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Determination of total phenol content and antioxidant activity of five medicinal plants growing in Bulgaria

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Interest in natural compounds with antioxidant activity as alternatives to commercial antioxidants has increased in recent years. Herbal extracts are well recognized sources of antioxidants. The phenols contained therein, including flavonoids, have been increasingly identified by many researchers as important dietary antioxidant factors. Studies have shown that there are differences in the content of bioactive substances in plants collected from different geographical regions. About 750 species in the Bulgarian flora are medicinal and have not yet been sufficiently studied. Because of this, in the present paper the total polyphenol content and antioxidant activity of some populations of *Equisetum arvense* L., *Equisetum telmateia* Ehrh., *Juniperus communis* L., *Lavandula angustifolia* Mill. and *Rosmarinus officinalis* L. were evaluated. In *Juniperus communis* the highest total phenol content of 946 mg GAE.kg^{-1} DM and antioxidant activity of 58.5 mmol TE.kg^{-1} DM were measured.

Keywords: total phenol content, antioxidant activity, *Equisetum, Juniperus communis, Lavandula angustifolia, Rosmarinus officinalis*

INTRODUCTION

Interest in natural compounds with antioxidant activity as alternatives to commercial antioxidants has increased in recent years. Herbal extracts are well recognized sources of antioxidants. The phenols contained therein have been increasingly identified by many researchers as important antioxidant factors. About 750 species in the Bulgarian flora are medicinal and have not yet been sufficiently studied. The subject of this study are five species: *Equisetum arvense* L., *Equisetum telmateia* Ehrh., *Juniperus communis* L., *Lavandula angustifolia* Mill. and *Rosmarinus officinalis* L. The most widely known phytochemical compounds of *E. arvense* are flavonoids, phenolic acids, alkaloids, phytosterols, tannins, and triterpenoids [1] and its extract exhibits significant antioxidant, anticancer, antimicrobial and many other effects [2, 3]. Studies have shown that there are differences in the content of bioactive substances in *E. arvense* plants collected in different geographical areas [4]. According to Radojevic *et al.* [5] *E. telmateia* has anti-inflammatory and antioxidant activity. The ethyl acetate fraction of needles from *J. communis* possesses high antioxidant and hepatoprotective properties [6] and the essential oil from them has strong disinfectant properties [7, 8]. The essential oil of *L. angustifolia* contains over 300 chemical compounds [9] and it has antibacterial [10], antimicrobial [11], antifungal [12] and antioxidant [13, 14] properties. The essential oil also has an antispasmodic effect [15] and analgesic activity [16]. According to the review by Andrade *et al.* [17], *R. officinalis* has a great pharmacological potential. Its essential oil has various pharmacological activities such as antibacterial [18], antidiabetic [19], anti- inflammatory [20, 21], antitumor [22-24] and antioxidant [25].

The plant species selected in the present study have long been known for their health potential and have been used in folk medicine and in the pharmaceutical and food industries. It is not yet clear, however, which fractions their useful properties are due to. This study aims to determine the amounts of total phenols and the corresponding antioxidant activity.

MATERIALS AND METHODS

Plant material and extract preparation

Plant parts of the studied species were collected from June to September in the 2018 growing season. The location of the plant populations is indicated in Table 1. To determine the total phenol content and antioxidant activity sterile non-reproductive stems of *E. arvense* and *E. telmateia*; leaves, unripe and ripe berries of *J. communis*; leaves of *R. officinalis* and flowers of *L. angustifolia* were used. Voucher specimens from the studied populations are kept in the herbarium of the Agricultural University in Plovdiv (SOA). 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.

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Table 1. Location of the plant populations

Plant population	Location	North	East	Elev., m a. s. l.
E. arvense	Eastern Balkan Range, Sinite Kamani Natural Park, Karandila area – near the Karandila bakery	42°42.852’	26°22.654’	915
E. telmateia	Eastern Balkan Range, Sinite Kamani Natural Park, Karandila area – near the Karandila bakery	42°42.582’	26°22.284’	915
J. communis	Eastern Balkan Range, Sinite Kamani Natural Park, Upper lift station	42°43.100’	26°21.619’	1015
L. angustifolia	Thracian Plain, Chirpan, Tselina village	42°07.497’	25°26.126’	153
R. officinalis	Thracian Plain, Stara Zagora, Trakia University	42°24.027’	25°34.192’	275

After filtration, the residue was washed up in triplicate. Finally, the extracts were adjusted to a concentration of 1 mg.ml^{-1} calculated on dry matter (DM).

Determination of total phenol content

The experimental procedure described by Anesini *et al*. [26] was applied for determination of total phenol content. 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) were 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 μg.ml^{-1} were used for calibration curve (R^2 = 0.9987). TPC of each sample was expressed as mmol GAE in 1 kg DM of plant extract.

Determination of antioxidant activity by DPPH method

The method described by Serpen *et al.* [27] was applied to measure the radical-scavenging potential of methanolic extracts obtained from the selected plants. To 2 ml of 100 μM solution of DPPH in methanol 20 μl of methanolic extract was added. Absorption at 517 nm was measured 30 min later. Since the composition of the extracts is complex, the results for their radical-binding capacity were compared with that 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 calibration curve (R^2 = 0.9989). The results were expressed as mmol Trolox equivalent in 1 kg DM of plant material.

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

Determination of the total phenol content

Total phenol content (TPC) in the studied plants varies within wide range: from 92± 8 to 946 ± 76 milligrams of gallic acid equivalents (GAE) in 1 g of dry matter (DM) of methanolic extract (Table 2). In *E. arvense* and *E. telmateia* the values are very close – 151± 12 and 148 ± 11 mg GAE.g^{-1} DM.

The highest total phenolic content is in *J. communis* leaves. In the various parts of the plant it varies within a wide range – from 170 ± 15 in ripe berries to 946 ± 76 mg GAE.g^{-1} DM in leaves – 5.6 times more compared to ripe berries and 2.5 times more compared to unripe berries. Compared to the other plants, the highest total phenol content is in juniper except for the ripe berries only. High phenol content was found in the *R. officinalis* leaves, too (365 ± 33 mg GAE.g^{-1} DM) 2.4 times more than the two horsetail species and 4 times more than *L. angustifolia.* The lowest total phenol content was found in *L. angustifolia* flowers – 92 ± 8 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
Equisetum arvense sterile stems	151 ± 12	18.9 ± 1.7
Equisetum telmateia sterile stems	148 ± 11	18.2 ± 1.7
Juniperus communis leaf	946 ± 76	58.5 ± 5.5
Juniperus communis ripe berry	170 ± 15	25.1 ± 2.3
Juniperus communis unripe berry	373 ± 34	46.3 ± 4.6
Rosmarinus officinalis leaf	365 ± 33	46.6 ± 4.3
Lavandula angustifolia flower	92 ± 8	13.4 ± 1.2

Kukrić *et al.* [2] reported that total phenol content in alcohol extracts of field horsetail is high 355.80 ± 17.8 mg GAE.g^{-1} of the dried extract. Quantitative and qualitative variations in the content of some phenol compounds present in *E. arvense* are possible owing to ecological and geographical factors. The highest concentration of phenol compounds in plant extracts was obtained by means of high-polarity solvents. Total phenol content is high in all *E. telmateia* extracts, among which methanolic extract – 262.7 mg GAE.g^{-1}, acetone extract – 145 mg GAE.g^{-1}, ethyl acetate extract – 159 mg GAE.g^{-1}. According to Radojevic *et al.* [5] methanol appears to be the best solvent for extracting phenol compounds from *E. telmateia*. The results obtained in the present study are lower than those reported in [2] and [5].

It has been found that total polyphenol content in various *J. communis* leaf extracts (aqueous fraction, hexane fraction, ethanol extract and ethyl acetate fraction) varies from 189.65 to 315.33 mg GAE.g^{-1}. Maximum phenol amount is found in the ethyl acetate fraction [6]. In methanolic extracts of branches of five *Juniperus* species from Turkey, the total polyphenol content varies from 170.43 ± 2.13 mg GAE.g^{-1} to 253.29 ± 3.16 mg GAE.g^{-1} extract. In their water extracts the content is lower and varies from 98.74 ± 0.49 mg GAE.g^{-1} to 212.88 ± 2.95 mg GAE.g^{-1} extract [28, 29]. According to Živić *et al.* [30] and other authors, the highest polyphenol concentrations are found in alcohol extracts. In studying ethanol extracts of *J. oxycedrus* and *J. communis* berries, 58.73 ± 0.14 and 189.82 ± 0.27 mg GAE.g^{-1} were obtained, respectively. Ethyl acetate and chloroform extracts showed significantly lower total phenol content compared to ethanol extracts. Total polyphenol content in methanolic extracts of ripe berries of the two *J. oxycedrus* subspecies from Turkey is between 5.14 ± 0.06 and 17.89 ± 0.23 mg GAE.g^{-1} extract [31]. The results obtained by us about the polyphenol content in methanolic extracts of ripe *J. communis* berry are close to those in [31], but the values for unripe berries and leaves are higher.

Ethanolic extracts of rosemary leaves were produced by maceration and percolation and different ethanol concentrations (30, 40, 50, 60, 70, 80, 90 and 96 %) were used for extraction. The most potent solvent concentration was 50 % for the evaluation of total phenols 47.39 ± 0.21 mg/ml rosemarinic acid equivalents. After maceration and stirring the total phenol content increases up to 212.5 ± 0.05 and 219.45 ± 0.05 mg/ml RAE [32]. Tawaha *et al.* [33] reported *R. officinalis* to be a good source of polyphenol compounds varying from 2.8 to 70.3 mg GAE.g^{-1} methanolic extract. Due to the positive linear relationship found between antioxidant activity and total phenol content for methanolic extracts, they established that phenolic compounds were the predominant antioxidant components in the studied plant species. Pérez *et al.* [34] confirmed that rosemary extracts could serve as electron donors and reacted with free radicals transforming them into more stable products, thus terminating radical chain reactions. Solvents considerably affect total phenolic concentration in extracts. Methanolic extracts show higher antioxidant activity and higher phenolic content regardless whether irradiated with gamma rays or not. Therefore, methanol is the most efficient solvent for extracting phenolic compounds from rosemary leaves. Ünver *et al.* [35] reported high polyphenol content in methanolic extracts of rosemary – 214.21 ± 1.14 mg GAE.g^{-1}. High polyphenol content of methanolic extracts from leaves of species from Lamiaceae family, in particular rosemary and lavender, have been found by Spiridon *et al.* [36]. Comparing these results with the results from the present study: 365 mg GAE.g^{-1} for extract from *R. officinalis* leaves, the species from Bulgaria demonstrates higher TPC values.

Ethanolic extracts of plant cell cultures of lavender contain 85.6 ± 5.3 mg GAE.g^{-1} total

phenols [37]. Total polyphenol content in alcohol extracts from the flowers of 5 lavender species from Romania has been established by Robu *et al.* [38]. The values vary from 74.98 to 89.88 mg.g^{-1} dry extract. The TPC results obtained in the present study are very close to theirs. Lower phenol content in the above-ground parts of *L. stricta* in Sothern Iran has been reported by Alizadeh *et al.* [39]. Total phenolic content varies from 61.05 to 64.45 mg GAE.g^{-1} DM.

Some authors have found higher phenolic and flavonoid content in lavender leaves compared to flowers. Due to the existing positive correlation of total phenolic content with the antioxidant activity (AA), leaves demonstrate higher AA than flowers [40, 41].

Determination of the antioxidant activity

Antioxidant activity (AA) of the plant species included in this study was expressed in mmol of Trolox equivalents (TE) in 1 kg DM of the methanolic extract. It ranged from 13.4 ± 1.2 to 58.5 ± 5.5 mmol TE.kg^{-1} DM (Table 2). From the studied plants the highest antioxidant activity was found for the extracts from *J. communis* leaves – 58.5 ± 5.5 mmol TE.kg^{-1} DM. Twice lower is AA of the ripe berries. Unripe berries of *J. communis* showed AA closer to that of leaves. *R. officinalis* leaf extracts also showed high AA (46.6 ± 4.3 mmol TE.kg^{-1} DM) – almost the same as that determined for the *J. communis* unripe berries.

L. angustifolia flower extracts showed the lowest AA – 13.4 ± 1.2 mmol TE.kg^{-1} DM. The difference between the AA values of *E. arvense* and *E. telmateia* is insignificant – 18.9 ± 1.7 and 18.2 ± 1.7 mmol TE.kg^{-1} DM.

E. arvense ethanolic extract belongs to the group of strong antioxidants due to the stable DPPH radical [2]. The greatest capacity for neutralizing DPPH radicals found by [5] for three different *E. telmateia* extracts was measured in its methanolic extract, which neutralizes 50 % of the free radicals in a very low concentration (33.4 µg/ml).

The results obtained about a number of plant species, [33], indicate *R. officinalis* to be one of the best sources of compounds removing free radicals.

According to Živić *et al.* [30] *J. communis* and *J. oxycedrus* berry extracts reveal significant AA. Compared to ethyl acetate and chloroform extracts, AA of the alcoholic extracts is the highest. Phenol-rich ethyl acetate fraction of the ethanolic extract of the leaves of *J. communis* has high AA, which determines its hepatoprotective potential [6].

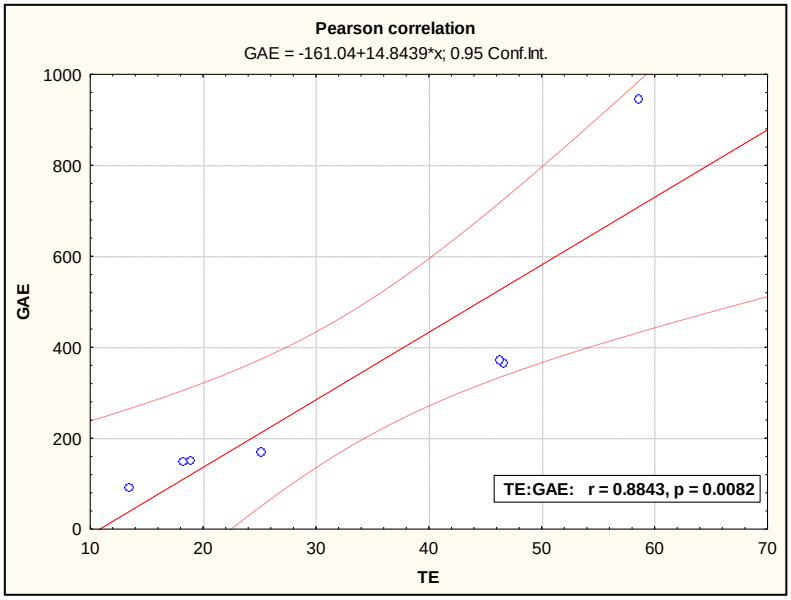

Figure 1. Pearson correlation between TPC and antioxidant activity, P ≤ 0.01 (2-tailed)

The present study has found a positive linear dependence between the AA and TPC values (Figure 1). Pearson correlation between the total phenol content and the antioxidant activity of the methanolic extracts was observed with a high positive coefficient r = 0.8843 (p ≤ 0.01), so these compounds are responsible for the antioxidant activity of the metanolic extracts of the tested plants. Most authors cited in this study, including [2, 5, 30, 33, 41, 42, etc.] also established such correlation.

CONCLUSION

In the present study the total phenol content and the antioxidant activity were measured by determination of the radical scavenging potential, thus bringing some clarity to the properties of the polar methanol extractions prepared from the tested medicinal plants.

The highest total phenol content and antioxidant activity were measured in *J. communis* leaves and unripe berries and *R. officinalis* leaves. The correlation between TPC and radical scavenging potential was found to be positive with high a correlation coefficient. Thus, although plant species from different families were tested, a positive relationship between the two determined parameters was confirmed.

The medicinal plants from populations in Bulgaria included in this study demonstrate high phenol content and antioxidant activity, therefore they can be used as substitutes of synthetic antioxidants in food products and additives used for people and animals and pharmaceuticals.

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