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Original Scientific Paper

Screening of plant extracts for antioxidant properties

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ABSTRACT: Antioxidant properties of total methanol extracts from 54 species of 30 families were studied. DPPH (1,1-diphenyl-2-picryl hydrazyl) radical was used for evaluation of free radical scavenging. Among tested species, the extracts of *Rumex crispus* (*radix*), *Rubus occidentalis* (*folia*), *Rumex alpinus* (*radix*), *Euphorbia helioscopia* (*herba*) and *Rubus idaeus* (folia), exhibited the strongest activity. Significant radical scavenging activity was found also in the extracts of *Echium vulgare* (*herba*), *Plantago arenaria* (*herba*), *Viola tricolor* (*folia*), *Pyrus communis* (*folia*), *Sideritis montana* (*folia*), *Betula pendula* (*folia*), *Achillea critmifolia* (*herba*), *Santolina rosmarinifolia* (*herba*), *Morus alba* (*folia*) and *Erigeron canadensis* (*herba*). Moderate activity was shown by extracts of *Forsythia* (*folia*), *Bryonia alba* (*folia*), *Hepatica nobilis* (*folia*), *Plantago cornuti* (*folia*), *Maclura cochinchinensis* (*folia*), *Cihorium inhybus* (*herba*) and *Caltha palustris* (*herba*).

Key words: antioxidant, DPPH, plant species

Abbreviation: DPPH (1,1-diphenyl-2-picrylhydrazyl)

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INTRODUCTION

Oxidative cell damages arising from free radicals are in the basis of many diseases. Thus, the intake of natural antioxidants is very important for humans. Medicinal and edible plants are rich source of such kind of compounds. Relatively new direction which is developed very fast is investigations concerning the antioxidant capacity of medicinal plants (Acuna *et al.* 2002; Manach *et al.* 2004; Bajpai *et al.* 2005). Survey studies in this direction have been made with Flora of India, China, South Africa and other countries (Qiong *et al.* 2004, Gacche & Dhole 2006; Atawodi 2005). Although a great world interest to antioxidant properties on the plants the investigations in Bulgaria are insufficient. There are only partly data (Ivanova *et al.* 2005; Marinova *et al.* 2005; Kiselova *et al.* 2006). From Vietnamese Flora many plant species have been investigated in the search for novel antioxidants, but there is still a demand to find more information concerning the antioxidant potential of plant species (Ogle *et al.* 2003; Thuong *et al.* 2006).

The evaluation of free radical scavenging activity of plant extracts have been extensively performed by DPPH (1,1-diphenyl-2-picrylhydrazyl) method (Cimpoiu 2006; Katalinic *et al.* 2006). DPPH is a purple colored radical that, after being reduced by an antioxidant turns into a yellow product.

The aim of present study is to analyze the 57 extracts of 54 plant species of 30 families for their free radical scavenging activity.

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Table1. Free radical scavenging activity of total methanol extracts of studied plant species

Family	Plant species	Plant part	IC_{50} µg/ml ± confidence intervals
Amaranthaceae	*Chenopodium bonus-henricus* L.	*radix*	>200
Amaryllidaceae	*Pancratium maritimum* L.	*bulbus*	>200
	Leucojum aestivum L.	*folia*	>200
Apiaceae	*Daucus carota* L.	*folia*	>200
	Crithmum maritimum L.	*folia*	>200
Araliaceae	*Hedera helix* L.	*folia*	>200
Asparagaceae	*Asparagus officinalis* L.	*folia*	>200
Asteraceae	*Tussilago farfara* L.	*flos*	194±51.58
	Erigeron canadensis L.	*herba*	95.26±45.62
	*Helichrysum arenarium (*L.) Moench	*herba*	>200
	Cihorium inhybus L.	*herba*	143.5±53.71
	Cihorium inhybus L.	*radix*	>200
	Alchemilla crithmifolia Walds. et Kit.	*herba*	77.32±54.32
	Santolina rosmarinifolia L.	*herba*	85.77±62.77
	Cardus nutans L.	*flos*	>200
Betulaceae	*Betula pendula* Roth.	*folia*	79.66±68.60
Bracicaceae	*Sisymbrium loeselii* L.	*herba*	>200
	Rhaphanus rathanistrum L.	*herba*	>200
	Brassica maritima Tardent	*folia*	>200
	Lepidium ruderale L.	*herba*	>200
	Berteroa incana (L.) DC.	*herba*	>200
Boraginaceae	*Echium vulgare* L.	*herba*	51.38±65.18
Caryophyllaceae	*Herniaria glabra* L.	*herba*	>200
	Stelaria media (L.)Vill	*herba*	>200
Cucurbitaceae	*Cucumis melon* L.	*semen*	>200
	Bryonia alba L.	*folia*	111.3±32.53
Euphorbiaceae	*Euphorbia helioscopia* L.	*herba*	49.52±12.05
Fabaceae	*Laburnum anagyroides* Medik.	*folia*	>200
Lamiaceae	*Sideritis montana* L.	*herba*	77.27±35.78
Liliaceae	*Muscary comosum* (L.)Miller.	*herba*	>200
Nitrariaceae	*Peganum harmala* L.	*herba*	>200
Moraceae	*Maclura cochinchinensis* (Lour.)DC*	*folia*	196±89.56
	Morus alba L.	*folia*	93.41±18.11
Oleaceae	*Jasminum fruticans* L.	*folia*	>200
Papaveraceae	*Fumaria officinalis* L.	*herba*	>200
	Corydalis bulbosa (L.)DC	*nerba*	>200

Family	Plant species	Plant part	IC_{50} μg/ml ± confidence intervals
Plantaginaceae	*Plantago cornuti* Gouan.	*folia*	165.2±81.42
	Plantago arenaria Walds. et Kit.	*herba*	55.53±31.63
	Forsythia Vahl., cultivated	*folia*	101.3±62.56
Polygonaceae	*Rumex alpinus* L.	*radix*	46.69±18.21
	Rumex crispus L.	*radix*	40.09±19.47
Ranunculaceae	*Aquilegia hybrida*	*herba*	>200
	Aquilegia hybrida	*radix*	>200
	Hepatica nobilis Schreb.	*folia*	140.3±42.16
	Caltha palustris L.	*herba*	189±98.60
Rosaceae	*Pyrus communis* L.	*folia*	64.33±19.47
	Prunus armeniaca L.	*semen*	>200
	Persica vulgaris Mill.	*semen*	>200
	Rubus occidentalis L.	*folia*	45.23±19.00
	Rubus ideaus L.	*folia*	50.72±10.76
Rutaceae	*Citrus limon* (L.) Burm.f.	*folia*	>200
Saxifragaceae	*Chrysosplenium alternifolium* L.	*herba*	>200
Solanaceae	*Physalis alkekengi* L.	*fruits*	>200
Vallerainaceae	*Valleriana offcinalis* L.	*radix*	>200
Verbenacea	*Verbena offcinalis* L.	*herba*	>200
Ulmaceae	*Ulmus campestris* L.	*folia*	>200
Violaceae	*Viola tricolor* L.	*folia*	60.64±16.87
	Viola tricolor L.	*herba*	>200
quercetin			3.15±1.1

*with Vietnamese origin

MATERIALS AND METHODS

Plant material. The plants used for this study were collected from natural habitats and from cultivated places of Bulgaria. Voucher specimens were deposited at the Herbarium of the Institute of Botany, Sofia (SOM).

Preparation of extracts

2g dry, ground plant material was extracted with 80% MeOH three times. After evaporation of the solvent the crude extract was subject to subsequent analysis.

Free radical scavenging activity determination

Different concentrations of extracts (10, 20, 50, 100, 200 and 300 μg/mL, in methanol) were added at an equal volume (2.5 mL) to methanolic solution of DPPH (0.3 mM, 1 mL). After 30min at room temperature, the Ab values were measured at 517 nm on a spectrophotometer (Jenway 6320D) and converted into the percentage antioxidant activity using the following equation: DPPH anti-radical scavenging capacity (%) = $[1\text{-}Ab_{\text{of sample}} - Ab_{\text{of blank}} / Ab_{\text{of control}}]100$. Methanol (1.0 mL) plus plant extract solution (2.5 mL) was used as a blank, while DPPH solution plus methanol was used as a control (5). The IC_{50} values were calculated by sigmoid non-linear regression model using plots, where the abscissa represented the concentration of tested plant extracts and the ordinate the average percent of scavenging capacity from three replicates (Software Prizm 3.00). IC_{50} values denote the concentration of sample required to scavenge 50% of DPPH radical.

RESULTS AND DISCUSSION

The results of the screening for free radical scavenging

activity of 57 extracts of 54 plant species are listed in Table 1. Free radical scavenging activity of total methanol extracts was quantitatively determined using a DPPH assay. The dosage of extract is expressed in μg of dry weight of the extract (compound) per mL of the assay mixture. IC_{50} value represents the concentration of test extract or compound where the inhibition of test activity reached 50%. Quercetin was employed as the reference compound. The IC_{50} value for DPPH of quercetin was 3.1 μg/mL. Plant extracts of *Rumex crispus* (*radix*), *Rubus occidentalis* (*folia*), *Rumex alpinus* (*radix*), *Euphorbia helioscopia* (*herba*) and *Rubus idaeus* (*folia*), ranked as the top five most active plant extracts, exhibited strong activity on scavenging DPPH radicals with the determined IC_{50} values 40.09, 45.23, 46.69, 49.52, 50.52 μg/mL, respectively. Another plant extracts of *Echium vulgare* (*herba*), *Plantago areanria* (*herba*), *Viola tricolor* (*folia*), *Pyrus communis* (*folia*), *Sideritis montana* (*herba*), *Betula pendula* (*folia*), *Achillea critmifolia* (*herba*), *Santolina rosmarinifolia* (*herba*), *Morus alba* (*folia*) and *Erigeron canadensis* (*herba*) also possessed significant activity and their IC_{50} values were between 50-100 μg/mL. The extracts of *Forsythia* (*folia*), *Bryonia alba* (folia), *Hepatica nobilis* (*folia*), *Plantago cornuti* (*folia*), *Maclura cochinchinensis* (*folia*), *Cichorium intybus* (*herba*) and *Caltha palustris* L. (*herba*) shown IC_{50} below 200 μg/mL. Little antioxidant activity (>200 μg/mL) was observed for the rest of extracts. There are few publications on antioxidant properties of widely known Bulgarian medicinal plants (Ivanova *et al.* 2005; Kiselova *et al.* 2006). The existing data give new information for antioxidant potential of plant species that have not been traditionally used as medicinal plants.

CONCLUSION

In conclusion during the screening of 57 extracts of 54 species in present work, the methanol extracts of *Rubus idaeus* (*folia*), *R. occidentalis* (*folia*), *Rumex crispus* (*radix*), *R. alpinus* (*radix*), *Euphorbia helioscopia* (*herba*), *Echium vulgare* (*herba*), *Plantago arenaria* (*herba*), *Viola tricolor* (*folia*), *Pyrus communis* (*folia*), *Sideritis montana* (*herba*), *Betula pendula* (*folia*), *Achillea critmifolia* (*herba*), *Santolina rosmarinifolia* (*herba*), *Maclura cochinchinensis* (*folia*), *Morus alba* (*folia*), *Erigeron canadensis* (*herba*) found to exhibit the strongest antioxidant activity and will be subject for future investigations.

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REZIME

Antioksidativna svojstva ekstrakata odabranih biljnih vrsta

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Izučavana su antioksidativna svojstva ukupnog metanolnog ekstrakta 54 vrsta biljaka iz 30 familija. DPPH je korišćen u proceni efikasnosti uklanjanja slobodnih radikala. Medju testiranim vrstama ekstrakti *Rumex crispus* (*radix*), *Rubus occidentalis* (*folia*), *Rumex alpinus* (*radix*), *Euphorbia helioscopia* (*herba*) i *Rubus idaeus* (*folia*) su pokazali najjaču aktivnost. Značajnu aktivnost su imali i ekstrakti vrsta *Echium vulgare* (*herba*), *Plantago arenaria* (*herba*), *Viola tricolor* (*folia*), *Pyrus communis* (*folia*), *Sideritis montana* (*folia*), *Betula pendula* (*folia*), *Achillea critmifolia* (*herba*), *Santolina rosmarinifolia* (*herba*), *Morus alba* (*folia*) and *Erigeron canadensis* (*herba*), dok je umerena aktivnost utvrđena za ekstrakte biljaka *Forsythia* (*folia*), *Bryonia alba* (*folia*), *Hepatica nobilis* (*folia*), *Plantago cornuti* (*folia*), *Maclura cochinchinensis* (*folia*), *Cichorium inhybus* (*herba*) and *Caltha palustris* (*herba*).

Ključne reči: antioksidansi, DPPH, biljke