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HERBAL ADAPTOGENS AND THEIR POTENTIAL ANTIOXIDANT PROPERTIES

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Herbal adaptogens and their potential antioxidant properties. – Z. Gogaľová, J. Poráčová, V. Sedlák, M. Konečná, M. Mydlárová Blaščáková, V. Mirutenko. *– Adaptogens represent herbs with pharmaceutical benefits due to their balancing, tonic and regulative functions. The antioxidant potential of adaptogens is rarely mentioned. The study investigated the antioxidant potential of three adaptogen extracts Aralia mandshurica* (Rupr. et Maxim)*, Schisandra chinensis* (Turcz.) *Baill. and Eleutherococcus senticosus* (Rupr. & Maxim.)*. The antioxidant activity of extracts was determined by the DPPH method. The results were evaluated by comparing of* IC_{50} *values. Aqueous and ethanol extract of Schisandra chinensis had the highest antioxidant activity.*
Key words: *Antioxidant activity, adaptogens, DPPH method,* $IC_{50,}$ *pharmaceutical benefits.*
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Фітоадаптогени та їх потенційні антиоксидантні властивості. – З. Ґоґальова, Я. Порачова, В. Седлак, М. Конечна, М. Мидларова Блашчакова, В. Мірутенко. *– Адаптогени представляють собою трави з корисними фармацевтичними властивостями завдяки їх компенсуючій, тонізуючій та регулюючій функціям. Антиоксидантний потенціал адаптогенів рідко згадується. У роботі досліджено антиоксидантний потенціал трьох адаптогенних екстрактів Aralia mandshurica (Rupr. Et Maxim), Schisandra chinensis (Turcz.) Baill. і Eleutherococcus senticosus (Rupr & Maxim.). Антиоксидантну активність екстрактів визначали методом DPPH. Результати оцінювали шляхом порівняння значень* IC_{50}*. Водний і спиртовий екстракт Schisandra chinensis мав найвищу антиоксидантну активність.*
Ключові слова: *антиоксидантна активність, адаптогени, метод DPPH,* IC_{50}*, корисні фармацевтичні властивості.*
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Introduction

Herbal adaptogens play an important role in the traditional medicine, because they contain phytochemicals which may have the potential therapeutic and preventive effect against various human diseases. Nowadays, diseases are commonly caused by stress and adaptogens could be a great benefit. History of adaptogens dates to ancient India and China. A Russian scientist Dr. Lazarev studied medicinal properties of herbal adaptogens in 1947. He was looking for substances which could improve body resistance, regulate the reaction of human adaptation to all types of stressors and help soldiers improve their performance on the battlefield. Breckham and Dardymov defined adaptogens as non-toxic substances with the potential resistance against multiple stressors (Ray et al. 2016).

Herbal adaptogens belong to medicinal plants. Their substances increase body's resistance to stressors, e.g. fatigue, anxiety and trauma. Adaptogens can eliminate negative effects of stress. Chen et al. (2008) asserts that adaptogens can enhance body's performance by metabolic regulators, e.g. the hypothalamic-pituitary-adrenal (HPA) axis hormones, neuropeptides, biogenic amines, nitric oxide and cytokines. It is assumed, that adaptogens have anti-inflammatory, antidepressant and antioxidant properties. Adaptogens influence the response of body to stressful stimuli by modulating the levels of corticosterone and biosynthesis of eicosanoids. *Aralia mandshurica* (Rupr. et Maxim), *Schisandra chinensis* (Turcz.) Baill. and *Eleutherococcus senticosus* (Rupr. & Maxim.) known as Siberian ginseng belong to the group of adaptogens, which contain phenolic compounds such as phenyl propanoids, phenylethane derivatives and lignans (Chen et al. 2008; Pawar and Shivakumar 2012).

Using of adaptogen extracts containing high levels of polyphenols may not only have adaptogen properties but the antioxidant components of herbal adaptogens may be used into drugs for the prevention and treatment of diseases caused by oxidative stress. Thus herbal adaptogens are a potential raw material for pharmaceutical industry.

Methods

On the base of many antioxidants studies dark colour fruits are potential source of natural antioxidants. We have decided to verify the potential antioxidant activity of *Aralia mandshurica* (Rupr. et Maxim), *Schisandra chinensis* (Turcz.) Baill. and *Eleutherococcus senticosus* (Rupr. & Maxim.) Maxim. known as Siberian ginseng.

Plant material (fruits) was collected in a period of September-October 2014 in Rokytov – locality of the Eastern Slovakia (210 m a.s.l., N 49°05'54", E 21°59'27") and stored at -20 °C until analysis. The average annual temperature is 7.6 °C and the average annual rainfall is 700 - 800 mm in the locality. For the determination of antioxidant properties of selected herbal adaptogens ethanol and aquaeous extracts of fruits *Aralia mandshurica*, *Schisandra chinensis* and *Eleutherococcus senticosus* were used. The extracts were prepared by extraction procedure described by Masteikova et al. 2008. We used distilled water to prepare aqueous extracts. In this way, we attempted to imitate common user preparation. For ethanol extracts we used 96.3% ethanol on the based its extraction propertie (Li et al. 2007). In our case, the extraction time was 24 hours and the extraction took place at room temperature (Masteikova et al., 2008).

The antioxidant activity of selected herbal adaptogens was determined by DPPH method (Brand a Williams 1995, Sachéz-Moreno a kol. 1998). The reaction between synthetic DPPH radical and antioxidants of extracts ran for 35 minutes – kinetic reaction was measured as decrease in absorbance at intervals 5 minutes. The activity of antioxidants in the analyzed ethanol and aquaeous extracts was presented by inhibition of DPPH radical and by value IC_{50} (Inhibitory Concentration). As a reference standard L-ascorbic acid was used.

Antioxidant activity was measured spectrophotometrically using spectrophotometer UV-1800 (Schimadzu, Japonsko). The extent of antioxidant activity in samples was detected by decolouration of reaction solutions i.e. by decreasing in absorbance at λ = 517 nm. The values of antioxidans activity were presented as an L-ascorbic acid equivalent and the results of IC_{50} values were reported in mg/ml.

Results

Fruits were selected on the base of their basic properties – dark colour of the fruits and their sweet – sour taste. The bitter taste indicates the presence of condensed tannins and the dark colour of the fruits indicates the presence of polyphenols – flavonoids (Okuda and Ito 2011).

Extraction temperature values significantly affect the leaching of certain bioactive substances. At lower temperatures, polyphenols extraction is more effective than at higher temperatures. The influence of higher temperature on lower flavonoid extraction was confirmed by Mikulajova et al. (2007). A decrease in absorbance was observed in all analysed samples and in both types of extracts. A more pronounced decrease in absorbance was measured in the first half of the measurement (at 0 minutes, 5 minutes, 10 minutes). In the second half of the measurement, the decrease in absorbance significantly slowed, respectively, stagnated at one absorbance value, what indicated finished reaction of active substance and DPPH radical. When comparing the absorbance of aqueous and ethanol samples, all analysed ethanol samples of the extracts achieved a higher absorbance compared to aqueous samples of the extracts. This is explained by the fact that ethanol increases leachate of bioactive substances, especially anthocyanins (Angela and Meireles 2008).

From the measured absorbance values we calculated the inhibition values of DPPH in aqueous and ethanol extracts. The percentage inhibition values characterize the ability of the monitored extracts to remove DPPH at a particular time. From the inhibition values obtained, we determined the average value for each sample that gave us information about the antioxidant potential of each analysed extract. Fig. 1 and Fig. 2 show the average percentage inhibition values for aqueous and ethanol extracts of selected herbal adaptogens.

We measured the absorbance of both types of extracts at wavelength λ = 517 nm to express the antioxidant activity by using the IC_{50} values. From the measured absorbance values, we calculated the percentage inhibition values of the extracts samples. The inhibition values were plotted into L-ascorbic acid calibration curve formula. Results were converted to 1 ml of extract in L-ascorbic acid equivalent (AAE).

Then we calculated IC_{50} values. The IC_{50} values give us information about the sample concentration that is capable of reduction 50% of DPPH radical. The antioxidant activity of the analysed sample of extract is higher, the IC_{50} is lower. The obtained results are summarized in Table 1 and Table 2.

Table 1 shows that the highst level of antioxidant activity in aqueous extracts had extract of *Schisandra chinensis*. This extract obtained the highest inhibition of DPPH radical among all analysed aqueous extracts. According to the lowest IC_{50} value, Schisandra's extract was the strongest antioxidant among all analysed aqueous extracts. The antioxidant activity of *Schisandra chinensis* ranged from 0.12 mg/ml AAE of extract. *Eleutherococcus senticosus* extracts had the second highest antioxidant activity. The antioxidant activity was 67.16 µg/ml AAE in the fruit extract. The water extract of *Aralia mandshurica* had the antioxidant activity value 0.059 mg/ml AAE of extract. In our measurements, all of the analyzed aqueous extracts were shown as extracts with the lowest antioxidant activity.

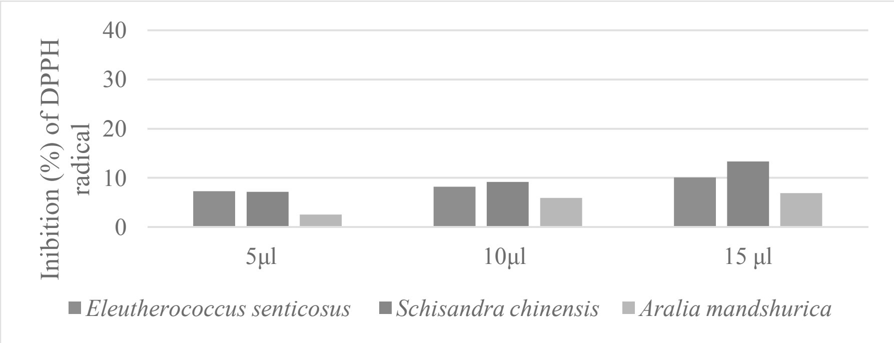

Fig. 1: The average percentage inhibition values of DPPH radical in aqueous samples of extracts

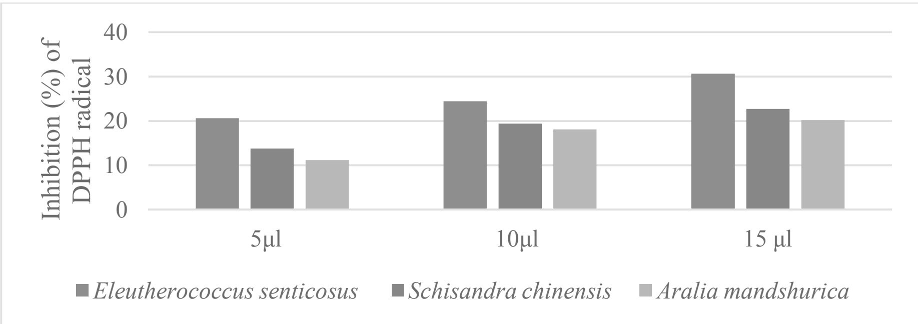

Fig. 2: The average percentage inhibition values of DPPH radical in ethanol samples of extracts

Table 1: Determination of antioxidant activity of aqueous herbal adaptogen extracts

Sample	Inhibition (%)	IC50 (µg/ml AAE)	Antioxidant activity (mg/ml AAE)
Schisandra chinensis	90.38	66.60	0.120
Eleutherococcus senticosus	83.45	67.16	0.112
Aralia mandshurica	39.82	74.40	0.059

Table 2: Determination of antioxidant activity of ethanol herbal adaptogen extracts

Sample	Inhibition (%)	IC50 (µg/ml AAE)	Antioxidant activity (mg/ml AAE)
Schisandra chinensis	90.83	66.63	0.121
Aralia mandshurica	78.08	67.62	0.106
Eleutherococcus senticosus	71.81	68.23	0.098

Table 2 shows that all analyzed ethanol extracts had some antioxidant activity. The highest antioxidant activity was measured in *Schisandra chinensis* fruit extract. The antioxidant activity in the extract represented 0.121 mg/ml AAE of extract. The second was the extract *Aralia madshurica* and the value of the antioxidant activity was 0.106 mg/ml AAE extract. The IC_{50} antioxidant activity of extract *Eleutherococcus senticosus* was 68.23 µg/ml AAE.

Antioxidant activity cannot be evaluated as completely complex because its value is always influenced by several factors. We take into account the impact of climatic conditions as a limited factor for the content of antioxidant substances. Very important is method of extract preparation and extraction conditions (e.g. extraction agent selection, temperature, time of extraction). Several methods are available to analyse the antioxidant potential of plant extracts, but all analyses do not have to detect all antioxidant substances in the extract. The storage of fresh plant material reduces antioxidant properties (Wang et al. 2011).

Both types of *Schisandra* fruit extracts achieved the highest antioxidant activity among all analyzed extracts. The fruits of *Schisandra* contain lignans and lignans are considered to be primary antioxidant substances of these fruits. Other substances that increase the antioxidant potential of these fruits include vitamin C and vitamin E (Wang et al. 2011). Cyanidin-3-O-(2"-O-xylosyl)-glucoside was identified in *Eleutherococcus senticosus* fruit. It is probably one of the major compound responsible for the antioxidant activity of *Eleutherococcus senticosus*. (Lee et al. 2003). As stated by Choi et al. (2007), various methods record different substances responsible for antioxidant activity, e.g. the DPPH method is based on detection of antioxidants that react with a stable DPPH radical.

Conclusions

The highest antioxidant activity of aqueous extracts was achieved by *Schisandra chinensis* (0.120 mg/ml AAE) and the lowest was detected for *Aralia mandshurica* (0.059 mg/ml AAE). Ethanol extracts had the highest antioxidant activity for *Schisandra chinensis* (0.121 mg/ml AAE) and *Aralia mandshurica* (0.106 mg/ml AAE). The fruit extract of *Eleutherococcus senticosus* had the lowest antioxidant activity.

The result show that examined plants *Aralia mandshurica, Schisandra chinensis* and *Eleutherococcus senticosus* have in addition to adaptive properties, antioxidative potential too. Perspective use of these plants can be applied in particular in the prevention and complex or complementary treatment of many diseases related to aging, neurodegenerative and cardiovascular diseases.

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