International Food Research Journal 22(1): 240-245 (2015)
Journal homepage: *http://www.ifrj.upm.edu.my*

Screening of total phenolic content and radical scavenging capacity of Bulgarian plant species

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Article history

Received: 21 April 2014
Received in revised form: 8 May 2014
Accepted: 16 May 2014

Keywords

Antioxidant activity
Polyphenolic content
Bulgarian plants

Abstract

The antioxidant activities of nine Bulgarian plants have been investigated, in the light of recent scientific developments, throughout the world due to their potent pharmacological activities and food viability. In the present study, the possible radical scavenging activity of *Clinopodium vulgare* L., *Cotinus coggygria* Scop., *Matricaria chamomilla* L., *Melissa officinalis* L., *Mentha peperita* L., *Origanum vulgare* L., *Salvia officinalis* L., *Sideritis scardica* Griseb. and *Thymus vulgaris* L. was investigated using different antioxidant methodologies: radical scavenging activity by DPPH and ABTS radicals and reducing power. The extracts have been performed of the dried powered samples using heat-reflux extraction with 96 % ethanol and methanol, respectively. The total polyphenol content of the extracts was investigated as well. The experiments conducted revealed that the investigated plant species are potential sources of polyphenolic compounds and antioxidant activity.

Introduction

It is known that antioxidants act as a defense mechanism that protect against oxidative damage, and include compounds to remove or repair damaged molecules. It can prevent/retard the oxidation caused by free radicals and sufficient intake of antioxidants is supposed to protect against diseases (Celiktar *et al*., 2007). Free radicals are not only produced naturally in the cell following a stress or respiration but also have been reported to be produced by radiation, bacterial and viral toxins, smoking, alcohol and psychological or emotional stress (Gamiotea-Tirro *et al.*, 2004).

Oxidative stress is a factor for many human diseases, as either a cause or an effect. It has been established that oxidative stress is among the major causative factors in induction of many chronic and degenerative diseases including atherosclerosis, diabetes mellitus, cancer, Parkinson's disease and immune dysfunction and is involved in aging (Halliwell, 2000; Metodiewa *et al*., 2000; Young *et al*., 2001). Plants are the source of medication for preventive, curative, protective or promotive purposes (Sidhu *et al.*, 2007). Epidemiological and *in vitro* studies on medicinal plants and vegetables strongly have supported the idea that plant constituents with antioxidant activity are capable of exerting protective effects against oxidative stress in biological systems (Block *et al.*, 1992; Ness *et al.*, 1997; Cao *et al.*, 2001).

Phenolic compounds with antioxidant activity, which are widely distributed in many fruits, vegetables, and tea, are believed to account mainly for the antioxidant capacity of many plants (Kaur *et al*., 2002; Vinson *et al*., 1998). Bulgaria constitutes an apt example where medicinal plants are widely used in everyday life as part of folk medicinal remedies. Bulgarian flora features a remarkable diversity and is a rich source of medicinal plants – 600 out of 3500 plant species recognized as medicinal and are traditionally used in ethnopharmacology and phytotherapy (Dimkov, 1979; Petkov, 1982). There are many reports in the literature about the antioxidant properties of medicinal plants (Zheng and Wang, 2001; Ivanova *et al.* 2005; Katalinic *et al*., 2006; Wojdylo *et al*., 2007), but there are only few papers reporting data about the antioxidant properties of Bulgarian herbs using methods such as DPPH and ABTS (Ivanova *et al.*, 2005; Kiselova *et al.*, 2006 Kratchanova *et al*., 2010; Mihaylova *et al*, 2013).

The aim of the current study was to evaluate the total polyphenolic content, antioxidant activity and reducing power of 9 Bulgarian plants, which are oft used in Bulgarian cuisine and folk medicine. In this regard, ethanol and methanol extracts of *Clinopodium vulgare* L., *Cotinus coggygria* Scop.,

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Table 1. Bulgarian plants investigated in the study and their usage in traditional phytotherapy

Plant	Common name	Part of plant tested	Medical use
Clinopodium vulgare L.	Wild basil	Leaves and flowers	Immunostimulant, cardio-tonic, verruca
Cotinus coggygria Scop.	Smoke tree	Leaves	Gastric and duodenal ulcer, antidiarrhetic, anti-inflammatory agent, paradontosis
Matricaria chamomilla L.	Chamomile	Flowers	Anti-inflammatory, antiseptic, sedative, throat and mouth inflammations, gastrointestinal disorders, influenza, pharyngitis, laryngitis
Melissa officinalis L.	Common balm	Leaves	Sedative, gastrointestinal disorders
Mentha piperita L.	Peppermint	Leaves	Spasmolytic, antiseptic, gastric disorders, indigestion, neuralgia, myalgia, antivomiting
Origanum vulgare L.	Wild marjoram	Leaves	Antitussive, expectorant, sedative, choleretic, cholagogue
Salvia officinalis L.	Sage	Leaves and flowers	Anti-inflammatory, antiseptic, inflammations of throat and mouth
Sideritis scardica Griseb.	Mountain tree	Leaves and flowers	Expectorant, antitussive, bronchitis, cough
Thymus vulgaris L.	Thyme	Leaves and flowers	Expectorant, spasmolytic, antibacterial, antitusive, asthma, emphysema, whooping-cough, diseases of respiratory tract

Matricaria chamomilla L., *Melissa officinalis* L., *Mentha peperita* L., *Origanum vulgare* L., *Salvia officinalis* L., *Sideritis scardica* Griseb., *Thymus vulgaris* L. were investigated.

Materials and Methods

Plant material

Leaves and flowers of *Clinopodium vulgare* L., *Cotinus coggygria* Scop., *Matricaria chamomilla* L., *Melissa officinalis* L., *Mentha peperita* L., *Origanum vulgare* L., *Salvia officinalis* L., *Sideritis scardica* Griseb., *Thymus vulgaris* L. (Table 1) as dry herbs were obtained from local pharmacies (Plovdiv, Bulgaria). After additional drying of the plant parts, leaves and stems were roughly grounded and stored in air-tight dark containers until extraction.

Preparation of the plant extracts

In order to obtain ethanol and methanol extracts of the investigated samples 0.5 g grounded plant material was placed in a round bottom flask and was subjected to heat-reflux extraction with 30 ml of the appropriate solvent in a water bath for 30 minutes. The final extracts were filtered with a Buchner funnel and the total volume was filled up to 30 ml with the solvent applied. The obtained extracts were stored at 4 °C without adding any preservatives until use.

Determination of total polyphenolic content (TPC)

The TPC was determined using Folin-Ciocalteu's phenol reagent method (Kujala *et al*., 2002) was expressed in terms of gallic acid equivalent (mg GAE)/ g dry weight (DW).

Antioxidant activity

DPPH radical scavenging activity

The DPPH radical scavenging activity was determined (Brand-Williams *et al*., 1995) after 30 min incubation at room temperature and the light absorption was measured by room temperature. The results were expressed as function of the concentration of Trolox (μM TE/g DW).

ABTS radical scavenging assay

The radicals scavenging activity of the extracts against radical cation ($ABTS^{\bullet+}$) was estimated according to a previously reported procedure with some modifications (Re et al. 1999). The results were expressed as TEAC value (μM TE/g DW).

Reducing power

The reducing power of the extracts was determined according to the method of Oyaizu (1986). Vitamin C was used as positive control.

Table 2. Total polyphenol content (mg GAE/g DW) of methanol and ethanol extracts of nine medicinal Bulgarian plants

Plant material/extract	Total polyphenolics content	
	ethanol extract	methanol extract
Wild basil	10.18 ± 0.68	27.37 ± 2.14
Smoke tree	114.73 ± 1.14	113.75 ± 8.79
Chamomile	4.50 ± 0.69	18.89 ± 0.45
Common balm	38.11 ± 0.46	37.52 ± 0.23
Peppermint	12.29 ± 0.91	33.53 ± 0.64
Wild marjoram	11.18 ± 0.96	22.45 ± 0.92
Sage	15.44 ± 0.69	25.67 ± 2.48
Mountain tree	18.68 ± 2.26	23.65 ± 0.09
Thyme	11.16 ± 2.76	25.20 ± 0.57

Statistical analysis

The presented results are average from two independent experiments carried out in triplicates. The results were expressed as mean ± SD analyzed using MS-Excel software.

Results and Discussion

Total polyphenolic content

Plants have been used traditionally for the treatment and prophylaxis of different disorders. Their protection effect has often been attributed to plant antioxidants such as polyphenols and vitamins C, E, β-carotene (Prior, 2003). Based on this in the present study the first step was to evaluate the total polyphenolic content of the obtained plant extracts.

The results for the both ethanolic and methanolic extracts are shown in Table 2. The established values among the studied Bulgarian plants varied in wide ranges from 4.50 ± 0.69 to 114.45 ± 0.92 mg GAE/g DW. However, using gallic acid as a standard, the highest polyphenol content of ethanol extracts was recorded by smoke tree 114.73 ± 1.14 mg GAE/ g DW and for methanol extracts again by smoke tree and by wild marjoram as well, respectively 113.75 ± 8.79 and 114.45 ± 0.92 mg GAE/ g DW. The established results were in agreement with the reported by Mihaylova and Georgieva (2013) - highest TPC in water extracts of smoke tree and wild marjoram - 40.60 and 30.17 mg GAE/g dw, respectively. Ivanova *et al*. (2005) by their investigation on the traditional prepared infusions of Bulgarian medicinal plants established extremely high TPC for the smoke tree extract compared to the 21 studied plants (923.33 ± 14.19 μM/QE).

In the present research the lowest polyphenol concentration among the examined ethanol and methanol extracts had the chamomile 4.50 ± 0.69 and 18.89 ± 0.45 mg GAE/ g DW, respectively, which correspond well with previously conducted investigations on this plant (Ivanova *et al.*, 2005; Mihaylova and Georgieva, 2013). Based on the results it can be assumed that in the plant extracts the TPC depends on the extraction solvent used. However the results were in favor of methanol as solvent. Regarding the smoke tree the both solvents were not distinguishable.

The reported from us results for the smoke tree extracts were not surprising because this plant is a common medicinal plant used in Balkan folk medicine, predominantly for external administration as an anti-haemorrhagic and wound-healing remedy (Landzhev, 2010). However, several authors report that aqueous infusions from leaves can be applied orally for treatment of throat and stomach inflammations, gastric ulcer, diarrhea and even diabetes mellitus. (Kultur, 2007; Novakovic *et al*., 2007; Dulger *et al.*, 2009). These findings highlighting the potential of the plant.

However, it is known that the polyphenols are the most abundant antioxidants in the diet. Since phenols and polyphenolic compounds have been shown to possess significant antioxidant capacities (Van Acker *et al*. 1996) and several studies have successfully correlated the polyphenolic content with the antioxidant activity (Tepe *et al*., 2006; Mihaylova *et al*., 2013), the next step was to evaluate the antioxidant activity of the studied extracts.

Investigation of antioxidant activity (AOA)

Many studies have shown that natural antioxidants in plants are closely related to their biofunctionalities such as the reduction of chronic diseases and other, which are often associated with the termination of free radical propagation in biological systems (Covacci *et al*., 2001). Thus, antioxidant capacity is widely used as a parameter to characterize food or medicinal plants and their bioactive components.

In order to investigate the antioxidant activity of the investigated plant extracts, in the present study have been conducted series of *in vitro* tests: 2,2-diphenil-1-picrylhydrazyl free radical scavenging, 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid free radical scavenging and reducing power. Antioxidant properties, especially radical scavenging activities, are very important due to the deleterious role of free radicals in foods and in biological systems. Excessive formation of free radicals accelerates the oxidation of lipids in foods and decreases food quality and consumer acceptance

Table 3. Antioxidant activity of methanol and ethanol extracts of nine Bulgarian plants, µMTE/ g DW

Plant material/ extract	$TEAC_{DPPH}$		$TEAC_{ABTS}$	
	ethanol extract	methanol extract	ethanol extract	methanol extract
Wild basil	9.59 ± 0.11	6.07 ± 0.23	0.44 ± 0.11	0.71 ± 0.05
Smoke tree	48.97 ± 0.25	61.27 ± 0.34	4.97 ± 0.45	4.54 ± 0.12
Chamomile	6.01 ± 0.57	4.65 ± 0.55	0.45 ± 0.56	0.18 ± 0.04
Common balm	17.65 ± 0.75	36.89 ± 0.12	0.97 ± 0.28	0.46 ± 0.01
Peppermint	7.86 ± 0.20	24.06 ± 0.47	0.39 ± 0.36	1.42 ± 0.15
Wild marjoram	7.94 ± 0.55	17.62 ± 0.20	0.44 ± 0.10	1.78 ± 0.17
Sage	8.67 ± 0.12	6.98 ± 0.16	0.66 ± 0.09	0.67 ± 0.05
Mountain tree	6.62 ± 0.11	8.94 ± 0.34	0.33 ± 0.08	0.29 ± 0.02
Thyme	11.00 ± 0.09	7.97 ± 0.78	0.53 ± 0.11	0.69 ± 0.01

(Min, 1998).

The results for scavenging of DPPH radical represented in table 3 were in range from 4.65 ± 0.55 to 61.27 ± 0.34 µMTE/ g DW. Higher TEAC value indicates that a sample has stronger AOA. Moreover high TEAC values correspond to a great extent to high polyphenolics content as well (Tables 2 and 3). The TEACDPPH values of ethanol extracts show that the highest antiradical capacity toward DPPH was measured for smoke tree 48.97 ± 0.25 µMTE/g DW and common balm 17.65 ± 0.75 µMTE/g DW. The highest TEAC values of methanol extracts were established for smoke tree 61.27 ± 0.34 µMTE/g DW and common balm 36.89 ± 0.12 µMTE/g DW.

Antioxidant activity of investigated plant extracts was determined as well with ABTS radical decolorization assay, by the intensity of green color, where the degree of color change is proportional to the concentration of present antioxidants. The results for scavenging of ABTS radical (Table 3) were in range from 0.18 ± 0.04 to 4.97 ± 0.45 µMTE/ g DW. The highest TEAC-values 4.97 ± 0.45 and 4.54 ± 0.12 µM TE/g DW were achieved for the both smoke tree extracts, followed by the wild majoram extract obtained with methanol (1.78 ± 0.17 µMTE/g DW). The reported results are in agreement with the total polyphenol content evaluation.

In order to achieve better assessment of the antioxidant potential, the nine ethanol and methanol extracts object in the present research were studied for their reducing power as well. Reduction properties of the extracts are mainly associated with the presence of reductons. It is believed that the AOA is based on the interruption of radical chain-reaction by giving electrons to free radicals, making them more stable end products.

For the purpose of the current study 10-fold dilutions of the extracts were preliminary prepared. The methanolic extracts of common balm and smoke tree were diluted 20-fold. As standard compound for comparison was used L-ascorbic acid. For the both type of extracts the best results were established for the smoke tree (Table 4). In Brief, the results revealed that the ethanol extract with the total highest polyphenolic content and the highest antioxidant potential according the applied methods was the smoke tree extract. The same trend was observed by the methanol extracts, where similar to the Cotinus coggygria results were established for wild marjoram and peppermint.

Table 4. The reducing power of ethanol and methanol extracts of the different plant, mg equivalents L-ascorbic acid/g DW

Plant material/ extract	Reducing power	
	ethanol extract	methanol extract
Wild basil	0.430 ± 0.05	0.629 ± 0.11
Smoke tree	1.363 ± 0.97	1.464 ± 0.15
Chamomile	0.223 ± 0.01	0.266 ± 0.28
Common balm	1.001 ± 0.66	0.994 ± 0.45
Peppermint	0.412 ± 0.12	0.785 ± 0.45
Wild marjoram	0.320 ± 0.15	1.212 ± 0.11
Sage	0.427 ± 0.29	0.622 ± 0.45
Mountain tree	0.302 ± 0.69	0.362 ± 0.85
Thyme	0.361 ± 0.72	0.562± 0.61

Conclusions

In the present study nine ethanol and methanol extracts from different traditionally used in Bulgaria plants were studied for their polyphenolic content and antioxidant potential. It was established that the extracting solvent affects significantly the total polyphenolic content and the antioxidant capacity measured. It can be concluded that several of the

investigated plant species are a promising source of polyphenolic compounds and free radical scavengers based on various *in vitro* assays. The extracts could be used as easily accessible source of natural antioxidants and therefore assumed as efficient preventive agents with potential for medicinal and commercial uses. However, further investigations are needed to evaluate the toxicity degree.

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