Antioxidant Activity and Total Polyphenol Content of Medicinal Herbs with Adaptogenic Effect to Human Body

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Abstract
Nowadays, medicinal herbs with an adaptogenic effect to human body start be very popular not only in medicine but also in food industry due to the high amount of bioactive compounds. The aim of this study was to determine antioxidant activity, total polyphenol, flavonoid and phenolic acid content of five plants with adaptogenic affect to human body: *Eurycoma longifolia* Jack., *Lepidium meyenii* Walp., *Turnera diffusa* Wild., *Tribulus terrestris* L. and *Rhodiola rosea* L. The antioxidant activity was detected by DPPH and phosphomolybdenum method, total polyphenol content with Folin – Ciocalteu reagent, flavonoids content by aluminium chloride method and total phenolic acid content by Arnova reagent. The highest antioxidant activity by DPPH and phosphomolybdenum method was determined in the sample of *Rhodiola rosea* L. (6.82 mg TEAC – Trolox equivalent antioxidant capacity per g of sample and 315.37 mg TEAC per g). In this sample was also measured the highest content of total polyphenols (76.64 mg GAE – gallic acid equivalent per g of sample) and phenolic acid (25.10 mg CAE – caffeic acid equivalent per g of sample). Total flavonoid content (20.80 mg QE – quercetin equivalent per g of sample) was the highest in sample of *Tribulus terrestris* L. Results showed that medicinal herbs with adaptogenic effect are rich for polyphenol compounds with antioxidant activity and can be use more in food industry as functional food additives.

Keywords: biological activity, flavonoids, phenolic acids, adaptogenic plants.

1. Introduction

Adaptogens are substances that enable the normalization of physiologic responses to various stressors, enhance work performance, and increase the stress tolerance of the body [1]. Various plants which have been used for hundreds of years in folk medicine show adaptogenic effect to human body. They are available as adaptogenic drugs which increase the body's resistance to physical, biological, emotional and environmental stressors in nonspecific manner. Also, increase of attention, end urance in fatigue, reduction of stress-induced impairments and disorders related to the neuroendocrine and immune systems to balance and maintenance of homeostasis in the body are reported [2,3]. Some herbalists claim that adaptogenic herbs are distinct from other substances in their ability to balance endocrine harmones and the immune system, and that they help [4]. A number of plants possess adaptogenic activity due to diverse classes of chemical compounds. *Eurycoma longifolia* Jack. (known as tongkat ali) is popular traditional herbal medicine, is a flowering plant of the family *Simaroubaceae*, native to Indonesia, Malaysia, Vietnam and also Cambodia, Myanmar, Laos and Thailand. The roots extract of this plant are used as folk medicine for sexual dysfunction, aging, malaria,

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cancer, diabetes, anxiety, aches, constipation, exercise recovery, fever and increased energy [5].
Lepidium meyenii Walp. is a biennial herbaceous plant from family *Brassica*e, which is cultivated mainly in the central Andes of Peru. The root extracts from this plant improving fertility, sexual performance, growth rate, antipostmenopausal osteoporosis, and improving ability in vitality and stress tolerance [6]. *Turnera diffusa* Willd. is a medicinal plant from family *Turneraceae* traditionally used as stimulant, diuretic and aphrodisiac; it is also commonly used for the preparation of infusions and liquors, and in the production of cosmetic products [7]. *Tribulus terrestris* L. deciduous herb of the *Zygophyllaceae* family is an important herb from Indian and Chinese traditional medicine literature for the treatment of various diseases especially ischemic heart diseases. It also has hypoglycemic, hypolipidemic, nephroprotective, aphrodisiac and immunomodulator activities [8]. *Rhodiola rosea* L. is a medicinal plant from the *Crassulaceae* family, with the main active substance salidroside, a phenylpropanoide with the stimulant and antistress actions [9].
The aim of this study was to determine antioxidant activity, total polyphenols, flavonoids, phenolics acid content in selected medicinal plants with adaptogenic effect to human body.

2. Materials and methods

2.1 Biological materials
The analyzed plants were purchased from local market (SK): *Eurycoma longifolia* Jack. – root, *Lepidium meyenii* Walp. – root, *Turnera diffusa* Wild. – leaves, *Tribulus terrestris* L. – fruit and *Rhodiola rosea* L – 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 0.2 g of sample was extracted with 20 ml of 80% ethanol for 2 hours. After centrifugation at 4000 g (Rotofix 32 A, Hettich, Germany) for 10 min, the supernatant was used for measurement (antioxidant activity, polyphenols, flavonoids, phenolic acids). Extraction was carried out in triplicate.

2.4 Radical scavenging activity – DPPH method
Radical scavenging activity of extracts was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH) [10]. 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 Phosphomolybdenum method
Phosphomolybdenum method of extracts was determined by the method of Prieto et al. [11] 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 [12] 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, [13]. 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 mg/g quercetin equivalents.

2.8. Total phenolic acid content

Total phenolic acids content was determined using method of Farmakopea Polska [14]. A 0.5 mL of sample extract was mixed with 0.5 mL of 0.5 M hydrochloric acid, 0.5 mL Arnova reagent (10% $NaNO_2$ +10% Na_2MoO_4), 0.5 mL of 1 M sodium hydroxide (w/v) and 0.5 mL of water. Absorbance at 490 nm was measured using the spectrophotometer Jenway (6405 UV/Vis, England). Caffeic acid (1 – 200 mg.L-1 , R^2 = 0.999) was used as a standard and the results were expressed in mg/g caffeic acid equivalents

2.9 Statistical analysis

All experiments were carried out in triplicate and the results reported are the results of those replicate determinations with standard deviations. Correlation coefficients were calculated by CORR analysis ($P \leq 0.05$) [15].

3. Results and discussion

3.1. Radical scavenging activity – DPPH method and phosphomolybdenum method

Results showed that all tested medicinal herbs exhibited antioxidant activity (Tab.1). By DPPH method the highest activity was determined in *Rhodiola rosea* L. (6.82 mg TEAC/g) followed by *Turnera diffusa* Wild., *Tribulus terrestris* L., *Lepidium meyenii* Walp. and *Eurycoma longifolia* Jack. By phosphomolybdenum method the best activity showed sample of *Rhodiola rosea* L. (315.37 mg TEAC/g) following by *Turnera diffusa* Wild., *Lepidium meyenii* Walp., *Eurycoma longifolia* Jack. and *Tribulus terrestris* L. Chiang et al. [1] reported that *Rhodiola rosea* showed strong antioxidant activity – singlet oxygen scavenging, H_2O_2 scavenging, hypochlorite scavenging, ferric reducing, ferrous chelating, and protein thiol protection activities. Zhou et al. [16] determined antioxidant activity of *Rhodiola* oligomeric proanthocyanidin by DPPH and found strong activity which was better like activity of vitamin C. Wong-Paz et al. [17] determined strong antioxidant activity in *Turnera diffusa*, and published results from 76 to 92% inhibition of DPPH radical. In our study extract from *Turnera* also exhibited strong activity in both used method for antioxidant activity. Strong activity was also determined in sample of *Lepidium meyenii* mainly in phosphomolybdenum method. Sandoval et al. [18] detected also strong activity of *Lepidium meyenii* by DPPH method which ranged depending on concentration of extract from 22% (0.03 mg/ml) to 71% (3 mg/ml). By DPPH method was good activity also found in sample of *Tribulus terrestris* – 5.53 mg TEAC/g. Similar results also published Zheleva-Dimitrova et al. [19] which found activity by DPPH method calculated as IC 50 between 2.84 to 4.56 mg/ml.

Table 1 Antioxidant activity of evaluated medicinal herbs

Sample	DPPH [mg TEAC/g]	Phosphomolybdenum method [mg TEAC/g]
Eurycoma	0.29 ±0.11	78.32 ±2.01
Lepidium	2.21 ±0.19	90.60 ±3.35
Turnera	6.60 ±0.74	165.06 ±2.52
Tribulus	5.53 ±0.22	70.90 ±1.58
Rhodiola	6.82 ±0.32	315.37 ±5.63

mean ± standard deviation; TEAC – trolox equivalent antioxidant capacity

3.2. Total polyphenol, flavonoid and phenolic acid content

Results of total polyphenol, flavonoid and phenolic acid content are presented in Tab. 2. The highest total polyphenols was determined in extract from *Rhodiola rosea* L. (76.64 mg GAE/g) followed by *Turnera diffusa* Wild., *Tribulus terrestris* L., *Lepidium meyenii* Walp. and *Eurycoma longifolia* Jack. Similar results also published Kumar et al. [20] which determined in *Rhodiola sp*. root 79.21 mg GAE/g total polyphenols. Vangalapati et al. [21] determined in *Tribulus* extract amount of total polyphenols between 5-25 mg GAE/g, which is comparable to our findings – 15.06 mg GAE/g in *Tribulus* sample.

The highest total flavonoids was determined in extract from *Turnera diffusa* Wild (42.00 mg QE/g) followed by *Tribulus terrestris* L., *Eurycoma longifolia* Jack., *Lepidium meyenii* Walp. and *Rhodiola rosea* L. Similar findings also reported Chai and Wong [22] which detected in *Turnera diffusa* leaves 53.11 mg QE/g flavonoids which was the highest value with compare to total

flavonoids in stem, root, flowers and fruits. Raj et al. [23] determined with compared to our results several higher amount of flavonoids in sample of *Rhodiola* – 269 mg QE/g which can be explain by used extraction process. In our study we used extraction with 80% ethanol in room temperature; in their study was used 80% ethanol – Soxhlet apparatus by which extracted higher amount of flavonoids compounds.

The highest total phenolic acids was determined in extract from *Turnera diffusa* Wild (28.35 mg CAE/g) followed by *Rhodiola rosea* L., *Eurycoma longifolia* Jack., *Tribulus terrestris* L. and *Lepidium meyenii* Walp. Szewczyk and Zidorn [24] reported that leaves from Turnera sp. Contain mainly ellagic acid and derivates of *p*-coumaric acid.

Statistically strong correlation ($P \leq 0.05$) was observed in our study between antioxidant activity by DPPH method and between total phenolic acid content ($r^2=0.782$) and between antioxidant activity by phosphomolybdenum method and phenolic acid content ($r^2=0.761$).

Table 2. Total polyphenol, flavonoid and phenolic acid content in evaluated medicinal herbs

Sample	Total polyphenol [mg GAE/g]	Total flavonoid [mg QE/g]	Total phenolic acid [mg CAE/g]
***Eurycoma longifolia* Jack.**	4.20 ±0.12	3.86 ±0.27	6.88 ±1.22
***Lepidium meyenii* Walp.**	9.92 ±1.04	2.82 ±0.11	0.94 ±0.11
***Turnera diffusa* Wild.**	40.04 ±2.52	42.00 ±2.41	28.35 ±2.52
***Tribulus terrestris* L.**	15.06 ±1.12	3.86 ±0.74	5.31 ±1.02
***Rhodiola rosea* L.**	76.64 ±3.04	2.64 ±0.85	25.10 ±2.52

mean ± standard deviation; GAE – gallic acid equivalent; QE – quercetin equivalent; CAE – caffeic acid equivalent

4. Conclusions

Results showed that medicinal herbs with adaptogenic effects are interesting source of bioactive compounds. Antioxidant activity by both used method was the highest in *Rhodiola rosea* L. and in the sample of *Turnera diffusa* Wild. was detected the highest amount of total polyphenols, flavonoids and phenolic acids. In future is also important try to find way how we can used more these herbs not only in folk medicine when is widely used but also in food industry and pharmacology.

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