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TOTAL ANTIOXIDANT ACTIVITY OF PLANTS OF *SYMPHYTUM* L. SPECIES

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The aim of this study to demonstrate the antioxidant potential of methanolic, ethanolic and aqueous extracts of plants of *Symphytum* L. species. The DPPH radical scavenging effect was assessed by the discoloration of methanol solution of 2.2-diphenyl-1-picrylhydrazyl after 10 minutes according to Brand-Williams et al. (1995). Total antioxidant activity (TAA) of above-ground part of *Symphytum asperum* Lepech. during vegetation was from 73.59% (at spring vegetation stage) to 79.65% (fruiatage) in methanolic extracts, from 17.53% (budding stage) to 36.31% (spring vegetation stage) – in ethanolic extracts, from 59.64% (spring vegetation stage) to 76.15% (stage of stem growth) – in water extracts. Methanolic extracts of *Symphytum caucasicum* Bieb. showed the TAA from 75.48% (budding stage) to 80.81% (spring vegetation stage), ethanolic extracts – from 22.61% (blossoming stage) to 73.99% (spring vegetation stage), water extracts – from 67.46% (budding stage) to 74.45% (blossoming stage). In plant raw material of *Symphytum* × *uplandicum* Nyman TAA was from 16.24% (blossoming stage) to 79.18% (budding stage) in methanolic extracts, from 5.64% (blossoming stage) to 21.06% (budding stage) – in ethanolic extracts, from 17.23% (stage of stem growth) to 60.58% (budding stage) – in water extracts. Maximal sign of radical inhibition was noticed in methanolic extracts of *Symphytum caucasicum* in stage of spring vegetation and minimal – in ethanolic extracts of *Symphytum* × *uplandicum* in blossoming stage. Obtaned data allow to use these plants as plant raw material with antioxidant potential.

Keywords: *Symphytum* spp.; antioxidant activity; DPPH-method; methanol; ethanol; aqueous extracts

Introduction

Medicinal plants have been used in the cure of human diseases due to their content of components with antioxidant value. Antioxidants are a group of different natural compounds in the human body which are important for protection against the harmful effects of free radicals (Tahirovic et al., 2014). Plants of genus of *Symphytum* L. are species widely used in traditional and folk medicine. Plant raw material of these plants is a rich source of biological activity compounds such as allantoin, mucopolysaccharides, flavones, steroidal saponins, alkaloids, macro elements, amino acids, phenolic acids with anticancer and antioxidant action (Roman et al., 2008; Vergun, 2008; Castro et al., 2001; Stef et al., 2010; Vergun and Rakhmetov, 2013). Savic et al. (2012) reported that ethanol extracts of the underground part of *Symphytum officinale* L. showed the DPPH radical scavenging 77.3%. The results obtained by Paun et al. (2012) showed over 80% DPPH inhibition by the concentrated extracts of underground part of *Symphytum officinale*. Puertas-Mejia et al. (2012) determined in the ethanol extracts of leaves the antiradical activity 49.94%. According to Stef et al. (2010) the antioxidant

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activity of extracts of *Symphytum officinale* was 36.3%. From the leaves *Symphytum asperum* Lepech. and *Symphytum caucasicum* Bieb. identified high molecular weight fractions with anticomplement and antioxidant activity which might be used as anti-inflammatory and wound-healing agents (Barbakadze et al., 2011). Alkan et al. (2014) obtained data that percentage of inhibition of the DPPH radical was varying from 38.72 to 85.09% in ethanolic extracts and from 21.68 to 52.19% in aqueous extracts of leaves.

In present study we are interested to quantify the total antioxidant capacity of above-ground part of three species of genus of *Symphytum*.

Materials and methodology

Plant raw material collected from the experimental collections of Cultural Flora department of M.M. Gryshko National Botanical Garden of the NAS of Ukraine. It were investigated dried abouve-ground part of plants of *Symphytum* species: *Symphytum officinale*, *Symphytum caucasicum*, *Symphytum* × *uplandicum* Nyman. Plant material was took at stage of spring vegetation, stem growth, budding, blossoming, fruitage. Investigation of total antioxidant activity (TAA) carried out in Institute of Biodiversity Conservation and Biosafety, Slovak University of Agriculture in Nitra (Slovak Republic). TAA of the methanolic, ethanolic and aqueous extracts was determined according to Brand-Williams et al. (1995) against DPPH radical (2.2-diphenyl-1-picrylhydrazyl) (Brand-Williams et al., 1995). The procedure of determination of optical density measured with spectrophotometer Genesis-20 at wavelength 515 nm. Dry mass (1 g) of investigated plants mixed with 25 mL of solvent. Extraction was carried out with methanol, ethanol and water during 12 hours with constant stirring on shaker. Antioxidant solution in solvent (0.1 mL) was added to 3.9 mL of methanol DPPH · solution (25 mg per 100 mL of methanol with further delution). Absorbance of radical solution was in range 0.700–0.800. Optical density of the solution was measured after adding sample immediately and after 10 min of incubation in thedark. Mean values of three replicates and standard deviations are given in Table 1–3.

Results and discussion

The antioxidant activity of plant extracts is of particular interest both of their beneficial physiological activity on human cells and the potential they have to replace synthetic antioxidants used in foodstuff (Amarowicz, 1999). Most of the methods of determination of total antioxidant activity characterize the ability of the tested compound or product to scavenge free radicals and to complex metal ions driving the oxidation process (Tirzitis and Bartosz, 2010). The DPPH method is rapid, simple, accurate and inexpensive assay for measuring the ability of different compounds to act as free radical scavengers (Marinova and Batchvarov, 2011).

For centuries, *Symphytum officinale* (comfrey) has been used as a traditional medicinal plant for the treatment of painful muscule and joint complaints. Comfrey has also been used in veterinary medicine. The therapeutic properties of comfrey are based on its anti-inflammatory and analgesic effect. These plants also stimulate granulation and tissue regeneration, and supports callus formation (Staiger, 2012).

As shown in Table 1 methanol extracts of above-ground part of *Symphytum asperum* was in range from 73.59 to 79.65 depending on stage of growth.

Aqueous extracts had antiradical scavenging in range from 59.64 to 76.15%. The lowest result of inhibition shown ethanol extracts of investigated plants during vegetation 17.53–36.31%. Methanolic extract showed the most antioxidant activity at the period of fruitage, ethanolic – at spring vegetation, aqueous – at stem growth.

Table 1 Antioxidant activity of different extracts of *Symphytum asperum* Lepech. during the vegetation, % of inhibition

Phase of growing	Methanol extracts		Ethanol extracts		Aqueous extracts	
	m	σ	m	σ	m	σ
Spring vegetation	73.59	1.03	36.31	2.89	59.64	0.47
Stem growth	78.04	3.23	26.78	1.29	76.15	0.34
Budding	78.73	1.12	17.53	0.88	72.67	2.70
Blossoming	75.92	0.98	25.16	1.84	62.99	1.13
Fruitage	79.65	0.74	19.97	0.34	67.65	1.04

m – mean values, σ – standard deviation

According to Vergun et al. (2014) antioxidant activity of shoots in the end of vegetation in extracts of *Symphytum asperum* was 87.34% (methanolic extracts) and 50.83% (water extracts).

In Table 2 represented the TAA of different extracts of plant raw material of *Symphytum caucasicum*. Methanol extracts inhibited solution of radical in range from 75.48 to 80.81%, ethanol extracts – from 22.61 to 73.99% and aqueous extracts – from 67.46 to 74.45%.

Table 2 Antioxidant activity of different extracts of *Symphytum caucasicum* Bieb. during the vegetation, % of inhibition

Phase of growing	Methanol extracts		Ethanol extracts		Aqueous extracts	
	m	σ	m	σ	m	σ
Spring vegetation	80.81	0.19	73.99	1.48	73.94	0.98
Stem growth	78.08	1.88	53.64	0.60	68.91	1.58
Budding	75.48	2.23	33.28	0.92	67.46	1.84
Blossoming	79.28	1.24	22.61	1.46	74.45	3.03
Fruitage	78.38	0.24	36.19	1.48	69.59	1.52

m – mean values, σ – standard deviation

As reported Badridze et al. (2013), the total antioxidant activity of leaves extracts of *Symphytum caucasicum* in ethanol was 27.5%. In methanolic extracts of leaves of these plants in the end of vegetation, as reported Vergun et al. (2014), inhibition of free radical was 52.64%, in water extracts – 57.43%. Methanolic extracts of above-ground part of *Symphytum caucasicum* during vegetation showed antioxidant activity 75–80%, aqueous – 67–74%. It should be noted for ethanolic extracts different signs for different stages. From the start of vegetation to blossoming stage antiradical activity decreases from 73.99 to 22.61% and in the stage of fruitage increase to 36.19%.

Table 3 has demonstrated that antioxidant activity of the methanol extracts of *Symphytum* uplandicum during vegetation was in range from 16.24 to 79.18, ethanol extracts – from 5.64 to 21.06 and aqueous extracts – from 17.23 to 60.58%.

Table 3 Antioxidant activity of different extracts of *Symphytum* × *uplandicum* Nyman during the vegetation, % of inhibition

Phase of growing	Methanol extracts		Ethanol extracts		Aqueous extracts	
	m	σ	m	σ	m	σ
Spring vegetation	77.95	1.70	10.61	1.10	55.47	1.40
Stem growth	67.44	0.66	11.99	0.54	17.23	2.57
Budding	79.18	1.46	21.06	0.46	60.58	1.04
Blossoming	16.24	0.92	5.64	0.32	53.87	0.88
Fruitage	26.37	2.51	5.67	0.40	37.86	2.81

m – mean values, σ – standard deviation

As showed our previous data the antioxidant activity of leaves of *Symphytum* × *uplandicum* in the end of vegetation was 58.32% in methanolic extracts and 58.98% – in water extracts (Vergun et al., 2014).

It's should be noted that among investigated plants *Symphytum* × *uplandicum* showed the most variable result in different extracts during vegetation. In spring vegetation in methanolic extracts it was 4.7 times more comparing with blossoming stage. In ethanolic extracts its increases from spring vegetation to budding and from budding to blossoming decreases again. In aqueous extracts peak of inhibition was noted in the budding stage, from budding to fruitage its decreases. The lowest sign identified in steam growth period.

Conclusions

Thus, the different extracts of dry plant raw material of *Symphytum* L. species were investigated by DPPH-method to identify antiradical scavenging. The TAA of methanolic extracts was in range from 16.24% in blossoming stage (*Symphytum* × *uplandicum*) to 80.81% in stage of spring vegetation (*Symphytum caucasicum*). The ethanolic extracts demonstrated the TAA in range from 5.64% in blossoming stage (*Symphytum* × *uplandicum*) to 73.99% in spring vegetation stage (*Symphytum caucasicum*). I the aqueous extracts was identified the TAA in range from 17.23% in stage of stem growth (*Symphytum* × *uplandicum*) to 76.15% in stage of stem growth (*Symphytum asperum*). Summarizing the obtained data it should be noted that for investigated plants of genus of *Symphytum* the radical inhibition by DPPH-method was 5.64–80.81% depending on extract solution that allows use it as potential antioxidant source.

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