



Original Research Article

Comparative study on the antioxidant activity of selected culinary plants growing in Bulgaria

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ABSTRACT

Keywords

Chrysanthemum balsamita,
Melissa officinalis,
Allium bulgaricum,
Culinary,
Polyphenol content,
Antioxidant activity

Culinary plant extracts prepared with 70% ethanol from three species *Chrysanthemum balsamita* L., *Melissa officinalis* L. and *Allium bulgaricum* L. growing in Bulgaria were screened for their *in vitro* antioxidant activity and total phenolic compounds content. Samples of fresh plant material were subjected to heat-reflux extraction and several complementary tests were used in order to determine the antioxidant activity of the extracts, namely ABTS radical scavenging, DPPH radical cation decolorization activity, ferric reducing antioxidant power (FRAP) assay and copper reduction assay (CUPRAC). The total polyphenolic content of the obtained extracts was executed spectrophotometrically using the Folin–Ciocalteu's phenol reagent. The concentration of polyphenolics was established to be in the range from 0.41 ± 0.08 to 2.71 ± 0.15 mg GAE/g fw. *M. officinalis* fresh leaves showed the strongest antioxidant activity according to all performed assays. Based on the results of the present study, it was revealed that the investigated Bulgarian culinary plants could be considered a potential source of polyphenolics and thus could be applied as antioxidants of natural origin in culinary.

Introduction

Herbal remedies have been used for thousands of years (Backer, 1965). Many plants, used as medical herbs, culinary spices or food ingredients have been recognized to have beneficial impact on health, e.g. antioxidant activity, digestive stimulation action, antimicrobial, hypolipidemic, antiinflammatory, antimutagenic effects and anticarcinogenic potential (Temple, 2000; Pandey and Rizvi,

2009). Some of them are applied to enhance flavour, to inhibit spoilage of foods, to improve biological activity, thus being an additional or a main ingredient in traditional meals or beverages (Temple, 2000; Alexieva, 2010; Sapundjieva *et al.*, 2012). Due to the specific content of antioxidants, plant extracts are of increasing interest for the food industry because they can be used as natural additives to improve the quality,

nutritional value and healthy potential of food. The antioxidant activity of plants depends on the type, quality, part (leaves, stalk, flower and seeds) of the plant, location of habitat, climatic conditions, soil characteristics, etc. Extraction method and solvent agent (water, alcohol, etc.) are also important factors, considerably effecting plant antioxidants capacity (Stoilova *et al.*, 2007; Maizura *et al.*, 2011; Petkova *et al.*, 2012; Vrancheva *et al.*, 2012).

Taking into account the increasing demand for natural ingredients that might be used as food additives, components of functional foods and for other applications (pharmaceutical, cosmetic, etc.), it is reasonable to revise the local plants by assessing their benefits using contemporary scientific analysis methods.

Costmary (*Chrysanthemum balsamita* L.) is a medicinal plant, belonging to the *Asteraceae* family. It is a large perennial plant of Asian origin with yellow flowers, grown in Europe and Asia since the Middle Ages (Bylaitè *et al.*, 2000). This plant has a hairy stem, complete shiny leaves, highly branched from the base and 70–120 cm height (Nickavar *et al.*, 2003; Hassanpouraghdam *et al.*, 2008). It has a characteristic odor due to its volatile oil constituents. The main compounds (above 3%) are: carvone (47.81%), α -thujone (12.56%), germacrene B (5.23%), benzaldehyde (4.64%) ethylbenzene (3.96%) and germacrene D (3.13%). In the traditional cuisine it is mainly used as a flavouring agent (Alexieva, 2010).

Allium bulgaricum (*Nectaroscordum siculum* Lindl. *Nectaroscordum siculum* ssp. *bulgaricum* (Janka) Stearn; *Allium s.ursium* var. *Dioscoridis*) is a plant from the family of *Amaryllidaceae*, subfamily *Allioideae*, *Allium* species. It is reported to grow in

Bulgaria, Caucas, Moldova, Romania, Turkey, Sicily and Malta (Hardalova *et al.*, 1994; Saukel *et al.*, 2003; Petrova and Vladimirov, 2010). In Bulgaria the plant can be found along the Black sea coastal area - Strandja region, in the Eastern Sredna Gora and in some areas of the Central and Eastern Stara Planina (Ozhatay, 2002; Radanova, 2006). The local name is “samardala” or “Bulgarian honey garlic”. *Allium bulgaricum* (samardala) is a glabrous plant, 50–100 (150) cm high. The plant is characterized by a powerful and heavy specific smell. It is poorly known in the other countries as a medical plant or as a culinary spice. It is more famous for the flowers in the gardens, than for its healing or flavouring properties. Traditionally it is used in salads or cooked meals (Alexieva, 2010). A common herb, lemon balm (*Melissa officinalis*) is native to southern Europe and northern Africa, Caucasus and northern Iran (Meftahizade *et al.*, 2010) and used to give a citrus flavor and aroma to foods and beverages. It has also been used as an herbal medicine to treat headaches, gastrointestinal disorders, nervousness and rheumatisms (Mimica-Dukic *et al.*, 2004). Like many herbs, the essential oil of lemon balm (*Melissa officinalis*), which is rich in aldehydes and terpenic alcohols (Robeiro *et al.*, 2004), is reported to have anti-microbial properties as well as a strong protective ability against lipid peroxidation (Mimica-Dukic *et al.*, 2004; Hedges and Lister, 2007). According to the Tinmaz *et al.* (2001) the highest essential oil ratio (0.14 %) is obtained from the plants, cut in the beginning of blooming. The main components in the top part of the plant are 39% citronellal, 33% citral (citronellol, linalool) and 2% geranial.

Successful determination of biologically active compounds from plant material is largely dependent on the type of solvent

used in the extraction procedure. Organic solvents such as ethanol, acetone, and methanol are often used to extract bioactive compounds (Eloff, 1998). Ethanol, however, is the most commonly used organic solvent by herbal medicine manufacturers because the final products can be safely used internally by consumers of herbal extracts (Low Dog, 2009).

Additionally, the bioactivity of plant extracts depends on the water and ethanol concentration used in the extraction process (Ganora, 2008). Although a great amount of research has been performed to determine the antibacterial activity of medicinal plants, optimal extraction of bioactive compounds has not been well established for most plants. Therefore, to maximize the recovery of plant antimicrobials for human consumption, establishing optimal and specific extraction condition using binary solvent system of ethanol and water is important (Wendakoon *et al.*, 2012; Ivanov *et al.*, 2014).

The aim of the present study was to establish the antioxidant potential and total polyphenolic content of herbs traditionally used in culinary technology in Bulgaria (*Chrysanthemum balsamita* L., *Melissa officinalis* L. and *Allium bulgaricum* L.) in order to compare their activity and thus to evaluate the benefits of their application in healthier and more nutritious recipes.

Materials and Methods

Plant material collection

Chrysanthemum balsamita L., *Melissa officinalis* L. and *Allium bulgaricum* L. were collected from their natural habitat in spring 2013, Bulgaria. The fresh plant material was cleaned and cut into pieces.

Preparation of plant extract

For each herb, separate samples of 0.5 g were placed in a round bottom flask and were subjected to heat-reflux extraction with 30 ml (1:20 v/v) of 70% ethanol (v/v) in a water bath for 30 minutes. The final extracts were filtered with a Buchner funnel and were stored at 4°C without adding any preservatives until use.

Total phenolic content (TPC)

The total polyphenol content was analyzed using the Folin-Ciocalteu method of Kujala *et al.* (2000) with some modifications. Each sample (1 ml) was mixed with 5 ml of Folin-Ciocalteu phenol reagent and 4 ml of 7.5 % Na₂CO₃. The mixture was vortexed well and left for 5 min at 50°C. After incubation, the absorbance was measured at 765 nm. The TPC in the extracts was expressed as mg gallic acid equivalent (GAE) per g fresh weight (fw).

Antioxidant capacity evaluation

DPPH radical scavenging activity

The ability of the extracts to donate an electron and scavenge DPPH radical was determined by the slightly modified method of Brand-Williams *et al.* (1995). Freshly prepared 4×10^{-4} mol methanolic solution of DPPH[•] was mixed with the samples in a ratio of 2:0.5 (v/v). The light absorption was measured at 517 nm. The DPPH radical scavenging activity was presented as a function of the concentration of Trolox - Trolox equivalent antioxidant capacity (TEAC) and was defined as the concentration of Trolox having equivalent AOA expressed as the $\mu\text{mol TE/g fw}$.

ABTS radical scavenging assay

The radicals scavenging activity of the extract against radical cation (ABTS^{•+}) was estimated according to a previously reported procedure with some modifications [Re et al., 1999]. Briefly, ABTS was dissolved in water to a 7 mM concentration. ABTS radical cation (ABTS^{•+}) was produced by reacting ABTS stock solution with 2.45 mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12–16 h before use. Afterward, the ABTS^{•+} solution was diluted with ethanol to an absorbance of 0.7 ± 0.02 at 734 nm and equilibrated at 30°C. After the addition of 1.0 mL of diluted ABTS^{•+} solution to 10 mL of samples, the absorbance reading was taken at 30°C after 6 min. The results were expressed as TEAC value ($\mu\text{mol TE/g fw}$).

Ferric-reducing antioxidant power (FRAP) assay

The FRAP assay was carried out according to the procedure of Benzie et al., (1999) with slight modification. The FRAP reagent was prepared fresh daily and was warmed to 37°C prior to use. 150 μl of plant extracts were allowed to react with 2850 μl of the FRAP reagent for 4 min at 37°C and the absorbance was recorded at 593 nm. The results were expressed as $\mu\text{M TE/g fw}$.

Copper reduction (CUPRAC) assay

The CUPRAC assay was carried out according to the procedure of Ak and Gülcin (2008). To a test tube were added 1 ml of CuCl_2 solution ($1.0 \times 10^{-2}\text{M}$), 1 ml of neocuproine methanolic solution ($7.5 \times 10^{-3}\text{M}$), and 1 ml NH_4Ac buffer solution (pH 7.0), and mixed; 0.1 ml of herbal extract (sample) followed by 1 ml of water were added (total volume = 4.1 ml), and mixed well. Absorbance against a

reagent blank was measured at 450 nm after 30 min. Trolox was used as standard and total antioxidant capacity of extracts was expressed as $\mu\text{M TE/g fw}$.

Statistical analysis

All measurements were carried out in triplicates. The results were expressed as mean \pm SD and statistically analyzed using MS-Excel software.

Results and Discussion

Total phenolic content

It is well-known that phenolic compounds contribute to the quality and nutritional value in terms of modifying color, taste, aroma, and flavor and also in providing health beneficial effects (Vaya et al., 1997). Therefore, the establishment of their presence in extracts of natural origin is an important matter.

The total phenolic content in the examined plant extracts using the Folin-Ciocalteu's reagent was evaluated to be in range from 0.41 ± 0.08 to 2.71 ± 0.15 mg GAE/g fw (Table 1). The highest concentration of polyphenols was measured by the ethanol extract of *M. officinalis*, while the lowest values were measured in the *A. bulgaricum* extract.

In comparison, Alexieva et al. (2013a) reported for water extract of *C. balsamita* after 15 min of thermal treatment 0.60 mg GAE/g fw. On the other hand the *A. bulgaricum* decoct was evaluated with 0.67 mg GAE/g fw polyphenolic content after 15 min of thermal treatment (Alexieva et al., 2013b). Wojdyło et al. (2007) established 13.2 ± 0.13 mg GAE/100 g dry weight, corresponding to 0.132 mg GAE/g dry weight for *M. officinalis* extract.

However, among the three investigated plants in this research, *M. officinalis* is the most studied one and in the literature there are results for comparing. The *A. bulgaricum* and *C. balsamita* are less investigated plant species. *C. balsamita* is relatively poorly known and studied; therefore comparison is difficult to be made.

Antioxidant activity (AOA)

Plants have been traditionally used for the treatment and prophylaxis of different disorders. The protection has often been attributed to plant antioxidants such as polyphenols and vitamins C, E, β -carotene (Prior, 2003). Polyphenols are the most abundant antioxidants in the diet. Their total dietary intake could be as high as 1 g/ day, which is much higher than that of all other classes of phytochemicals and known dietary antioxidants – about 10 times higher than the intake of vitamin C and 100 times higher than the intakes of vitamin E and carotenoids (Scalbert *et al.*, 2005).

In order to investigate the AOA of the studied plants extracts, experiments with two stable radicals DPPH[•] and ABTS^{•+} were conducted. In addition the ferric reducing antioxidant power (FRAP) and copper reduction (CUPRAC) assays were also performed. The results were expressed as TEAC-value (Table 2).

The reducing ability of the extracts according the ABTS assay ranged from 5.80 ± 0.25 to 36.54 ± 0.37 $\mu\text{M TE/g fw}$ (Table 2). In this assay, ABTS is oxidized by peroxy radicals or other oxidants to its radical cation, ABTS^{•+}, which is intensely colored, and AOC is measured as the ability of test compounds to decrease the color reacting directly with the ABTS^{•+} radical (Prior *et al.*, 2005). Significant radical scavenging activity was evident in the *M.*

officinalis extract (36.54 ± 0.37 $\mu\text{M TE/g fw}$); while the lowest was detected in the *A. bulgaricum* extract (5.80 ± 0.25 $\mu\text{M TE/g fw}$). The *C. balsamita* extract showed moderate values (11.61 ± 0.25 $\mu\text{M TE/g fw}$). All the results obtained were in accordance with the polyphenolic content establishment.

The DPPH assay as commonly used for fast evaluation of the antioxidant capacity due to the simplicity of the assay was also applied in the present investigation. The obtained results were reported in Table 1. The highest values were found to be 31.17 ± 0.13 $\mu\text{M TE/g fw}$ (Table 2). Thus the ethanol extract of *M. officinalis* definitely affirmed with the highest antioxidant activity compared the other. The lowest values were estimated by the extract of *A. bulgaricum* leaves- 4.77 ± 0.88 $\mu\text{M TE/g fw}$.

The ferric reducing ability of plasma (FRAP) method is used for the assay of antioxidants in botanicals. The reaction measures reduction of ferric 2,4,6-tripyridyl-s-triazine (TPTZ) to a colored product. The results of the investigated plant extracts were in range from 7.16 ± 0.06 to 21.05 ± 1.6 $\mu\text{M TE/g fw}$. The *M. officinalis* showed the highest antioxidant activity (Table 2).

The CUPRAC assay is based on the reduction of Cu (II) to Cu (I) by the combined action of all antioxidants (reducing agents) in a sample. The established in this study results ranged from 7.70 ± 0.14 to 50.50 ± 1.39 $\mu\text{M TE/g fw}$, as the best value was measured by *M. officinalis* extract in accordance with the other conducted assays (Table 2).

Several other studies have been carried out in order to evaluate the antioxidant activity of the studied plants. In comparison, Nikolova and Dzhurmanski (2009) reported

for a methanol extract of *C. balsamita* growing in Bulgaria according the DPPH assay IC50 value of 42.73 µg/ml. Koksals et al. (2011) estimated the following IC50 value results for a methanol extract of *M. officinalis* growing in Turkey - 202.7 µg/ml. The TEAC_{FRAP} value of *A. bulgaricum* decoct was established to be 6.06 µMTE/g FW by Alexieva et al. (2013a) and the TEAC_{CUPRAC} value of *C. balsamita* water extract was assessed by Alexieva et al. (2013b) on 5.93 µMTE/g FW. However, comparing their results with the reported in the present study the differences could be

due to the origin country, season of harvesting and the method of extraction.

In brief, the present study proved that the studied plants can be a suitable culinary ingredient which could help prevent many stress related negative effects on the human body. All investigated extracts possessed free radical-scavenging activity, but at different levels. However, *M. officinalis* was revealed as the most promising source of substances with antiradical activity.

Table.1 Total phenolic content (mg GAE/g fw) of ethanol extracts from culinary plants growing in Bulgaria

Plant ethanol extract	Total phenolic content
<i>A. bulgaricum</i>	0.41 ± 0.08
<i>C. balsamita</i>	0.89 ± 0.12
<i>M. officinalis</i>	2.71 ± 0.15

Table.2 Antioxidant activity of ethanol extracts from culinary plants growing in Bulgaria (µM TE/g fw)

Plant ethanol extract	TEAC _{DPPH}	TEAC _{ABTS}	TEAC _{FRAP}	TEAC _{CUPRAC}
<i>A. bulgaricum</i>	4.77 ± 0.88	5.80 ± 0.25	7.16 ± 0.06	7.70 ± 0.14
<i>C. balsamita</i>	10.16 ± 0.27	11.61 ± 0.25	17.43 ± 1.1	17.99 ± 0.4
<i>M. officinalis</i>	31.17 ± 0.13	36.54 ± 0.37	21.05 ± 1.6	50.50 ± 1.39

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