ANTIOXIDANT POTENTIAL OF MEDICINAL HERBS FROM THE NORTHERN BLACK SEA COASTAL ZONES

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ABSTRACT

Aqueous-alcoholic extracts of 32 plants used in Bulgarian phytotherapy for treatment of respiratory, gastro-intestinal and other inflammatory disorders and widely distributed in the Northern Black Sea coastal zones were screened in vitro for antioxidant activity and phenolic compounds content. The antioxidant potential presented as UAE (Uric acid equivalents) of the plant extracts was determined using the ABTS cation radical decolorization method. The content of total polyphenols was measured spectrophotometrically according to the Folin-Ciocalteu procedure and calculated as quercetin equivalents (QE). Four Bulgarian medicinal plants were established to have very high antioxidant properties: *Fragaria vesca*, *Hypericum perforatum*, *Agrimonia eupatoria* and *Rubus sp. diversa* (UAE above 10 mM); another 10 plants had intermediate antioxidant capacity - higher than 4mM; and other 5 - higher than 2 mM. Interestingly, those plants comprised 58% of all studied plant species and the high antioxidant potential of these medicinal plants could be a major factor contributing to their healing properties. Polyphenol content varied from $39,52 \pm 15,17 \mu\text{M}$ to $2930,70 \pm 62,01 \mu\text{M}$. A positive correlation ($r=0.98$) between antioxidant activity and polyphenol content was found, suggesting that the antioxidant capacity of the aqueous-alcoholic plant extracts is mainly due to their polyphenol content.

Key words: Bulgarian medicinal plants, aqueous-alcoholic extracts, antioxidant activity, total polyphenol content

INTRODUCTION

Medicinal plants, known also as medicinal herbs, include a variety of plants used in medicinal or veterinary practice for prophylaxis and treatment of diseases - generally the term "medicinal plant" refers to the application of a plant. They are also often known as drug- or poison- plants since the medical usefulness is often only a matter of the dilution factor in which the active ingredient is introduced into or onto the human body. Since thousands of years ago people were apparently aware of hundreds of medically active compounds that were directly derived from plants. Such biologically active compounds from plant origin may range from alkaloids to glycosides, vitamins, waxes, tanning matters, essential oils, coumarins, lipids, pigments, saponins, phytoncides, or flavonoids. A number of medicinal plants are recognized nowadays as functional foods, food additives and a source for nutraceuticals, because of their active ingredients, and others are used by the pharmaceutical and cosmetic industries. Medicinal properties of plants are often described as anti-inflammatory, bactericide, bacteriostatic, immunostimulating

or other, and are related to the induction of resistance to various inflammatory diseases - gastrointestinal, urinary tract infections, respiratory or skin diseases etc. Part of the medicinal herbs' properties are attributed to the antioxidant activities of their constituents, including vitamins A, C and E and polyphenols and various concepts dominate assigning the biological activity of polyphenols to their properties as reducing agents, hydrogen donors, metal chelators and radical quenchers (9,11) or immunostimulators (1,2).

Despite the wide distribution of Bulgarian medicinal plants, only few recent systematic studies explore their antioxidant potential (6-8) and no investigation had been carried out since now on the distribution of plant species with high antioxidant properties. The present study aims at screening the in vitro antioxidant activity and polyphenol content of medicinal plants widely distributed in the Northern wet Black Sea regions and used in Bulgarian phytotherapy for treatment of respiratory, gastrointestinal and other inflammatory disorders.

MATERIALS AND METHODS

Plants

Thirty two Bulgarian medicinal plants were selected based on two major criteria: (i) established wide everyday use by the Bulgarian population for treatment of inflammatory

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conditions of various etiology and (ii) distribution in the vicinity of the town of Varna and the Northern Black sea coastal zones (3-5). The plants available on the market without prescription (commercial products of "Thalloderma" Pharmaceutical Laboratories) were collected from the region in the summer of 2008.

Plant extracts

Plant extracts were prepared as described earlier (8), with variations in the solvent type and concentration. Briefly, 250 mg dried plant material was blended into a fine powder and extracted 3 times (3 min each) with 40% (v/v) ethanol/water. Plant material/solvent ratio was kept to 1:20 w/v (0.250g:5ml). The supernatants from each extraction step were combined (3.5ml from the first step, 4.5ml from the second and 5ml from the third - 13 ml totally) and were diluted with 40% (v/v) ethanol/water to total volume of 25ml. Antioxidant activity and total polyphenol content were measured in a clear filtrate.

Antioxidant activity of plant extracts

The antioxidant activity of plant extracts was determined by the ABTS (2,2'-azinobis (3- ethylbenzothiazoline- 6-sulfonic acid)) radical decolorization assay (10). The extend of decolorization as percentage inhibition of the pre-formed ABTS+ radical cation, proportional to the concentration of antioxidants, was calculated relative to the reactivity of uric acid as a standart. Results are presented as meansuric acid equivalents (mM UAE) $\pm$ S.D. (n, number of independent experiments = 3-6). ABTS, uric acid and potassium persulfate were purchased from Aldrich Chemical Company, Inc., Milwaukee, USA. Measurements were performed using a Perkin Elmer spectrophotometer.

Total polyphenol content

Phenolic compounds were assayed, according to the spectrophotometric method of Singleton and Rossi (12). Samples (7 μ l, five replicates) were introduced into test tubes; 1000 μ l of Folin-Ciocalteu's reagent diluted ex tempore with distilled water I ratio 1:10, and 800 μ l NaHCO₃ (7.5%) were added. The tubes were mixed and incubated at 60°C for 10 min. Absorption at 760 nm was measured (Perkin Elmer spectrophotometer). The total phenolic content was expressed as means quercetin equivalents (μ M QE) $\pm$ S.D. Quercetin and Folin-Ciocalteu's reagent were obtained from Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany.

RESULTS AND DISCUSSTION

Measurements of antioxidant activity and polyphenol content of aqueous-alcoholic extracts demonstrated considerable variations within the plant species: from 0,28 $\pm$ 0,16 mM UAE for *Humulus lupulus* to 16,13 $\pm$ 0,28 mM for *Fragaria vesca* (Table 1). Similar variations were evident from our previous studies (5,6) of water-phase antioxidant activity of medicinal plants. Totally, very high antioxidant properties were established for aqueous-ethanolic extracts

of four Bulgarian medicinal plants: *Fragaria vesca*, *Hypericum perforatum*, *Agrimonia eupatoria* and *Rubus sp. diversa* (UAE above 10 mM); another 10 plants had intermediate antioxidant capacity - higher than 4 mM; and other 5 - higher than 2 mM. Interestingly, those plants comprised as much as 58 % of all studied plant species. The high antioxidant potential of these medicinal plants could be a major factor contributing to their healing properties.

There were variations in the polyphenol content as well: from 39,52 $\pm$ 15,17 μ M for *Valeriana officinalis* to 2930,70 $\pm$ 62,01 μ M QE for *Fragaria vesca*, and the higher polyphenol concentrations were measured for the extracts with higher antioxidant activity. A positive correlation ($r=0.98$) between antioxidant activity and polyphenol content was found (Fig. 1), suggesting that the antioxidant capacity of the aqueous-alcoholic plant extracts is mainly due to their total polyphenol content. Similarly, a high positive correlation was established earlier for aqueous-methanolic extracts of 23 Bulgarian herbs (8) and for the water infusions of those plants (6,7).

Fig. 1. Correlation between the antioxidant activity and total polyphenol content of aqueous-ethanolic extracts (40%, v/v) of 32 medicinal plants from the Northern

CONCLUSION

Medicinal plants of North Black Sea coastal zones represent an excellent source of polyphenols with more than a half of all studied plant species exhibiting considerable antioxidant capacity. Our findings along with the numerous properties attributed to plant polyphenols give reason to suggest that the high polyphenol content of Bulgarian herbs is the major factor contributing to plants' healing properties.

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Table 1. Antioxidant activity and total polyphenol content in aqueous-ethanolic extracts (40%, v/v) of 32 medicinal plants from the Northern Black sea coastal zones.

Plant name	Part of plant tested	Antioxidant activity means $\pm$ S.D. [mM UAE]	Total polyphenols means $\pm$ S.D. [μ M QE]
<i>Fragaria vesca</i> L.	Leaves	16,13 $\pm$ 0,28	2930,70 $\pm$ 62,01
<i>Hypericum perforatum</i> L.	Aerial parts	13,49 $\pm$ 0,29	2585,49 $\pm$ 21,46
<i>Agrimonia eupatoria</i> L.	Aerial parts	11,59 $\pm$ 0,29	2095,11 $\pm$ 52,13
<i>Rubus sp. diversa</i>	Leaves	10,14 $\pm$ 0,28	1741,94 $\pm$ 43,26
<i>Sambucus ebulus</i> L.	Fruits	7,22 $\pm$ 0,31	1587,92 $\pm$ 32,45
<i>Salvia officinalis</i> L.	Leaves	5,80 $\pm$ 0,04	1308,72 $\pm$ 22,33
<i>Crataegus monogyna</i> Jacq.	Leaves, flowers	4,62 $\pm$ 0,06	985,13 $\pm$ 40,21
<i>Frangula alnus</i> Mill.	Roots	4,51 $\pm$ 0,32	1033,71 $\pm$ 207,02
<i>Sambucus nigra</i> L.	Flowers	4,38 $\pm$ 0,09	893,88 $\pm$ 108,53
<i>Crataegus monogyna</i> Jacq.	Fruits	4,37 $\pm$ 0,05	1058,60 $\pm$ 41,25
<i>Populus alba</i> L.	Leaves, flowers	4,36 $\pm$ 0,22	940,01 $\pm$ 22,33
<i>Polygonum aviculare</i> (L.) L.	Aerial parts	4,17 $\pm$ 0,12	801,02 $\pm$ 34,14
<i>Arctium lappa</i> L.	Roots	4,13 $\pm$ 0,05	440,06 $\pm$ 127,99
<i>Helichrysum arenarium</i> (L.) Moench.	Flowers	4,05 $\pm$ 0,82	803,67 $\pm$ 21,46
<i>Matricaria chamomilla</i> L.	Flowers	2,60 $\pm$ 0,05	639,92 $\pm$ 25,10
<i>Pulmonaria officinalis</i> L.	Leaves	2,39 $\pm$ 0,11	782,43 $\pm$ 24,53
<i>Asparagus officinalis</i> L.	Aerial parts	2,29 $\pm$ 0,15	378,55 $\pm$ 52,92
<i>Achillea millefolium</i> L.	Flowers	2,10 $\pm$ 0,02	558,49 $\pm$ 108,97
<i>Taraxacum officinalis</i> L.	Aerial parts	2,05 $\pm$ 0,11	805,62 $\pm$ 114,44
<i>Plantago mayor</i> L.	Leaves	1,60 $\pm$ 0,14	508,92 $\pm$ 45,17
<i>Apium graveolens</i> L.	Leaves	1,62 $\pm$ 0,06	348,70 $\pm$ 74,92
<i>Mentha spicata</i> L.	Leaves	1,61 $\pm$ 0,02	546,98 $\pm$ 61,33
<i>Verbena officinalis</i> L.	Aerial parts	1,60 $\pm$ 0,08	710,02 $\pm$ 67,67
<i>Galega officinalis</i> L.	Aerial parts	1,11 $\pm$ 0,02	426,77 $\pm$ 64,39
<i>Urtica dioica</i> L.	Leaves	1,04 $\pm$ 0,13	204,03 $\pm$ 54,00
<i>Ononis spinosa</i> L.	Roots	0,88 $\pm$ 0,03	204,03 $\pm$ 22,33
<i>Astragalus glycyphyllos</i> L.	Aerial parts, fruits	0,74 $\pm$ 0,03	308,16 $\pm$ 15,33
<i>Prunus spinosa</i> L.	Fruits	0,72 $\pm$ 0,05	293,82 $\pm$ 42,93
<i>Capsella bursa-pastoris</i> (L.) Medic.	Aerial parts	0,66 $\pm$ 0,05	202,65 $\pm$ 37,18
<i>Taraxacum officinalis</i> L.	Roots	0,64 $\pm$ 0,02	243,01 $\pm$ 140,70
<i>Valeriana officinalis</i> L.	Roots	0,59 $\pm$ 0,04	39,52 $\pm$ 15,17
<i>Humulus lupulus</i> L.	Flowers	0,28 $\pm$ 0,16	78,73 $\pm$ 50,20

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