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Chemical composition and chosen bioactive properties of *Panax quinquefolius* extracts

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Panax quinquefolius L. (Araliaceae) is considered to be a medicine having potential to prevent and treat various human diseases. This study determined the chemical composition (flavonoids, o-dihydroxyphenols – by the means of spectrophotometry and ginsenosides – by HPLC and HPLC-PDA/ESI/MS) of ethanol and hexane extracts of above- and underground parts of plants. Extracts obtained from roots and leaves of *P. quinquefolius* were also analyzed by GC-MS, and their antibacterial and antimutagenic activities were evaluated.

The ginseng root ethanol and hexane extracts detected 55 and 32 volatile compounds, the leaf extracts revealed 45 and 33 compounds, respectively. The content of flavonoids and *o*-dihydroxyphenols in ginseng leaves was higher than in its roots, while a reverse tendency was observed in the content of ginsenosides. Ginsenosides R_{b1} and R_e were the main compounds of American ginseng roots, whereas ginsenosides R_{b2}, R_d and R_e were the main ones in leaves.

It was found that extracts tested were characterized by their antibacterial action. The strongest action on Gram-positive bacteria was shown by ethanol and hexane extracts of *P. quinquefolius* roots, while the other extracts had a weak effect. Hexane extracts of *P. quinquefolius* roots also showed strong antimutagenic activity (at the concentration of 1.0 mg/ml) – with *S. typhimurium* TA 1535 from the collection of the ATCC and sodium azide as a mutagen.

Key words: American ginseng, content of active substances, antibacterial activity, antimutagenic activity

INTRODUCTION

Panax quinquefolius L., known as American ginseng (Araliaceae), is one of twenty species of *Panax* genus [1]. This valuable medicinal plant, originated from shady forests of North America, have been harvested from its native state and cultivated in Canada and the USA for over 100 years. Recently, attempts of its cultivation have been made in many other countries (China, Australia, New Zealand, France, Holland, and Poland) [1, 2]. Its relative, *Panax ginseng* C. A. Meyer, has been used as a traditional medicine in the Far East for thousands of years. At the same time, wild American ginseng was apparently used by many Indian tribes to increase mental powers, woman fertility, and to treat headache, cramps, fevers, rheumatism, and cough [1]. Many scientists are of the opinion that American ginseng is less stimulating than the Asian one [1, 3]. Ginseng possesses multiple constituents and multiple actions. *Panax quinquefolius* is used as a mild tonic; however, its mechanism of action remains unclear [4]. It has been considered to be an adaptogen, aphrodisiac, antidepressant, effective in memory stimulation, having potential to prevent and treat heart and circulatory system diseases. American ginseng roots inhibit the formation of lipid peroxides in the cardiac muscle or the liver, by influencing enzyme function, decreasing blood coagulation, cholesterol and sugar levels in blood, and stimulating the immune system [5].

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Recent literature reports that the root extracts of American ginseng and its derivatives possess immunopotentiating, antineoplastic, and antiapoptotic properties [6]. American ginseng extract is also capable of suppressing chromosomal aberration induced by mitomycin C (MMC) in mice [7]. Other study demonstrates that oral administration of *Panax ginseng* extract results in significant reduction of tumor incidences, its burden, size and weight in mammals [8] as well as chemo-preventive effects [9].

Approximately 200 substances have been isolated from ginseng including ginsenosides, polyacetylenes, alkaloids, polysaccharides, oligosaccharides, oligopeptides, phenolic compounds, lipids, vitamins, and minerals [10–12]. Nonvolatile ginsenosides are believed to be the main pharmacologically active ginseng constituents [4, 9]. Most ginsenosides are composed of a dammarane skeleton (17 carbons in a four-ring structure) with various sugar moieties (e. g. glucose, rhamnose, xylose, and arabinose) attached to the C-3 and C-20 positions. Ginsenosides are named as 'R_x', where the 'R' stands for the root and the 'x' describes the chromatographic polarity in an alphabetical order. Over 30 ginsenosides have been identified and classified into two categories: the 20(S)-protopanaxadiol (PPD) (e. g. R_{b1}, R_{b2}, R_c, R_d) and the 20(S)-protopanaxatriol (PPT) (R_e, R_{g1}, R_{g2}, R_{h1}). The difference between PPTs and PPDs is the presence of a carboxyl group at the C-6 position in PPDs [1, 11]. Generally, saponins are considered to have antimutagenic activity both *in vitro* and *in vivo*. They stimulate inhibition of several tumor cell lines growth [13], reverse transformation in culture Morris hepatoma cells and ginsenoside R_{g1} – immunomodulatory action in mice [13]. These active ingredients also have antioxidant, antiinflammatory, antiapoptotic and immunostimulant properties. Phyto-active compounds occur both in foliage and roots, but traditionally only roots are used [1]. Thus, limited data on American ginseng leaf extract composition and biological effects are available [11, 14, 15]. Despite the fact that ginseng is becoming a world-famous medicinal plant, its bio-active properties are still not scientifically proved.

Therefore, the objectives of this study were to determine the content of active compounds as well as antibacterial and antimutagenic effects of *Panax quinquefolius* roots and leaves, the parts used to treat patients suffering from cold and cancer.

EXPERIMENTAL

Plant material

Leaves and roots of three-year-old *Panax quinquefolius* from experimental cultivation of the Department of Medicinal and Industrial Plants, University of Life Sciences in Lublin, were collected in September 2011 for the study. Seeds of *Panax quinquefolius* used for plantation were supplied by Prof B. F. Zilkey (Delhi Research Station, Ontario, Canada), where this plant was identified. Voucher specimens (number N-100-SKK-05, N-100SKK-06) are deposited at the Department of Medicinal and Industrial Plants, University of Life Sciences in Lublin. After collection, the plant material was dried at 38 °C in a drying chamber and finally properly powdered.

Plant extracts

Extracts for the study were obtained by extraction of 100 g of powdered raw material (roots and leaves of *Panax quinquefolius* L.) using hexane (1 : 5) and 50% aqueous-ethanol (1 : 5). Obtained extracts were condensed in a rotation evaporator up to 100 ml or dried. After solvent evaporation at 60 °C, dry matter in hexane and ethanol extracts (Table 1) was determined yielding a very dense extract (considering it as solvent-free) and the results were calculated on the extract.

Table 1. **Dry matter content in ethanol and hexane extracts from American ginseng roots and leaves**

Extract	Concentration, mg/g	
	Root	Leaves
Hexane	23 ± 1.0[z]	40 ± 1.0
Ethanol	183 ± 1.527	214 ± 0.001

[z] Data are means ± SE.

Chemical composition

The liquid extracts were subsequently subjected to GC-MS screening analysis. In the case of ethanol extracts the following procedure was carried out in order to perform the GC-MS analysis of lipophilic fraction. Aliquot of 1 ml of ethanol 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, then the vial was shaken again. The solution remained in the vial until the separation of fractions. Organic fraction was collected into glass vials sealed with a Teflon stopper. After solvent evaporation at 60 °C, dry matter was determined in ethanol extract. Salted fraction was subjected to GC-MS screening analysis.

Raw alcohol fraction was phytochemically tested 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 [16], according to the modified Polish Pharmacopoeia VI [17] 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 the means of spectrophotometry, according to the Folin-Ciocalteu method as reported by Singleton and Rossi [18].

Particular ginsenosides (R_{b1}, R_{b2}, R_{g1}, R_d, R_e, R_c) were determined by HPLC method according to the previously described procedures [2]. Additionally, screening of ginseng roots extract components was performed by comparison of their retention times and MS spectra for HPLC separations of the studied extract and standard solutions of ginsenosides (Roth). A qualitative interpretation of the isolated components was made by comparison of the achieved mass spectra for particular separated substances with the spectra and molecular weight for earlier described saponins of *Panax* species [19].

HPLC-PDA/ESI/MS

A LC system consisting of a Finnigan Surveyour pump equipped with a gradient controller, an automatic sample injector, and a PDA detector were used. The separation was performed on a 250 mm × 4 mm i. d., 5 μm, Eurospher 100 C_{18} column (Knauer, Germany). A mobile phase consisted of 0.5% acetic acid in water (B), and 0.05% acetic acid in acetonitrile (A) was used for the separation. The flow rate was kept constant at 0.6 ml/min for a total run time of 110 min. The system was run with the following gradient program: 0.00–15 min, isocratic 20% A; 45 min, 46% A; 50 min, 55% A; 50–90 min, isocratic 55% A; 95 min, 90% A; 95–100 min, isocratic 90% A; 105 min, 20%vA; and 105–110 min, isocratic. The sample injection volume was 10 μl. A Thermo Finnigan LCQ Advantage Max ITMS with an electrospray ion source was coupled to the HPLC system described above. The samples were introduced on column via an automatic sampler injector or direct injection by a syringe pump at a flow rate of 5 μl/min. The spray voltage was set to 4.2 kV, and the capillary offset voltage was set to –60 V. All spectra were acquired at a capillary temperature of 220 °C. The calibration of mass range (400–2 000 Da) was performed in negative ion mode. Nitrogen was used as the sheath gas, and the flow rate was 0.9 l/min. The maximum ion injection time was set to 200 ms.

Statistical analysis

Results achieved were performed in three replications and statistically processed using variance analysis. Confidence intervals were estimated using Tuckey's test at 5% significance level.

GC-MS

The quantitative and qualitative determination of the lipophilic fraction of *Panax quinquefolius* extracts was made using a gas chromatograph (Varian 4000 GC/MS/MS). The carrier gas was helium with the splitting ratio of 1 : 1 000 and capillary flow rate of 0.5 ml/min. Oven temperature was held isothermal at 50 °C 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 was directly injected. The GC-MS data were obtained on a Varian 4 000 MS/MS detector (scan range: 40–1 000 Da, scan time 0.8 s). For component separation 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). A qualitative analysis was based on the comparison of the retention time, relative to C_6–C_{40} *n*-alkanes, and mass spectra with the corresponding data of the 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 the means of gas chromatography / quadrupole mass spectroscopy [19]. 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 the American Type Culture Collection (ACCT, Rockville, MD, USA), the 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 the Institute of Natural Fibres 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 ones: *Escherichia coli* (PZH 028B6), *Klebsiella pneumoniae* 231 and *Pseudomonas aeruginosa* 85/2.

Determination of the minimum inhibitory concentration

Determination was carried out by the use of a liquid Antibiotic Medium 1 agar substrate (Merck, Darmstadt, Germany). Liquid and dry ethanol and hexane extracts (after dissolving them in DMSO) were added to 10 ml of a liquid agar substrate on Petri dishes of 10 cm in 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 the initial extract per 1 ml. After thorough substrate mixing and solidification on the surface of the liquid, cultures were inoculated with bacterial strains, tested by the means of calibrated loop. Before plating 24-hour cultures of tested smooth strains they were lately diluted in a liquid Antibiotic Medium 1 to get the number from 10^4 to 10^5 cells in 1 ml. Incubation of samples was carried out for 24 hours at 37 °C, and then the lowest concentration of the tested extract was determined (MIC – minimum inhibitory concentration) completely inhibiting the development of bacteria tested strains (no growth of bacteria in the inoculation site). Each assay was done in triplicate.

Antimutagenic assays

Antimutagenic action was determined using the Ames test [21]. The 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 the following concentrations: 5.0, 2.5 and 1.0 mg of the initial extract per 1 ml. The investigated extracts were dissolved in DMSO in an amount of 100 mg/ml, and then in the same solvent the corresponding dilution of the extract was performed. To an Erlenmeyer flask containing 10 ml of cooled to 50 °C top layer medium (medium B composed of 60 mg agar, sodium chloride 50 mg, distilled water 10 ml), the following substrates in an 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 10 h, diluted in proportion 1 : 10 in nutrient medium).

An experimental design is shown in Table 2. Then 1.5 ml of medium B with adequate substrates were placed on the surface of 10 ml of solid medium of lower layer in a 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 × 7 H_2O 1 g, citric acid × 1 H_2O 10 g, acid anhydrous potassium phosphate 50 g, sodium-ammonium phosphate × 4 H_2O 17.5 g and distilled water 67 ml. Sodium azide was used in a form of aqueous solution in a concentration of 1 mg/ml. After preparation, plates were incubated at 37 °C for 72 hours, protected from drying. Then, the number of colonies of *S. typhimurium* grown on the plates was determined and the study 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 shows antimutagenic activity. However, in the case of growth a similar number of colonies on the plate to the one resulted from the control of growth in the presence of mutagen *S. typhimurium* (plate 2) (that is more than 1 000 colonies on the plate) suggested that the extract did not exhibit antimutagenic activity. Each assay was done in triplicate.

RESULTS AND DISCUSSION

Table 3 lists concentrations of flavonoids, o-dihydroxyphenols and ginsenosides in ethanol *P. quinquefolius* extracts, and Table 4 lists the results of HPLC-PDA/MS screening analyses of root extract. Previous experiments [3, 10] showed the presence of rutin, kaempferol and quercetin as well as protokatechuic, gentisic, *p*-coumaric, caffeic and ferulic acids in roots, and hyperoside, quercitrin and isoramnetin-3-rutinoside as well as protokatechuic, gentisic, *p*-coumaric, caffeic, salicylic and *p*-hydroxybenzoic acids in ginseng leaves. In our experiment, concentration of flavonoids and o-dihydroxyphenols in ginseng leaves, determined by the spectrophotometric method, was higher than in roots (Table 3).

The total content of ginsenosides in *P. quinquefolius* roots ranges from 1 up to 10% [2–4, 14]. The ginsenoside composition of leaves and roots is different (Table 4), as in Xie et al. [14] and Ligor et al. [11] studies. In our experiment, ginsenoside R_{b1} and R_e were the main compounds of American ginseng

Table 2. **Experimental design and number of *S. typhimurium* TA 1535 from the collection of the ATCC (29629) colonies**

Number and name of the plate	Substrates added to the surface medium B	Number of *S. typhimurium* colonies on the plate
1. Growth control of *S. typhimurium*	I, IV	<100*
2. Growth control of *S. typhimurium* in the presence of mutagen (sodium azide)	I, II, IV	>1 000
3. Investigated extracts (5.0 mg per plate)	I, II, III, IV	>1 000
4. Investigated extracts (2.5 mg per plate)	I, II, III, IV	>1 000
5. Investigated extracts (1.0 mg per plate)	I, II, III, IV	>1 000
6. Reference extract *Dracocephalus moldavica* (10.0 mg per plate)	I, II, III, IV	<100*

I – a mixture of histidine and biotine, II – a solution of sodium azide, III – a solution of the test extract, IV – dilution of *S. typhimurium* culture, * – spontaneous revertants.

Table 3. **The content of flavonoids and o-dihydroxyphenols in ginseng hexane and ethanol extracts and particular ginsenosides concentration in American ginseng ethanol extracts**

Extract	Flavonoids concentration, mg/g	
	Roots	Leaves
Hexane	0.002 ± 0.000 ^{z}a	0.040 ± 0.001b
Ethanol	0.098 ± 0.001 a	5.14 ± 0.001b
Extract	**O-dihydroxyphenols concentration, mg/g**	
	Roots	**Leaves**
Hexane	0.002 ± 0.000 ^{z}a	0.04 ± 0.0009b
Ethanol	0.295 ± 0.009a	1.915 ± 0.003a
Ginsenosides content, mg/g	**Roots**	**Leaves**
R_{b1}	16.590 ± 0.61a	0.740 ± 0.14b
R_{b2}	0.905 ± 0.105a	12.290 ± 0.62b
R_{g1}	0.840 ± 0.04a	0.355 ± 0.075a
R_c	2.470 ± 0.45a	0.495 ± 0.095b
R_d	6.855 ± 0.725a	6.380 ± 0.41a
R_e	11.165 ± 0.105a	4.075 ± 0.315b
Ginsenosides totally	38.825 ± 0.745a	24.335 ± 0.805b

^{z} Data are means ± SE; Values designated with the same letters (a, b, c) within the row do not significantly differ at 5% error (Tuckey test).

Table 4. **Results of HPLC-PDA/MS screening analysis of *Panax quinquefolium* roots**

Compound	R_t, min	λ_{max}, nm	MS spectra
R_{b1}	37.4	205	1 167[a] (100), 1 186 (75), 613 (70)
R_{b2}	39.5	205	1 137[a] (100), 598 (73), 1 138 (35)
R_{g1}	25.6	205	859[a] (100), 1 005 (95), 860 (40)
R_c	38.5	205	1 137[a] (100), 598 (61), 1 138 (35)
R_d	42.1	205	1 005[a] (100), 1 892 (35), 1 006 (34)
R_e	26.7	205	1 005[a] (100), 1 006 (45), 1 478 (21)

roots, whereas ginsenosides R_{b2}, R_d and R_e were the main ones in leaves. The contribution of both R_{b1} and R_e to the total content of ginsenosides in roots was 42.7 and 28.8%, respectively. Ginsenoside R_d was detected at a lower concentration than R_{b1} and R_e and its contribution to the total content amounted to 17.7%. We recorded that the amount of particular ginsenosides in roots was similar to that reported by Gafner et al. [4] and Wang et al. [14]. The authors in question and we recorded clear domination of R_{b1} and R_e or R_d. The lowest abundance measured in our experiment (among determined panaxadiols) was for R_{b2} and R_{g1} saponins. The contributions of ginsenosides in the leaf extract were different from those mentioned before. Ginsenoside R_{b2} was detected at a concentration of 12.29 mg g^{-1}. The contribution of this compound was about 50.5% to the total content. Comparing the contribution of R_d, we found that its level was significantly higher in leaves (26.2%) than in root (17.7%). Similarly as in Xie et al. [15], Ligor et al. [11] and Wang et al. [14] studies, other ginsenoside contributions to the total concentration were as follows: R_e 16.8%, R_c 2.0%, R_{b1} 3.0%, R_{g1} 1.5%. Generally, American ginseng roots contained higher amount of ginsenosides belonging to PPTs (group of saponins with 20(S)-protopanaxatriol skeleton) in comparison to the leaves, thus the ratio of PPTs (protopanaxatriol) to PPDs (protopanaxadiol) tested in the case of roots reached 0.44, being twice as low in its leaves.

In total, 72 volatile compounds were detected, 55 of which occurred in the root hexane extract, 32 of which were found in the root ethanol extract, and 45 of which were identified in hexane and 33 in ethanol extracts of ginseng leaves, respectively (Table 5). The primary compounds (concentration

Table 5. **Results of GC-MS screening of extracts from above- and underground parts of American ginseng**

Constituents	Lit. RI	Exp. RI	Concentration, %			
			Roots		Leaves	
			H*	E*	H	E
α-Pinene	939	939	0.5	–*	0.4	–
β-Pinene	979	982	0.3	–	0.6	–
p-Cymene	1 025	1 027	tr*	–	–	–
Limonene	1 029	1 030	tr	–	1.1	–
γ-Terpinene	1 060	1 060	–	–	0.5	–
Undecane	1 100	1 096	–	–	1.0	–
Tetrahydro-lavandulol	1 162	1 153	–	–	0.4	–
trans-Verbenol	1 145	1 154	0.6	–	–	–
Menthone	1 153	1 165	0.7	–	3.6	–
Verbenone	1 205	1 219	tr	–	–	–
Pulegone	1 237	1 258	–	–	1.6	–
Bornyl acetate	1 289	1 294	0.6	2.3	–	1.5
α-Cubebene	1 351	1 351	tr	–	–	–
α-Longipinene	1 353	1 359	–	–	0.8	–
Isoledene	1 376	1 373	–	–	0.8	–
α-Ylangene	1 375	1 377	0.2	–	–	–
α-Copaene	1 377	1 381	0.3	–	–	–
β-Burbonene	1 388	1 389	0.1	–	–	–
β-Elemene	1 391	1 396	0.3	–	–	–
Tetradecane	1 400	1 398	tr	5.8	–	–
α-Gurjunene	1 410	1 413	0.9	0.8	–	3.9
β-Funebrene	1 415	1 419	0.2	–	–	–
(*E*)-Caryophyllene	1 419	1 427	0.6	0.5	0.3	0.6
β-Copaene	1 432	1 441	1.0	–	–	0.4
Aromadendrene	1 441	1 452	0.1	–	–	–
(*Z*)-β-Farnesene	1 443	1 459	0.6	1.4	–	0.8
α-Humulene	1 455	1 469	0.7	–	–	0.6
γ-Gurjunene	1 477	1 486	3.1	–	2.0	1.2

Table 5. **Continued**

Constituents	Lit. RI	Exp. RI	Concentration, %			
			Roots		Leaves	
			H*	E*	H	E
α-Amorphene	1 485	1 490	tr	1.1	0.2	0.7
β-Selinene	1 490	1 504	1.6	0.9	0.9	0.8
α-Selinene	1 498	1 510	–	0.9	0.6	0.6
Butylated hydroxytoluene	1 516	1 513	30.3	–	48.2	–
Phenol,2,4-bis(1,1-dimethylethyl)-	1 519	1 519	0.3	4.8	0.7	5.8
δ-Cadinene	1 523	1 526	0.4		0.2	–
δ-Amorphene	1 512	1 529	1.0	1.0	0.4	0.7
trans-Calamenene	1 529	1 533	tr		–	–
(*E*)-γ-Bisabolene	1 531	1 534	–	1.0	1.7	0.7
2(4H)-Benzofuranone,5,6,7,7a-tetrahydro-4,4,7-a-trimethyl-	1 471	1 549	0.2	–	–	–
α-Calacorene	1 546	1 554	0.2	–	–	–
Germacrene B	1 561	1 559	1.5	–	3.4	–
α-Copaen-11-ol	1 541	1 572	tr	–	0.4	–
Ledol	1 569	1 581	0.8	–	-	–
Longipinanol	1 569	1 582	–	–	0.4	–
Spathulenol	1 578	1 589	3.4	3.7	1.4	3.6
Caryophyllene oxide	1 583	1 592	0.8	0.5	0.5	0.8
Globulol	1 585	1 597	1.2	–	0.3	–
Hexadecane	1 600	1 600	–	1.0	–	1.2
Salvial-4(14)-en-1-one	1 595	1 604	–	1.1	1.1	2.1
Thujopsan-2-alpha-ol	1 587	1 605	2.5	–	–	–
Viridiflorol	1 593	1 617	2.5	0.7	0.7	0.8
Humulene epoxide II	1 608	1 623	1.5	0.5	0.5	1.0
I		1 630	2.4	4.9	1.1	3.2
Aristolene epoxide	1 724	1 632	1.4	–	–	–
Aromadendrene oxide-(2)	1 641	1 647	1.0	–	0.2	0.3
Epoxy allo-alloaromadendrene	1 636	1 662	1.3	0.7	0.5	0.3
Selina-3,11-dien-6-alpha-ol	1 644	1 672	3.0	–	0.8	–
Cedr-8(15)-en-10-ol	1 652	1 674	0.9	–	–	–
14-Hydroxy-9-epi-(E)-caryophyllene	1 670	1 687	0.3	0.4	0.1	–
Apiole	1 678	1 700	–	0.8	0.4	0.6
Khusinol	1 680	1 709	2.7	1.9	1.0	1.2
2-Methyl-4-(2,6,6-trimethylcyclohex-1-enyl)but-2-en-1-ol	1 735	1 713	0.2	0.8	0.3	1.4
8-Cedren-13-ol	1 689	1 724	1.1	–	0.6	–
14-Hydroxy-α-humulene	1 714	1 742	–	0.9	0.4	0.3
Oplopanone	1 740	1 752	–	–	0.1	–
Cyclocolorenone	1 761	1 769	12.1	5.5	5.3	3.5
II	2 119	1 836	0.1	–	2.1	–
2-Pentadecanone,6,10,14-trimethyl-	1 809	1 845	–	1.8	1.1	2.7
III	2 317	1 868	–	10.2	2.1	22.1
Fluorensadiol	1 870	1 873	0.3	–	1.1	–
Methyl hexadecanoate	2 210	1 929	–	3.3	–	4.2
IV		1 968	–	8.0	–	9.5
Ethyl hexadecanoate	1 993	1 996	–	7.6	–	7.1
Falcarinol	2 036	2 051	14.0	1.1	–	–
9,12-Octadecadienioc acid, methyl ester	2 113	2 159	–	3.9	–	2.3

* E – ethanol, H – hexane, n. i. – not identified, tr – trace (<0.1%), – – not determined, RI – retention indices.

Mass spectra of unknowns identified tentatively:

GC/MS, 70eV, 220°C, m/z (rel. int.):

I: no M^+, 204(1), 157(3), 131(6), 120(2), 118(2), 116(3), 106(3), 104(5), 93(4), 91(8), 81(3), 79(5), 76(4), 67(4), 55(3), 44(48), 40(100), 38(19);

II: no M^+, 206(1), 123(9), 109(7), 97(7), 96(8), 95(25), 83(9), 81(17), 79(7), 71(7), 70(4), 69(15), 68(14), 67(26), 57(14), 56(3), 55(14), 44(37), 41(16), 40(100), 39(10);

III: no M^+, 223(2), 207(1), 167(2), 150(6), 149(64), 105(1), 104(4), 95(1), 93(2), 77(3), 76(6), 69(3), 57(12), 56(3), 55(3), 50(3), 44(40), 40(100), 39(6);

IV: no M^+, 288(2), 214(2), 212(2), 165(1), 163(4), 93(2), 78(3), 77(3), 74(4), 58(2), 55(3), 45(11), 44(69), 40(100).

>5.0%, calculated as a percentage peak area of GC-MS) detected in hexane root extracts were as follows: butylated hydroxytoluene, cyclocolorenone and falcarinol (panaxynol), whereas butylated hydroxytoluene and cyclocolorenone in the hexane extract of leaves. Butyl octyl ester and butyl-2-ethylhexyl ester of 1,2-benzenedicarboxylic acid (phthalates) and hexadecanoate ethyl were the main compounds found in ethanol extracts of American ginseng leaves and roots. Four unknown compounds were detected and their mass spectra are shown under Table 5.

Antibacterial activity of ethanol and hexane extracts made of *Panax quinquefolium* leaves and roots is expressed as MIC values (Table 6) and antimutagenic activity of the studied extracts is shown in Table 7. All extracts are characterized by a quite high biological activity (Tables 6 and 7). Our studies indicate that both ethanol and hexane extracts acted strongly on Gram-positive than Gram-negative bacteria. Contrary to Ludwiczuk et al. [3] experiments, the strongest action on Gram-positive bacteria was shown by ethanol extracts of *P. quinquefolius* root (MIC values: 5–10 mg/ml) when compared with corresponding leaf extracts (MIC values: >100 mg/ml). Ethanol extract was effective in the case of *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Klebsiella pneumonia* and *Bacillus subtilis*. Moreover, ethanol extracts of roots showed stronger antibacterial action compared with hexane ones (from 10 to 75 mg/ml). Extracts inhibited microorganism growth by the means of different mechanisms, which are partially due to the hydrophobic nature of some components. As a result, they can show greater interaction with a cell membrane lipid bilayer, affecting both respiratory chain and energy production [22]. Interference with bacterial enzyme systems can also be a potential mechanism of action. In the case of Gram-negative bacteria, all tested extracts exhibited weak activity (MIC > 100 mg/ml), which was in accordance with Ludwiczuk et al. [3] studies. The presence of a high content of sesquiterpenoid cyclocolorenone (5–12%) and polyacetylene falcarinol (1–14%) in the studied extracts made from roots contributed to express anti-bacterial activity investigated in the present study. Cyclocolorenone is a sesquiterpene ketone with the proven antibacterial and antifungal activity, found also in *Magnolia* and *Solidago* species [23, 24]. The higher amount of this compound was found in ethanol and hexane extracts of ginseng roots (12.1 and 5.3%, respectively), whereas in leaf extracts its content was distinctly lower. It is worth to underline that the cyclocolorenone content was clearly connected with the antibacterial activity of the ginseng extracts studied. What is more, Jacyno et al. [23] observed that cyclocolorenone was extremely effective against Gram-positive bacteria, which was in accordance with our findings (Table 6). High content of falcarinol in ginseng root extracts (1.1–14.1%) also was responsible for their stronger antibacterial activity. Falcarinol, synonymous with panaxynol, has been variously reported as a skin sensitizer and irritant. It reportedly is a common constituent of many plant parts (from Apiaceae family), but is especially well known from roots of ginseng [25]. This polyacetylenic compound and its derivatives are known to have antifungal activity as well as are toxic to bacteria [26, 27]. Furthermore, apart from the biologically active substances given above, terpenenes or terpenoids are also active

Table 6. **Values of the minimal inhibitory concentration for dry ethanol and hexane extracts from American ginseng leaves and roots**

Bacteria	MIC in mg/ml			
	Ethanol extract		Hexane extract	
	Roots	Leaves	Roots	Leaves
Staphyllococcus aureus ATCC 6538P	0.92	>21.4	0.23	>4
Enterococcus faecalis ATCC 8040	1.83	>21.4	0.58	>4
Bacillus subtilis ATCC 1633	0.92	>21.4	0.23	>4
Escherichia coli PZH 028 B6	>18.3	>21.4	>2.3	>4
Klebsiella pneumoniae 231 UM	>18.3	>21.4	>2.3	>4
Pseudomonas aeruginosa 85/2 IWNRZ	>18.3	>21.4	>2.3	>4

Table 7. **Antimutagenic activity of *Panax quinquefolium* roots and leaves extracts and the reference extract**

Tested extracts	Growth control of *S. typhimurium* TA 1535 from the collection of the ATCC (29629) (spontaneous mutations)	Growth control of *S. typhimurium* TA 1535 from the collection of the ATCC (29629) in the presence of mutagen	Concentration of the liquid extract on a plate (mg/ml)		
			5.0	2.5	1.0
Hexane extracts					
Roots	51*	>1 000*	1*	1*	15*
Leaves	53	>1 000	>1 000	>1 000	>1 000
Ethanol extracts					
Roots	96	>1 000	>1 000	>1 000	>1 000
Leaves	64	>1 000	>1 000	>1 000	>1 000
Reference extract *Dracocephalus moldavica*	38	>1 000	39	>1 000	>1 000

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

against bacteria, whereas flavonoids are synthesized by plants in response to microbial infection and are effective against a broad range of microorganisms. Flavonoids activity probably resulted from their capacity to form complexes with extracellular soluble proteins, which bind with bacterial cell walls. Some lipophilic flavonoids also caused the rupture of microorganism plasma membrane [28]. Stronger antibacterial activity could be associated also with higher triterpene saponins content of ginseng roots, which was in accordance with Aydin and Bagcigil [12] findings.

Ames Salmonella mutagenicity assay is a short-term bacterial reverse mutation assay specifically designed to detect mutagenicity in a wide range of chemical substances that can produce genetic damage leading to gene mutation. In the present study, the antimutagenic effect of hexane and ethanol extracts of American ginseng was studied using the Ames test. Results presented in Table 7 indicate that only hexane extracts of *Panax quinquefolius* roots reflected antimutagenic activity. That extract exhibited antimutagenic activity already at a concentration of 1.0 mg/ml. All ethanol extracts and hexane extract of aboveground parts of American ginseng, in the applied range of concentrations, did not show antimutagenic activity. Antimutagenic action of the reference extract of *Dracocephalus moldavica* obtained under the same conditions of experiment indicates the correctness of the applied technology research (Table 7). These results were in accordance with the previous results showing that *Panax ginseng* extract could suppress tumor promoting activity, induce apoptosis in cancer cells, interfere with cell cycle progression, enhance immune activity and suppress tumour angiogenesis [13, 29]. Apart from saponins [8], flavonoids, phenolics acids and terpenoids are also phytochemicals that possess proven antimutagenic properties, in addition to a wide range of biological activities [30]. Strongly antimutagenic hexane extracts of American ginseng roots contained high amount of falcarinol and BHT. It was found out that polyacetylenes of the falcarinol-type have shown many interesting bioactivities including anti-inflammatory, immune stimulatory, antiplatelet-aggregatory effects and antitumor activity as well as activity against bacteria and mycoplasma [31, 32]. Falcarinol and its derivatives have also been shown to possess cytotoxic [25, 33] and antimutagenic activity *in vitro* [27]. In recent study falcarinol has showed cytotoxic action against various human tumor cell lines and a biphasic effect on cell proliferation of primary mammalian cells at low concentrations [34]. The discussed compound had a protective effect toward the development of colon cancer and other types of cancer. In Purup et al. [32] studies falcarinol had potential to modify cellular survival (having significant inhibitory effects, especially on cancer cell proliferation). On the other side, butylated hydroxytoluene (BHT), a phenol derivative naturally occurring in *Spilanthes acmella* or *Mesembryanthemum crystallinum* hexane extracts, has been widely used for many years as an antioxidant which preserves and stabilizes freshness, nutritive value, flavour and colour of food and animal feed products [35]. Probably due to free radical trapping activity BHT has been shown to inhibit the carcinogenicity of a variety of carcinogens in different tissues in mice and rats [36]. In recent studies [37] BHT was shown to be a very strong antimutagen. Those two components together with ginseng saponins could be responsible for the antimutagenic property of hexane extracts of roots.

CONCLUSIONS

Hexane and ethanol extracts obtained from American ginseng roots are proved to possess beneficial properties and may be safely used in treatment of some diseases. The results showed that hexane extracts of American ginseng roots possess a significantly stronger antimutagenic and antimicrobial activity than its leaves. On the basis of our results it may be concluded that there is a direct relationship between contents of ginsenosides and its bio-activity. However, a strong antimutagenic effect of hexane extracts of American ginseng roots merits attention; if it should be confirmed in studies other than in bacterial systems, it could open up new prospects for the use of this natural substance in the field of human health.

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PANAX QUINQUEFOLIUS CHEMINĖ SUDĖTIS IR KAI KURIOS BIOAKTYVUMO SAVYBĖS

S a n t r a u k a

Panax quinquefolius L. yra vaistinis augalas, tinkantis įvairių žmogaus ligų prevencijai ir gydymui. Darbe buvo tiriama šio augalo cheminė sudėtis naudojant etanolinius ir heksaninius viršutinių ir požeminių augalo dalių ekstraktus. Be to, buvo vertintas antibakterinis ir antimutageninis aktyvumas.