Screening of radical scavenging activity and polyphenol content of Bulgarian plant species

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ABSTRACT

Background: Discovery of new plant species with antioxidant properties is a priority of many research teams. Most of the species included in this study are unstudied for antioxidant properties, but they are taxonomically related to reference plants with well-documented antioxidant activity. **Materials and Methods:** Free radical scavenging activity of plant extracts was evaluated using a 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. An aluminum chloride colorimetric method was used for flavonoid determination. The amount of phenolic compounds in the extracts was estimated by using the Folin–Ciocalteu reagent. **Results:** As a result of screening, it was found that the significant antioxidant properties possess several unstudied until now plant species (*Veronica bellidioides L., V. kellereri* Deg. et *Urm, V. vindobonensis* (M. Fisher) M. Fisher, *V. beccabunga L., V. rhodopaea L., V. austriaca* (Velen.) Degen*., Clinopodium vulgare L., Stachys recta L., Clematis vitalba L., and Xeranthemum annum L.*). The antioxidant potential of the new species is comparable to that of reference medicinal plants. **Conclusions:** The existing data presented here provide new information for antioxidant potential of plant species that have not been traditionally used as medicinal plants.

Key words: Asteraceae, DPPH, flavonoids, phenols, Salvia, Veronica

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INTRODUCTION

Recently, there is an increasing interest toward plant extracts as a potential source of naturally occurring new antioxidants. The evaluation of antioxidant properties of plant extracts has been extensively performed by 1,1-diphenyl-2-picrylhydrazyl (DPPH) assays. This is a quick, reliable, and reproducible method.[1-4] It has been observed that phenols and flavonoids contribute significantly to the antioxidant capacity of plant extracts.[5-7] That is why many research groups investigated the connection between the antioxidant capacity and polyphenol content of plants.[7-10] In most cases, the surveys have been related to traditionally used medicinal plants.[6,11] However, there are a lot of species taxonomically related to medicinal plants that have not been evaluated for their antioxidant capacity.

Previously, we have performed phytochemical studies on the genus *Veronica* and *Salvia*.[12,13] We have identified flavonoid compounds from these species. However, we have not studied their biological activity.

This study deals with estimation of antioxidant potential, total phenolic content, and total flavonoid content of extracts from 38 plant species, the most of which are unstudied for antioxidant properties, with a aim to discover potential extracts as newly sources of antioxidant activity.

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MATERIALS AND METHODS

Plant material

The plants used for this study were collected from natural habitats in Bulgaria. Voucher specimens were deposited at the Herbarium of the Institute of Biodiversity and Ecosystem Research, Sofia (SOM). Data about pharmacological activity and main group biologically active compounds of reference medicinal plants are presented in Table 1.

Preparation of extracts

Two grams of air-dried ground plant material was extracted with 80% methanol three times. The combined MeOH

Table 1: Pharmacological properties and main group components of reference medicinal plants[14]

Plant species	Biological activity	Active compounds
Lamiaceae		
Salvia officinalis	Anti-inflammatory, antimicrobial, diuretic, expectorant, and astringent activities	Terpenes, and essential oil flavonoids
Stachys officinalis	Antispasmodic, anti-inflammatory, and appetizer	Alkaloids, essential oil, flavonoids, and tannins
Plantaginaceae		
Veronica officinalis	Anti-inflammatory, antiseptic, expectorant, and diuretic	Iridoids glycosides, flavonoids saponins, chlorogenic and caffeic acids
Ranunculaceae		
Clematis vitalba	Anti-inflammatory, antimicrobial, and local analgesic	Lactones, saponins, sterols, and chrologenic acid
Asteraceae		
Arctium lappa	Diuretic, diaphoretic, a blood purifying agent, and rheumatic pain	Terpenes, tannins, and caffeic acid

extracts were evaporated under vacuum to give crude MeOH extracts that were subject to subsequent analysis.

DPPH radical scavenging activity

The DPPH radical scavenging method was used for the determination of the antioxidant capacity of the extracts.[2] Different concentrations of the plant extract (10, 20, 50, 100, 200, and 300 μg/ml, in methanol) were added at an equal volume (2.5 ml) to methanol solution of DPPH (0.3 mM, 1 ml). After 30 min 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 antiradical scavenging capacity (%) = $[1 - (Ab_{of\ sample} - Ab_{of\ blank})/Ab_{of\ control}] \times 100$. 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. The IC_{50} values were calculated by the 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 (Software Prizm 3.00). IC_{50} values denote the concentration of the sample required to scavenge 50% of DPPH radicals.

Flavonoid content

The content of flavonoids was determined by a pharmacopeia method (1989) using rutin as a reference compound.[7] One milliliter of the plant extract in methanol (10 g/l) was mixed with 1 ml aluminum trichloride in ethanol (20 g/l) and diluted with ethanol to 25 ml. The absorption at 415 nm was noted after 40 min at room temperature. Blank samples were prepared from 1 ml plant extract and one drop of acetic acid, and diluted to 25 ml. The absorption of rutin solutions was measured under the same conditions. Standard rutin solutions were prepared from 0.05 g rutin. All determinations were carried out in duplicate. The amount of flavonoids in plant extracts in rutin equivalents (RE) was calculated by the following formula: $X = (A \times m_0 \times 10)/(A_0 \times m)$, where: X is the flavonoid content, mg/g plant extract in RE; A is the absorption of plant extract solution; A_0 is the absorption of standard rutin solution; m is the weight of plant extract, g; and m_0 is the weight of rutin in the solution, g.

Phenol content

The amount of total phenolics in the extracts was determined by the Folin-Ciocalteu procedure, using gallic acid as the standard.[4,15] Distilled water (2 ml) was combined with 0.5 ml of sample, 200 μl of Folin–Ciocalteu's reagent, and 2 ml of Na_2CO_3 (6%). After incubation at room temperature for 40 min, the absorbance of the mixture was measured at 765 nm against a blank without the sample. Quantification was done on the basis of the standard curve of gallic acid. Results were expressed as milligrams of gallic acid equivalents (GAE) per gram of extracts.

Statistical analysis

All of the experiments were carried out in triplicate. The content of total phenols and flavonoids was presented as mean ± SD. The correlation coefficient was calculated using MS Excel software (CORREL statistical function).

RESULTS AND DISCUSSION

Methanol extracts of 38 species listed in Table 2 were examined for their antiradical potential using a DPPH assay. Most of species included in this study are unstudied for antioxidant properties, but they are taxonomically related to reference plants (*Salvia officinalis, Salvia tomentosa, Stachys officinalis,* and *Veronica officinalis*) with well-documented antioxidant activity. Among the plants screened, the extracts of *Salvia scabiosifolia*, *Veronica chamaedrys, Clinopodium vulgare*, *Veronica vindobonensis, Salvia ringens, Veronica bellidioides, Tanacetum macrophyllum, Veronica persica, Veronica rhodopaea*, *Stachys recta*, *Clematis vitalba*, and *Vetronica beccabunga* are the most active and their IC_{50} values are 17.72, 21.65, 22.33, 24.48, 25.71, 31.52, 37.85, 37.85, 39.21, 39.79, 41.17, 46.73, and 47.85 μg/ml, respectively. Other plant extracts of *Salvia pratensis, S. nemorosa, Veronica kellereri, V. austriaca,* and *Xeranthemum annum* also possessed significant activity and their IC_{50} values were between 50 and 100 μg/ml. The extracts of *Veronica polita, V. teucrium,* and *V. hederifolia* have shown IC_{50} values below 200 μg/ml. Little antioxidant activity (>200 μg/ml) was observed for

Table 2: Antiradical activity, total flavonoids, and phenols of methanol extracts of the studied plant species

Plant species	Plant parts	IC_{50} µg/ml DPPH	Total flavonoids (mg/g extract) in QE	Total phenols (mg/g extract) in GAE
Lamiaceae				
Salvia officinalis L.	Inflor.	35.72	2.24 ± 0.2	98.86 ± 5.9
Salvia tomentosa Mill.	Inflor.	11.32	3.83 ± 0.5	94.42 ± 4.6
Salvia ringens Sibth et Sm.	Inflor.	25.71	1.17 ± 0.6	48.54 ± 5.7
Salvia nutans L.	Inflor.	>200	2.05 ± 0.4	30.51 ± 2.4
Salvia pratensis L.	Inflor.	86.20	2.44 ± 0.6	21.44 ± 3.2
Salvia nemorosa L.	Inflor.	93.72	0.90 ± 0.1	36.41 ± 4.7
Salvia scabiosifolia Lam.	Inflor.	19.72	3.31 ± 0.4	59.42 ± 7.1
Stachys officinalis (L.) Trev.	Aerial	>200	1.05 ± 0.4	33.75 ± 5.4
Stachys annua L.	Aerial	>200	1.59 ± 0.2	37.16 ± 6.9
Stachys recta L.	Aerial	41.17	2.72 ± 0.7	45.52 ± 4.2
Stachys milani Petr.	Aerial	>200	2.21 ± 0.5	40.36 ± 3.6
Stachys balcanica Ball.	Aerial	>200	1.59 ± 0.3	41.78 ± 3.2
Stachys germanica L.	Aerial	>200	1.51 ± 0.4	37.23 6.2
Stachys bulgarica (Deg. Et Neič.) Hay.	Aerial	>200	1.60 ± 0.2	15.16 ± 5.1
Stachys alpina L.	Aerial	>200	0.86 ± 0.7	24.37 ± 3.9
Stachys sylvatica L.	Aerial	>200	0.60 ± 0.5	17.34 ± 2.8
Clinopodium vulgare L.	Aerial	22.33	2.41 ± 0.5	41.83 ± 6.5
Plantaginaceae				
Veronica officinalis L.	Aerial	17.59	3.63 ± 0.2	64.26 ± 3.7
Veronica kellereri Deg. et Urm.	Aerial	56.93	2.06 ± 0.3	59.99 ± 7.2
Veronica serpylifolia L.	Aerial	>200	2.62 ± 0.2	33.84 ± 5.6
Veronica persica Poir.	Aerial	39.21	3.24 ± 0.1	45.34 ± 7.1
Veronica bellidioides L.	Aerial	31.52	1.77 ± 0.4	78.22 ± 7.4
Veronica polita Fries.	Aerial	104.4	1.92 ± 0.6	23.77 ± 3.4
Veronica vindobonensis (M. Fisher) M. Fisher	Aerial	24.48	2.27 ± 0.3	56.32 ± 6.3
Veronica chamaedrys L.	Aerial	21.65	2.19 ± 0.3	45.43 ± 4.9
Veronica becabunga L.	Aerial	47.85	0.93 ± 0.4	49.27 ± 4.7
Veronica teucrium L.	Aerial	112.8	1.92 ± 0.4	32.98 3.8
Veronica rhodopoea (Velen.) Degen.	Aerial	39.79	3.85 ± 0.3	79.93 ± 8.2
Veronica hederifolia L.	Aerial	124	1.50 ± 0.2	23.12 4.1
Vetronica austriaca L.	Aerial	71.12	1.58 ± 0.6	37.32 ± 3.6
Veronica urticifolia Jacq.	Aerial	>200	0.87 ± 0.2	34.43 3.4
Ranunculaceae				
Clematis vitalba L.	Leaves	46.73	3.16 ± 0.4	15.77 ± 2.9
Pulsatilla montana (Hoppe) Reichenb.	Aerial	>200	2.39 ± 0.6	19.94 ± 23.5
Asteraceae				
Tanacetum macrophilum (Waldst. and Kit.) Schultz	Inflor.	37.85	2.89 ± 0.1	57.47 ± 8.7
Echinops sphaerocephalus L.	Inflor.	>200	0.76 ± 0.3	18.75 4.3
Arctium lappa L.	Aerial	>200	1.78 ± 0.2	16.93 ± 3.9
Carthamus lanatus L.	Inflor.	>200	1.01 ± 0.4	18.99 ± 4.6
Xeranthemum annum L.	Aerial	98.86	2.36 ± 0.4	19.50 3.2

Inflor., Inflorescences; QE, quercetin equivalents; GAE, gallic acid equivalents

15 extracts. These results show that free radical scavenging effects (IC_{50} values) of part of newly studied species were similar to those of the reference plants: *Salvia officinalis* (IC_{50} = 35.72), *Salvia tomentosa* (IC_{50} = 11.32), and *Veronica officinalis* (IC_{50} = 17.59) which defines them as perspective objects for further research.

The content of phenolic compounds in the studied methanol extracts expressed in GAE, varied between 9.91 and 98.86 mg/g. The highest amounts were found in the extracts of *Salvia officinalis* and *Salvia tomentosa*. A high content of phenolic compounds also exhibited the extracts of *Veronica bellidioides*, *V. rhodopaea*, *V. officinalis*, *V. kellereri,* *Salvia scabiosifolia,* and *Tanacetum macrophilum.*

The level of flavonoids, expressed in quercetin equivalents in mg/g of plant extract, varied from 0.6 to 3.8 mg/g. The highest amounts were found for the extracts of *Salvia tomentosa*, *S. scabiosifolia*, *Veronica officinalis*, *V. persica*, *Tanacetum macrophyllum,* and *Clematis vitalba.*

Although in some cases the extracts with strong antiradical activity are abundant in flavonoids or phenolic compounds, statistically significant correlation between these indicators is not established. The correlation coefficient (*R*) between a DPPH assay and data of flavonoid content of the

studied extracts was only 0.286 while between free radical scavenging activity and total phenolic compounds was 0.560. The received result that the antioxidant properties of the studied extracts are correlated more strongly with the content of phenolics than that of flavonoids is in agreement with finding by other authors.[5,7,16]

According to the received results, it was concluded that 16 plant extracts (*Veronica bellidioides, V. persica, V. rhodopaea*, *V. vindobonensis, V. chasmaedrys*, *V. beccabunga, V. kellereri, V. austriaca, Salvia scabiosifolia*, *S. ringens*, *S. pratensis*, *Clinopodium vulgare*, *Tanacetum macrophyllum, Stachys recta*, *Clematis vitalba,* and *Xeranthemum annum*) of 38 tested have potent antioxidant activity, achieved by scavenging abilities observed against DPPH. The existing data give new information for the antioxidant potential and polyphenol content of plant species that have not been traditionally used as medicinal plants.

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