, the oil of *A. grandifolia* was evaluated for free radical scavenging and antienzymatic potentials *via* inhibition of acetylcholinesterase (AChE) and α-amylase enzymes.

2. MATERIALS AND METHODS

2.1. Instrumentation

Agilent 5975 GC-MSD system (Agilent, USA; SEM Ltd., Istanbul, Turkey) was equipped with the HP-Innowax FSC column (60 m × 0.25 mm id with 0.25 μm film thickness, Agilent, USA). The GC-FID analysis was carried out with capillary GC using an Agilent 6890N GC system (SEM Ltd., Istanbul, Turkey). Microtiter plate assays were performed with Biotek Powerwave XS microplate reader. Ultrapure water (0.05 μS/cm) was obtained from a Direct-Q® Water Purification System (Germany).

2.2. Chemicals

Hydrochloric acid, *n*-hexane, dimethyl sulfoxide (DMSO) (Sigma-Aldrich, Germany), anhydrous sodium sulfate (Fluka, Germany), iodine (ACS reagent), potassium iodide (Saint Louis, USA), methanol (Sigma-Aldrich, Poland), potassium persulfate (Sigma-Aldrich, Saint Louis, USA), sodium phosphate and disodium phosphate were of analytical grade. A C_8–C_{40} *n*-alkane standard solution was purchased from Fluka (Buchs, Switzerland). Gallic acid (GA), (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), soluble starch, acarbose, α-amylase from porcine pancreas (Type VI-B, EC 3.2.1.1), Tris (hydroxymethyl) aminomethane (ACS reagent), acetylcholinesterase (AChE) from *Electrophorus electricus* (Type VI-S), bovine serum albumin (BSA), acetylthiocholine iodide (ATCI), 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB), galanthamine from *Lycoris* sp. were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.3. Plant Material

Aerial parts of *A. grandifolia* were collected in period of flowering in Antalya province of Turkey, on June 2016. The plant material was dried under the shade. Botanical identification was performed by Dr. Münevver Arslan (Directorate of Institute of Forest, Soil and Ecology Research, Eskişehir/Turkey). Voucher specimen is deposited at the Herbarium of the Forest Regional Department (Eskişehir).

2.4. Hydrodistillation of Essential Oil

Aerial parts of *A. grandifolia* were subjected to hydrodistillation (3 h) in a Clevenger type apparatus to yield essential oils (9^{th} Eu.Ph.) [37].

2.5. Gas-Chromatography - Mass Spectrometry (GC/MS)

The GC/MS analysis was carried out with an Agilent 5975 GC-MSD system (Agilent, USA; SEM Ltd., Istanbul, Turkey). HP-Innowax FSC column (60 m × 0.25 mm, 0.25 μm film thickness, Agilent, USA) was used with a helium carrier gas at 0.8 mL/min. GC oven temperature was kept at 60ºC for 10 min and programmed to 220ºC at a rate of 4ºC/min, kept constant for 10 min at 220ºC, and then programmed to increase at a rate of 1ºC/min to 240ºC. The oils were analyzed with a split ratio of 40:1. The injector temperature was 250ºC. Mass spectra were taken at 70 eV and the mass range was from m/z 35 to 450.

2.6. Gas Chromatography (GC-FID)

The GC-FID analysis was carried out with capillary GC using an Agilent 6890N GC system (SEM Ltd., Istanbul, Turkey). Flame ionization detector (FID) temperature was set at 300ºC in order to obtain the same elution order with GC/MS. Simultaneous injection was performed using the same column and appropriate operational conditions.

2.7. Identification and Quantification of Compounds

Identification of the volatile constituents was achieved as reported previously [38]. Briefly, identification of the individual compounds was based on the following: (i) comparison of the GC/MS Relative Retention Indices (RRI) of the compounds on polar column determined relative to the retention times of a series of *n*-alkanes (C_8-C_{40}), with those of authentic compounds or literature data; (ii) computer matching with commercial mass spectral libraries: MassFinder software 4.0 [39], Adams Library [40], Wiley GC/MS Library (Wiley, New York, USA) and Nist Library, and comparison of the recorded spectra with literature data Confirmation was also achieved using the in-house "Başer Library of Essential Oil Constituents" database, obtained from chromatographic runs of pure compounds performed with the same equipment and conditions.

2.8. Free radical scavenging activity

DPPH assay: The hydrogen atoms or electrons donation ability of the essential oil was evaluated according to bleaching of purple colored DPPH stable radicals using method of Brand-Williams [41] with slight modifications. The DPPH solution (0.08 mg/mL, in methanol) was freshly prepared daily, kept in the dark at 4°C between the measurements. Gallic acid (standard) was used as positive control. The solutions of the oil (10 mg/mL) and gallic acid (0.1 mg/mL) were prepared in methanol. 100 μL of the sample (oil or standard) solution and 100 μL DPPH solution were pipetted by multichannel automatic pipette (Eppendorf Research® plus, Germany) into 96-flat bottom well plate cells and allowed to stand in the dark for 30 min. The control well contained 100 μL methanol (instead of the sample) mixed with 100 μL of DPPH. The decrease in the absorbance was recorded at 517 nm. Experiments were performed in triplicate. The free radical scavenging activity of the samples was expressed as percentage of inhibition calculated according to Eq. (1):

$$\%\, I\, h = \left(\frac{Abs_{control} - Abs_{sample}}{Abs_{control}}\right) \times 100, \quad (1)$$

where, $Abs_{control}$ is the absorbance of the control (containing all reagents except the test sample), Abs_{sample} is the absorbance of the sample with added DPPH. The IC_{50} values were obtained by plotting the DPPH scavenging percentage of each sample against the sample concentration. Data were analyzed using the SigmaPlot software (Version 12.0).

Trolox Equivalent Antioxidant Capacity (TEAC) assay: $ABTS^{\bullet+}$ free radical cation scavenging activity of the oil was tested according to the procedure described by Re et al. [42] with slight modifications. 7 mM ABTS and 2.5 mM $K_2S_2O_8$ dissolved in 10 mL ultrapure water were allowed to stand in dark for 16 h at room temperature to create $ABTS^{\bullet+}$ free radical cation. Prior to the assay, $ABTS^{\bullet+}$ solution was diluted with absolute ethanol to an absorbance between 0.7 - 0.8 at 734 nm. The solutions of the oil (10 mg/mL) and Trolox (3.0; 2.0; 1.0; 0.5; 0.25; 0.125 mM) were prepared in MeOH. 10 µL of the sample solution was mixed with 990 µL $ABTS^{\bullet+}$ solution. 10 µL MeOH instead of sample or standard mixed with $ABTS^{\bullet+}$ solution was used as control. Gallic acid (0.1 mg/mL) was used as positive control. Decrease in the absorbance after 30 minutes of incubation was recorded at 734 nm to get linear Trolox equation. $ABTS^{\bullet+}$ scavenging activity of the samples was expressed as Trolox equivalent antioxidant capacity and calculated using linear equation obtained for Trolox (y = 23.224 – 1.7141, r^2 = 0.9989). Calibration curve obtained for Trolox is presented on Figure 1.

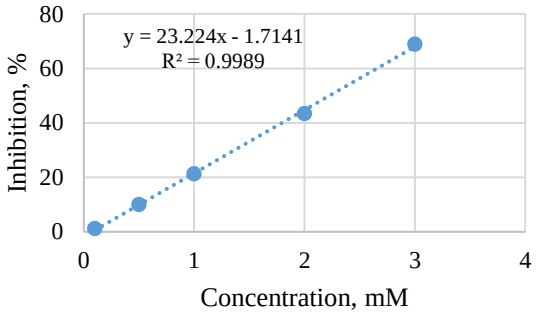

Figure 1. Calibration curve obtained for Trolox dilutions

2.9. α-Amylase Inhibition Assay

The activity of α-amylase under effect of EO and ME was measured using Caraway-Somogyi iodine/potassium iodide (IKI) method [43] with slight modifications. The substrate solution (0.05 %) was prepared by dissolving of soluble potato starch (10 mg) in 20 mL ultrapure water then boiling for 10 min and cooling to room temperature before use. As a positive control experiment, acarbose (0.01-0.1 mg/mL in buffer) was used. The experiment was carried out as follow: 20 mM sodium phosphate buffer (pH 6.9) was pipetted in the 96-well flat bottom plates with multichannel automatic pipette (Eppendorf Research® plus, Germany), then 25 µL sample solution and 50 µL α-amylase (0.8 U/mL in buffer) were added and incubated for 10 min at 37ºC. After incubation, 50 µL substrate solution was added to the mixture. The mixture was subjected to a second incubation for 10 min at 37ºC. The reaction was stopped by addition of 25 µL HCl (1 M). Finally, 100 µL IKI reagent was added to the wells. The sample blanks contained all reaction reagents and 50 µL buffer instead of enzyme. The control wells contained all reaction reagents and 25 µL solvent (instead of the sample solution). The absorbance values were recorded for the sample and blank at 630 nm. The percentage inhibition of the α-amylase activity (Inh %) was calculated according to Eq. 2.

$$\%Inh = \left[\frac{(Abs_{control} - Abs_{control\ blank}) - (Abs_{sample} - Abs_{sample\ blank})}{Abs_{control} - Abs_{control\ blank}}\right] \times 100 \qquad (2)$$

where $Abs_{control}$ and $Abs_{control\ blank}$ are the absorbance values of the control and its blank, Abs_{sample} and $Abs_{sample\ blank}$ are the absorbance values of the sample and its blank.

2.10. Acetylcholinesterase Inhibition Assay

Acetylcholinesterase (AChE) inhibition of the oil was evaluated using Ellman's method as previously reported [44] with slight modification. Three buffers were used: (**A**) 50 mM Tris-HCl (pH=8.0, in ultrapure water); (**B**) 0.1 % BSA in buffer A; (**C**) 0.1 M NaCl and 0.02 M $MgCl_2$•$6H_2O$ in buffer A. In the 96-well flat bottom plates, 25 µL sample (essential oil or standard), 50 µL buffer B and 25 µL AChE (0.22 U/mL in buffer A) solution were pipetted with 8-channel automatic pipette (Eppendorf Research® plus, Germany) and incubated for 15 min at 25ºC. Then, 125 µL Ellman's reagent DTNB (3.0 mM in buffer C) and 25 µL substrate ATCI (15 mM, in ultrapure water) were added. Hydrolysis of ATCI was monitored by the formation of the yellow 5-thio-2-nitrobenzoate anion as a result of the reaction of DTNB with thiocholines, catalyzed by enzymes at 412 nm utilizing a 96-well microplate reader (Biotek Powerwave XS, USA). The mixture allowed to stand 15 min at 25ºC and the absorbance was recorded at 412 nm. Similarly, a blank (for eliminating the colors of the samples) was prepared by adding sample solution to all reaction reagents and 25 µL buffer instead of enzyme. The control wells contained all the reagents without the sample (the solvents of the samples instead were added). Galanthamine hydrobromide from *Lycoris* sp. (0.1 mg/mL) was used as positive control. The percentage inhibition was calculated according to equation (Eq 3):

$$\%Inh = \left[\frac{(Abs_{control} - Abs_{control\ blank}) - (Abs_{sample} - Abs_{sample\ blank})}{Abs_{control} - Abs_{control\ blank}}\right] \times 100 \quad (3)$$

where $Abs_{control}$ and $Abs_{control\ blank}$ are the absorbance of the control and its blank, Abs_{sample} and $Abs_{sample\ blank}$ are the absorbance of the sample and its blank. Data obtained from *in vitro* enzyme inhibition assays were expressed as the mean standard error (±SEM).

2.11. Statistical analysis of data

Data obtained from antioxidant and enzyme inhibition experiments were expressed as mean standard error (±SEM). IC_{50} values were estimated using a nonlinear regression algorithm.

3. RESULTS AND DISCUSSION

In literature it could be found highlighting promising phytochemical properties and biological activities of diverse *Achillea* species [10, 16, 45]. The main objective of the present work was to evaluate chemical composition and confirm the biological potent of volatile metabolites of *A. grandifolia*. Namely, there was carried out gas-chromatographic investigation of the essential oil composition. Subsequently, the oil was subjected to evaluations for free radical scavenging activity against model radicals (DPPH and ABTS). Another goal was to determine enzyme inhibitory activities, involved into digestion of carbohydrates (α-amylase) and neurodegenerative process in brain (acetylcholinesterase).

3.1. Essential oil Chemical Composition

The hydrodistillation of *A. grandifolia* aerial parts resulted with yellowish essential oil (0.08 % yield) with specific odor. Gas-chromatographic analysis of the oil resulted with 56 compounds, which belong to monoterpene hydrocarbons, oxygenated monoterpenes, oxygenated sesquiterpene, fatty acids and phenylpropanoids. The list of detected compounds with their relative retention indices, relative percentages and method of identification is given in Table 1 in order of their elution on the HP-Innowax FSC column. Gas-chromatographic profile of *A. grandifolia* oil is presented in Figure 1. The oil was characterized with high abundance of the oxygenated monoterpenes (76.2 %) with α-terpinyl acetate

(54.1 %), *cis*-piperitone oxide (7.5 %) and borneol (3.1 %) as main constituents. The monoterpene hydrocarbons were presented by *p*-cymene (17.7 %) and α-terpinene (2.2 %). Oxygenated sesquiterpenes, fatty acids and their esters, and phenylpropanoids were detected in scarce amounts.

Table 1. Chemical composition of *Achillea grandifolia* essential oil.

No	RRI	Compound	%	ID method
1	1032	α-Pinene	0.1	c,d,e
2	1035	α-Thujene	t	c,d,e
3	1076	Camphene	0.4	c,d,e
4	1188	α-Terpinene	2.2	c,d,e
5	1213	1,8-Cineole	1.7	c,d,e
6	1255	γ-Terpinene	0.2	c,d,e
7	1280	*p*-Cymene	17.7	c,d,e
8	1285	Isoamyl isovalerate	t	c,d,e
9	1286	2-Methyl butyl 2-methyl butyrate	t	d,e
10	1290	Terpinolene	t	c,d,e
11	1296	Octanal	0.1	c,d,e
12	1303	Amyl isovalerate	t	c,d,e
13	1474	*trans*-Sabinene hydrate	t	c,d,e
14	1483	Octyl acetate	t	c,d,e
15	1494	(Z)-3-Hexenyl 3-methylbutyrate (=*(Z)-3-hexenyl isovalerate*)	t	d,e
16	1541	Benzaldehyde	0.1	c,d,e
17	1553	Linalool	0.1	c,d,e
18	1556	*cis*-Sabinene hydrate	0.1	c,d,e
19	1562	Octanol	t	c,d,e
20	1568	1-Methyl-4-acetylcyclohex-1-ene	t	e
21	1571	*trans*-*p*-Menth-2-en-1-ol	1.0	c,d,e
22	1582	*cis*-Chrysanthenyl acetate	t	c,d,e
23	1590	Bornyl acetate	0.6	c,d,e
24	1611	Terpinen-4-ol	0.6	c,d,e
25	1617	Lavandulyl acetate	0.1	c,d,e
26	1638	*cis*-*p*-Menth-2-en-1-ol	0.8	c,d,e
27	1648	Myrtenal	t	c,d,e
28	1651	Sabinaketone	t	d,e
29	1670	*trans*-Pinocarveol	t	c,d,e
30	1689	*trans*-Piperitol (=*trans*-*p*-Menth-1-en-3-ol)	0.3	c,d,e
31	1706	α-Terpineol	0.1	c,d,e
32	1709	α-Terpinyl acetate	54.1	c,d,e
33	1719	Borneol	3.1	c,d,e
34	1735	Piperitone oxide-I (= *cis*-Piperitone oxide)	7.5	c,d,e
35	1758	*cis*-Piperitol	0.9	c,d,e
36	1864	*p*-Cymen-8-ol	0.2	c,d,e
37	1889	Ascaridole	2.1	c,d,e
38	1902	Benzyl isovalerate	t	c,d,e
39	1948	*trans*-Jasmone	0.1	d,e
40	1969	*cis*-Jasmone	1.8	d,e
41	2008	Caryophyllene oxide	0.1	c,d,e
42	2110	4-Hydroxy-4-methyl-cyclohex-2-enone	0.5	e
43	2113	Cumin alcohol	0.1	c,d,e
44	2144	Spathulenol	1.0	c,d,e
45	2181	Isothymol (=*2-Isopropyl-4-methyl phenol*)	t	c,d,e
46	2186	Eugenol	0.2	c,d,e
47	2192	Nonanoic acid	0.2	c,d,e
48	2198	Thymol	0.2	c,d,e
49	2221	Isocarvacrol (=*4-Isopropyl-2-methyl phenol*)	t	c,d,e
50	2239	Carvacrol	0.4	c,d,e

No	RRI	Compound	%	ID method
51	2298	Decanoic acid	0.1	c,d,e
52	2300	Tricosane	0.1	c,d,e
53	2308	(*E*)-Methyl jasmonate	0.2	d,e
54	2503	Dodecanoic acid	0.1	c,d,e
55	2655	Benzyl benzoate	t	c,d,e
56	2931	Hexadecanoic acid	0.5	c,d,e
		Total	**99.7**	
		Monoterpene hydrocarbons	20.6	
		Oxygenated monoterpenes	76.2	
		Oxygenated sesquiterpenes	1.1	
		Fatty acids and esters	0.9	
		Phenylpropanoids	0.2	
		Others	0.7	

[a] Relative Retention Indices calculated against n-alkanes (C_9-C_{40}) on HP-Innowax column; **%** calculated from FID data; [c] Identification based on retention index of genuine compounds on the HP-Innowax column; [d] Identification on the basis of computer matching of the mass spectra from Başer; [e] Tentative identified on the basis of computer matching of the mass spectra from Adams, MassFinder, Wiley, and NIST libraries; **t** Trace (< 0.1 %).

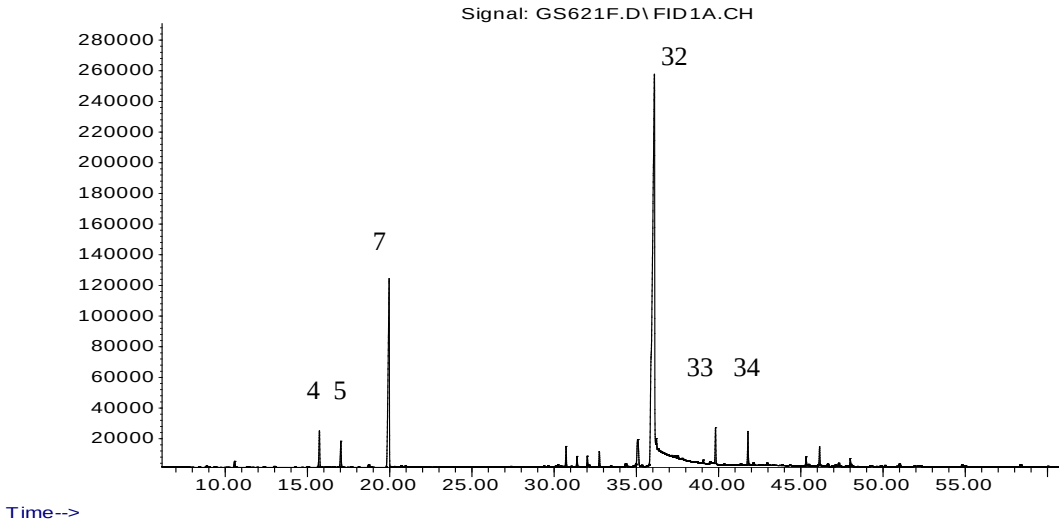

Figure 1. Gas-chromatographic profile of the essential oil of *Achillea grandifolia*. Numeration of the peaks is depicted according to the list of detected compounds in Table 1.

There are several reports in the literature dealing with the essential oils of diverse *A. grandifolia* from different countries. It was interesting to compare the chemical profile of *A. grandifolia* essential oil with those reported earlier (Table 2).

Table 2. Major constituents of *Achillea grandifolia* essential oils according to literature survey

***A. grandifolia* locality and collection year**	**Main constituents (%)**	**Ref.**
Serbia (2007)	1,8-cineole (28.8), camphor (22.8), borneol (4.9)	[30, 31]
Greece (1992)	*cis*- and *trans*-thujone (20)	[31, 32]
Turkey / Izmir (2004)	piperitone (34.0), carvacrol (7.0), *p*-cymene (5.0)	[33]
Turkey / Aydin (2004)	1,8-cineole (32.0), piperitone (18.7), *p*-cymene (10.0)	[33]
Turkey/ Antalya (2016)	α-terpinyl acetate (54.1), *p*-cymene (17.7), *cis*-piperitone oxide (7.5), borneol (3.1)	present work

As can be seen in Table 2, in all the essential oils of *A. grandifolia* reported earlier, the oxygenated monoterpenes had the first rank from frequency point of view. So, if all reported data is taken into consideration, we can assume that oxygenated monoterpenes are characteristic constituents of the volatiles of *A. grandifolia*.

3.2. Antioxidant Activity of the Essential Oil

Antioxidant activity assessments were performed *in vitro* by using non-enzymatic systems employing different model substrates: stable free radical $DPPH^{\bullet}$ and cation radical $ABTS^{+\bullet}$. The essential oil demonstrated free radical scavenging activity (52.07 % of inhibition) against DPPH and moderate Trolox-equivalent antioxidant capacity (0.62 mM) against ABTS (Table 3). Results of antioxidant activity assays showed that the oil of *A. grandifolia* could be considered as a source of effective antioxidants.

3.3. Inhibitory effects of the Essential Oil on Pancreatic α-Amylase Activity

The evidenced-based therapeutic usage of many plants is scarce. Nowadays, there is demand for new effective and safe natural products with hypoglycemic property. The efficacy of known synthetic hypoglycemic products is debatable. So, the plants reputed for their antidiabetic effect should be verified either experimentally or clinically. The essential oil of *A. grandifolia* was *in vitro* evaluated for hypoglycemic activity *via* inhibition of the porcine pancreatic α-amylase. The oil showed significant inhibitory activity (Inh. > 85.0 %). Serial dilution method revealed that the oil inhibition potential was an IC_{50} of 0.15 mg/mL (Table 3). It seems to be that *A. grandifolia* can be considered as perspective potential source of natural phytochemicals with hypoglycemic effect.

Table 3: The biological activity of *Achillea grandifolia* essential oil

	DPPH Inh., %	**TEAC, mM**	**α-Amylase, IC_{50}, mg/mL**	**AChE, IC_{50}, mg/mL**
Essential oil	52.07[§]	0.62[§]	0.15[§]	1.77[§]
Gallic acid	90	-	-	-
Acarbose[d]	-	-	0.08	-
Galanthamin				0.006

[§] the deviation from the mean is <0.01 of the mean value

Earlier, antidiabetic phytochemicals from different *Achillea* species have been proved to be effective agents. The hypoglycemic effect of *A. ligustica* All. *n*-hexane extract was reported to have high antidiabetic effect [46]. *A. biebersteinii* Afan. reduced significantly fasting blood glucose levels, improved oral glucose tolerance, tended to raise serum insulin levels, enhanced regeneration of β-cells, and seemed to be more effective than the reference glibenclamide [45]. Abd-Alla reported about *in-vitro* α-amylase inhibitory reducing starch-induced postprandial glycemic effects of sesquiterpene lactones belonging to germacranolide and guaianolide classes and obtained from *A. biebersteinii* [14, 47].

3.4. Inhibitory Effects of the Essential Oil on Acetylcholinesterase Activity

In literature there is information about anti-AChE activity of *A. falcata*, and *A. filipendulina* essential oils suggesting a potential use as adjuvant therapy in neurodegenerative conditions such as Alzheimer's disease [48, 51, 52]. Above-mentioned data about anti-AChE potential of *Achillea* species prompted us to subject *A. grandifolia* oil to investigation against AChE. In scope of the present work, the oil of *A. grandifolia* was evaluated for antineurodegenerative potential *via* inhibition of AChE enzyme *via* microtiter plate test based on Ellman's colorimetric assay. The microtiter plate assay revealed that the oil inhibited AChE with > 82 %. Therefore, we performed dilution of the oil to determine IC_{50} value (Table 3).

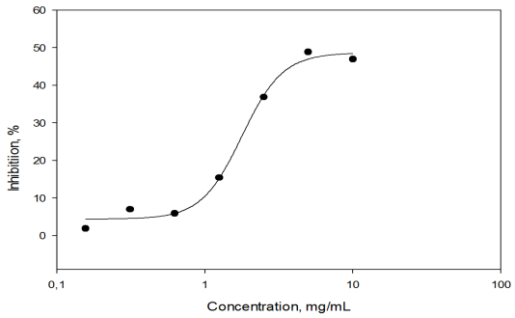

Figure 2. Plotting of inhibition percentage of *Achillea grandifolia* oil against concentration.

Subsequent dilution of the oil and plotting the inhibition percentage of each diluted sample against the concentration allowed us to determine its inhibiting potential as an IC_{50} of 1.77 mg/mL (Figure 2).

Two oxygenated monoterpenes esters from *A. falcata* oil *trans*-sabinyl formate and tiglate have been reported to have moderate acute toxicity and acetylcholinesterase inhibitory activities [48]. The main constituents of the oil of *A. filipendulina* (borneol, bornyl acetate, geraniol, 1,8-cineole and farnesol) have earlier been reported as recognized pesticides [53-58]. Their insecticidal effect was related with neurotoxic mode of action through anti-AChE effect on enzyme. In the present work, in part 3.1 we have reported that the oil of *A. grandifolia* contain major constituents such as α-terpinyl acetate, α-terpineol, borneol, 1,8-cineole. In literature, these constituents have recognized insecticidal activity [59, 60], which related with their inhibitory effect on cholinesterase enzyme. In addition, it should be noted that the synergistic action of the volatile constituents identified in the oil could contribute to their AChE inhibitory ability [53].

4. CONCLUSION

According to phytochemical study, we herein disclose the diversity of the chemical composition of *A. grandifolia* essential oil from Turkey. The oil was found as source of valuable secondary metabolites: oxygenated monoterpenes and monoterpene hydrocarbons. In scope of the present study, the biological potent encompass antioxidant, antidiabetic and antiacetylcholinesterase activities. It seems, that *A. grandifolia* can be considered as valuable source of bioactive components useful in combating various diseases related with disbalance in oxidation and antioxidation system, inflammation, skin disease, neurodegenerative problems as well as in the provision of cheap, safe and natural phytoparmaceuticals.

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