Biological Activity of Selected Plants with Adaptogenic Effect

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Abstract

The aim of this study was to determine biological activity of plants with adaptogenic effect: *Panax ginseng* Mayer., *Withania somnifera* L., *Eleuterococcus senticosus* Rupr. et Maxim., *Astragallus membranaceus* Fisch. and *Codonopsis pilosulae* Franch. The antioxidant activity was detected by DPPH and phosphomolybdenum method, total polyphenol content with Folin – Ciocalteu reagent, flavonoids content by aluminium chloride method. The detection of antimicrobial activity was carried out by disc diffusion method against three species of Gram-negative bacteria: *Escherichia coli* CCM 3988, *Salmonella enterica* subsp. *enterica* CCM 3807, *Yersinia enterocolitica* CCM 5671 and two Gram-positive bacteria: *Bacillus thuringiensis* CCM 19, *Stapylococcus aureus* subsp. *aureus* CCM 2461. Results showed that plants with adaptogenic effect are rich for biologically active substances. The highest antioxidant activity by DPPH method was determined in the sample of *Eleuterococcus senticosus* (3.15 mg TEAC – Trolox equivalent antioxidant capacity per g of sample) and by phosphomolybdenum method in the sample of *Codonopsis pilosulae* (188.79 mg TEAC per g of sample). In the sample of *Panax ginseng* was measured the highest content of total polyphenols (8.10 mg GAE – galic acid equivalent per g of sample) and flavonoids (3.41 μg QE – quercetin equivalent per g of sample). All samples also showed strong antimicrobial activity with the best results in *Panax ginseng* and *Withania somnifera* in particular for species *Yersinia enterocolitica* CCM 5671 and *Salmonella enterica* subsp. *enterica* CCM 3807. The analyzed species of plant with high value of biological activity can be used more in the future, not only in food, but also in cosmetics and pharmaceutical industries.

Keywords: antioxidant activity, adaptogens, polyphenols, flavonoids, antimicrobial activity

1. Introduction

Adaptogens are medicinal plants that enhance the ‘‘state of non-specific resistance’’ of an organism to stress, augmenting resistance to physical, biological, chemical and psychological stresses, and increasing concentration, performance and endurance during fatigue [1].

Adaptogens typically act upon the neuroendocrine immunologic system, which is an all-encompassing description of how the immune system and brain interact with hormones. Plants with adaptogenic effect reduce stress reactions in the alarm phase, or retard/prevent the exhaustion phase, and thus provide a certain degree of protection against long-term stress [2]. Several numbers of drugs whose adaptogenic activity has been proven or reported includes, among others, the plant drugs *Withani*a, *Rhodiol*a, *Ginseng*, *Eleutherococcus*, *Astragallus* and *Codonopsis*. *Withania* has immunomodulatory, anti-inflammatory but most significantly adaptogenic effects, which may result from the complex of the many steroidal withanolides found in the root of

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the herb [2]. The adaptogenic properties of *Ginseng* are believed to be due to its effects on hypothalamic pituitary adrenal axis, resulting in elevated plasma corticotropin and corticosteroids levels [3]. *Eleutherococcus* helps enhance the body's immune system and improve rehabilitation of any physiological, biochemical or immunological defects. Biological and pharmacological effects of *Eleutherococcus* include antioxidant, antidiabetes, anticancer, antiinflammatory, immunoregulatory and immunomodulating, antimicrobial and antiviral activities [4]. *Astragallus* is proved to have the function of enhancing immunity, anti-inflammatory, antivirus, antitumor, antioxidant and delaying senescence [5]. *Codonopsis* contains a variety of active ingredients, such as polysaccharides, phenols, saponins, alkaloids and so on. *Codonopsis* polysaccharide is an important active ingredient and possesses antioxidant, immune-enhancing, antitumor and other pharmacological activities [6]. Plants with adaptogenic effect are rich for biologically active compounds specially polyphenols, which have multiple biological effects, including antioxidant activity. The antioxidant activity of polyphenols is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donators, and singlet oxygen quenchers. In addition, they have a metal chelation potential [7]. The polyphenol compounds are increasingly of interest in the food industry because they retard oxidative degradation of lipids, inhibit bacterial and yeast activity and thereby improve the quality and nutritional value of food [8].

The aim of this study was to evaluated antioxidant and antimicrobial activity of selected plants with adaptogenic effect to human body.

2. Materials and methods

2.1 Biological materials

The plants with adaptogenic effect were purchased from market. It was used: *Panax ginseng* Mayer. – root; *Withania somnifera* L. – root; *Eleuterococcus senticosus* Rupr. et Maxim. – root; *Astragallus membranaceus* Fisch. – root and *Codonopsis pilosulae* Franch. – root. Before the analysis samples were pulverized in the mortar.

2.2 Chemicals

All chemicals were analytical grade and were purchased from Reachem (Slovakia) and Sigma Aldrich (USA).

2.3 Sample preparation

An amount of 0.1 g of sample was extracted with 20 ml of 80% ethanol for 24 hours. After centrifugation at 4000 g (Rotofix 32 A, Hettich, Germany) for 10 min, the supernatant was used for measurement (antioxidant activity, polyphenols, flavonoids). Extraction was carried out in triplicate.

2.4 Radical scavenging activity

Radical scavenging activity of extracts was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH) [9]. The sample (0.4 ml) was mixed with 3.6 ml of DPPH solution (0.025 g DPPH in 100 ml methanol). Absorbance of the reaction mixture was determined using the spectrophotometer Jenway (6405 UV/Vis, England) at 515 nm. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (10-100 mg/L; R^2=0.989) was used as the standard and the results were expressed in mg/g Trolox equivalents.

2.5 Reducing power

Reducing power of extracts was determined by the phosphomolybdenum method of Prieto et al. [10] with slight modifications. The mixture of sample (1 ml), monopotassium phosphate (2.8 ml, 0.1 M), sulfuric acid (6 ml, 1 M), ammonium heptamolybdate (0.4 ml, 0.1 M) and distilled water (0.8 ml) was incubated at 90°C for 120 min, then rapidly cooled and detected by monitoring absorbance at 700 nm using the spectrophotometer Jenway (6405 UV/Vis, England). Trolox (10-1000 mg/L; R^2=0.998) was used as the standard and the results were expressed in mg/g Trolox equivalents.

2.6 Total polyphenol content

Total polyphenol content extracts was measured by the method of Singleton and Rossi [11] using Folin-Ciocalteu reagent. 0.1 ml of each sample was mixed with 0.1 ml of the Folin-Ciocalteu reagent, 1 ml of 20% (w/v) sodium carbonate, and 8.8 ml of distilled water. After 30 min. in darkness the absorbance at 700 nm was measured using the spectrophotometer Jenway (6405 UV/Vis, England). Gallic acid (25-300 mg/L; R^2=0.998)

was used as the standard and the results were expressed in mg/g gallic acid equivalents.

2.7 Total flavonoid content

Total flavonoids were determined using the modified method of Willett, [12]. 0.5 ml of sample was mixed with 0.1 ml of 10% (w/v) ethanolic solution of aluminium chloride, 0.1 ml of 1 M potassium acetate and 4.3 ml of distilled water. After 30 min. in darkness the absorbance at 415 nm was measured using the spectrophotometer Jenway (6405 UV/Vis, England). Quercetin (0.5-20 mg/L; R^2=0.989) was used as the standard and the results were expressed in μg/g quercetin equivalents.

2.8 Microbial strains

Five strains of microorganisms were tested in this study, Gram-negative bacteria: *Escherichia coli* CCM 3988, *Salmonella enterica* subsp. *enterica* CCM 3807, *Yersinia enterocolitica* CCM 5671 and two Gram-positive bacteria: *Bacillus thuringiensis* CCM 19, *Stapylococcus aureus* subsp. *aureus* CCM 2461. All tested strains were collected from the Czech Collection of microorganisms. The bacterial suspensions were cultured in the nutrient broth (Imuna, Slovakia) at 37°C.

2.9 Disc diffusion method

Antimicrobial activity of each plant extract was determined by a disc diffusion method. Briefly, 100 μl of the test bacteria were grown in 10 ml of fresh media until they reached a count of approximately 10^5 cells/ml. Then 100 μl of the microbial suspension was spread onto Mueller Hinton agar plates. The extracts were tested using 6 mm sterilized filter paper discs. The diameters of the inhibition zones were measured in millimeters. All measurements were to the closest whole millimeter. Each antimicrobial assay was performed in at least triplicate. Filter discs impregnated with 10 μl of distilled water were used as a negative control.

2.10 Microbroth dilution method

MICs were determined by the microbroth dilution method according to the Clinical and Laboratory Standards Institute recommendation [13] in Mueller Hinton broth (Biolife, Italy). Briefly, the DMSO plant extracts solutions were prepared as serial two-fold dilutions obtaining a final concentration ranging between 0.5-512 μg/ml. After that each well was inoculated with microbial suspension at the final density of 0.5 McFarland. After 24 h of incubation at 37°C, the inhibition of microbial growth was evaluated by measuring the well absorbance at 450 nm in an absorbance microplate reader Biotek EL808 with shaker (Biotek Instruments, USA). The 96 microwell plates were measured before and after experiment. Differences between both measurements were evaluated as growth. Measurement error was established for 0.05 values of absorbance. Wells without plant extracts were used as negative controls of growth. Pure DMSO was used as negative control. This experiment was done in eight-replicates for a higher accuracy of the MICs of used medical plant extracts.

2.11 Statistical analysis

Differences in absorbance between the measurements before and after the analysis were expressed as a set of binary values. These values were assigned to exact concentrations. The following formula was created for this specific experiment: value 1 (inhibitory effect) was assigned to absorbance values lower than 0.05, while value 0 (no effect or stimulant effect) was assigned to absorbance values higher than 0.05. For this assigned to absorbance values higher than 0.05. For this statistical evaluation the probit analysis in Statgraphics software was used.

3. Results and discussion

3.1 Radical scavenging activity and reducing power

DPPH$^{\bullet}$ radical discoloration degree is attributed to hydrogen donating ability of tested compound [9]. The DPPH$^{\bullet}$ radical efficiency values of plants analyzed in this study are presented in Figure 1.

The best activity was detected in the sample of *Eleuterococcus* 3.15 mg TEAC/g and *Panax ginseng* 2.68 mg TEAC/g. In sample of *Withania* was found the lowest activity (0.02 mg TEAC/g).

Kim et al. [14] also determined antiradical activity of *Eleuterococcus* ethanol, butanol and water extract from root, stem and leaf by DPPH method. All plant parts and extracts showed strong activity which was greater than activity of α-tocopherol. Chung et al. [15] reported that antioxidant activity of *Panax ginseng* root is 3-5 fold lower with

compare to the fruit. It was suggested that the electron donating capacity is associated with bioactive compounds, reflecting the reducing power of antioxidant activity. Antioxidants can be reductants, and inactivation of oxidants by reductants can be described as redox reactions in which one reaction species is reduced at the expense of the oxidation of the other. The presence of reductants, such as antioxidant substances in the samples, causes the reduction of the Mo^{VI} to Mo^{V}. The reducing power of the plant extracts increased with increasing concentration, which suggests that the electron donating ability of the extracts is concentration dependent [16]. Reducing power results are presented in Figure 2. The best reducing power was evaluated in sample of *Codonopsis* (188.80 mg TEAC/g) and *Astragalus* (80.91 mg TEAC/g). Chang-Sion and Sung-Jin [17] reported that *Codonopsis* extract is rich for antioxidant compounds with strong reducing power and also found that *Codonopsis* extract significantly reduces the concentration of NO (nitric oxide) generated by LPS (lipopolysaccharide) raw cells and shows strong inhibition of iNOS expression (inducible nitric oxide synthase). Li et al. [18] reported that *Codonopsis* root can effectively protect against hydroxyl-induced DNA damage. One mechanism of protective effect may be radical-scavenging which is via donating hydrogen atom ($H^{\bullet}$), donating electron. Its antioxidant ability can be mainly attributed to the existences of flavonoids or phenolic acids.

3.2 Total polyphenol a flavonoid content

Polyphenols have been implicated in antioxidant activity of medicinal and culinary herbs, fruits and vegetables and derived beverages [19]. Table I shows total polyphenol content evaluated plants with adaptogenic effect. The highest value of polyphenols was measured in *Panax* extract – 8.11 mg GAE/g and *Astragalus* – 6.33 mg GAE/g. Bouazis et al. [20] determined polyphenols content in *Astragalus* and also confirmed high value of these compounds (874 pyE – propygallol equivalent/100 g), and also reported that methanol gives the highest extraction yields of phenolics, followed by ethyl acetate. Water and hexane was less efficient in their study.

According to Chung et al. [15] the dominant compounds from polyphenol groups are chlorogenic acid, gentisic acid, *p*- and *m*-coumaric acid, and rutin. In *Astragalus* caffeic acid, chlorogenic acid, gentisin and emodin can be found from polyphenol groups [18].

The content of flavonoids (Table1) in observed plants ranged from 0.11 to 3.41 µg QE/g, with the best results in sample of *Panax* and *Codonopsis*. Chung et al. [15] described 23 phenolic compounds in *Panax* fruit, leaves and root and reported that in leaves are dominant phenolic acid, whereas in root flavonoinds, mainly naringenin.

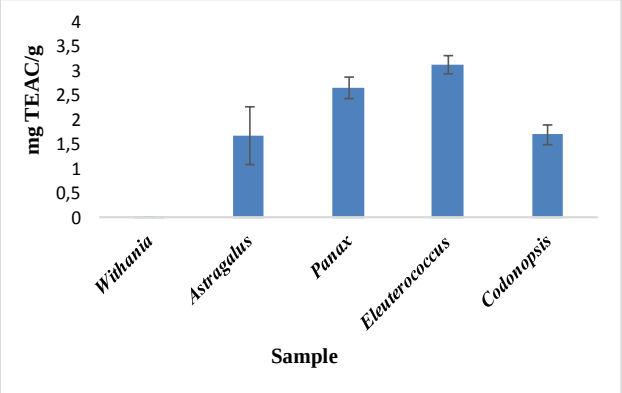

Figure 1. Radical scavenging activity of selected plants with adaptogenic effect

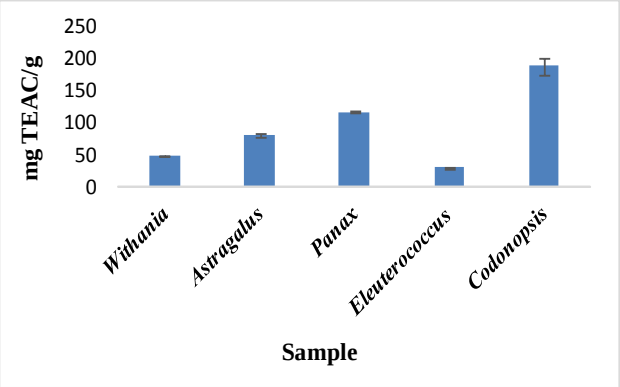

Figure 2. Reducing power of selected plants with adaptogenic effect

Table 1. Total polyphenol and flavonoid content of selected plants with adaptogenic effect

Sample	Total polyphenol content [mg GAE/g]	Total flavonoidd content [µg QE/g]
Withania	4.44 ±0.65	0.11 ±0.05
Astragalus	6.33 ±1.29	0.78 ±0.18
Panax	8.11 ±0.36	3.41 ±0.43
Eleuterococcus	2.92 ±0.64	1.21 ±0.16
Codonopsis	5.81 ± 1.08	1.80 ±0.25

± standard deviation; GAE – galic acid equivalent; QE – quercetin equivalent

3.3. Antimicrobial activity

In our study of plants with adaptogenic effect: *Panax ginseng* Mayer., *Withania somnifera* L., *Eleuterococcus senticosus* Rupr. et Maxim., *Astragallus membranaceus* Fisch. and *Codonopsis pilosulae* Franch. were tested against four different strains of Gram-negative bacteria: *Escherichia coli* CCM 3988, *Salmonella enterica* subsp. *enterica* CCM 3807, *Yersinia enterocolitica* CCM 5671 and two Gram-positive bacteria: *Bacillus thuringiensis* CCM 19, *Stapylococcus aureus* subsp. *aureus* CCM 2461. with disc-diffusion and MIC methods.

The results of disc diffusion method showed that *Withania somnifera* exhibited the highest antibacterial activity with 8.33 resp. 6.67 mm zone of inhibition against *Y. enterocolitica*, *Salmonella enterica* subsp. *enterica* and *Bacillus thuringiensis* and *Panax ginseng* exhibited the highest antibacterial activity with 5.33 mm zone of inhibition against *Salmonella enterica* subsp. *enterica* (Figure 2).

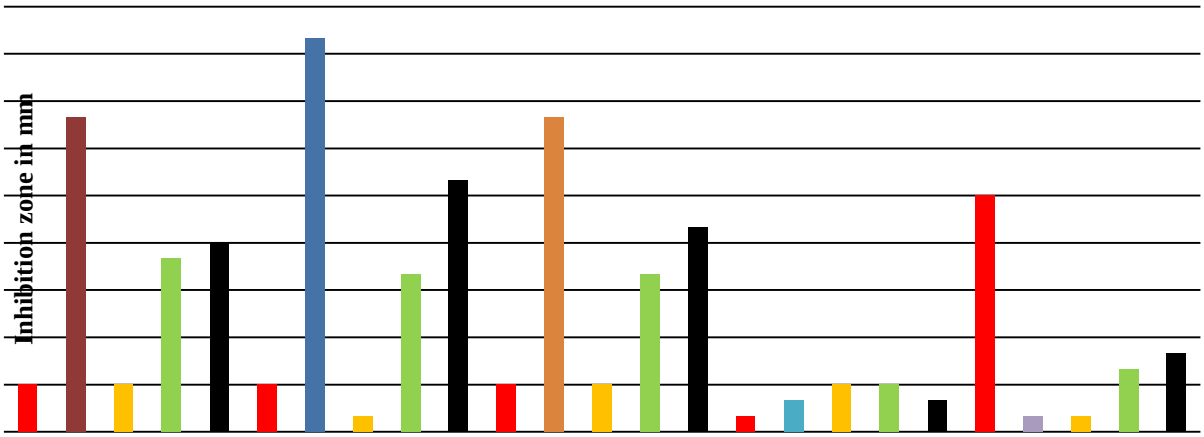

Figure 3 Antibacterial activity of plants with adaptogenic effect detected by disc diffusion method
EX1-Astragallus membranaceus; EX2-Withania somnifera; EX3-Codonopsis pilosulae; EX4-Eleuterococcus senticosus; EX5-Panax ginseng

Table 2. MIC of plants with adaptogenic effect in µg/ml

Microorganisms	Extract									
	EX1		EX2		EX3		EX4		EX5	
	MIC50	MIC90	MIC50	MIC90	MIC50	MIC90	MIC50	MIC90	MIC50	MIC90
EC	12.79	13.60	12.79	13.60	> 34.13	> 34.13	17.07	19.05	8.53	9.54
YE	12.79	13.60	12.79	13.60	51.29	81.69	12.79	13.60	12.79	13.60
BT	8.53	9.54	12.79	13.60	> 34.13	> 34.13	17.07	19.05	12.79	13.60
SA	12.79	13.60	17.07	19.05	> 34.13	> 34.13	6.40	6.82	12.79	13.60
SE	12.79	13.60	12.79	13.60	> 34.13	> 34.13	12.79	13.60	12.79	13.60

EX1-Astragallus membranaceus; EX2-Withania somnifera; EX3-Codonopsis pilosulae; EX4-Eleuterococcus senticosus; EX5-Panax ginseng

Minimal inhibition concentration of *Astragallus membranaceus* ranged from 8.53 in MIC50 resp. 9.54 in MIC 90 to 12.79 resp. 13.60 µg/ml, MIC of *Withania somnifera* ranged from 12.79 in MIC50 resp. 13.60 in MIC 90 to 17.07 resp. 19.05 µg/ml and MIC of *Panax ginseng* from 8.53 resp. 9.54 to 12.79 resp. 13.60 µg/ml (Table 2).

The best antimicrobial activity according to MIC was found in *Eleuterococcus senticosus* against *Stapylococcus aureus*.

In study of Balachandar et al. [21] the extracts were tested in disc diffusion assays against Diarrheal bacterial pathogens *Escherichia coli*, *Salmonella enteritidis*, *Shigella* and *Campylobacter*. The results of antibacterial

activity revealed that all the extract showed good inhibitory activity against all the tested pathogens. Methanolic extract of *W. somnifera* showed potent antibacterial activity against Gram-positive clinical isolates [22].

The antibacterial activity of polysaccharides extracted from desulfurized *C. pilosula* was higher than that of polysaccharides extracted from sulfur-fumigated herbs, with the MIC values of 35 and 70 mg/ml, respectively [23].

In addition, the essential oil of *Eleuterococcus senticosus* showed remarkable antimicrobial activity against *Kocuria rhizophila* (MIC=125 µg/ml), *Micrococcus luteus* (MIC=500 µg/ml), and *Escherichia coli* (MIC=63 µg/ml) [24].

In study of Lee et al. [25] all ginseng extracts inhibited the growth of *Bacillus cereus, Salmonella enteritidis, Escherichia coli* O157:H7 and *Listeria monocytogenes*.

4. Conclusions

In conclusion, our results indicate that plants with adaptogenic effect to human body are rich for biologically active compounds with antioxidant and antimicrobial activity. These findings can be interesting for food industry, whereas these plants can be used for enriched dairy products, mainly bakery products and beverages.

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