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. rhodopea 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

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. It has been observed that phenols and flavonoids contribute significantly to the antioxidant capacity of plant extracts. That is why many research groups investigated the connection between the antioxidant capacity and polyphenol content of plants. In most cases, the surveys have been related to traditionally used medicinal plants. 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. 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 polyphenolic 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.

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
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: \( X = \frac{(A \times m_0 \times 10)}{(A_0 \times m)} \), 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. Distilled water (2 ml) was combined with 0.5 ml of sample, 200 μl of Folin–Ciocalteu’s reagent, and 2 ml of \( \text{Na}_2\text{CO}_3 \) (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 scabissifolia, Veronica chamaedrys, Clinopodium vulgare, Veronica vindobonensis, Salvia rigens, Veronica bellidiformis, Tanacetum macrophyllum, Veronica persica, Veronica rhodopaica, Stachys recta, Clematis vitalba, and Vetrosnica 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 annuum also possessed significant activity and their \( IC_{50} \) values were between 50 and 100 μg/ml. The extracts of Veronica polita, V. tenuirost, and V. hederae folia have shown \( IC_{50} \) values below 200 μg/ml. Little antioxidant activity (>200 μg/ml) was observed for other plant species.

### Table 1: Pharmacological properties and main group components of reference medicinal plants

| Plant species | Biological activity | Active compounds |
|---------------|---------------------|------------------|
| **Lamiaceae** |                     |                  |
| *Salvia officinalis* | Anti-inflammatory, antimalarial, diuretic, expectorant, and astringent activities | Terpenes, essential oil, flavonoids |
| *Stachys officinalis* | Antispasmodic, anti-inflammatory, and appetite | Alkaloids, essential oil, flavonoids, and tannins |
| **Plantaginaceae** |                     |                  |
| *Veronica officinalis* | Anti-inflammatory, antiseptic, expectorant, and diuretic | Iridoids glycosides, flavonoids, monoterpenes, and caffeic acids |
| **Ranunculaceae** |                     |                  |
| *Clematis vitalba* | Anti-inflammatory, antimalarial, and local analgesic | Lactones, saponins, steroids, 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. 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 (%) = \( [1 – (\text{Ab}_{\text{of sample}} – \text{Ab}_{\text{of blank}})/\text{Ab}_{\text{of standard}}] \times 100 \). The amount of total phenolics in the extracts was calculated by the sigmoid non-linear regression model using plots, where the abscissa represents 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 pharmacopoeia method (1989) using rutin as a reference compound. 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 content of flavonoids was determined by a pharmacopoeia method (1989) using rutin as a reference plant species.
Table 2: Antiradical activity, total flavonoids, and phenols of methanol extracts of the studied plant species

| Plant species               | Plant parts | IC<sub>50</sub> 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 scabiosa* 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 serpyllifolia* 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 belldiioides* 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                          | 56.52 ± 6.3                       |
| *Veronica chamaedrys* L.  | Aerial      | 21.65                      | 2.19 ± 0.3                          | 45.43 ± 4.9                       |
| *Veronica bacabunga* 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 hederafolia* L. | Aerial      | 124                       | 1.50 ± 0.2                          | 23.12 ± 4.1                       |
| *Veronica 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* (Walld. 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 annuum* 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<sub>50</sub> values) of part of newly studied species were similar to those of the reference plants: *Salvia officinalis* (IC<sub>50</sub> = 35.72), *Salvia tomentosa* (IC<sub>50</sub> = 11.32), and *Veronica officinalis* (IC<sub>50</sub> = 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. rhodopoea*, *V. officinalis*, *V. kellereri*, *Salvia scabiosa* 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. scabiosa*, *Veronica officinalis*, *V. persica*, *Tanacetum macrophilum*, 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 plant species that have not been traditionally used as medicinal plants.

According to the received results, it was concluded that 16 plant extracts (Veronica bellidioideis, V. persica, V. rhodopea, V. vindobonensis, V. chasmaedrys, V. becabunga, V. kellereri, V. austriaca, Salvia scabiosifolia, S. ringens, S. pratensis, Clinopodium vulgare, Tanacetum macrophyllum, Stachys recta, Clematis vitalba, and Xeranthemum annuum) 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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