Evaluation of the antioxidant capacity of aqueous extracts of fresh Chrysanthemum balsamita L. leaves growing in Bulgaria

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Abstract: The antioxidant activity of aqueous extracts of fresh leaves of costmary (Chrysanthemum balsamita L.) was investigated. The effect of the extraction time on total phenolic compounds (TPhC) and antioxidant activity (ABTS -, CUPRAC- and FRAP - assays) was studied. Results demonstrated highest levels of TPhC (0.60 GAE/g fresh plant weight), CUPRAC (5.93 µMTE/g FW), ABTS (10.68 µMTE/g FW), and FRAP (7.35 µMTE/g FW) at 15 min of thermal treatment. A high correlation between antioxidant capacities of fresh leaves of Chrysanthemum balsamita and their total phenolic contents (R=0.78-0.99) was established.

Key words: Chrysanthemum balsamita L., antioxidant capacity, phenolic content

INTRODUCTION

Aromatic and spicy plants are a substantial source of natural compounds with various beneficial properties. Such herbs have been used since ancient ages for various applications, such as flavoring of foods, healing of diseases, and formulation of fragrances.

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 'forgotten 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 (4). This plant has a hairy stem, complete shiny leaves, highly branched from the base and 70-120 cm height (6, 7, 8). It has a characteristic odor due to its volatile oil constituents. The main compounds (above 3 %) were: carvone (47,81 %), α -thujone (12,56 %), germacrene B (5,23 %), benzaldehyde (4,64 %) ethylbenzene (3,96 %) and germacrene D (3,13 %). Costmary has different phytochemicals such as; oxygenated terpenes (73,5 %), hydrocarbon terpenes (14,6 %), aromatic hydrocarbons (5,7 %), aromatic oxygenated (4,7 %) and other components (1,5 %) (1). It is also rich in phenylpropane derivatives, flavones, sesquiterpene lactones, tannins and oligoelements (5).

Considering the very limited information concerning the antioxidant capacity of the plant, the basic aim of the research was to determine the total phenolic content as well as the free radical scavenging activity of *Chrysanthemum balsamita*.

MATERIALS AND METHODS

Plant material - Chrysanthemum balsamita L. was collected in May 2013 from the Plovdiv region, Bulgaria.

Extract preparation

The applied method is decoction – extracting by boiling herbal and plant material. 2.5 g of the fresh plant sample are boiled for 10, 15, and 20 min respectively in 50 ml of distilled water. The resulting solution is being filtered before analyzed.

Determination of total phenolics

A modified Kujala et al. (2010) method with Folin- Ciocalteu's reagent was used for the determination of the total polyphenolic content (TPhC). Gallic acid was employed as a calibration standard and the results were expressed as mg gallic acid equivalents (mg GAE) per gram of plant fresh weight.

Determination of antioxidant activity

-DPPH • radical scavenging assay

Antioxidant activity is described as having activity against the stable form of the synthetic product DPPH • (2,2-diphenyl-1-pikrilhidrazil; Sigma-Aldrich, Steinheim,

Germany) by the method of Brand-Williams et al. [1995] with slight modifications. Briefly, a freshly prepared 12.10-5 M solution of DPPH • (in methanol) is mixed with the sample in a ratio of 2:0.5. Light absorption is measured at a wavelength of 517 nm using a spectrophotometer (Spectrostar Nano, BMG Labtech). The absorption of samples (free sample) is measured with respect to the corresponding extractant for a certain period of time.

- Ferric-reducing antioxidant power assay (FRAP)

The FRAP assay was carried out according to the procedure of Benzie & Strain (1996) with slight modification. FRAP assay measures the change in absorbance at 593 nm owing to the formation of a blue colored Fe (II)-tripyridyltriazine compound from colorless oxidized Fe (III) form by the action of electron donating antioxidants. Briefly, the FRAP reagent was prepared from 300 mM acetate buffer (pH 3.6), 10 mM TPTZ solution in 40 mM HCl, and 20 mM iron (III) chloride solution in proportions of 10:1:1 (v/v), respectively. The FRAP reagent was prepared fresh daily and was warmed to 37 °C in a water bath prior to use. One hundred and fifty microliters of plant extracts were allowed to react with 2850 μ l of the FRAP reagent solution for 4 min at 37 °C and the absorbance of the reaction mixture was recorded at 593 nm. The results were expressed as μ M TE/g FW.

- CUPRAC assay

The CUPRAC assay was carried out according to the procedure of Apak et al. (2004) with modifications. To a test tube were added 1 mL of CuCl2 solution $(1.0 \times 10^{-2}$ M), 1 mL of neocuproine methanolic solution $(7.5 \times 10^{-3}$ M), and 1 mL NH4Ac 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 herbal extracts was measured as mM trolox equivalent.

Absorbance was measured at 450 nm against a reagent blank (Ak and Gulcin, 2008). Increased absorbance of the reaction mixture indicates increased reduction capability.

Statistical analysis

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

RESULTS AND DISCUSSION

Total phenolics

It is well-known that phenolic compounds contribute to quality and nutritional value in terms of modifying color, taste, aroma, and flavor and also in providing health beneficial effects (10).

The total phenolic contents in the examined plant extracts using the Folin-Ciocalteu's reagent was expressed in terms of gallic acid equivalents. The values obtained for the concentration of total phenols were expressed as mg of GAE/g PW (Table 1). The total phenolic contents in the examined extracts ranged from $0,48 \pm 0,02$ to $0,60 \pm 0,04$ mg GAE/g PW. The highest concentration of phenols was measured in the 15min decoction extract.

Table 1. Total polyphenolic content of different extracts of Chrysanthemum balsamita

Plant extract	TPC, mg GAE/g FW		
Decoction 10min	$0,48 \pm 0,02$		
Decoction 15min	0,60 ± 0,04		
Decoction 20min	0,51 ± 0,05		

Antioxidant activity

Table 2 shows the CUPRAC, ABTS, and FRAP assay results of aqueous extracts of *Chrysanthemum balsamita*. The antioxidant capacities are given as Trolox equivalents, in the units of µlmol TE per gram plant weight. In present study, all extracts possessed free radical-scavenging activity but at different levels.

Table 2. Antioxidant activity of aqueous extracts of *Chrysanthemum balsamita* using three different complementary assays (DPPH, ABTS, FRAP)

Plant extract	TEAC _{ABTS} ,	TEAC _{FRAP} ,	TEAC _{CUPRAC} ,
	µM TE/g FW	µM TE/g FW	µM TE/g FW
Decoction 10min	9,78 ± 0,13	6,22 ± 0,04	5,36 ± 0,04
Decoction 15min	10,68 ± 0,25	7,35 ± 0,12	5,93 ± 0,07
Decoction 20min	9,43 ± 0,22	7,18 ± 0,21	5,21 ± 0,38

-ABTS

The reducing ability of the extracts was in range from $9.43 \pm 0.22 \ \mu\text{M}$ TE/g FW to $10.68 \pm 0.25 \ \mu\text{M}$ TE/g FW. Significant ABTS free radical scavenging activity was evident in the 15 min decoction of leaves ($10.68 \pm 0.25 \ \mu\text{M}$ TE/g FW), while the lowest was detected in the 10 min decoction extraction ($9.43 \pm 0.22 \ \mu\text{M}$ TE/g FW). All the results correspond to those in the other conducted assays.

-FRAP

The antioxidant potential of *C. balsamita* extracts was estimated from their ability to reduce TPRZ-Fe (III) complex to TPTZ-Fe (II). Increasing absorbance indicates an increase in reductive ability. The FRAP values of the studied extracts were calculated and the results are presented in Table 2. In accordance with the ABTS assay and the total polyphenolics, the highest value in the FRAP method was obtained in the 15min decoction extract of fresh costmary leaves- $7.35 \pm 0.12 \ \mu M \ TE/g \ FW$.

-CUPRAC

The cupric ion (Cu2+) reducing ability of various extracts of *C. balsamita* leaves is shown in Table 2. Among all the extracts the 15 min decoction of leaves showed the highest CUPRAC value $-5.93 \pm 0.07 \mu$ M TE/g FW. The results of this assay correspond well to the already mentioned results pursuant to the other methods.

Correlations

Correlation analysis between total phenolics and antioxidant capacity are shown in table 3. The coefficient between the ABTS and the CUPRAC assay showed a positive significant correlation (r= 0.9971). High correlation coefficients have also been reported between the TPhC and CUPRAC, ABTS and TPhC, and FRAP and TPhC (r= 0.9042; r= 0.8691; and r= 0.7871 respectively). However, lower less significant but still positive correlations have been established between the FRAP and CUPRAC assays and FRAP and ABTS assays (r= 0.4483 and r= 0.3790 respectively).

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Correlation	ABTS	TPhC	FRAP	CUPRAC
coefficients				
ABTS	-	0,8691	0,3790	0,9971
TPhC	0,8691	-	0,7871	0,9042
FRAP	0,3790	0,7871	-	0,4483
CUPRAC	0,9971	0,9042	0,4483	-

Table 3. Correlation coefficients (r) for relationships between assays

CONCLUSION

The results obtained confirmed costmary to be a source of phenolic compounds with high antioxidant activity. Boiling up to 15 min led to higher antioxidant capacity of the decoction extracts and highest results being estimated.

The outcomes of this study show the great potential of *Chrysanthemum balsamita* for the development of foods rich in compounds with antioxidant properties. During cooking and preparing aqueous decoctions best antioxidant activity is achieved at 15 min of heating. Therefore, further investigation is needed to isolate and identify the antioxidant compounds present in the aqueous extracts.

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