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Chemical characterization, antioxidant activity, α-amylase and acetylcholinesterase inhibitory potential of *Angelica pancicii* Vandas ex Velen

[Caracterización química, actividad antioxidante, potencial inhibidor de α-amilasa y acetilcolinesterasa de *Angelica pancicii* Vandas ex Velen]

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Abstract: Comparative study GC-FID/MS of essential oils of fruits, leaves and roots of the endemic plant *Angelica pancicii* Vandas ex Velen. revealed a significant difference in their chemical composition. The enantiomeric purity of the main component in the fruit oil (+)-β-phellandrene was also confirmed. In addition, imperatorin, isoimperatorin, oxypeucedanin, oxypeucedanin hydrate, angeloylpangelin and umbelliprenin were isolated from the fruit hexane extract. The content of these coumarins in the hexane extracts from different plant parts was further determined by HPLC. The essential oils and hexane extracts were assessed for their antioxidant potential and inhibitory effect towards α-amylase and acetylcholinesterase enzymes. The fruit and leaf essential oils (> 80%) as well as the fruit hexane extract (> 62%) significantly inhibited acetylcholinesterase enzyme. Distinguish free radical scavenging properties were detected for the leaf (Inh. 95.0 ± 2.2 %) and the root (Inh. 66.0 ± 2.4 %) extracts.

Keywords: *Angelica pancicii*; Essential oils; Coumarins; Antioxidant activity; α-amylase inhibiton; Acethylcholinesterase inhibition

Resumen: Estudio comparativo GC-FID / MS de aceites esenciales de frutas, hojas y raíces de la planta endémica *Angelica pancicii* Vandas ex Velen revelaron una diferencia significativa en su composición química. También se confirmó la pureza enantiomérica del componente principal del aceite de fruta (+)-β-felandreno. Además, se aislaron imperatorina, isoimperatorina, oxipeucedanina, hidrato de oxipeucedanina, angeloilpangelina y umbeliprenina del extracto de hexano del fruto. El contenido de estas cumarinas en los extractos de hexano de diferentes partes de la planta se determinó adicionalmente mediante HPLC. Los aceites esenciales y extractos de hexano se evaluaron por su potencial antioxidante efecto inhibidor de las enzimas-α-amilasa y acetilcolinesterasa. Los aceites esenciales de frutas y hojas (> 80%), así como el extracto de hexano de frutas (> 62%) inhibieron significativamente la enzima acetilcolinesterasa. Se detectaron propiedades de captación de radicales libres diferenciadas para los extractos de hoja (Inh. 95,0 ± 2,2%) y de raíz (Inh. 66,0 ± 2,4%).

Palabras clave: *Angelica pancicii*; Aceites esenciales; Cumarinas; Actividad antioxidante; Inhibición de la α-amilasa; Inhibición de la acetilcolinesterasa.

INTRODUCTION

In the last decades, there is a growing interest in the investigation of aromatic, spicy, and medicinal plants in order to find new, effective and safe therapeutic agents for the treatment of oxidative diseases, metabolic disorders and neurodegenerative diseases (Williams *et al*., 2011; Charles, 2013; Seo *et al*., 2013; Upadhyay, 2016; Sarikurkcu *et al*., 2017; Patel *et al*., 2018; Uysal *et al*., 2019; Karakaya *et al*., 2020). The genus *Angelica* L. (Apiaceae) is one of the most important genera of medicinal plants used in traditional medicinal systems of the Far East and certain Western countries (Sarker & Nahar, 2004). Many species of this genus such as *A. dahurica* (Kim *et al*., 2002; Seo *et al*., 2013), *A. archangelica* (Sigurdsson & Gudbjarnason, 2007), *A. officinalis* (Senol *et al*., 2011), *A. sylvestris* var. *sylvestris* (Orhan *et al*., 2016), *A. gigas* (Sowndhararajan & Kim, 2017), *A. pubescens* (Guo *et al*., 2018), *A. purpurascens* (Karakaya *et al*., 2020), etc. have been studied for their antioxidant potential and neurobiological effects on memory enhancement through cholinesterase inhibition, while the investigations on their antidiabetic properties by inhibition of α-amylase are scarce (Park *et al*., 2011; Guo *et al*., 2018). Antioxidant, anti-inflammatory, antimicrobial, immunotoxic, insecticidal, etc. activities have been also reported for the essential oils from *Angelica* species (Sowndhararajan *et al*., 2017). Coumarins (particularly furanocoumarins), the most characteristic chemical markers of the genus are considered as leading active components of the plants (Sarker & Nahar, 2004; Bruni *et al*., 2019).

Angelica pancicii Vandas ex Velen. is a perennial species, endemic for the Balkan Peninsula (Peev, 1982; Assyov *et al*., 2012). Roots of *A. pancicii* are known as a household remedy for complications such as hypertension (Davidov & Yavashev, 1939; Stojanov & Kitanov, 1960). Literature survey showed scarce data for essential oil and coumarin content of *A. pancicii* (Botcheva, 1972; Simonović *et al*., 2014; Mileski *et al*., 2017). Recently, polar (ethanol, methanol and aqueous) extracts of *A. pancicii* aerial parts and roots have been studied for their antioxidant, antibacterial, antifungal and antiquorum sensing potential (Mileski *et al*., 2017). There are no data regarding the biological activity of essential oil and less polar extracts of *A. pancicii.*

The aim of this study was to give new results concerning antioxidant, α-amylase and acetylcholinesterase inhibitory potential of essential oils and hexane extracts obtained from the leaves, fruit and roots of *Angelica pancicii* as well as to obtain new data on their chemical composition.

MATERIALS AND METHODS

Plant Material

A. pancicii was collected from Rila Mts. (Chakalitza hut, GPS: 42.992175ºN 23.312152ºE) in Bulgaria in 2016. The plant material was separated into leaves, fruits and roots, air-dried and kept in a dark and cool place until extraction. The plant species was identified by Dr Ina Aneva and a voucher specimen (SOM 1372) was deposited in the Herbarium of the Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences.

Extraction

Essential oils were obtained from fruit (60 g), leaves (100 g) and roots (40 g), separately by hydrodistillation using a Clevenger-type apparatus for 2.5 hours. The yields of the resulting essential oils were 1.29, 0.07 and 0.13 %, respectively.

The air-dried plant parts separately (10 g) were extracted with 200 mL of *n*-hexane in a Soxhlet apparatus for 4 hrs. The extracts were concentrated under vacuum at 40°C by using a rotary evaporator and stored at +4°C in dark until use. The yields of the hexane extracts from fruit, leaves and roots were 9.1, 3.3 and 3.6%, respectively.

Gas-Chromatographic analysis and identification of compounds

Chemical composition of the essential oils was determined using GC-FID/MS techniques. GC/MS analysis was performed with an Agilent 5975 GC-MSD system (Agilent Technologies, Santa Clara, CA, USA). An Innowax FSC column (60 m × 0.25 mm, 0.25 □m film thickness) was used with He as carrier gas (0.8 mL/min). GC oven temperature was kept at 60°C for 10 min, increased to 220°C at a rate of 4°C/min, kept constant at 220°C for 10 min, and then increased to 240°C at a rate of 1°C/min. The split ratio was adjusted to 40:1, and the injector temperature was 250°C. Mass spectra were collected at 70 eV with a mass range from *m/z* 35 to 450. GC-FID analysis was performed using an Agilent 6890N GC system. To obtain the same elution order as with GC/MS, the line was splitted for FID and MS detectors. Flame ionization detector (FID) temperature was 300°C. The identification of the volatile constituents was based on computer matching of their mass spectra with commercial mass spectral

libraries: MassFinder software 4.0, Adams Library, Wiley GC/MS Library (Wiley, New York, NY, USA), and NIST Library; 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. 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. Relative percentage amounts of the separated compounds were calculated from FID chromatograms.

GC/MS analysis on chiral column of β-phellandrene in the fruit essential oil of A. pancicii

Enantiomeric status of β-phellandrene was determined using LIPODEX G (25 m × 250 µm x 0.13 µm) column with He (5 mL/min) as a carrier gas. Flow rate was 80.028 cm/sec. Temperature programm was as follow: 100 min at 60°C, 5°C/min to 140°C then 40°C/min to 200°C, 2.5 min at 200°C, total 120 min. Injection was performed in split mode with ratio 40:1. Injection temperature was 230°C. Mass spectra were collected at 70 eV with a mass range from *m/z* 35 to 450. FID temperature was 250°C.

Isolation of the individual compounds

A portion of the fruit hexane extract (50 mg) was separated by preparative TLC (SiO_2, glass plate, Merck, 20 × 20 cm) with hexane/diethyl ether (2:1, 3 developments) and UV monitored at 254 and 366 nm. The zones, containing coumarins were scratched and eluted with mixture of hexane/diethyl ether (1:1). The isolated compounds imperatorin (**1,** 2 mg), isoimperatorin (**2**, 3 mg), oxypeucedanin (**3**, 5 mg), oxypeucedanin hydrate (**4,** 1 mg), angeloylpangelin (**5**, 2 mg), and umbelliprenin (**6**, 1 mg) were identified by comparison of their ^{1}H NMR data with those in the literature and with authentic standards.

Quantitative determination of coumarins by HPLC

The HPLC equipment was a Waters HPLC system (Waters, 2795) with a Waters binary pump, an auto-sampler, a column oven, and a Waters 2487 Dual wavelength absorbance detector. The LiChrospher 100 RP-18 column (5 µm, Merck) was tested with a guard column that was filled with the same stationary phase. A (20% CH_3OH in H_2O) and B (CH_3CN) were used as the mobile phase under gradient conditions (0 min, 70% A; 25 min, 40% A; 26 min, 20% A; 35 min, 0% A; and 40 min, 60% A) to analyze the samples. The analysis was carried out at a flow rate of 0.8 mL/min and the total run time was 60 min. The detection wavelength was set at 310 nm and the sample injection volume was 10 µL. The peak identification was based on the retention time (Rt) of the standard compounds, as follows: oxypeucedanin hydrate (3.6 min), oxypeucedanin (11.4 min), imperatorin (18.3 min), isoimperatorin (22.4 min), angeloylpangelin (26.5 min), and umbelliprenin (31.4 min). The correlation coefficients (R^2) were higher than 0.99 (five concetrations in three replicates each) and the relative standard deviations (% RSD) were < 5% confirming the linearity and repeatability of the method for each compound. All samples were run in triplicate and quantification was carried out using external standards. The content of each compound was calculated and expressed as mg/g on dry extract (DE).

Free radical scavenging activity tests

DPPH test

The free radical scavenging activity was measured by using the bleaching of purple-coloured stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical according to the method of Brand-Willams et al. (Brand-Williams *et al*., 1995) with slight modifications. The DPPH solution (0.08 mg/mL) was prepared in methanol daily and stored in the dark at 4°C. The stock solutions of the essential oils (10 mg/mL), hexane extracts (10 mg/mL) and reference compounds gallic acid (0.1 mg/mL), BHT (1 mg/mL) and ascorbic acid (0.1 mg/mL) were prepared in methanol. In the experiment, into cells of 96-flat bottom well microplate 100 µL of the sample solution and 100 µL DPPH solution were transferred by automatic pipette and incubated in the dark for 30 min. The control well contained 100 µL methanol (instead of the sample) mixed with 100 µL of DPPH. The absorbance was recorded at 517 nm. Gallic acid, BHT and ascorbic acid were used as positive control. The free radical scavenging activity of the samples was expressed as the percentage of inhibition calculated according to the equation:

$$\%\ Inh = \left(\frac{Abs_{control} - Abs_{sample}}{Abs_{control}}\right) \times 100,$$

where $Abs_{control}$ is the absorbance of the control (containing all reagents except the test compound)

and 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 analysed using the SigmaPlot software (Version 12.0). Experiments were performed in triplicate.

TEAC (Trolox equivalent antioxidant capacity) test

ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) free radical cation scavenging activities of the essential oils were evaluated according to the procedure reported earlier (Re *et al*., 1999) with slight modifications. Details of the experiments as follow: the solution of ABTS (7 mM) was prepared in $K_2S_2O_8$ (2.5 mM) in 10 mL ultrapure water by incubating (16 h) in the dark at room temperature to create $ABTS^{\bullet+}$. Before the experiment, the $ABTS^{\bullet+}$ solution was diluted with absolute ethanol to an absorbance 0.7–0.8 at 734 nm. The solutions of the essential oils (30 mg/mL), hexane extracts (10 mg/mL), gallic acid (1 mg/mL) and Trolox (3.0; 2.0; 1.0; 0.5; 0.25; 0.125 mM) were prepared in MeOH. In the experiment, the oil or Trolox solution (10 μL) were mixed with $ABTS^{\bullet+}$ solution (990 μL) in cells of 96-deep well plate. As a control, 10 μL of MeOH was mixed with $ABTS^{\bullet+}$ solution. Trolox was used for creating of calibration curve and obtaining the linear equation. The mixtures were incubated (30 min) in the dark at room temperature. After incubation, the aliquots (300 μL) of the mixtures were transferred into 96-well flat bottom microplate and decrease in the absorbance was recorded at 734 nm. The percentage of inhibition was calculated using linear equation obtained for Trolox. The experiments were performed in triplicate. $ABTS^{\bullet+}$ scavenging activity of the samples was expressed as Trolox equivalent antioxidant capacity.

Microtiter assay for determination of α-amylase inhibition

The inhibitory effect of the samples on α-amylase was evaluated using the iodine/potassium iodide (I/KI) method (Yang *et al*., 2012). In the experiment, 25 μL sample solution (essential oil (5 mg/mL), hexane extract (5 mg/mL) or reference compound (1 mg/mL) and 50 μL α-amylase (0.8 U/mL in 20 mM of sodium phosphate buffer pH = 6.9) were pipetted into wells of 96-well flat bottom plate and incubated for 10 min at 37°C. The reaction was initiated with addition of 50 μL starch solution (0.05% in ultrapure water). After incubation (10 min at 37°C) the reaction was stopped by adding of hydrochloric acid (25 μL, 1 M). Finally, 100 μL of I/KI was pipetted to the wells. The sample blanks contained all reaction reagents and 50 μL of buffer instead of enzyme. The control wells contained all the reagents without the sample. Acarbose was used as reference antidiabetic compound. The inhibition of □-amylase by the tested samples was monitored by the formation of the dark color as a result of reaction between non-hydrolysed starch and iodine, at 412 nm utilizing a microplate reader. The percentage inhibition was calculated according to above mentioned equation.

Microtiter assay for determination of acetylcholinesterase inhibition

Acetylcholinesterase (AChE) inhibition of the samples was evaluated using Ellman's method (Ellman *et al*., 1961) 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 $MgCl_2 \bullet 6H_2O$ in buffer A. In experiment, 25 μL of the sample (10 mg/mL), 50 μL of buffer B, and 25 μL of AChE (0.22 U/mL in buffer A) solution were pipetted with 8-channel automatic pipette (Eppendorf Research® plus, Germany) into wells of the 96-well flat bottom plate. After 15 min incubation (at 25°C) the Ellman's reagent DTNB (3.0 mM, 125 μL) and the substrate ATCI (15 mM, 25 μL) 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 enzyme at 412 nm utilizing a microplate reader (Biotek Powerwave XS, USA). The mixture was allowed to stand 15 min at 25°C, and the absorbance was recorded at 412 nm. Similarly, a blank (for eliminating the colors of the samples) was prepared by adding the sample solution to all reaction reagents and 25 μL of buffer instead of enzyme. The control wells contained all the reagents without the sample. Galanthamine hydrobromide solution (0.1 mg/mL) was used as positive control. The percentage inhibition was calculated according to equation:

$$\%\, Inh = \left(\frac{(Abs_{control} - Abs_{control\ blank}) - (Abs_{sample} - Abs_{sample\ blank})}{Abs_{control} - Abs_{control\ blank}} \right) \times 100,$$

where $Abs_{control}$ and $Abs_{control\ blank}$ are the absorbances of the control and its blank, and Abs_{sample} and $Abs_{sample\ blank}$ are the absorbances of the sample and its blank. Data obtained from *in vitro* enzyme inhibition assays were expressed as the mean standard error (± SEM). The samples demonstrated inhibition more than 50% have been subjected to serial dilution and tested again for activity to determine IC_{50} values.

RESULTS AND DISCUSSION

GC-FID/MS analysis of *A. pancicii* fruit, leaf and root essential oils led to detection of 170 components in concentration more than 0.1%, accounting 84.9 - 98.9% of the total oils (Table No. 1). As can be seen, the studied essential oils differed significantly in their chemical composition. Thus, fruit essential oil was rich in monoterpene hydrocarbons (84.3%), while sesquiterpenoids dominated in leaf and root oils (64.0 and 71.7%, respectively). Sesquiterpene hydrocarbons prevailed in the leaf oil (38.9%) unlike the root oil where oxygenated sesquiterpenoids were the main class of compounds (45.2%). (+)-β-Phellandrene (69.1%) was the principal component in the fruit oil, and its enantiomeric purity was confirmed by GC/MS with a chiral column (Figure No. 1). It is worth mentioning that none of the identified compounds in the leaf and root oils reached 10%. Germacrene D (9.7%), δ-cadinene (6.6%), caryophyllene oxide (6.1%) and β-caryophyllene (4.3%) were detected in substantial amounts in the leaves, and elemol (9.8%), kessane (9.6%), γ-eudesmol (5.3%), β-eudesmol (5.3%) and T-cadinol (4.9%) – in the roots of the plant.

Table No. 1
Chemical composition of *Angelica pancicii* fruit, leaf and root oils

RRIexp.	RRI lit.	Compound	%			RRI exp.	RRI lit.	Compound	%		
			fruit	leaf	root				fruit	leaf	root
1032	1008–1039*	α-Pinene	**5.3**	0.3	1.5	1738	1670-1740*	*p*-Mentha-1,5-dien-8-ol		0.4	
1035	1012–1039*	α-Thujene	t	0.1		1740	1686–1753*	α-Muurolene		0.6	
1048	1048**	2-Methyl-3-buten-2-ol	t		t	1740	1702-1772*	*cis*-α-Bisabolene			1.5
1076	1043–1086*	Camphene	0.6	t	0.1	1741	1698–1748*	β-Bisabolene			1.3
1093	1056–1106*	Hexanal		0.1	0.2	1742	1686–1743*	β-Selinene		0.5	
1118	1085–1130*	β-Pinene	0.4	0.1	0.1	1743	1734–1803*	α-Cadinene			0.5
1132	1098–1140*	Sabinene	0.3	0.1	t	1744	1696–1748*	α-Selinene			0.1
1146	1110–1150*	δ-2-Carene			0.2	1748	1689-1748*	Piperitone	0.1		
1159	1122–1169*	δ-3-Carene			0.1	1755	1692-1757*	Bicyclogermacrene		0.6	
1174	1140–1175*	Myrcene	2.2	2.1	0.9	1758	1668-1771*	*cis*-Piperitol	0.1		1.3
1176	1148–1186*	α-Phellandrene	2.3	0.1	0.1	1763	1763***	α-Alaskene	0.2		
1177	1177**	β-Terpinene		2.9		1771	1726–1773*	γ-Bisabolene			0.6
1183	1183**	*p*-Mentha-1,7(8)-diene	0.5			1773	1735–1782*	δ-Cadinene	t	**6.6**	3.5
1187	1187***	o-Cymene		0.2		1776	1722–1774*	γ-Cadinene		0.3	1.7
1188	1154–1195*	α-Terpinene			t	1781	1781***	Zonarene			0.1
1194	1163–1208*	Heptanal			0.1	1783	1748–1783*	β-Sesquiphellandrene	0.3		
1195	1167–1197*	Dehydro-1,8-cineole			t	1786	1779**	Kessane		0.1	9.6
1200	1200**	Sylvestrene		0.1		1786	1743-1788*	*ar*-Curcumene	0.1		0.2
1203	1178-1219*	Limonene	3.5	1.4	2.4	1796	1750-1800*	Selina-3,7(11)-diene		1.7	
1218	1188-1233*	**(+)–β-Phellandrene**	**69.1**	0.4	0.4	1800	1782-1820*	*cis*-Sabinol		0.1	
1225	1118-1160*	(*Z*)-3-Hexenal		0.5		1802	1747-1805*	Cumin aldehyde	0.6		
1244	1232**	Amyl furan		0.1	0.2	1809	1765-1811*	Citronellyl butyrate		0.7	
1246	1211-1251*	(*Z*)-β-Ocimene		0.3		1811	1814**	*p*-Mentha-1,3-dien-7-al	0.3		

RRIexp.	RRI lit.	Compound	%			RRI exp.	RRI lit.	Compound	%		
			fruit	leaf	root				fruit	leaf	root
1255	1222-1266*	γ-Terpinene		0.1	0.1	1814	1814**	*p*-Mentha-1,5-dien-7-ol	0.1		
1266	1232-1267*	(*E*)-β-Ocimene		0.4		1823	1823**	*p*-Mentha-1(7),5-dien-2-ol	0.3		
1278	1244-1279*	*m*-Cymene		0.1	t	1827	1770-1834*	(*E*,*E*)-2,4-Decadienal			0.2
1280	1246-1291*	*p*-Cymene	1.9	1.1	0.2	1834	1834***	Citronellyl isovalerate		1.3	
1290	1261-1300*	Terpinolene	t	0.1	t	1853	1800-1853*	*cis*-Calamenene			0.3
1296	1267-1312*	Octanal		0.1	0.7	1864	1813-1865*	*p*-Cymen-8-ol		0.2	
1299	1295**	2-Methylbutyl isovalerate		0.4		1870	1807-1873*	Hexanoic acid			0.6
1300	1300**	Tridecane	t			1900	1854-1928*	*epi*-Cubebol		0.3	0.7
1303	1303**	Amyl isovalerate		0.2		1901	1910***	Neophytadiene isomer I		2.5	
1308	1308***	*cis*-2-(2-Pentenyl)furan		t		1941	1893–1941*	α-Calacorene		1.7	0.2
1327	1323**	3-Methyl-2-butenol			t	1945	1945**	1,5-Epoxy-salvial(4)14-ene		0.4	
1348	1345**	6-Methyl-5-hepten-2-one		0.1		1957	1884-1964*	Cubebol		0.9	1
1360	1316-1377*	Hexanol			t	1958	1892-1958*	(*E*)-β-Ionone		0.5	
1362	1331-1369*	*cis*-Rose oxide		0.1		1984	1984**	γ-Calacorene		0.4	
1368	-	7-Methyl-4-octyl acetate		t	0.1	2001	2001**	Isocaryophyllene oxide		0.5	
1376	1341-1386*	*trans*-Rose oxide		t		2008	1936-2023*	Caryophyllene oxide	t	**6.1**	0.5
1398	1374-1415*	2-Nonanone		0.1	0.1	2037	2016-2043*	Salvial-4(14)-en-1-one		1.3	0.2
1400	1370-1414*	Nonanal		0.1	0.1	2071	2003-2071*	Humulene epoxide-II		1.7	0.2
1413	1413**	Rose furan		t		2077	2076**	Tridecanol			0.7
1429	1405-1431*	Perillen		0.2		2080	2019-2090*	Cubenol			0.3
1441	1407-1463*	(*E*)-2-Octenal			0.1	2081	2081**	Humulene epoxide-III		0.6	
1443	1434**	2,5-Dimethylstyrene		t	0.1	2084	2011-2089*	Octanoic acid			0.6
1452	1452**	α,*p*-Dimethylstyrene		0.1		2096	2043-2103*	Elemol			9.8
1466	1438-1480*	α-Cubebene		0.1	t	2105	2108***	Muurola-4,10(14)-dien-1-ol		0.2	
1467	1446-1478*	6-Methyl-5-hepten-2-ol			t	2110	2110**	Salviadienol		0.4	
1483	1459-1491*	Octyl acetate		0.1		2113	2108-2013**	Cumin alcohol	0.3		
1497	1462-1522*	α-Copaene		2.6	0.2	2127	2089-2121*	10-*epi*-γ-Eudesmol			0.5
1500	1500*	Pentadecane			0.1	2140	2096-2131*	Hexahydrofarnesylacetone		1.8	
1507	1455-1514*	(*E*,*E*)-2,4-Heptadienal		0.1		2144	2074-2150*	Spathulenol		3.3	
1528	1528**	α-Bourbonene		0.2		2179	2123-2174*	Tetradecanol			0.8
1535	1496-1546*	β-Bourbonene		1.9		2185	2147–2199*	γ-Eudesmol			5.3
1545	1534-1580*	*cis*-α-Bergamotene			0.1	2187	2184**	T-Cadinol			4.9
1548	1509-1569*	(*E*)-2-Nonenal			0.5	2202	2202***	Germacrene D-4α-ol			0.8
1549	1518-1560*	β-Cubebene		0.4		2205	2204**	Eremoligenol			1
1560	1555–1645*	*cis*-p-Menth-2-en-1-ol	0.1	0.2	1.5	2209	2209**	T-Muurolol		0.8	3.2
1571	1557-1625*	*trans*-*p*-Menth-2-	0.1	0.3	1.8	2210	2210***	α-Guaiol			0.6

RRIexp.	RRI lit.	Compound	%			RRI exp.	RRI lit.	Compound	%		
			fruit	leaf	root				fruit	leaf	root
		en-1-ol									
1587	1587**	β-Funebrene	0.1			2219	2219**	δ-Cadinol			0.4
1590	1549-1597*	Bornyl acetate	0.1	0.3	1.8	2237	2231**	Valerianol			1
1594	1594***	*trans*-β-Bergamotene			0.6	2239	2140-2246*	Carvacrol	t		
1595	1563-1607*	Isothymol methyl ether		0.1		2240	-	Acorenone B	0.3		
1600	1565-1608*	β-Elemene		0.5	0.2	2245	2127-2223*	Acorenol B	2.9		
1602	1550-1608*	β-Copaene		1.1	0.1	2245	2231-2278**	Torilenol		1.5	
1604	1563-1607*	Thymol methyl ether		0.6		2248	2171-2248*	Bulnesol			0.8
1611	1564-1630*	Terpinen-4-ol				2250	2186–2250*	α-Eudesmol			1.7
1612	1569-1632*	β-Caryophyllene	0.3	**4.3**	0.3	2255	2180–2255*	α-Cadinol			2.8
1613	1574-1647*	β-Cedrene	0.6			2257	2196–2272*	β-Eudesmol			5.3
1614	1620**	10-*epi*-Acora-2,4-diene	0.3			2260	2260**	Alismol			0.6
1638	1565-1645*	*cis*-*p*-Menth-2-en-1-ol	0.1			2262	-	Gossonorol			0.3
1655	1595-1662*	(*E*)-2-Decenal			0.3	2268	2265**	*cis*-Guai-9-en-11-ol			0.6
1659	1647-1689*	γ-Gurjunene		0.3		2307	-	Eudesma-4(15),7-diene-1-ol isomer		1.6	
1668	1627–1668*	(*Z*)-β-Farnesene	0.8		0.6	2341	-	14-Oxo-α-muurolene		0.3	
1683	1665-1691*	*trans*-Verbenol	t			2355	-	Ambrettolide			0.3
1687	1637-1689*	α-Humulene		2.4	1.1	2368	2351-2402*	Eudesma-4(15),7-diene-1-β-ol		2.4	0.2
1688	1688**	Selina-4,11-diene		0.2		2384	2341-2392*	Hexadecanol			0.6
1690	1644-1690*	Cryptone	3.4			2392	2392-2396*	Caryophyllenol II		0.7	
1693	1674-1708*	β-Acoradiene	0.1			2535	-	14-Hydroxy-α-muurolene		0.1	
1695	1643-1684	(*E*)-β-Farnesene			0.3	2607	2607***	14-Hydroxy-δ-cadinene		0.2	
1695	-	Amorpha-4,11-diene			0.3	2622	2510-2633*	Phytol		1.6	
1704	1682-1704*	γ-Curcumene	0.1			2670	2634-2719*	Tetradecanoic acid		0.3	
1704	1655-1714*	γ-Muurolene	0.5	2.3	0.5			**Monoterpene hydrocarbons**	84.3	8.5	6.1
1725	1696-1735*	Verbenone	0.1					**Oxygenated monoterpenes**	4.8	3.3	6.5
1726	1696-1743*	α-Zingiberene	0.1					**Sesquiterpene hydrocarbons**	4.1	38.9	26.5
1726	1676-1726*	Germacrene D	0.4	**9.7**	2			**Oxygenated sesquiterpenes**	3.3	25.1	45.2
1730	-	Guaioxide			2.5			**Others**	2.4	9.1	6.2
1737	1713-1748*	(*Z*,*E*)-α-Farnesene			0.5			**Total**	**98.9**	**84.9**	**90.5**

The obtained data for *A. pancicii* were compared with those published for the essential oil content of the aerial parts of species from North Macedonia (Mileski *et al*., 2017) and Serbia (Simonović et al., 2014). The fruit essential oil was similar to that from aerial parts of *A. pancicii* collected from Vidlic Mts. (Serbia), which was rich in monoterpenoids and *β*-phellandrene (54.9%) as the major constituent (Simonović *et al*., 2014). The essential oil from aerial parts of *A. pancicii* gathered from Pelister Mts., North Macedonia (Mileski *et al*., 2017) was characterized with the domination of

sesquiterpenoids, which corresponded to the chemical composition of the leaf oil in this study. The observed differences in the chemical composition of the essential oils are probably due to the inhomogeneity of the samples than to environmental conditions.

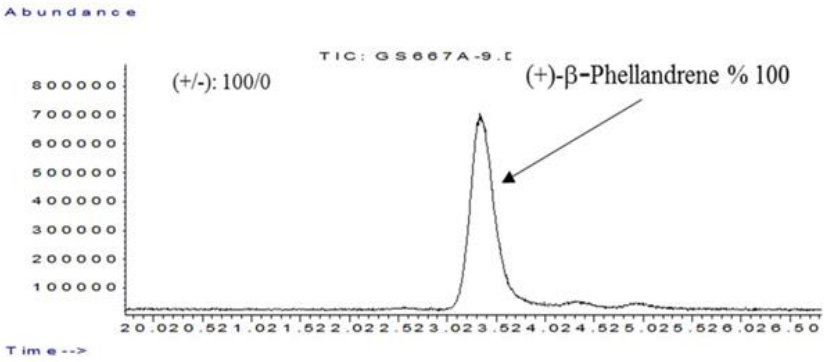
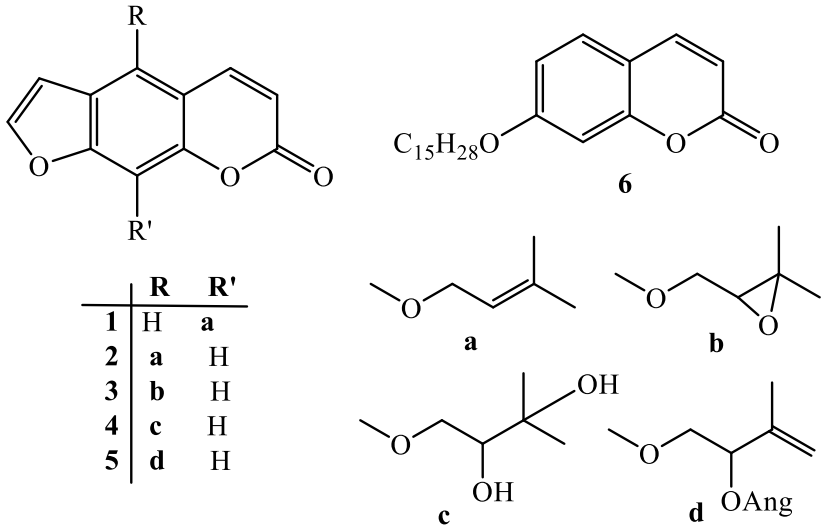


Figure No. 1
Enantiomeric distribution of β-phellandrene in the fruit essential oil of *A. pancicii*



	R	R'
1	H	**a**
2	**a**	H
3	**b**	H
4	**c**	H
5	**d**	H

Figure No. 2
Structures of the isolated compounds

Preparative TLC of the hexane extract obtained from fruit of the plant afforded 6 compounds. Comparison of their ^{1}H NMR data with those published in the literature as well as with authentic standards allowed the identification of isoimperatorin (**1**) (Seo et al., 2013), imperatorin (**2**) (Seo *et al*., 2013), oxypeucedanin (**3**) (Seo *et al*., 2013), oxypeucedanin hydrate (**4**) (Seo *et al*., 2013), angeloylpangelin (**5**) (Botcheva, 1972) and umbelliprenin (**6**) (Murphy *et al*., 2004) (Figure No . 2). Furanocoumarins **1 - 5** were identified in the fruit and roots of *A. pancicii* from Bulgarian origin (Botcheva, 1972). Oxypeucedanin (**3**) and oxypeucedanin hydrate (**4**) were reported for the species collected in N. Macedonia (Mileski *et al*., 2017). It should be pointed out that umbelliprenin (**6**) has been isolated for the first time from *A. pancicii*. Up to now, this 7-prenyloxycoumarin has been found in four *Angelica* species only, namely *A. pubescens* (Hata *et al*., 1981), *A. pachycarpa* (Méndez &

Castro-Poceiro, 1983), *A. sylvestris* (Murphy *et al*., 2004; Sarker *et al*., 2005), *A. archangelica* (Waksmundzka-Hajnos *et al*., 2004; Taddeo *et al*., 2017).

The amount of the individual compounds (**1** - **6**) in different plant parts was further determined by HPLC (Table No. 2). The leaves were poor in the content of furanocoumarins. Oxypeucedanin (**3**) was the main component in the fruit and roots. It is worth noting that the content of oxypeucedanin hydrate (**4**) in the roots was 15 times higher in comparison with that in the fruit. The amount of imperatorin (**1**), isoimperatorin (**2**) and angeloylpangelin (**5**) prevailed in the roots, while umbelliprenin (**6**) dominated in the fruit extract.

Table No. 2
Amount of the individual compounds in the hexane extracts [mg/g DE]

Compound	Fruit	Leaf	Root
Imperatorin (**1**)	87.9 ± 0.5	nd	95.3 ± 0.7
Isoimperatorin (**2**)	32.5 ± 0.3	0.7 ± 0.2	44.5 ± 0.5
Oxypeucedanin (**3**)	326.7 ± 1.3	1.9 ± 0.3	261.9 ± 1.5
Oxypeucedanin hydrate (**4**)	6.4 ± 0.5	1.9 ± 0.2	103.2 ± 0.8
Angeloylpangelin (**5**)	27.4 ± 0.4	nd	58.5 ± 0.5
Umbelliprenin (**6**)	48.2 ± 0.6	8.2 ± 0.4	2.1 ± 0.3

nd: Not determined

The antioxidant potential of the extracts was evaluated against DPPH and ABTS radicals and the results are presented in Table No. 3. *Angelica pancicii* essential oils and extracts did not demonstrate noteworthy activity in these tests. The leaf extract was found to be the best DPPH radical scavenger (IC_{50} 1.2 ± 0.06 mg/mL), while Trolox equivalent antioxidant capacity of leaf and root extracts was found to be equal (0.5 mM). The obtained results in DPPH assay were lower in comparison with those published for the polar methanol, ethanol and aqueous extracts from the aerial parts (IC_{50} 0.26 - 0.29 mg/mL) and roots (IC_{50} 0.40 - 0.47 mg/mL) of the plant from N. Macedonia (Mileski *et al*., 2017). The relatively low antioxidant potential of the studied extracts could be explain with the absence of compounds with free phenolic groups, which are essential for the quenching of the generated ABTS and DPPH free radicals in *in vitro* systems.

The hexane extracts and essential oils obtained from the leaves, fruit and roots of *A. pancicii* were also evaluated for their antidiabetic inhibitory activity (Table No. 3) using the iodine/potassium iodide (IKI) method (Yang *et al*., 2012). The studied extracts were not active or demonstrated a weak inhibitory effect (% Inh. up to 31.9%) towards *α*-amylase (from porcine pancreas) at a concentration of 5 mg/mL in comparison with the reference compound acarbose (90% at a concentration of 1 mg/mL). However, the highest activity of the root extract could be attributed to the presence of the highest content of imperatorin and oxypeucedanin hydrate, for which a good inhibition of *α*-amylase has been already reported (Shalaby *et al*., 2014).

The hexane extracts and essential oils were further investigated for their potential to inhibit acetylcholinesterase enzyme (AChE) using Ellman's method (Table No. 3). The essential oils were more active than the hexane extracts, and fruit and leaf oils demonstrated remarkable inhibition of AChE (Inh. 82.0 ± 2.4 and 88.3 ± 3.2%, respectively). The significant anti-AChE activity of the fruit oil (IC_{50} 1.9±0.1 mg/mL) could be linked to the presence of monoterpenoids predominately (84.3%, Table No. 3). These components have been reported to possess anti-acetylcholinesterase properties (Wojtunik-Kulesza *et al*., 2019). Moreover, the main component in this oil, β-phellandrene was found to be a good inhibitor of AChE with IC_{50} value of 120 μg/mL (Bonesi *et al*., 2010). The leaf essential oil demonstrated the highest (88.3%) inhibitory effect on AChE activity, but 50% ihhibition (IC_{50}) of the AchE was achieved at a concetration of 3.0 ± 0.2 mg/mL. If we look at the composition, 38.9% of the leaf oil of *A. pancicii* comprised of the sesquiterpene

hydrocarbons with germacrene D, β-caryophyllene, caryophyllene oxide and δ-cadinene as major constituents. In the literature there are approved reports about the correlation between AChE inhibitory activity and essential oils rich in germacrene D (Tel *et al*., 2010; Rahali *et al*., 2017), β-caryophyllene (Savelev *et al*., 2004; Loizzo *et al*., 2008; Sharma *et al*., 2016) and δ-cadinene (Loizzo *et al*., 2010). It was estimated that above-mentioned literature sources support our idea about the leaf oil anti-AChE activity to be linked to the presence of those sesquiterpenes in the oil of *A. pancicii*.

Table No. 3
Biological activity tests result for *A. pancicii* essential oils and hexane extracts

Sample	Plant part	DPPH		TEAC	α-Amylase	AChE	
		Inh %	IC_{50} [mg/mL]	mM	Inh %	Inh %	IC_{50} [mg/mL]
Essential oil	Fruit	N/A	-	N/A	N/A	82.0 ± 2.4	1.9 ± 0.1
	Leaf	20.5 ± 1.1	>10	N/A	27.5 ± 0.9	88.3 ± 3.2	3.0 ± 0.2
	Root	14.2 ± 2.0	>10	N/A	20.3 ± 1.3	50.8 ± 6.5	≥10
Hexane extract	Fruit	34.1 ± 1.0	>10	0.06 ± 0.02	20.3 ± 0.5	62.9 ± 2.0	1.62 ± 0.14
	Leaf	95.0 ± 2.2	1.20 ± 0.06	0.50 ± 0.02	7.3 ± 0.7	N/A	-
	Root	66.0 ± 2.4	9.65 ± 0.15	0.50 ± 0.05	31.9 ± 1.1	47.1 ± 2.6	>10
Gallic acid		90	0.002±0.0002	2.8			
BHT		75	0.5±0.04				
Ascorbic acid		60	0.03±0.002				
Acarbose					90		
Galanthamine						88	0.006±0.0004

The hexane extracts from fruit and roots also exhibited good anti-AChE activity (62.9 and 47.1% of inhibition, respectively), while the leaf extract was inactive at the tested concentration. The determined 50% of AchE (IC_{50} 1.62 ± 0.14 mg/mL) of the fruit extract was remarkable. The inhibitory activity may be related to coumarin content, which can inhibit the peripheral anionic site of AChE, which is responsible for binding acetylcholine to the enzyme to begin the hydrolytic reaction at the catalytic site (Anand *et al*., 2012). Literature survey showed that imperatorin, oxypeucedanin and oxypeucedanin hydrate had moderate to significant inhibition against AChE and BChE (Senol *et al*., 2011; Seo *et al*., 2013; Karakaya *et al*., 2020).

CONCLUSION

The results of this study revealed a significant variability in the essential oil and coumarin composition between the different plant parts of *A. pancicii*, which reflected on their biological activity too. Among the studied samples, leaf and root extracts possessed the highest DPPH radical scavenging activity and TEAC. All extracts and essential oils demonstrated a weak inhibitory effect towards α-amylase and a good to significant inhibitory activity against acetylcholinesterase. The fruit essential oil and hexane extract were found to be the best acetylcholinesterase inhibitors and therefore, they could represent promising therapeutic agents for neurodegenerative disorders.

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