DIPSACUS FULLONUM L. LEAVES AND ROOTS – IDENTIFICATION OF THE COMPONENTS OF THE ESSENTIAL OILS AND ALPHA-AMYLASE INHIBITORY ACTIVITIES OF METHANOLIC EXTRACTS

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Abstract: *Dipsacus fullonum* L. syn. *D. sylvestris* is a biennial plant from the *Dipsacaceae* family. It grows in Europe, North Africa and Asia Minor. It has been used in folk medicine mainly to treat Lyme disease. This study was undertaken to identify and compare the components of the essential oils from dried and fresh roots, which are usually applied as medicinal agents, as well as those of the essential oils from dried and fresh leaves, used less frequently. The essential oils from the *Dipsacus fullonum* leaves and roots were analyzed by the GC and GC-MS analysis. 18 components were identified in the essential oil from the fresh leaves, whereas 37 were recognized in the oil from the dried leaves; 10 components were identified in the essential oil from the fresh roots and 40 in that from the dried roots. Phytol was the main component in the essential oil from the leaves, whereas n-hexadecanoic acid was predominant in the roots. The α -amylase inhibition has been proven for methanolic extracts of *D. fullonum* leaves and roots. The strongest activity was demonstrated by the extract from fresh leaves of *D. fullonum* and showed an effect about one hundred times weaker than acarbose.

Keywords: essential oil composition; methanolic extract; alpha-amylase inhibition

The lifestyle of present-day societies, leisure activities, development of tourism, expanding into new, once inaccessible, terrains increases the risk of getting infected with the microorganisms transmitted by ticks. Ticks are a natural reservoir and vector of Lyme disease, anaplasmosis, tick-borne encephalitis, tularemia, Q fever, babesiosis, and rickettsiosis. Dangerous, often undiagnosed for years, they are diseases difficult to treat. In traditional medicine, alcoholic and water extracts of the *Dipsacus* species are used in the therapy of Lyme disease and coinfections. They are usually multi-component dietary supplements that are supposed to have anti-inflammatory, antimicrobial and immunostimulatory effects.

The studies conducted to date have mainly concerned the *D. asper* species and, to a lesser extent, *D. japonicus*, *D. azureus*, *D. laciniatus*, *D. xerox*, found in North America and Asia, in which compounds from the groups of iridoids, triterpenes, phenolics, alkaloids, and sterols have been identified. For many of them, antimicrobial, antiviral, anti-inflammatory, cytoprotective and antinociceptive effects have been proven. Experiments on rats have also demonstrated therapeutic effects against Alzheimer's disease. The results of the studies may provide an explanation for

the effectiveness of extracts from the *Dipsacus* species in the treatment of Lyme disease and coinfections. The species native to North Africa, Asia Minor and some part of Europe – *D. fullonum* – has not been examined well. The foregoing research has indicated the presence of only a few iridoids (cantleyoside, loganin, sweroside, sylvestroside I-IV) and phenolic acids. In medicine, in turn, the root and herb are applied. In folk medicine, it has long been used externally for small wounds, eczema, and rheumatism (1).

This phytochemical studies have included identification and a comparison of the components of the essential oils from the dried and fresh roots and leaves of the biennial plants of *D. fullonum*. The enzymatic reactions occurring during drying and storage influence the qualitative and quantitative composition of the compounds in the raw material. Inhibition of alpha-amylase was determined for extracts of fresh or dried leaves and roots.

MATERIALS AND METHODS

Plant materials

The *Dipsacus fullonum* Moench. leaves and roots were collected in second growing season Herb

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Production Company "Flos" Mokrsko 118; 98-345 Mokrsko, Poland in 2015. The voucher specimen (No. 48) was deposited at the Department of Pharmacognosy, Poznań University of Medical Sciences, Poland.

Essential oil extraction

The essential oil was obtained by hydrodistillation of the fresh and dried leaves or roots using a Deryng apparatus. 50 g of the material was poured with 500 mL of distilled water, and heated for 3 h after the content of the flask was brought to the boil. 3 mL of the fluid flowed into the receiver per minute. 30 min after the distillation finished the volume of the essential oil was read. The extraction was performed three times.

GC-MS analysis

The identification of the essential oil compounds was performed using a Varian 4000 GC-MS instrument equipped with a Varian VF-5ms silica column (30 mm × 0.25 mm × 0.39 mm No. CP8944). Helium was used as the carrier gas at a flow rate of 1.0 ml/min with a split ratio equal to 1 : 50. The oven temperature was raised from 40°C to 280°C at a rate of 10°C/min. The injector temperature was kept at 220°C. The detector temperatures were as follows: ion trap 220°C, manifold 50°C, transfer line 280°C. The compounds were identified by comparing their retention time and EI (70 eV) mass spectra with standards from the NIST MASS SPECTRA LIBRARY. Semi-quantitative data were obtained from relative peak area percentages.

Inhibition assay for pancreatic α -amylase activity

The fresh (100 g) and dried (10 g) leaves and roots of *D. fullonum* were extracted with 200 mL of methanol for 1h using an ultrasonic bath. The extraction was carried out at a temperature of 50°C, 1000 W ultrasonic power, frequency of 37 kHz (Elma S180 H). The methanolic extracts from leaves (DL₁ – fresh, DL₂ – dried) and roots (DK₁ – fresh, DK₂ – dried) were concentrated to dry mass and used to prepare a concentration of 0.625 µg/mL to 5.0 µg/mL in methanol.

The α -amylase inhibitory activity was performed by Keharom et al. (2).

The starch (Starch, from potato, Sigma-Aldrich, St. Louis, USA) solution (1%) was prepared by boiling and stirring 0.5 g of starch in 50 mL of sodium phosphate buffer (0.02 M, buffer components were of analytical grade) for 5 min. The solution of pancreatic α -amylase (α -Amylase from porcine pancreas, type VI – B, = 10 units/mg Sigma-

Aldrich Chemical Co, St. Louis, USA) was obtained by added 0.01g of the enzyme to 10 mL of the sodium phosphate buffer. The colour reagent was the solution containing 3,5-dinitrosalicylic acid (0.1 g) (DNS, Sigma Aldrich Chemical Co, St. Louis, USA), sodium potassium tartrate tetrahydrate (2.99 g) (Sigma-Aldrich Chemical Co, St. Louis, USA), sodium hydroxide (POCH. S.A., Poland) (0.16 g) and phosphate buffer (completed to 10 mL).

Dimethyl sulfoxide (DMSO, POCH. S.A. Poland) served as the solvent for the extracts and a concentration of 0.625 µg/mL to 5.0 µg/mL was obtained. Twenty five microliters of each plant extract and 25 µL of the enzyme were mixed in a 96-well plate and incubated for 10 min at 25°C. Then 25 µL of the starch solution were added and the mixture was similarly incubated under identical conditions. Addition of 50 µL of a dinitrosalicylic color reagent stopped the reaction. The mixture was incubated for 20 min at 85°C (Incubator CLN32STD POL-EKO Aparature, Poland). Absorbance was measured at 540 nm (Spectrophotometer Thermo Fisher SCIENTIFIC Multiskan Go, Finland) after the mixture had been cooled to room temperature. The mixture without the plant extract, which replaced DMSO, was used as a control. Blank samples (devoid of the enzyme which had been replaced with the buffer solution) were used to correct the absorption of the mixture. The positive control consisted of acarbose (Acarbose, PHR1253 Sigma-Aldrich, USA) solution at concentrations of 0.25 µg/mL to 1.0 µg/mL. The inhibition percentage of α -amylase was calculated by the following formula:

$$I_{\alpha\text{-amylase}} \% = 100 \times (\Delta A_{\text{control}} - \Delta A_{\text{sample}}) / \Delta A_{\text{control}}$$

Statistical analyses

The statistical analysis was performed with the use of the Microsoft Excel 2007 software. The data obtained in this study were expressed as the mean of three replicates. The significant difference was accepted at p value = 0.05. The Simple linear regression was used for the analysis of the activity of α -amylase, and the coefficient of determination (R^2) was calculated.

RESULT AND DISCUSSION

Composition of essential oils from the *D. fullonum* drugs

In this study, the chemical composition of the essential oils from the dried and fresh *D. fullonum* roots and leaves was investigated. The essential oil was obtained by hydrodistillation with a 0.6% yield for the dried and 0.5% for the fresh leaves, and 0.5%

Table 1. Compounds identified in the essential oils from the dried and fresh *D. fullonum* roots and leaves using GC-MS.

No.	Compounds	Retention time [min]	Kovats Retention Index	Root Fresh RA[%]	Root Dried RA[%]	Leave Fresh RA[%]	Leave Dried RA[%]	Molecular formula
1.	4-methyl-2-oxovaleric acid	7.521	927		0.180			C ₆ H ₁₀ O ₃
2.	Pyridine, 3-ethyl-	8.001	954	0.547	5.136		0.795	C ₇ H ₉ N
3.	Ethanone, 2,2-dihydroxy-1-phenyl	8.163	961			0.550		C ₈ H ₈ O ₃
4.	Benzaldehyde	8.295	969		1.154		0.624	C ₇ H ₆ O
5.	Pyridine, 3-ethenyl-	8.401	974	0.666	2.658		0.451	C ₇ H ₇ N
6.	Isopropylbenzene	8.826	994		0.240			C ₉ H ₁₂
7.	2-Pentene, 5-(pentyloxy)	8.925	999				0.269	C ₁₀ H ₂₀ O
8.	Furan, 2-pentyl	9.183	1015	0.290	1.023			C ₉ H ₁₄ O
9.	2,4-heptadienal	9.419	1029				0.374	C ₇ H ₁₀ O
10.	ERrans-2-(2-pentenyl)furan	9.498	1035		0.831			C ₉ H ₁₂ O
11.	Octanal	9.633	1042				0.147	C ₈ H ₁₆ O
12.	4-penten-1-ol, 3-methyl-, acetate	9.651	1044			0.806		C ₈ H ₁₄ O ₂
13.	2,4-Nonadienal, (E,E)	10.035	1066		0.838		0.497	C ₉ H ₁₄ O
14.	4-ethyl-2-hexynal	10.090	1069				0.303	C ₈ H ₁₂ O
15.	1,3,5-Cycloheptatriene	11.278	1143		1.581			C ₇ H ₈
16.	Bicyclo octa-1,3,5-trien-7-ol	12.229	1202			2.009	0.473	C ₈ H ₈ O
17.	Benzaldehyde, 4-methyl	12.249	1204	0.435	1.523			C ₈ H ₈ O
18.	Pyridine, 5-ethenyl-2-methyl-	12.799	1243		0.404			C ₈ H ₉ N
19.	3,5-Octadien-2-one	13.127	1266		0.702			C ₈ H ₁₂ O
20.	3-Nonen-1-ol	13.526	1292	0.957				C ₉ H ₁₈ O
21.	2-Nonen-1-ol	13.543	1293				0.614	C ₉ H ₁₈ O
22.	Phenylethyl alcohol	13.868	1318		0.388			C ₈ H ₁₀ O
23.	2,6-Nonadienal, (E,Z)-	15.354	1430		2.095			C ₉ H ₁₄ O
24.	6-Nonenal, (E)-	15.612	1451		1.271			C ₉ H ₁₆ O
25.	Cyclodecanol	17.294	1588		0.703			C ₁₀ H ₂₀ O
26.	7-Oxabicycloheptane, 1-methyl-4-(2-methyloxiranyl)-	17.485	1603				0.234	C ₁₀ H ₁₆ O ₂
27.	Pentanoic acid, 2-methyl, methyl ester	17.771	1618				0.217	C ₇ H ₁₄ O ₂
28.	1-Cyclohexene-1-carboxaldehyde, 2,6,6-trimethyl	17.835	1635			0.682	0.584	C ₁₀ H ₁₆ O
29.	Terrein	17.906	1642		0.352			C ₈ H ₁₀ O ₃
30.	1,2,2,6,8-Pentamethyl-7-oxabicyclo dec-8-en-10-one	17.954	1645				0.435	C ₁₄ H ₂₂ O ₂
31.	Bicyclo[3.1.1]hept-3-en-2-ol, 4,6,6-trimethyl	18.455	1690		0.477			C ₁₀ H ₁₆ O
32.	2,6-Octadienal,3,7-dimethyl, (Z)-	18.463	1690		0.456			C ₁₀ H ₁₆ O
33.	2,3-Octanedione	18.534	1696				0.144	C ₈ H ₁₂ O ₂
34.	2,6-Octadien-1-ol, 3,7-dimethyl, (E)-	18.879	1729		2.389			C ₁₀ H ₁₈ O
35.	1-Cyclohexene-acetaldehyde, 2,6,6-trimethyl-	19.120	1751				0.140	C ₁₁ H ₁₈ O
36.	Isoquinoline	19.303	1768		0.516			C ₉ H ₇ N
37.	2,6-Octadienal,3,7-dimethyl-	19.504	1787		1.936			C ₁₀ H ₁₆ O
38.	2,4-Pentadien-1-ol, 3-pentyl-,(2Z)-	20.574	1891		0.549			C ₁₀ H ₁₈ O

Table 1. Continued.

No.	Compounds	Retention time [min]	Kovats Retention Index	Root Fresh RA[%]	Root Dried RA[%]	Leave Fresh RA[%]	Leave Dried RA[%]	Molecular formula
39.	2-methoxy-4-vinylphenol	21.009	1935		0.428			C ₉ H ₁₀ O ₂
40.	2,4-Nonadienal, (E,E)-	21.254	1961		0.344			C ₉ H ₁₄ O
41.	2,4-Decadienal	21.265	1962		0.242			C ₁₀ H ₁₆ O
42.	Cyclohexa-1,3-diene, 5,6-diethyl-	22.092	2048				0.353	C ₁₀ H ₁₆
43.	Cyclohexane, cyclopropylidene	22.161	2056		0.556	5.198	2.896	C ₉ H ₁₄
44.	Eugenol	22.384	2080		1.006			C ₁₀ H ₁₂ O ₂
45.	2-Buten-1-one, 1-(2,6,6-trimethyl-1,3-cyclohexadiene-1-yl)	23.239	2175		0.578	1.182	0.879	C ₁₃ H ₁₈ O
46.	1,5-Cyclohexadiene-1-methanol, 4-(1-methylethyl)-	23.692	2226				0.154	C ₁₀ H ₁₆ O
47.	1H Cycloprop[e]azulene	24.145	2280		0.605			C ₁₅ H ₂₄
48.	2-Buten-1-one, 1-(2,6,6-trimethyl-1-cyclohexen-1-yl)	24.235	2289				0.205	C ₁₃ H ₂₀ O
49.	4-(2, 6, 6-trimethylcyclohexa-1,3-dienyl) but-3-en-2-one	24.848	2364				0.138	C ₁₃ H ₁₈ O
50.	3-Buten-2-one, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)	26.430	2566			3.211	1.533	C ₁₃ H ₂₀ O
51.	3-Buten-2-one, 4-(2,6,6-trimethyl-7-oxabicyclohept-1-yl)	26.526	2578			0.795	0.403	C ₁₃ H ₂₀ O ₂
52.	α-Farnesene	27.153	2660				0.185	C ₁₅ H ₂₄
53.	α-Bisabolene epoxide	27.459	2700				0.233	C ₁₅ H ₂₄ O
54.	Naphthalene, 1,2, 3,5,6, 8a-hexahydro-4,7-dimethyl-1 (1-methylethyl)-, (1S-cis)	27.619	2718			0.864		C ₁₅ H ₂₄
55.	1,2,4-Cyclopentanetrione, 3-(2-pentenyl)-	27.631	2789				0.711	C ₁₀ H ₁₂ O ₃
56.	(-)-Spathulenol	29.353	2899		1.356			C ₁₅ H ₂₄ O
57.	Indole-5-carboxylic acid, 1,2,3-trimethyl-	30.996	3028			1.420		C ₁₂ H ₁₃ NO ₂
58.	Cyclopentaneacetic acid, 3-oxo-2-(2-pentenyl)-, methyl ester	31.239	3045			1.537		C ₁₃ H ₂₀ O ₃
59.	Indole-5-carboxylic acid, 1-ethyl-2,3-dimethyl	32.866	3144		7.677	1.114		C ₁₃ H ₁₅ NO ₂
60.	6-Pentadecen-1-ol	33.181	3212				1.443	C ₁₅ H ₃₀ O
61.	9-Eicosyne	36.259	3304			1.470		C ₂₀ H ₃₈
62.	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	36.275	3306				1.279	C ₂₀ H ₄₀ O
63.	6,10,14-Trimethyl-2-pentadecanone	36.383	3310	0.809			1.093	C ₁₈ H ₃₆ O
64.	Pentadecanoic acid	36.939	3329		1.225			C ₁₅ H ₃₀ O ₂
65.	Hexadecanal, 2-methyl-	38.206	3374				0.376	C ₁₇ H ₃₄ O
66.	8-Hexadecenal, 14-methyl	38.349	3379			2.876	1.635	C ₁₇ H ₃₂ O
67.	Pentadecanoic acid, 14-methyl, methyl ester	38.478	3384		0.624			C ₁₇ H ₃₄ O ₂
68.	n-Hexadecanoic acid	39.513	3417	2.928	15.997	2.014	2.337	C ₁₆ H ₃₂ O ₂
69.	9,12,15-Octadecatrienoic acid, methyl ester	42.522	3502	3.212	0.650	6.057		C ₁₉ H ₃₂ O ₂

Table 1. Continued.

No.	Compounds	Retention time [min]	Kovats Retention Index	Root Fresh RA[%]	Root Dried RA[%]	Leave Fresh RA[%]	Leave Dried RA[%]	Molecular formula
70.	Tetrahydropyranyl ether of citronellol	42.583	3503				0.310	C ₁₅ H ₂₈ O ₂
71.	2,6-Dodecadien-1-ol, 3,7,11-trimethyl-	42.687	3506				0.310	C ₁₅ H ₂₈ O
72.	9,12-Octadecadienoic acid	43.360	3522		2.530			C ₁₈ H ₃₂ O ₂
73.	11,14,17-Eicosatrienoic acid, methyl ester	43.514	3526		15.859			C ₂₁ H ₃₆ O ₂
74.	Phytol	43.893	3534			61.083	72.311	C ₂₀ H ₄₀ O
75.	Pregnanetriol	46.826	3600		0.442			C ₂₁ H ₃₆ O ₃
76.	Tetracontane, 3,5,24-trimethyl-	48.093	3625	0.294				C ₄₃ H ₈₈
77.	Total [%]			9.844	77.521	92.868	95.085	

for the dried, and 0.4% v/w for the fresh roots. It was found to be transparent, yellow in color and have a pleasant odor. Its chemical composition was analyzed by the GC and GC-MS analyses. The results have been listed in Table 1.

On the basis of the retention time and mass spectra, 18 components were identified in the essential oil from the fresh leaves and 37 in that from the dried leaves. Ten components were found in the oil from the fresh roots and 40 in that from the dried ones. In the case of the essential oil from the fresh and dried leaves, it constituted 92.87%, 95.09% of the total amount, respectively, and in the essential oil from the fresh and dried roots, it amounted to 9.8%, 77.5% of the total amount, respectively. The low percentage of compounds in the essential oil from fresh roots may result from the formation of non-volatile artifacts after distillation. The semi-quantitative data were obtained from relative peak area percentages. 9,12,15-octadecatrienoic acid, methyl ester (3.21%), n-hexadecanoic acid (2.93%) were the major constituents of the essential oil from the fresh root whereas in the essential oil from the dried root the following were prevalent: n-hexadecanoic acid (16%), 11,14,17-eicosatrienoic acid methyl ester (15.86%) and indole-5-carboxylic acid, 1-ethyl-2,3-dimethyl (7.68%), pyridine, 3-ethyl (5.14%), pyridine, 3-ethenyl- (2.66%), 9,12-octadecadienoic acid (2.53%), 2,6-octadien-1-ol, 3,7-dimethyl (2.39%), 2,6-nonadienal (2.10%). The following were determined in an amount exceeding 1%: 2,6-octadienal,3,7-dimethyl (1.94%), 1,3,5-cycloheptatriene (1.58%), benzaldehyde, 4-methyl (1.52%), spathulenol (1.36%), 6-nonenal (1.27%), pentadecanoic acid (1.23%), eugenol (1.01%).

In the essential oils from the leaves the content of phytol was dominant; there was 61.08% of this compound in the oil from the fresh leaves and 72.31% in that from the dried ones. In the oil from the fresh leaves 9,12,15-octadecatrienoic acid, methyl ester (6.06%), cyclohexane (5.20%), 3-buten-2-one, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl) (3.21%), 8-hexadecenal, 14-methyl (2.88%), n-hexadecanoic acid (2.01%), bicyclo octa-1,3,5-trien-7-ol (2.01%) were additionally determined. Cyclopentaneacetic acid 3-oxo-2-(2-pentyl)-methyl ester; indole-5-carboxylic acid 1,2,3-trimethyl; 2-buten-1-one 1-(2,6,6-trimethyl-1,3-cyclohexadiene-1-yl); indole-5-carboxylic acid 1-ethyl-2,3-dimethyl and 9-eicosane were determined slightly above 1%. Apart from phytol, n-hexadecanoic acid (2.34%) and 8-hexadecenal, 14-methyl (1.64%), 3-buten-2-one, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl) (1.53%), 6-Pentadecen-1-ol (1.44%), 3,7,11,15-tetramethyl-2-hexadecen-1-ol (1.28%), 6,10,14-trimethyl-2-pentadecanone (1.09%) were also present in the oil from the dried leaves (Table 1).

This data can be helpful to the explanation antimicrobial activity of *D. fullonum* and the development of new therapeutic forms. What draws attention in the examined essential oils from both the fresh and dried leaves is the high content of phytol – a branched-chain unsaturated diterpene alcohol, about 0.4% for dried leaves and 0.3% for fresh. It is a precursor for vitamins E and K₁. Its antinociceptive, anti-inflammatory, antiallergic and antioxidant activity is well-known. Phytol probably reacts with free radicals to a less reactive structure and protects the lipid biomolecules. This action is important in the treatment of *Borrelia burgdorferi* co-infections, for example by *Bartonella* sp., which causes neuro-

logic symptoms, and *Babesia* sp. leading to hemolytic anemia. In thermal models and formalin tests on mice phytol significantly reduced the paw licking time, both in the neurogenic and inflammatory phases (3). Phytol is used in cosmetics and as immunoadjuvants in vaccine formulations (4, 5). In antioxidant activity tests (TBARS, OH scavenging activity and scavenging activity of Nitric Oxide) phytol demonstrated a strong effect similar to Trolox (a synthetic analog of α -tocopherol) (3).

Phytol is a compound known for antimicrobial properties. Numerous studies have confirmed its activity against, for example, *Escherichia coli*, *Candida albicans*, *Aspergillus niger* (6, 7). In the oil from the dried roots of *Dipsacus*, the following compounds dominated: n-hexadecanoic acid; 11,14,17-eicosatrienoic acid methyl ester and indole-5-carboxylic acid 1-ethyl-2,3-dimethyl, which are also known for the antimicrobial activity and may be

responsible for the medicinal activity of the raw material to a large extent (8, 9). Next, 9,12,15-octadecatrienoic acid methyl ester and n-hexadecanoic acid, identified in the fresh root of *D. fullonum*, are some of the compounds responsible for the antimicrobial activity of *Andrographis paniculata* (10).

Inhibition of α -amylase

The natural sources of the α -amylase inhibitors are important in traditional medicine to treat type 2 diabetes. Application thereof allows to control hyperglycemia and counteract diabetes-related complications. Inhibition of α -amylase delays glucose absorption. Polyphenolic compounds and essential oils are characterized by strong antioxidant activity; their ability to inhibit the activity of amylase and glycosidase has also been described.

The species of the *Dipsacus* genus, including *D. fullonum*, contain iridoids, triterpenoids, and caf-

Table 2. α -Amylase inhibitory activities and IC₅₀ values of the *Dipsacus fullonum* extracts and acarbose.

Extract/compound	Concentration (μ g/mL)	Inhibitory percentage ($\pm$ SD)	IC ₅₀ (μ g/mL)
DL1	10	2.48 $\pm$ 0.09	94.20
	40	15.83 $\pm$ 0.57	
	60	27.12 $\pm$ 1.42	
	80	44.31 $\pm$ 1.55	
	100	53.60 $\pm$ 0.89	
DL2	10	5.50 $\pm$ 0.36	86.01
	40	20.97 $\pm$ 0.51	
	60	31.13 $\pm$ 0.94	
	80	49.78 $\pm$ 1.05	
	100	57.95 $\pm$ 1.18	
DK1	10	5.61 $\pm$ 0.46	92.73
	40	12.75 $\pm$ 0.27	
	60	29.09 $\pm$ 0.81	
	80	46.06 $\pm$ 1.11	
	100	54.58 $\pm$ 1.12	
DK2	10	2.29 $\pm$ 0.29	143.10
	40	8.75 $\pm$ 0.59	
	60	19.91 $\pm$ 0.41	
	80	27.67 $\pm$ 0.42	
	100	33.92 $\pm$ 0.37	
Acarbose	0.25	31.44 $\pm$ 0.14	0.69
	0.5	45.74 $\pm$ 0.21	
	1.0	61.05 $\pm$ 0.17	
	1.5	81.11 $\pm$ 0.11	

DL1 - methanolic extract of fresh *D. fullonum* leaves, DL2 - methanolic extract of dried *D. fullonum* leaves, DK1 - methanolic extract of fresh *D. fullonum* roots, DK2 - methanolic extract of dried *D. fullonum* roots

feic acid derivatives. Their antioxidant and antimicrobial activity have been proven, too.

The aim of this study was to determine the inhibition of porcine pancreatic α -amylase by *D. fullonum* roots and leaves extracts, and the results have been shown in Table 2.

The strongest activity was demonstrated by the extract from fresh leaves of *D. fullonum* $IC_{50} = 86.01 \mu\text{g/mL}$. Those from dried leaves and roots displayed comparable activity ($IC_{50} = 94.20 \mu\text{g/mL}$ and $92.73 \mu\text{g/mL}$, respectively). The extract from the fresh roots showed the weakest activity ($IC_{50} = 143.10 \mu\text{g/mL}$). The reference substance was acarbose, for which IC_{50} was $0.69 \mu\text{g/mL}$. To obtain activity similar to a single 50 mg dose of acarbose, it would be necessary about 6.5 g extracts of the DL_1 , DL_2 , DK_1 , and about 10 g of DK_2 .

CONCLUSION

Determining the composition of the essential oils from the leaves and roots of *D. fullonum* may help to explain the antimicrobial activity of the materials applied against Lyme disease. The studies conducted by Liebold et al. have proven that dichloromethane and ethyl acetate extracts are effective, whereas aqueous alcoholic ones are non-active (11). The investigation into the composition of the essential oil suggests numerous compounds of antimicrobial activity which may play an important role in the antimicrobial activity, especially that they will rather extract with solvents of lower polarity, such as dichloromethane or ethyl acetate, and contribute to the proven antimicrobial activity of iridoid glycosides present in the root of *D. fullonum*.

The extracts from *D. fullonum* inhibited α -amylase, yet the activity was 100 times weaker than that of acarbose. Similar strength of activity in relation to acarbose was demonstrated by aqueous extracts from the Cinnamon Bark species, which are traditionally applied to support treatment of diabetes (12).

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