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Full Length Research Paper

Antibacterial and antimutagenic activity of extracts aboveground parts of three Solidago species: *Solidago virgaurea* L., *Solidago canadensis* L. and *Solidago gigantea* Ait.

Barbara Kołodziej[1]*, Radosław Kowalski[2] and Bogdan Kędzia[3]

[1]Department of Industrial and Medicinal Plants, University of Life Sciences, Lublin, Poland.
[2]Department of Analysis and Evaluation of Food Quality, Central Laboratory of Agroecology, University of Life Sciences, Lublin, Poland.
[3]Institute of Natural Fibres and Medicinal Plants, Department of Pharmacology and Experimental Biology, Poznań, Poland.

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Hexane and ethanolic extracts obtained from aboveground parts of three Solidago species (*Solidago virgaurea* L., *Solidago canadensis* L. and *Solidago gigantea* Ait.) were analyzed by gas chromatography-mass spectrometry (GC-MS). Thirty nine and forty nine volatile compounds were detected, respectively in ethanolic and hexane extracts of *S. virgaurea*, sixty two and forty six in *S. canadensis* extracts, respectively and seventy three and forty five compounds in the ethanolic and hexane extracts of *S. gigantea*. Moreover, evaluation of the content of total secondary metabolites (flavonoides, o-dihydroxyphenols) was made. It was found that both ethanolic and hexane extracts showed an antibacterial activity and acted strongly to Gram-positive (*Staphyllococcus aureus*, *Staphyllococcus faecalis* and *Bacillus subtilis*) than Gram-negative (*Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*) bacteria. Hexane extract of *S. canadensis* acted the strongest of Gram-positive bacteria (minimal inhibitory concentration (MIC) values: 5 to10 mg/ml), while the other hexane extracts showed rather weak effect (MIC values: 100 and > 100 mg/ml). In the case of ethanolic extracts relatively strong activity against Gram-positive bacteria were found in *S. gigantea* and *S. canadensis* (MIC values: from 10 to 50 mg/ml). In general, alcohol extracts had slightly stronger antibacterial properties compared with lipophilic ones. However, hexane extracts of the *S. virgaurea*, *S. canadensis* and *S. gigantea* herb exhibited antimutagenic activity (at a concentration of 2.5 mg/ml), whereas ethanolic extracts, in the applied range of concentrations, did not show antimutagenic activity.

Key words: *Solidago virgaurea, Solidago canadensis, Solidago gigantea,* antibacterial activity, antimutagenic activity, chemical composition.

INTRODUCTION

Goldenrod is a perennial medicinal plant belonging to Asteraceae family. In Poland four representatives of Solidago occur naturally. *Solidago virgaurea* L. is native to Europe and the others, *Solidago canadensis* L., *Solidago gigantea* Ait. (syn. *S. serotina* Ait.) and *Solidago graminifolia* (L) Salisb. were introduced from North America and are widely distributed in most European countries (Hiller and Bader, 1996; Kalemba and Thiem, 2004; Johnson et al., 2007). The medicinal raw material known as *S. viragureae herba* includes only aboveground, flowering parts of *S. virgaurea* L, whereas *Solidaginis herba* includes *S. gigantea* and *S. canadensis*

*Corresponding author. E-mail: barbara.kolodziej@up.lublin.pl. Tel: +48814456679.

Abbreviations: GC-MS, Gas chromatography-mass spectrometry; **MIC,** minimal inhibitory concentration; **DMSO,** dimethyl sulfoxide; **DNA,** deoxyribonucleic acid.

(Polish Pharmacopoeia VIII, 2008). Goldenrod contains flavonoids (on an average: 1.5% in *S. virgaurea*, 2.4% in *S*. *canadensis* - mainly rutoside and 3.9% in *S. gigantea* herb - mainly quercitrin) and anthocyanidins (Hiller and Bader, 1996). Flavonoids, especially quercetin and derivatives, inhibit the enzyme neutral endopeptidase, which is responsible for the interaction of the atrial natriuretic peptide and thus regulates the formation of urine via the excretion of sodium ions. This can be interpreted as the basis of enhanced urinary flow therapy (Apati et al., 2003). The flavonoids from *S. virgaurea* and *S. canadensis* caused also increased excretion of calcium with urine (Chodera et al., 1991). Saponins, flavonoids and caffeic acid esters from Solidago inhibited the activity of leucocyte elastase, a protease involved in the progression of inflammation.

The ester saponins increased permeability of cells and stimulated the synthesis and release of glucocorticoids in the adrenal glands (Melzig et al., 2000). Other constituents include diterpenes (*cis*- and *trans*-clerodane), triterpene saponins of the oleanane type up to 2%, bisdesmosidic phenol glycosides leiocarposide (0.08 to 0.48% only in *S. virgaurea*), phenolics acids and essential oil – 0.4 to 1.5% (Kalemba et al., 1993; Wichtl, 1994; Hiller and Bader, 1996; Kalemba, 2000; Lück et al., 2000; Apati et al., 2003; Wang et al., 2006; Tyszkiewicz, 2008; Starks et al., 2010). Solidago raw material has been traditionally used to treat urinary tract, nephrolithiasis and prostate. In folk medicine the drug is used as a so-called “blood-purifying” agent in gout, rheumatism, arthritis, eczema and other skin disorders. Saponins and polysaccharides of *S. viragurea* demonstrated significant antitumour effects as well as non-specific immunostimulation brought about by the saponins has been suggested as the mechanism of action (Wichtl, 1994; Bader et al., 1996). The ester saponins have been shown to have an antimycotic action on human pathogenic fungi (Bader et al., 1987; Wang et al., 2006). Antibacterial, analgesic, antioxidant, spasmolytic, sedative and hypotensive activities have been also reported (Wichtl, 1994; Kalemba, 2000; Thiem and Goślińska, 2002; Demir et al., 2009; Deepa and Ravichandiran, 2010; Starks et al., 2010). Such herbal preparations with a rather complex action spectrum are especially recommended for treatment of infections and inflammations, to prevent formation of kidney stones and to help remove urinary gravel (Melzig, 2004). Despite such intensive study of the raw material evidence of a broad spectrum of activities, there is no information on mutagenicity of *Solidago herba* (Tyszkiewicz, 2008). Ames-*Salmonella* test system is the important *in vitro* bacterial test system commonly used for determining the antimutagenic properties of natural or synthetic materials obtained from various sources (Gulluce et al., 2010; Ajith and Janardhanan, 2011). Therefore, the aim of the study was to determine flavonoids, o-dihydroxyphenols and volatile compounds as well as antibacterial and antimutagenic properties of hexane and ethanolic extracts of three Solidago species (*S. virgaurea* L., *S. canadensis* L. and *S. gigantea* Ait.).

MATERIALS AND METHODS

Plant material

Aboveground parts (stems, leaves and inflorescences) of three Solidago species (*S. virgaurea*, *S. gigantea* and *S. canadensis*) from experimental cultivation of the Department of Medicinal and Industrial Plants, University of Life Sciences in Lublin, were collected for the study. Plants were authenticated by the Prof. Z. Warakomska, taxonomist of the University of Life Sciences in Lublin (Poland). Voucher specimens (number N-100SKK-07, N-100SKK-08 and N-100SKK-09) are deposited at the Department of Medicinal and Industrial Plants, University of Life Sciences in Lublin. Plant material was collected in August 2009 and after that dried at 40°C in a drying chamber and then properly powdered.

Plant extracts

Extracts for the study were obtained by extraction of 100 g of powdered raw material (three Solidago species) using hexane (1:5) and 50% aqueous-ethanol (1:5). Achieved extracts were condensed in a rotation evaporator up to 100 ml. Moreover, dry matter after solvent evaporation at 60°C was determined in hexane and ethanolic extracts (results in Table 2).

Chemical composition

The liquid extracts were subsequently subjected to GC-MS screening analysis. In the case of ethanolic extracts the following procedure was carried out in order to perform the screening GC-MS analysis of lipophilic fraction. Aliquot of 1 ml of ethanolic extract was placed in a glass vial (20 ml) containing 0.5 ml of hexane. The vial was shaken and 15 ml of saturated water solution of NaCl was added and the vial shaken again. The solution remained in the vial until the separation of fractions. Organic fraction was collected to glass vials sealed with a Teflon stopper. Then, dry matter after solvent evaporation at 60°C was determined in ethanolic extract (Table 2). Moreover, salted fraction was subjected to GC-MS screening analysis. Raw alcohol fraction was phytochemically assessed for the presence of the following secondary metabolite group: flavonoids and o-dihydroxyphenols. Flavonoids (flavonoles recalculated onto quercetin) were determined by the means of spectrophotometry, using Christ-Müller’s method (1960), according to modified Polish Pharmacopoeia VI (2002) procedure (based on measuring the absorbance of a colored complex of flavonoids with aluminum chloride). The o-dihydroxyphenols (given as caffeic acid) were determined by spectrofotometric means, according to the Folin-Ciocalteu method as reported by Singleton and Rossi (1965). Results achieved were performed in three replications and statistically processed using variance analysis. Confidence intervals were estimated using Turkey’s test at 5% significance level.

Gas chromatography-mass spectrometry (GC-MS)

The quantitative and qualitative determination of lipophilic fraction of Solidago extracts was made using a GC (Varian 4000 GC/MS/MS) – data from GC-MS screening in Table 3. The carrier gas was helium with the splitting ratio of 1:1000 and capillary flow rate of 0.5 ml/min. Oven temperature was held isothermal at 50°C

Table 1. Testing scheme of antimutagenic properties.

S/N	Number and name of plate	Substrates added to the surface medium B
1	Growth control of *S. typhimurium*	I, IV
2	Growth control of *S. typhimurium* in the presence of mutagen (natrium azide)	I, II, IV
3	Investigated extracts (5.0 mg per plate)	I, II, III, IV
4	Investigated extracts (2.5 mg per plate)	I, II, III, IV
5	Investigated extracts (1.0 mg per plate)	I, II, III, IV
6	Reference extract *Dracocephalus moldavica* (10.0 mg per plate)	I, II, III, IV

I - mixture of histidine and biotine; II - solution of sodium azide, III - solution of the test extract, IV - dilution of *S. typhimurium* culture.

for 1 min and then programmed to increase at a rate of 4°C/min to 250°C. The samples were diluted with n-hexane and 0.1 µl were directly injected. The GC-MS data were obtained on a Varian 4000 MS/MS detector (scan range: 40 to 1000 u, scan time 0.8 s). For separation of components a fused-silica capillary column VF-5 ms poly (5% phenyl-95%-dimethylsiloxane) was used (30 m ×0.25 mm inside diameter, film thickness of 0.25 µm; Varian, USA). Qualitative analysis was based on the comparison of the retention times, relative to C_6-C_{40} n-alkanes and of the mass spectra with the corresponding data of components of reference oils and pure compounds whenever possible. Mass spectra were compared with those of mass spectra libraries (NIST, 2002; Mass Spectra Library, USA) and from Adams' Identification of essentials oil components by GC /quadrupole mass spectroscopy (2004). The percentage of volatile compounds of the extract was presented, assuming that the sum of peak areas for all identified constituents was 100%.

Antibacterial assays

Bacterial strains

Tests were performed on six bacteria reference strains obtained from American Type Culture Collection (ACCT, Rockville, MD, USA), National Institute of Hygiene (PZH, Warsaw, Poland), Public Clinic Hospital of Poznań University of Medical Sciences (UM, Poznań Poland), strain *Pseudomonas aeruginosa* was isolated from plant material in Institute of Natural Fibers and Medicinal Plants (IWNRZ, Poznań, Poland). Three Gram-positive microorganism strains were used for microbial assays *Staphyllococcus aureus* (ATCC 6538P), *Staphyllococcus faecalis* (ATCC 8040) and *Bacillus subtilis* (ATCC 1633) as well as three Gram-negative: *Escherichia coli* (PZH 028B6), *Klebsiella pneumoniae* 231 and *Pseudomonas aeruginosa* 85/2.

Determination of the minimal inhibitory concentration

Determinations were carried out using liquid antibiotic medium 1 agar substrate (Merck, Darmstadt, Germany). Liquid and dry alcohol and lipophilic extracts were added to 10 ml of liquid agar substrate on Petri dishes of 10 cm diameter, achieving the following concentrations: 1.0, 2.5, 5.0, 7.5, 10.0, 25.0, 50.0, 75.0 and 100.0 mg of initial extract per 1 ml. After thorough mixing and solidification of the substrate on the surface of liquid, cultures were inoculated with bacterial strains tested using a calibrated loop. Before plating 24 h cultures of smooth strains tested were diluted in a liquid antibiotic medium in such a way to get the number from 10^4 to 10^5 cells in 1 ml. Incubation of samples was carried out for 24 h at 37°C and then determined the lowest concentration of extract tested MIC completely inhibiting the development of the tested strains of bacteria (no growth of bacteria in the inoculation site). Each assay was done in triplicate.

Antimutagenic assays

Antimutagenic action was determined using a microbiological Ames Test (Maron and Ames, 1983). A histidine dependent standard strain *Salmonella typhimurium* TA 1535 from the collection of the ATCC (29629) with reversion leading to forming of strain independent from histidine presence is inducted by defined mutagenic substances. Sodium azide was used as a standard mutagen.

Antimutagenic testing was conducted at following concentrations: 5.0, 2.5 and 1.0 mg of initial extract per 1 ml. The investigated extracts were dissolved in dimethyl sulfoxide (DMSO) amount of 100 mg/ml, and then performed in the same solvent the corresponding dilution of this extract. To Erlenmeyer flask containing 10 ml cooled to 50°C top layer medium (medium B composed of 60 mg agar, sodium chloride 50 mg and distilled water 10 ml) following substrates in the amount of 0.1 ml were added: mixture of histidine and biotine, sodium azide, solution of investigated extracts and diluted standard strain of *S. typhimurium* standard (cultivated 24 h, diluted in proportion 1:10 in nutrient medium). Experimental design is shown in Table 1. Then 1.5 ml of medium B with adequate substrates was placed to the surface of 10 ml of solid medium of lower layer in Petri plate (medium A containing 150 mg of agar, 0.2 ml of VBME salts (50^x), 0.5 ml of 40% glucose, 9.3 ml of distilled water) and was distributed uniformly on the plate surface.

A mixture of histidine and biotine contained 96 mg of L-histidine hydrochloride (Merck, Darmstadt, Germany), 124 mg D-biotine (Sigma- Aldrich, Steinheim, Germany) and 10 mg of distilled water. The mixture of VBME salt (50^x) consisted of the following ingredients: magnesium sulphate x 7 H_2O 1 g, citric acid x 1 H_2O 10 g, acid anhydrous potassium phosphate 50 g, sodium-ammonium phosphate x 4 H_2O 17.5 g and distilled water 67 ml. Sodium azide was used in a form of aqueous solution in concentration of 1 mg/ml. After preparation, plates were incubated at 37°C for 72 h, protected from drying. Then, number of colonies of *S. typhimurium* grown on the plates (Table 5) was determined and studies results were interpreted. If in the plates containing 5.0, 2.5 and 1.0 mg of investigated extracts were the same number of *S. typhimurium* colonies as by growth control (spontaneous revertants) occurred (below 100 colonies on plate 1), it was assumed that the investigated extract show antimutagenic activity. However, in the case of the growth a similar number of colonies on the plate as in the control of growth in the presence of mutagen *S. typhimurium* (plate 2), that is more than 1000 colonies on the plate, it was assumed that the extract does not exhibit antimutagenic activity. Each assay was done in triplicate.

Table 2. Dry matter, flavonoids and o-dihydroxyphenols contents in hexane and ethanolic Solidago extracts.

Extract	Dry matter concentration (mg/g)		
	S. virgaurea	***S. canadensis***	***S. gigantea***
Hexane	35±0.577[z]	98±0.580	89±0.01
Ethanol	118±0.578	156±0.567	156±0.577
Flavonoids concentration (mg/g)			
Hexane	0.22±0.002[za]	0.040±0.001[b]	0.15±0.002[a]
Ethanol	0.80±0.001[a]	1.76±0.002[b]	4.57±0.03[c]
O-dihydroxyphenols concentration (mg/g)			
Hexane	0.12±0.002[za]	0.04±0.0009[b]	0[c]
Ethanol	0.19±0.003[a]	0.29±0.013[a]	0.40±0.171[b]

[z]Data are means ±SE; Values designated with the same letters (a, b, c) within line do not significantly differ at 5% error (Turkey test).

Table 3. Results of GC-MS screening of extracts from three Solidago species.

Compound	Lit. RI*	Exp. RI	Percentage					
			S. virgaurea		***S. canadensis***		***S. gigantea***	
			He*	**E***	**He**	**E**	**He**	**E**
α-pinene	939	938	0.8	7.0	0.1	9.5	0.1	0.5
β-pinene	979	982	0.3	-*	tr*	-	tr	-
Decane	1000	993	-	-	0.3	-	0.5	-
p-cymene	1025	1027	-	-	tr	-	0.1	-
Limonene	1029	1030	-	-	0.1	-	tr	-
1,8-cineole	1031	1034	-	-	tr	-	0.1	-
Trans-verbenol	1145	1151	-	-	0.1	-	0.1	-
Tetrahydro-lavandulol	1162	1153	0.8	-	-	-	-	-
Menthone	1153	1163	0.7	-	-	-	-	-
Borneol	1169	1179	-	0.7	0.1	1.0	0.3	0.2
Verbenone	1205	1219	-	-	0.1	-	0.1	-
Bornyl acetate	1289	1292	-	1.8	0.6	11.8	0.9	3.3
δ-elemene	1341	1294	-	1.1	-	1.3	-	0.2
α-cubebene	1351	1351	-	-	0.1	-	0.1	-
α-ylangene	1375	1374	-	-	tr	-	0.06	-
α-copaene	1377	1380	-	-	0.1	-	0.1	-
β-bourbonene	1388	1389	-	-	0.1	-	0.1	-
β-elemene	1391	1393	-	-	0.4	-	0.2	-
tetradecane	1400	1398	-	1.9	tr	0.6	0.1	0.4
α-gurjunene	1410	1413	-	0.8	0.1	1.1	1.2	0.3
β-funebrene	1415	1417	-	-	tr	-	0.1	-
(*E*)caryophyllene	1419	1427	0.3	0.6	0.1	0.8	0.2	0.2
β- copaene	1432	1439	0.1	0.5	0.1	0.7	0.2	0.2
Aromadendrene	1441	1452	-	-	tr	-	0.1	-
(*Z*)β-farnesene	1443	1458	-	-	tr	-	0.1	-
α-humulene	1455	1467	-	0.5	0.1	0.3	0.1	0.1
γ-gurjunene	1477	1485	1.9	0.8	0.4	1.2	3.1	2.2
γ-muurolene	1480	1488	-	0.2	-	0.5	-	0.4
α-amorphene	1485	1490	-	0.2	tr	0.3	0.1	0.1

Table 3. Contd.

Germacrene D	1485	1494	-	0.2	0.1	0.3	0.4	0.1
β-selinene	1498	1504	2.1	0.9	1.6	5.2	1.1	0.5
γ-amorphene	1496	1505	-	0.9	0.1	1.2	0.2	0.3
α-selinene	1498	1510	0.4	-	0.4	-	0.4	-
Trans-cycloisolongifol-5-ol	1514	1514	-	-	-	-	0.1	-
2,4-di-tert-butylphenol	1519	1518	-	1.7	0.1	1.1	0.2	0.9
1,1-dimethylethyl-phenol,2,4-bis	1519	1520	1.1	-	-	-	-	-
γ-cadinene	1514	1526	0.3	0.6	0.1	0.7	0.5	0.3
δ-amorphene	1512	1530	0.4	0.4	0.2	0.3	0.5	0.2
Trans-calamenene	1529	1534	-	-	0.1	-	0.1	-
(2,6,6-trimethyl-2-hydroxycyclohexylidene) acetic acid lactone	1548	1549	-	-	tr	-	0.2	-
α-calacorene	1546	1553	-	0.1	tr	0.1	0.1	tr
Germacrene B	1561	1559	0.2	0.4	tr	0.5	0.1	0.2
α-copaen-11-ol	1541	1567	3.0	-	0.1	-	-	-
ledol	1569	1580	-	0.5	0.1	0.7	0.1	1.7
Longipinanol	1569	1581	0.5	-	-	-	1.22	-
Spathulenol	1578	1588	12.8	7.5	0.5	1.8	5.5	6.6
caryophyllene oxide	1583	1593	3.8	4.7	1.4	6.6	2.7	2.4
globulol	1585	1599	0.3	0.4	0.2	0.4	0.3	1.0
thujopsan-2-α-ol	1587	1603	2.2	0.4	1.0	0.2	2.5	0.3
cyclohexene, 6-(2-butenyl)-1,5,5-trimethyl(*E*)-	2044	1605	-	1.7	-	5.6	-	3.8
Viridiflorol	1593	1617	2.0	-	0.4	-	4.2	-
Humulene epoxide II	1608	1623	4.5	4.3	0.7	2.7	1.6	2.4
I*		1629	5.0	2.9	0.9	3.5	2.2	2.0
Aristolene epoxide	1724	1632	-	-	0.1	-	0.2	-
Aromadendrene oxide-(2)	1678	1647	0.9	1.2	0.2	1.6	0.4	0.7
Epoxy allo-alloaromadendrene	1636	1661	2.5	1.7	0.5	1.0	1.1	1.2
Selina-3,11-dien-6-alpha-ol	1644	1670	5.3	3.3	1.3	4.4	2.7	3.4
Cedr-8(15)-en-10-ol	1652	1674	-	-	0.8	-	1.8	-
14-hydroxy-9-epi-(E)-caryophyllene	1670	1686	0.6	2.0	0.7	2.6	0.1	2.0
II*		1694	-	-	0.2	-	0.2	-
Apiole	1678	1702	0.7	-	0.4	-	0.3	-
Khusinol	1680	1708	6.8	2.9	1.1	3.0	3.2	3.2
2-methyl-4-(2,6,6-trimethylcyclohex-1-enyl)but-2-en-1-ol	1735	1714	1.9	0.5	0.5	0.6	0.8	0.7
8-cedren-13-ol	1689	1724	0.6	-	0.3	-	0.2	-
14-hydroxy-α-humulene	1714	1740	2.5	0.5	0.6	0.7	0.3	0.2
Oplopanone	1740	1751	1.5	0.4	0.2	0.5	0.7	0.3
Cyclocolorenone	1761	1769	4.0	1.9	1.2	2.0	38.3	40.6
14-hydrohy-α-muurolene	1780	1779	-	tr	0.2	tr	0.2	tr
III		1783	1.2	0.8	1.0	1.1	1.2	0.6
IV		1835	3.2	-	1.1	-	1.5	-
6,10,14-trimethyl- 2-pentdecanone	1847	1843	5.4	1.5	0.7	0.6	0.7	0.2
V		1847	5.1	1.2	1.5	2.5	2.2	1.9
VI		1868	3.0	6.6	0.3	2.1	0.4	2.0
VII		1880	4.70	-	1.19	-	1.87	-
VIII		1921	-	0.3	0.3	tr	0.1	tr
Methyl palmitate	1928	1926	-	0.4	0.1	tr	0.1	tr

Table 3. Contd.

III		1783	1.2	0.8	1.0	1.1	1.2	0.6
IV		1835	3.2	-	1.1	-	1.5	-
6,10,14-trimethyl- 2-pentdecanone	1847	1843	5.4	1.5	0.7	0.6	0.7	0.2
V		1847	5.1	1.2	1.5	2.5	2.2	1.9
VI		1868	3.0	6.6	0.3	2.1	0.4	2.0
VII		1880	4.70	-	1.19	-	1.87	-
VIII		1921	-	0.3	0.3	tr	0.1	tr
methyl palmitate	1928	1926	-	0.4	0.1	tr	0.1	tr
β-bisabolenal	1770	1935	-	0.8	0.1	tr	0.1	tr
IX		1949	-	-	0.4	-	0.2	-
X		1966	-	4.9	-	1.9	-	0.6
palmitoleic acid	1984	1977	-	-	14.1	-	1.0	-
ethyl hexadecanoate	1993	1995	-	8.1	-	2.0	-	4.2
falcarinol	2036	2043	-	-	tr	-	-	-
kaurene	2043	2055	-	-	-	-	0.2	-
methyl linoleate	2098	2086	-	0.9	0.5	0.7	0.2	0.3
XI		2144	-	1.8	46.4	2.6	0.4	0.9
methyl(*Z*)-5,11,14,17-eicosatetraenoate	2231	2167	-	3.6	-	1.2	-	0.5
XII		2173	-	-	6.5	-	-	-

*E, ethanol; He, hexane; tr, trace (<0.1%); -, not detected; Lit. RI, retention indices from literature; Exp. RI, experimental retention indices; I-XII – unknown compounds.

RESULTS AND DISCUSSION

Table 2 lists concentrations of flavonoids and o-dihydroxyphenols. The flavonoids are one of the most numerous and widespread groups of natural constituents in the plant kingdom, some of them due to inhibit free radical-mediated processes as well as diuretic and anti-inflammatory action. As a rule flavonoids have low toxicity which, combined with high antioxidant, antiallergic, antiviral, antimutagenic and anticarcinogenic capacity makes these compounds extremely useful as pharmaceutical agents (Nijveldt et al., 2001). Similarly as in Hiller and Bader (1996) study the highest content of flavonoids and o-dihydroxyphenols in ethanolic extracts were stated in *S. gigantea* herb, the lowest being in *Solidaginis viragureae herba*. In the case of hexane extracts the highest amount of investigated secondary metabolites was found in *S. viragureae herba*, the lowest one are in *S. gigantea and S. canadensis* herb (Table 2). The same level of active compounds content in *S. viragaurea* herb were obtained by Lück et al. (2000), Kołodziej (2002) and Kalemba et al. (1993). It is worth to emphasize, that stated flavonoids content was in accordance with requirements of Polish Pharmacopoeia VIII (2008).

In total 88 volatile compounds were detected: thirty nine and forty nine in ethanolic and hexane extracts of *S. virgaurea*, sixty two and forty six in *S. canadensis* extracts, respectively, and seventy three and forty five compounds in the etanolic and hexane extracts of *S. gigantea*. Results of GC-MS screening indicated that extract from *S. virgaurea* was dominated by spathulenol, humulene epoxide II, selina-3,11-dien-6-alpha-ol and khusinol (Table 3). Hexane and ethanolic extracts from *S. canadensis* were rich in caryophyllene oxide as well as oleic acid and unknown compound which mass spectrum was marked as XI (hexane extract) and α-pinene, bornyl acetate (ethanolic extract), whereas *S. gigantea* extracts were extremely rich in cyclocolorenone as well as in spathulenol, γ-gurjunene, selina-3,11-dien-6-alpha-ol and khusinol, like in Johnson et al. (2007) and Wang et al. (2006) studies. Kalemba et al. (2001) found in *S. gigantea* essential oil similar amounts of cyclocolorenone (8 to 32%), spathulenol (1 to 1.9%), and γ-gurjunene (2.1 to 2.5%). What is more, twelve unknown compounds were detected and their mass spectra are shown in Figure 1. Also, additionally about 5% of artifact, phthalate (from the environment), was identified in the extracts. Antibacterial activity of ethanolic and hexane extracts made of *S. virgaurea, S. canadensis* and *S. gigantea* is expressed as MIC values presented in Table 4 and antimutagenic activity of studied extracts is shown in Table 5. As follows from the Tables 4 and 5, all the extracts are characterized by quite high biological activity. Similarly as in Thiem and Goślińska (2002) study, ethanolic extracts of *S. virgaurea* plants and in Kalemba (2000) experiments its essential oil, showed a moderate bactericidal activity.

The results were also in accordance with the antibacterial potential of naturally growing *S. virgaurea*

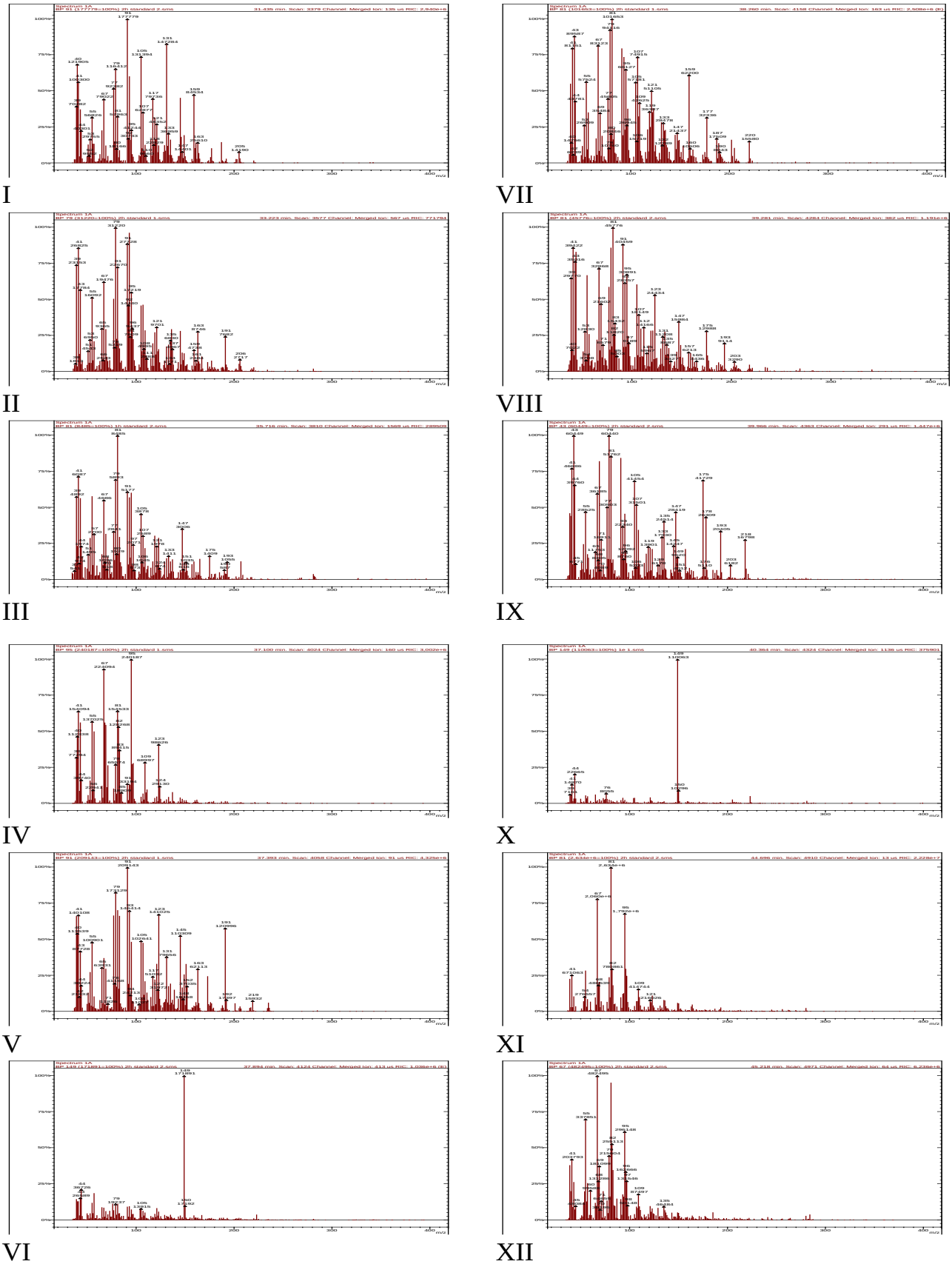

Figure 1. Mass spectra of unknown constituents.

Table 4. MICs obtained for alcoholic and lipophilic extracts from *Solidago virgaurea, Solidago gigantea* and *Solidago canadensis herba.*

Bacteria	Chloramphenicol	Antibacterial activities (MIC in mg/ml)											
		Ethanolic extract						Hexane extract					
		S. v.*		S. c.		S. g.		S. v.*		S. c.		S. g.	
		LIQ[a]	D	LIQ	D	LIQ	D	LIQ	D	LIQ	D	LIQ	D
Staphyllococcus aureus ATCC 6538P	0.005	25	2.95	50	7.8	25	3.9	100	3.5	10	0.98	>100	>8.9
Staphyllococcus faecalis ATCC 8040	0.005	50	5.9	75	11.7	50	7.8	100	3.5	10	0.98	>100	>8.9
Bacillus subtilis ATCC 1633	0.005	50	5.9	10	1.56	10	1.56	>100	>3.5	5	0.49	100	8.9
Escherichia coli PZH 028 B6	0.005	100	11.8	>100	>15.6	>100	>15.6	>100	>3.5	>100	>9.8	>100	>8.9
Klebsiella pneumoniae 231 UM	0.05	100	11.8	>100	>15.6	100	15.6	>100	>3.5	>100	>9.8	>100	>8.9
Pseudomonas aeruginosa 85/2 IWNRZ	0.05	75	8.85	75	11.7	75	11.7	100	3.5	100	9.8	>100	>8.9

*S. v. – *Solidago virgaurea*, S. c. – *Solidago canadensis*, S. g. – *Solidago gigantea;* [a]LIQ – liquid extract; D- dry extract.

Table 5. Antimutagenic activity of tested extracts and reference extract.

Tested extract	Growth control of *S. typhimurium* (spontaneous mutations)	Growth control of *S. typhimurium* in the presence of mutagen	Concentration of the liquid extract on a plate (mg/ml)		
			5.0	2.5	1.0
Hexane extracts					
S. virgaurea	29*	>1000*	1	0	800
S. canadensis	52	>1000	53	90	450
S. gigantea	61	>1000	53	78	>1000
Ethanolic extracts					
S. virgaurea	59	>1000	>1000	>1000	>1000
S. canadensis	48	>1000	>1000	>1000	>1000
S. gigantea	90	>1000	>1000	>1000	>1000
Reference extract *Dracocephalus moldavica*	38	>1000	39	>1000	>1000

* Number of *S. typhimurium* colonies on the plate.

plants against the same strains of bacteria (Thiem and Goślińska, 2002; Thiem et al., 2001; Demir et al., 2009). Our studies indicate that both ethanolic and hexane extracts acted strongly to Gram-positive than Gram-negative bacteria. In the case of hexane extracts the strongest of Gram-positive bacteria acted hexane extract of *S. canadensis* (MIC values: 5 to 10 mg/ml), while the other hexane extracts showed a weak effect (MIC values: 100 and > 100 mg/ml). In the case of ethanolic extracts relatively strong activity against Gram-positive bacteria was found in *S. gigantea* and *S. canadensis* (MIC values: 10 to 50 mg/ml). The mechanisms by which extracts can inhibit the growth of microorganisms are varied, and can be

Meyers (2010) experiment *S. canadensis* extract inhibited *S. aureus*, in a similar way as in our study (Table 4). As Kalemba (2000) stated antimicrobial activity of Solidago constituents was based on inhibition of bacterial cell division and possibly damage the plasma membrane or intracellular membranes and then cell lysis.

The presence of soluble phenolic and polyphenolic compounds, including phenolic acids as well as flavonoids (Table 2), volatile terpenes (Table 3) and other phyto-constituents may be the contributing components for the expressed anti-bacterial activity investigated in the present study. For example Ahmad et al. (1993) stated that terpenenes or terpenoids are active against bacteria. Furthermore, Dixon et al. (1983) found that flavonoids are synthesized by plants in response to microbial infection and are effective against a broad range of microorganisms. Their activity is probably due to their capacity to form complexes with extracellular soluble proteins, which bind to the bacterial cell wall. Some lipophilic flavonoids can also cause rupture of the plasma membrane of microorganisms (Tsuchiya et al., 1996). Furthermore, besides the biologically active substances given above, significant contents of cyclocolorenone (38.3% in the case of hexane and 40.6% in ethanolic extract) in *S. gigantea*, a sesquiterpene ketone with antibacterial and antifungal activity (Kalemba and Thiem, 2004; Kalemba, 2000; Starks et al., 2010) were found, which may also have an effect on higher antibacterial activity of these investigated extracts (Table 4). Similarly as in Brantner (1999) studies in the case of Gram-negative bacteria all the tested extracts exhibited weak activity (MIC values: 75 to> 100 mg/ml). Moreover, as in Deepa and Ravichandiran (2010) experiment alcohol extracts show stronger antibacterial action compared with a hexane ones.

Mutagenic and carcinogenic agents are omnipresent in our environment and their elimination seems to be very difficult. The results presented in the Table 5 indicate that hexane extracts of the *S. virgaurea*, *S. canadensis* and *S. gigantea* herb showed antimutagenic activity. The mutagen used to determine the antimutagenic activity of the test extracts in this study was NaN_3, which is known as a powerful mutagen in several organisms including bacteria, plants and animals (Grant and Salamone, 1994; Sadiq and Owais, 2000; Gulluce et al., 2010). Previous studies clearly showed that the mutagenicity of sodium azide is mediated through the production of an organic metabolite of azide called L-azidoalanine (Sadiq and Owais, 2000; Gulluce et al., 2010). All the three extracts obtained from Solidago could suppress mutagenic effect induced by sodium azide on TA1535 at a concentration of 2.5 mg/ml. The exact mechanism by which the extracts showed antimutagenicity is unknown. However, the antimutagenic activity of the extracts against direct acting mutagen probably due to the direct inactivation of the mutagen by complex formation with the ingredients present in the extracts. Antimutagenic effect can also be speculated by protecting the bacterial genome from the directly acting mutagens. This protection can be rendered by rapid elimination of mutagens from bacteria before their interaction with the deoxyribonucleic acid (DNA), which may be mediated by facilitating or stimulating the transmembrane export system in bacteria (Ajith and Janardhanan, 2011). Ethanolic extracts, in the applied range of concentrations, did not show antimutagenic activity.

Conclusions

The findings of the present study support the beneficial effects of the Solidago extracts against the pathogenic organisms as well as their laudable antimutagenic activity. Since major attention has been recently devoted to natural antimutagenic and antimicrobial factors that could lower the rates of mutation and bacterial infection by including them in dietary products, this activity is valuable towards an extension of the employ of that crude drug as new valuable ingredients for food and/or nutriceutical, cosmeceutical support in the promotion of health, besides its consolidated traditional use.

REFERENCES

Ahmad A, Mahmoud A, Williams H, Scott A, Reibebspies J, Mabry T (1993). New sesquiterpene a-methylenelactones from the Egyptian plants *Jasonia candicans*. J. Nat. Prod., 56: 1276-1280.

Ajith TA, Janardhanan KK (2011). Antimutagenic effect of *Phellinus rimosus* (Berk) Pilat against chemical induced mutations of histidine dependent *Salmonella typhimurium* strains. Food Chem. Toxic., 49: 2676-2680.

Adams R (2004). Identification of essential oil compounds by gas chromatography/quadrupole mass spectroscopy. Carol Stream. IL, Allured Publishing Corporation, p. 456.

Apati P, Kristo T, Szőke E, Kery A (2003). Comprehensive evaluation of different *Solidaginis herba* extracts. Proc. Int. Conf. on MAP, Acta Hortic., 597: 69-73.

Bader G, Binder K, Hiller K, Ziegler-Böhme H (1987). The antifungal action of triterpene saponins of *Solidago virgaurea* L. Pharmazie, 42: 140.

Bader G, Plohmann B, Franz G, Hiller K (1996). Saponin from *Solidago virgaurea L*. Possible Agents for Therapy of Cancer? 44th Annual Congress of the Society for Medicinal Plant Research and a Joint Meeting with the Czech Biotechnology Society. Abstracts of Lectures and Poster Presentations, Prague, September 3-7, SL14.

Brantner A (1999). The antimicrobial activity of Solidago-containing phytourologica. Drogen-report, 12: 27-28.

Chodera A, Dabrowska K, Sloderbach A, Skrzypczak L, Budzianowski J (1991). The effect of flavonoids fraction of Solidago species on diuretic and electrolytes concentration. Acta Pol. Pharm. Drug Res., 48: 5-6, 35-37.

Christ B, Müller KH (1960). Determination of the amount of flavonol derivatives in drugs. Archiv. Pharm., 293: 1033-1042.

Deepa N, Ravichandiran V (2010). Antimicrobial activity of *Solidago canadensis* L. Int. J. Res. Pharm. Sci., 1(4): 411-413.

Demir H, Açik L, Bali EB, Koç LY, Kaynak G (2009). Antioxidant and antimicrobial activities of *Solidago virgaurea* extracts. Afr. J. Biotechnol., 8(2): 274-279.

Dixon R, Dey P, Lamb C (1983). Phytoalexins: Enzymology and Molecular Biology. Adv. Enzymol. Relat. Areas Mol. Biol., 55: 1-69.

Frey F, Meyers R (2010). Antibacterial activity of traditional medicinal plants used by Haudenosaunee peoples of New York State. BMC

Complement. Altern. Med., 10: 64 [Online]. Available at: http://www.biomedcentral.com/1472-6882/10/64. Accessed on 1 May 2011.
Grant WF, Salamone MF (1994). Comparative mutagenecity of chemicals selected for test in the International Program on Chemical Safety's collaborative study on plant systems for the detection of environmental mutagens. Mutat. Res., 310: 187-209.
Gulluce M, Agar G, Baris O, Karadayi M, Orhan F, Sahin F (2010). Mutagenic and antimutagenic effects of hexane extract of some *Astragalus* species grown in the eastern Anatolia region of Turkey. Phytother. Res*.,* 24: 1014-1018.
Hiller K, Bader G (1996). Goldruten-kraut. Goldruten-kraut. Goldenrod herb. The genus Solidago, a pharmaceutical evaluation. Portrait of a medicinal plant. Zeitschrift für Phytother., 17: 123-130.
Johnson RH, Hull-Sanders HM, Meyer GA (2007). Comparison of foliar terpenes between native and invasive *Solidago gigantea*. Biochem. Syst. Ecol., 35: 821-830.
Kalemba D (2000). Constituents and biological activity of the essential oils of some Solidago and Artemisia species. Scientific Papers Technical University of Lodz, 857: 1-118.
Kalemba D, Góra J, Kurowska A, Lis A, Lasocka I (1993). Polyphenolic compounds in four *Solidago* species. Acta Pol. Pharm. Drug Res., 50(6): 441-442.
Kalemba D, Marschall H, Bradesi P (2001). Constituents of the essential oil of *Solidago gigantea* Ait. (giant goldenrod). Flavour Fragr. J., 16: 19-26.
Kalemba D, Thiem B (2004). Constituents of essential oils of four micropropagated Solidago species. Flavour Fragr. J., 19: 40-43.
Kołodziej B (2002). The effect of soil material and nitrogen fertilization on growth and development of goldenrod (*Solidago virga-aurea* L.). Folia Hortic., 14(1): 187-193.
Lück L, Schenk R, Weber K, Bader G, Abel G (2000). Wild origins of *Solidago virgaurea* L. – biomass yield and variability of morphological and phytochemical characteristics. Z. Arzn. Gew. Pfl., 1: 20-27. (Translate).
Maron D, Ames B (1983). Revised methods for the Salmonella mutagenicity test. Mutat. Res., 113: 173-215.
Melzig M, Löser B, Bader G, Papsdorf G (2000). European goldenrod as anti-inflammatory drug. Zeitschrift für Phytotherapie, 21: 67-70.
Melzig M (2004). European goldenrod – a classical exponent in the urological phytotherapy. Wiener Medizinische Wochenschrift, 154: 21-22, 523-527.
Nijveldt R, van Nood E, van Hoorn D, Boelens P, van Norren K, van Leeuwen P (2001). Flavonoids: a review of probable mechanisms of action and potential applications. Am. J. Clin. Nutr., 74(4): 418-425.
Nicolson K, Evans G, O'Toole P (1999). Potentiation of methicillin activity against methicillin-resistant *Staphylococcus aureus* by diterpenes. FEMS Microbiol. Lett., 179: 233-239.
Polish Pharmacopoeia VI (2002). Warsaw, Polish Pharm. Soc., p. 151.
Polish Pharmacopoeia VIII (2008). Warsaw, Polish Pharm. Soc., pp. 2911-2914.
Sadiq MF, Owais WM (2000). Mutagenicity of sodium azide and its metabolite azidoalanine in *Drosophila melanogaster*. Mutat. Res., 469: 253-257.
Singleton VL, Rossi JA (1965). Colorimetry of total phenolics with phosphomolybdic-phosphoturgstic acid reagents. Am. J. Enol. Viticult., 1(16): 44-58.
Starks C, Williams R, Goering M, O-Neil-Johnson M, Norman V, Hu J, Garo E, Hough G, Rice S, Eldrige G (2010). Antibacterial clerodane diterpens form goldenrod (*Solidago virgaurea*). Phytochemistry, 71: 104-109.
Thiem B, Goślińska O (2002). Antimicrobial activity of *Solidago virgaurea* L. from *in vitro* cultures. Fitoterapia, 73: 514-516.
Thiem B, Wesołowska M, Skrzypczak L, Budzianowski J (2001). Phenolic compounds in two Solidago L. species from *in vitro* culture. J. Acta Polon. Pharm., 58: 277-281.
Tsuchiya H, Sato M, Miyazaki T, Fujiwara S, Tanigaki S, Ohyama M (1996). Comparative study on the antibacterial activity of phytochemical flavanones against methicillin-resistant *Staphylococcus aureus*. J. Ethnopharm., 50: 27-34.
Tyszkiewicz E (2008). Assessment report on *Solidago virgaurea* L., herba. EMEA, Evaluat. Med. Hum. Use, pp. 1-31. [Online]. Available at: http://www.emea.europa.eu. Accessed on 1 May 2011.
Wang KJ, Li N, Chen LZ, Yu XP (2006). Chemical constituents and antifungal activity of essential oil from *Solidago canadensis*. J. Plant Res. Environ*.,* 15(1): 34-36.
Wendakoon C, Sakaguchi M (1995). Inhibition of amino acid decarboxylase activity of *Enterobacter aerogenes* by active components in spices. J. Food Prot., 58: 280-283.
Wichtl M (1994). *Virgaureae herba* – Golden rod. In: Herbal drugs and phytopharmaceuticals. Medpharm GmbH, Stuttgart, pp. 530-533.