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Chemical Composition and Acetylcholinesterase Inhibition Activity of Volatile Oil from Leaves of *Arbutus unedo* L. from Croatia

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The volatile oil from leaves of *Arbutus unedo* L. from Croatia was subjected to chemical composition and acetylcholinesterase inhibition activity. The tested volatile oil was dominated by nonterpene compounds, with (*E*)-2-decenal, nonanal, nonanoic acid and octanol as main compounds. Terpene compounds were identified in lower quantity, with linalool and α-terpineol as main ones. Results showed low to moderate acetylcholinesterase (AChE) inhibition potential of tested oil, for tested concentration of 1 mg/mL.

Keywords: *Arbutus unedo* L., Volatile oil, GC-MS, Ellman.

INTRODUCTION

The genus Arbutus belonging to Ericaceae family. *Arbutus unedo* L., commonly known as strawberry-tree, is an evergreen shrub or small tree, native in the Mediterranien region. Almost all parts of the *A. unedo* plant (leaves, fruits, bark, roots and honey) have been used in traditional medicine for the treatment of many different diseases. Many ethnobotanical studies confirmed biological potential of *A. unedo* extracts in the treatment of kidney, gastrointestinal, dermatologic, urological, cardiovascular and hypertensive diseases and diabetes [1,2]. The medicinal characteristics of this plant is related to pharmacologically active compounds which are present in different parts of plant. A lot of study has been performed about phytochemical composition of different parts of *Arbutus unedo*. Among them, only two studies have been performed regarding chemical composition of volatile oil from leaves of *A. unedo* [3,4].

The aim of this work was to determinate the chemical composition as well as acetylcholinesterase inhibition activity of volatile oil from leaves of Croatian *Arbutus unedo*. To our best of knowledge this is the first report about chemical composition of volatile oil from leaves of *Arbutus unedo* from Croatia as well as the first record about acetylcholinesterase (AChE) inhibition activity of this volatile oil.

EXPERIMENTAL

Plant material (fresh leaves) was collected in October 2015 near Split, Croatia. Plant material was identified by a botanist dr. Mirko Ruscic, associate professor, Department of Biology, Faculty of Science, Split, Croatia. A voucher specimen of plant material was deposited in herbarium at the Department of Biochemistry, Faculty of Chemistry and Technology, Split, Croatia.

Volatile oils isolation: The fresh leaves of *Arbutus unedo* L. were subjected to hydrodistillation using a Clevenger-type apparatus for 3 h. The obtained volatile oils were stored in a sealed vial, under -20 °C until use.

Gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) analyses: GC and GC-MS analyses of isolated volatile oils were performed using Agilent Technologies (Palo Alto, CA, USA) Gas Chromatograph, model 7890A, equipped with flame ionization detector and mass detector, model 5975C, with polar capillary column HP-FFAP (Free Fatty Acid Phase, Hewlett Packard; 50 m × 0.32 mm i.d., film thickness 0.52 μm). Temperature program for HP-FFAP column was: 70 °C isothermal for 4 min, than increased to 180 °C at a rate of 4 °C min^{-1} and subsequently held isothermal for 15 min. Carrier gas was helium at flow rate 1 mL/min, injector temperature was 250 °C, injected volume 1 μL;

split ratio of 1:50; FID detector temperature was 300 °C. Mass spectrometer ionization voltage was 70 eV, mass scan range: 30-300 mass units and ion source temperature was 280 °C.

Compounds identification and quantification: Identification of volatile compounds were based on comparison of compounds mass spectra with databases (Wiley 275 library-Wiley, New York, NY, USA) and comparison of their retention indices (relative to series of *n*-alkanes C_9-C_{40}), with internal database retention indices and literature retention indices using NIST2002 (National Institute of Standards and Technology, Gaithersburg, MD, USA) [5,6]. The internal database of compounds was created during previous analyses from authentic compounds obtained commercially and from more than thousand volatile oils obtained during our previous studies. The percentages of components were calculated as mean values from the GC and GC-MS peak areas.

Acetylcholinesterase inhibition potential of volatile oil: Acetylcholinesterase (AChE) inhibition potential of *Arbutus unedo* volatile oil isolated from leaves was carried out by a slightly modified Ellman method [7]. A run consisted of 180 µL of phosphate buffer (0.1 M, pH 8), 10 µL of DTNB (at a final concentration of 0.3 mM prepared in 0.1 M phosphate buffer pH 7 with 0.12 M sodium bicarbonate added for stability), 10 µL of sample solution (dissolved in 80 % EtOH) and 10 µL of AChE/BChE solution (with final concentration 0.03 U/mL). Reactants were mixed in a 96-well plate wells and reaction was initiated by adding 10 µL of acetylthiocholine iodide iodide (ATChI), to reach a final concentration of 0.5 mM. As a negative control, 80 % EtOH was used instead of sample solution. Non-enzymatic hydrolysis was also monitored by measurement of two blank runs for each run. All spectrophotometric measurements were performed at 405 nm and at room temperature for 6 min periods. The results are expressed as percentage inhibition of enzyme activity. The experiment was performed in triplicate and the results were expressed as mean.

RESULTS AND DISCUSSION

The yield of 0.06 % was obtained from *Arbutus unedo* volatile oil isolated from fresh leaves. Sixty volatile oil compounds, representing 93.7 % of total oil, were identified (Table-1). The principal oil compounds were nonterpene compounds, with (*E*)-2-decenal (14.1 %), nonanal (9.5 %), nonanoic acid (8.1 %) and octanol (5.2 %) as main compounds. Terpene compounds were identified in lower quantity, with linalool (4.4 %) and α-terpineol (2.9 %) as main ones. Other volatile oil compounds were identified in lower quantity.

This is the first record about chemical composition of volatile oil from leaves of *A. unedo* of Croatian origin. Previously, the volatile oil composition of *A. unedo* leaves from Algeria and Turkey were performed [3,4]. The chemical composition of volatile oil from leaves of *A. unedo* from Algeria oil was characterized by a high content of pamitic acid (35.2 %) and linoleic acid (18.8 %) and the presence of heavy aliphatic hydrocarbons. According to authors this oil belongs to palmitic acid, linoleic acid and *p*-cresol, 2,6-di-*tert*-butyl-chemotype of *A. unedo* oil. The principal components of Turkish oil were (*E*)-2-decenal (12.0 %), α-terpineol (8.8 %), hexadecanoic acid (5.1 %) and (*E*)-2-undecenal (4.8 %). The results of this study

TABLE-1
CHEMICAL COMPOSITION OF *Arbutus unedo* VOLATILE OIL

Identified compound	KI	%
α-Pinene	1045	0.1
Ethyl butanoate	1047	1.2
Ethyl 2-methylbutanoate	1053	0.7
Ethyl 3-methylbutanoate	1064	0.5
Hexanal	1092	0.7
Undecane	1100	0.8
2-Pentanol	1112	0.4
p-Xylene	1140	0.5
Ethyl 2-butenoate*	1159	2.9
4-Methyl-3-penten-2-one	1165	0.4
Heptanal	1167	0.9
Isopentyl alcohol	1174	0.4
Limonene	1240	1.2
Octanal	1289	1.6
cis-3-Hexenyl acetate*	1305	0.9
2,2,6-Trimethyl cyclohexanone	1310	0.8
2-Heptenal*	1316	0.5
Hexanol	1320	0.4
3-Hexen-1-ol*	1400	0.6
Nonanal	1402	9.5
3,5,5-Trimethyl-3-cyclohexene-1-one	1410	1.4
2-Octenal*	1429	0.6
trans-Linalool oxyde	1450	0.4
Etanoic acid	1462	1.8
Heptanol	1467	0.3
3-Hexenyl-2-methylbutanoate*	1480	0.9
Nonane, 1,1-diethoxy	1515	0.7
Propanoic acid	1547	0.7
Vitispirane	1551	1.4
Linalool	1557	4.4
3,4-Dimethyl 2-pentene*	1561	0.6
Octanol	1564	5.2
2-Undecanone	1579	1.0
β-Cyclocitral	1635	1.5
(E)-2-Decenal	1647	14.1
3-Methyl butanoic acid	1651	0.6
Methyl benzoate	1653	2.4
α-Terpineol	1701	2.9
4-Ketoisophorone	1705	0.4
Verbenone	1710	0.5
2-Undecanal	1736	2.0
Methyl salicylate	1773	1.1
Nerol	1808	0.5
2-Tridecanon	1812	1.3
(*E,E*)-2,4-Decadienal	1814	0.2
Ethyl salicylate	1820	0.4
(*E*)-β-Damascenone	1826	0.6
Hexanoic acid	1849	0.7
Geraniol	1859	1.4
p-Cymen-8-ol	1862	0.2
Geranyl acetone	1867	0.4
Benzyl alcohol	1883	0.5
Ethyl hydrocinnamate	1889	0.5
Heptanoic acid	1944	1.0
β-Ionone	1948	1.2
Nerolidol	2018	0.6
Octanoic acid	2050	2.4
3-Hexenyl benzoate*	2127	1.6
Ethyl cinnamate*	2129	2.2
Nonanoic acid	2150	8.1
Total		93.7

KI = Retention indices relative to C_9-C_{40} *n*-alkanes on polar HP-FFAP column, *Correct isomer is not identified.

showed that the Croatian *A. unedo* volatile oil isolated from leaves is more similar to Turkish oil than to Algerian oil.

Acetylcholinesterase inhibition activity of *A. unedo* volatile oil was also tested. This is the first record about AChE inhibition activity of this oil. AChE inhibition activity was tested by Ellman method [7]. Results showed low to moderate AChE activity of tested oil (48.5 %) for tested concentration of 1 mg/mL (0.045 mg/mL in reaction system) (Table-2). For comparison, eserine as referent compound showed 92.2 % inhibition of AChE for tested concentration of 0.1 mg/mL (4.5 µg/mL in reaction system). Among volatile compounds identified as components of tested oil, several compounds were previously tested on AChE inhibition. Among them, β-ionone, verbenone, geraniol and α-terpineol showed good AChE inhibition potential [8-13]. These compounds or synergism of these compounds are probably responsible for low to moderate, but noticeable, AChE inhibition activity of tested oil.

TABLE-2
ACETYLCHOLINESTERASE INHIBITION POTENTIAL OF *Arbutus unedo* VOLATILE OIL

	AChE inhibition (%)
Volatile oil[a]	48.5
Eserine[b]	92.9

[a]Tested concentration was 1 mg/mL (0.045 mg/mL in reaction system); [b]Tested concentration was 0.1 mg/mL (4.5 µg/mL in reaction system)

Conclusion

The volatile oil from leaves of *Arbutus unedo* from Croatia was subjected to chemical composition and acetylcholinesterase inhibition activity. The tested volatile oil was dominated by nonterpene compounds, with (*E*)-2-decenal (14.1 %), nonanal (9.5 %), nonanoic acid (8.1 %) and octanol (5.2 %) as main compounds. Terpene compounds were identified in lower quantity, with linalool (4.4 %) and α-terpineol (2.9 %) as main ones. Acetylcholinesterase inhibition activity of tested volatile oil showed low to moderate AChE inhibition activity (48.5 %) for tested concentration of 1 mg/mL.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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