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Chemical composition, antioxidant activity and total phenol content of six vascular medicinal plants

N. H. Grozeva*, M. A. Gerdzhikova, M. T. Tzanova

Faculty of Agriculture, Trakia University, Studentski grad Str., 6000 Stara Zagora, Bulgaria

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The Bulgarian flora is rich in medicinal plants, the annually collected and exported herbs are used on the Bulgarian and international markets as a raw material for a number of medicinal, cosmetic and other objectives. Despite the exceptional biodiversity and significant resources, the antioxidant potential of Bulgarian medicinal plants is still insufficiently explored. Data on the chemical composition of a number of medicinal wild plants are not complete. The aim of this study was to determine the chemical composition, antioxidant activity and total phenol content of the aerial parts of *Artemisia annua* L. (sweet wormwood), *Artemisia vulgaris* L. (common mugwort), *Prunus laurocerasus* L. (cherry laurel), *Tanacetum vulgare* L. (common tansy), *Urtica dioica* L. (common nettle) and *Verbascum densiflorum* Bertol. (denseflower mullein) from their populations in the Thracian Lowland. The Weende method was used to determine crude protein, crude fat, crude fiber, ash, and nitrogen free extracts (NFE). The antioxidant activity was tested by determining the radical scavenging capacity of the selected species by the DPPH method and the total phenol content - by using Folin-Ciocalteu reagent and gallic acid as a standard.

Keywords: total phenol content, antioxidant activity, chemical composition, *Artemisia, Prunus laurocerasus, Tanacetum vulgare, Urtica dioica, Verbascum densiflorum*

INTRODUCTION

The Bulgarian flora is rich in medicinal plants, the annually collected and exported herbs are used on the Bulgarian and international markets as a raw material for a number of medicinal, cosmetic and other objectives. Despite the exceptional biodiversity and significant resources, the antioxidant potential of Bulgarian medicinal plants is still insufficiently explored. Data on the chemical composition of a number of medicinal wild plants in Bulgarian flora are not complete.

Artemisia annua L. (Asteraceae) is spread throughout the country from sea level up to 1000 m above sea level. Various researchers reported that the species have several biological activities such as antioxidant, antispasmodic, antimicrobial, insecticidal, anticancer, antifungal, cytotoxic [1-8]. According to Ćavar *et al.* [9] variability of chemical composition of essential oil of *A. annua* depends on the geographical origin and stage of plant development.

Artemisia vulgaris L. (Asteraceae) is widespread in Bulgaria from 0 to 1000 m above sea level. Aerial parts of the species contain polysaccharides which are employed to treat numerous diseases and carbohydrates extracted from this plant exhibit several beneficial properties [10]. However, the main polysaccharide in the infusion is inulin-type fructan [11]. According to Temraz and El-Tantawy [12] *A. vulgaris* extract possesses antioxidant activity which might be helpful in preventing or slowing the progress of various oxidative stress-related diseases.

Prunus laurocerasus L. (Rosaceae) is naturally distributed in the Central and Eastern Balkan range and the Strandja. It is cultivated for landscaping parks and gardens throughout the country. The fruits of the species contain vitamins (A, C and D) with high antioxidant activity and abundant phenolic compounds (phenolic acids, flavonoids, flavonols, anthocyanin, tannins and lignin) [13-15]. Fatty acids in seeds [16] and essential oil constituents in leaves and fruits [17] have been determined.

In Bulgaria, *Tanacetum vulgare L.* (Asteraceae) grows in all phytogeographical areas from 0 to 2000 m above sea level. It is often grown as an ornamental plant, too. The essential oils of the species find application as cardial, stomach remedies and are used as a food preservative and containing bitter substances and sesquiterpene lactones exhibit cytotoxicity, antimicrobial activity, and regulate growth [18]. In Bulgaria, the dry leaves and flowers of *T. vulgare* are used as spasmodic, antiseptic means and for protection against dandruff [19]. The extract from the aerial parts of the species has been reported to exhibit antitumor [20], anti-inflammatory [21] and antioxidant [22, 23] properties.

Urtica dioica L. (Urticaceae) is widespread in ruderalized terrains throughout the country from 0 to 1700 m. Its leaves have high levels of protein,

* To whom all correspondence should be sent:
E-mail: grozeva@uni-sz.bg

vitamins, nine carotenoids [24-28]. The antimicrobial and antioxidant activities have been studied [29-31]. But, to our knowledge, there is no data available on the antioxidant activities of *U. urens* from Bulgaria.

Verbascum densiflorum Bertol. (Scrophulariaceae) is widespread in Bulgaria from 0 to 2000 m above sea level. The flowers of the species are used for treatment of sore throat, chills, phlegm congestion [32]. Both flowers and leaves possess mildly demulcent, expectorant, and astringent properties [32]. Phytochemical investigations of the flowers have shown the presence of flavonoids, iridoids, phenolic acids, saponins, amino acids and free sugars [33-35].

The aim of this study was to determine the chemical composition, antioxidant activity and total phenol content of the aerial parts of *Artemisia annua* L., *Artemisia vulgaris* L., *Prunus laurocerasus* L., *Tanacetum vulgare* L., *Urtica dioi*ca L. and *Verbascum desniflorum* Bertol. from their populations in the Thracian Lowland.

MATERIALS AND METHODS

Plant material and extract preparation

Plant parts of the studied species were collected in the 2018 growing season from their natural populations. They were dried in shade at 20 - 24 °C, ground in a mechanical grinder (final powder size less than 400 μm) and stored at 18 - 20 °C. The extractions were performed by maceration of 1 g of powdered plant material in 10 ml of methanol at room temperature for 7 days. After filtration, the residue was washed up in triplicate. The collected methanol extracts were concentrated to a final volume of ca. 7 ml by a rotary evaporator under vacuum at 30 °C. The dry matter (DM) of these methanol extracts was determined gravimetrically by drying 1 ml of each extract at 120 °C for 6 hours. Finally, the extracts were adjusted to 1 $mg.ml^{-1}$ calculated on DM by diluting of the concentrated extracts.

Chemical composition, g kg^{-1} DM, determined by the Weende method, includes the following determinations: crude protein – by Kjeldahl method [36]; crude fat – by Soxhlet method [37]; crude fiber [38]; ash [39]; and NFE was calculated by the formula: 1000 – (crude protein + crude fat + crude fibre + ash).

Determination of total phenol content

The experimental procedure described by Anesini *et al.* [40] was applied for determination of total phenol content (TPC). Briefly, 1 ml of the methanolic extract was mixed in separate tubes with 5.0 ml of Folin-Ciocalteu's reagent (1:10 dilution with water of the commercial reagent). Then, 4 ml of 7.5 % Na_2CO_3 aq (w/v) was added and the tubes were left at room temperature for one hour. The absorbance at 765 nm was measured against water. Each sample was analyzed in triplicate. Gallic acid (Sigma-Aldrich, St. Louis, MO) solutions in methanol ranging from 2 to 60 $\mu g.ml^{-1}$ were used for a calibration curve (R^2 = 0.9987). Total phenol content (TPC) of each sample was expressed as mg gallic acid equivalents (GAE) in 1 g DM of plant extract.

Determination of antioxidant activity by DPPH method

The method described by Serpen *et al.* [41] was applied to measure the radical-scavenging potential of methanolic extracts obtained from the tested plant species. Briefly, to 2 ml of 100 μM solution of DPPH in methanol was added 20 μl of methanolic extract. Absorption at 517 nm was measured 30 minutes later. Since the composition of the extracts is complex, the results for their radical-binding capacity were compared with those of Trolox (water-soluble analogue of Vitamin E) and calculated by regression analysis from the linear dependence between concentration of Trolox and absorption at 517 nm. Trolox standard was purchased from Sigma-Aldrich (St. Louis, MO). Standard solutions in methanol ranging from 1 to 50 μmol l^{-1} were used for a calibration curve (R^2 = 0.9989). The results were expressed as mmol of Trolox equivalents (TE) in 1 kg DM of the methanolic extract.

Statistical data analysis

The statistical analyses were performed using Statistica 6 for Windows. All analytical determinations were performed in triplicate and the mean values ± standard deviation (SD) were reported.

RESULTS AND DISCUSSION

Chemical composition in the above-ground biomass of the tested plants

The results from the complete chemical analysis show that in the studied six plants nitrogen free extracts (NFE) predominate (Table 1). No significant differences were observed between the NFE values in the various plants. The highest values were determined in *P. laurocerasus, T. vulgare* and *V. densiflorum* flower (604.38; 594.71; 592.67 g kg^{-1} DM, respectively).

Table 1. Chemical composition of the tested plants, g kg^{-1} DM

Plant	Crude protein, g kg^{-1}	Crude fat, g kg^{-1}	Crude fiber, g kg^{-1}	Ash, g kg^{-1}	NFE, g kg^{-1}
Artemisia annua	180.94 ± 15	32.26 ± 3	125.29 ± 10	110.13 ± 9	551.38 ± 52
Artemisia vulgaris	183.42 ± 16	44.46 ±	131.58 ± 10	99.56 ± 7	540.98 ± 52
Prunus laurocerasus	103.27 ± 9	34.99 ± 3	146.81 ± 12	110.56 ± 9	604.38 ± 58
Tanacetum vulgare	106.97 ± 9	27.34 ± 2	186.72 ± 14	84.27 ± 7	594.71 ± 58
Urtica dioica	160.73 ± 13	22.99 ± 2	81.71 ± 6	166.88 ± 13	567.68 ± 53
Verbascum densiflorum leaf	125.32 ± 9	9.82 ± 1	242.29 ± 20	57.75 ± 5	564.82 ± 53
Verbascum densiflorum flower	110.10 ± 7	16.58 ± 1	231.35 ± 18	49.31 ± 5	592.67 ± 57

In the other plants NFE vary within a close range – from 567.68 to 540.98 g kg^{-1} DM. In the *V. densiflorum* leaves lower NFE content was found, but higher crude protein, crude fiber and ash content compared to flowers. The two wormwood species demonstrated close NFE values – 551.38 and 540.98 g kg^{-1} DM.

Crude protein content varies from 103.27 to 183.42 g kg^{-1} DM. High protein values are typical of *A. vulgaris*, *A. annua* and *U. dioica* – 183.42; 180.94; 160.73 g kg^{-1} DM, respectively. The two horsetail species exhibit almost the same crude protein content. The protein content is lower in *V. densiflorum* leaf – 125.32 g kg^{-1} DM. In *P. laurocerasus, T. vulgare, V. densiflorum* flower low crude protein values were established – 103.27; 106.97 and 110.10 g kg^{-1} DM.The highest crude fiber content was found in *V. densiflorum* – 242.29 g kg^{-1} DM in leaves and 231.35 g kg^{-1} DM in flowers. Medium is the position of *T. vulgare* and *P. laurocerasus* (186.72; 146.81 g kg^{-1} DM) followed by the two wormwood species (131.58; 125.29 g kg^{-1} DM). In *U. dioica* the lowest crude fiber value was recorded – 81.71 g kg^{-1} DM.

Mineral substances (ash) values range from 49.31 to 166.88 g kg^{-1} DM. The highest ash content is shown by *U. dioica.* Similar are the values of *P. laurocerasus, A. annua, A. vulgaris* and *T. vulgare* – 110.56; 110.13; 99.56; 84.27 g kg^{-1} DM, respectively. The lowest values were recorded in *V. densiflorum* flower – 49.31 g kg^{-1} DM, and slightly higher – in *Verbascum densiflorum* leaf – 57.75 g kg^{-1} DM.

The chemical composition of the 6 plant species included in this study is characterized by the lowest crude fat content. Higher values were found in *A. vulgaris, P. laurocerasus* and *A. annua* (32.26; 44.46; 34.99 g kg^{-1} DM). The lowest crude fat content is observed in *V. densiflorum* – 16.58 g kg^{-1} for flowers and 9.82 g kg^{-1} DM for leaves. With values of 27.34 and 22.99 g kg^{-1} DM, *T. vulgare* and *U. dioica* occupy medium position by that indicator.

For the two wormwood species included in the study, *A. vulgaris* and *A. Annua*, close values of the tested chemical composition parameters were found. The obtained crude protein and ash values of *A. annua* are higher than those published by Iqbal *et al.* [46] and lower for crude fat and fiber.

Various researchers report high protein content in nettle leaves. In addition to the higher protein level, nettle has a better amino acid profile than most other leaf vegetables [57]. According to Sidaoui *et al.* [30] protein content is 15.75 %. The results from the chemical analysis by Adhikari *et al.* [58] show 33.8 % crude protein, as well as high ash (16.2 %), crude fat (3.6 %) and crude fiber (9.1 %) content. The crude protein values obtained in our study are lower compared to [58] and similar to [30]; for ash, crude fiber and fat they are close and higher for NFE compared to [58].

Total phenol content and antioxidant activity of the tsted plants

Total phenol content (TPC) varies from 152 ± 11 to 591 ± 56 mg GAE.g^{-1} DM of methanolic extracts in the plants studied (Table 2). The values for *A. annua* and *A. vulgaris* are similar: 270 ± 22 and 282 ± 25 mg GAE.g^{-1} DM.

Table 2. Total phenol content and antioxidant activity of the tested plants, (n = 3)

Plant	mg GAE.g^{-1} DM	mmol TE.kg^{-1} DM
Artemisia annua stems	270 ± 22	27 ± 3
Artemisia vulgaris stems	282 ± 25	39 ± 3
Prunus laurocerasus leaves	324 ± 28	47 ± 4
Tanacetum vulgare leaves	525 ± 44	62 ± 5
Tanacetum vulgare flowers	291 ± 27	61 ± 5
Urtica dioica leaves	591 ± 56	45 ± 5
Verbascum densiflorum leaves	330 ± 29	35 ± 3
Verbascum densiflorum flowers	152 ± 11	14 ± 2

Differences in the phenol content were found in the different parts of *T. vulgare* and *V. densiflorum*. Higher TPC content is typical for the leaves of these plants – 525 ± 44 and 330 ± 29 mg GAE.g^{-1} DM. In flowers TPC is twice lower. From the plants included in the study, the total phenol content is the highest in the *U. dioica* leaves – 591 ± 56 mg GAE.g^{-1} DM. Over 500 mg GAE.g^{-1} DM were found in *T. vulgare* leaves as well. In the other plants TPC vary from 270 ± 22 to 330 ± 29 mg GAE.g^{-1} DM – from 2.2 to 1.6 times lower compared to *U. dioica* and *T. vulgare.* With TPC value of 152 ± 11 mg GAE.g^{-1} DM, *V. densiflorum* flowers are determined to have the lowest TPC – from 3.4 to 3.9 times lower.

Antioxidant activity (AA) varies from 14 ± 2 to 62 ± 5 mmol TE.kg^{-1} DM (Table 2). *T. vulgare* leaf and flower extracts demonstrate the highest AA. The AA values for the different plant parts are very close – 61 ± 5; 62 ± 5 mmol TE.kg^{-1} DM. With regard to TPC big differences between leaf and flower were observed, with leaf values being 1.8 times higher. High AA is demonstrated by the methanolic extracts of *P. laurocerasus* and *U. dioica* – 47 ± 4 and 45 ± 5 mmol TE.kg^{-1} DM. Due to the established highest phenol content for nettle leaves, it is expected it to reveal the highest AA compared to the other plants included in the study. Probably for nettle AA phenol content is primarily accountable, while in *T. vulgare* and *P. laurocerasus* other biologically active substances also have an effect.

Lower AA values were determined in *A. annua, V. densiflorum* leaves and *A. vulgaris.* AA found for *A. annua* is lower than that of *A. vulgaris*, which corresponds to the lower TPC of that plant. The lowest AA is exhibited by the *V. densiflorum* flower extracts – 2.5 times lower than that of the leaves of the same plant.

Phenols are plant ingredients with important significance for antioxidant activity. There is a strong positive relation between total phenols and antioxidant activity of many plant species due to the high reactivity of the phenol group that participates in the following reaction:

$$\text{Ar-OH} + \text{R.} \rightarrow \text{Ar-O.} + \text{RH}$$

Single electron delocalization makes this reaction thermodynamically favorable. A reaction turns the phenolic group into a stable quinone structure [42]. According to some authors, what makes phenol compounds good antioxidants is that they are efficient hydrogen donors [43]. Temraz and El-Tantawy [12] found that total phenol content in *A. vulgaris* water extract is 19 ± 0.16 mg GAE.g^{-1} plant extract. The extraction method and the solvent used play a key role in extracting phenols from the plant material. According to Skowyra *et al.* [44] the ethanol extract of *A. annua* leaves contains 23.36 ± 0.92 mg GAE.g^{-1} DM. In another study about methanolic and acetone extracts from *A. annua* leaves [45] TPC values of 384.1 ± 6.7 and 521.2 ± 5.4 mg GAE.100 g^{-1} DM, respectively, have been determined. Higher values have been reported by [46]. In different extracts TPC values vary from 90.12 to 134.50 mg GAE.g^{-1} DM with the highest ones having been reported for the methanolic extract. The TPC values obtained in the present study (270 ± 22 mg GAE.g^{-1} DM) are higher that the cited ones.

Antioxidant activity of *A. vulgaris* can be useful for preventing or delaying the development of various diseases related to oxidative stress [12]. *A. annua* extract can be used as a substitute of synthetic antioxidants [44].

A number of authors report TPC and AA in *P. laurocerasus* fruit [13-15]. For *P. laurocerasus* leaves, depending on the extraction technique, Karabegović *et al.* [47] found a fluctuation of TPC from 119.4 to 85.4 mg GAE.g^{-1} DM, and of AA – from 124.5 to 108.1 μg/ml. They noted down a high correlation among TPC, AA and total flavonoid content with 0.945 and 0.985 ratio. The TPC values

determined in the present study are higher compared to the results presented in [47].

Methanolic extract from the above-ground parts of *T. vulgare* shows AA with a value of 37 ± 1.2 μg.ml^{-1}. The revealed strong antioxidant activity of the extract and the isolated active ingredients come to support the traditional medicinal applications of the plant in healing wounds, rheumatoid arthritis and other inflammatory conditions [48].

According to Bączek *et al.* [49] *T. vulgare* and *T. balsamita* extracts have antioxidant potential values established by the DPPH method of 13.59 and 13.86 μmol TE.g^{-1} DM, respectively, and can be used in the pharmaceutical and food industries as antiseptics and preservers. Tansy extracts can be effectively used as an antioxidant in rapeseed oil [22, 50]. Ivănescu *et al.* [51] report values of *T. vulgare* TPC from Romania 26.37 mg GAE.g^{-1} DM and AA 242.8 μg.ml^{-1}. In the present study extracts from both *T. vulgare* leaves and flowers exhibit the highest AA compared to the other plants.

Alan *et al.* [52] used various extract solvents in order to determine antioxidant activity of three *Verbascum* species. According to them, methanol and water extracts exhibit greater antioxidant activity compared to the other extracts. The study by Saltan *et al.* [53] also shows that methanolic extracts from various *Verbascum* species reveal good antioxidant activity. AA of *Verbascum* species is mainly determined by the secondary metabolite verbascoside, and methanolic extracts show the strongest antioxidant activity in various *in vitro* methods [54].

The results from the study by Sidaoui *et al.* [30] reveal that the phenol content is 11.62 mg GAE.g^{-1} DM, whereas AA is 8.11 mM.g^{-1} DM. In a publication by Mzid *et al.* [31] TPC values of 31.41 mg GAE.g^{-1} DM and of AA 560 mmol Trolox.g^{-1} of DM in *U. dioica* alcohol extract have been obtained. According to Biesiada *et al.* [55] from Poland the average TPC content in the methanolic extract of nettle leaves is 14.47 mg.g^{-1} DM and AA is 26.5 μM TE.g^{-1} DM. The values stated by Ozkan *et al.* [56] about TPC and AA in methanolic extracts of nettle leaves from Turkey are 332.19 mg GAE.g^{-1} DM and 40.59 mM TE.g^{-1} DM, respectively. Total phenol content in nettle extracts from Serbia amounts to 208.37 mg GAE.g^{-1} DM, while antioxidant activity measured by using the DPPH and ABTS methods has IC_{50} values of 31.38 and 23.55 μg mL^{-1}, respectively [27]. The TPC values of 591 ± 56 mg GAE.g^{-1} DM obtained in the present study are higher than the cited ones.

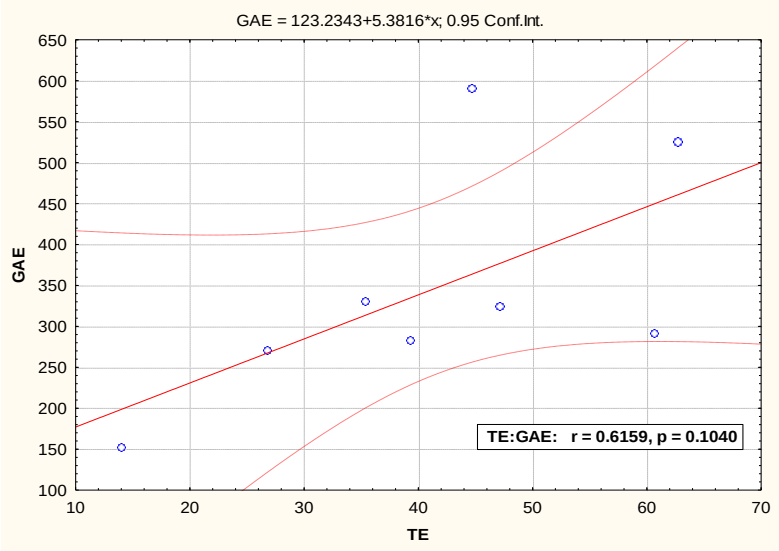

Figure 1. Pearson correlation between TPC and antioxidant activity, P ≤ 0.05 (2-tailed)

In the present study a positive linear dependence was found between the antioxidant activity and total phenol content (Figure 1). Pearson correlation measures the linear correlation between two variables. In the present study, the correlation between the values of TPC and the values of AA of the tested methanolic extracts was observed. The calculated correlation coefficient was positive with a value of 0.6159 (p ≤ 0.05). A conclusion could be drawn that these compounds are responsible for the antioxidant activity of the metanolic extracts of the tested plants.

CONCLUSION

The highest crude protein content was found in *A. vulgaris* and *A. annua*, crude fat – in *A. vulgaris*, crude fiber – in *V. densiflorum*, ash – in *U. dioica* and nitrogen free extracts – in *P. laurocerasus*.

The highest total phenol content was measured in *U. dioica* leaves and antioxidant activity – in *T. vulgare* leaves and flowers.

Although Pearson correlation was evaluated between total phenol content and radical scavenging potential measured at extracts from different plant species, the correlation coefficient observed had a large, positive value. This once again confirmed the strong influence and a high contribution of the total phenolic content to the antioxidant activity of methanolic plant extracts.

Referring to the results obtained in the present study, the tested plant species can be used in further works of researching of their qualities as health-promoting additives or preservatives in the pharma-; food- and cosmetic industries.

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