BIOLOGICA NYSSANA

5 (1) • September 2014: 47-51

Original Article

Received: 3 September 2014
Revised: 5 September 2014
Accepted: 10 September 2014

Antioxidant potential of *Tanacetum vulgare L.* extracts

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***Abstract*:**

Stojković, M., Mitić, S., Pavlović, J., Stojanović, B., Paunović, D.: ***Antioxidant potential of Tanacetum vulgare L. extract. Biologica Nyssana, 5 (1), Septmeber 2014: 47-51.***

Antioxidant activity of *Tanacetum vulgare* L. extracts was determined. Areal plant parts (leaves and flowers) were dried, grinded and extracted with five different solvents: methanol, ethanol, acetone, water and isopropanol. Total phenols and total flavonoids were determined and four *in vitro* antioxidant assays were applied. The best extraction medium (considering only phenols and flavonoids) was methanol and 62.7 mg galic acid equivalents per gram of dry weight were found (for leaves extract). Comparing amounts of phenolic compounds found in the extracts and its antioxidant potential with other herbal teas and extracts, it may be concluded that *T. vulgare* is a plant species destitute with phenolic compounds. Obtained results suggest that phenolic compounds, present in the plant tissues, are carriers of antioxidant properties.

Key words: antioxidant, flavonoid, extrac, phenol, *Tanacetum vulgare*

***Apstract*:**

Stojković, M., Mitić, S., Pavlović, J., Stojanović, B., Paunović, D.: ***Antioksidantni potencijal ekstrakta biljne vrste Tanacetum vulgare L. Biologica Nyssana, 5 (1), Septmeber 2014: 47-51.***

Određivana je antioksidantna aktivnost ekstrakta biljne vrste *Tanacetum vulgare* L. Nadzemni delovi ove biljne vrste (listovi i cvetovi) su sušeni, samleveni i ekstrakovani sa pet različitih rastvarača (metanol, etanol, aceton, voda i izopropanol). Određen je sadržaj totalnih fenola i totalnih flavonoida kao i antioksidantna aktivnost pomoću četiri *in vitro* metoda. Kao najbolje ekstrakciono sredstvo (za fenole i flavonoide) pokazao se metanol i nađeno je 62.7 mg ekvivalenata galne kiseline po gramu suve biljne mase (u ekstraktu lista). Ako nađeni sadrđaj fenola i flavonoida uporedimo sa sličnim ekstraktima ili biljnim čajevima, možemo zaključiti da je biljna vrsta *T. vulgare* relativno siromašna fenolnim jedinjenjima. Dobijeni rezultati sugeriču da su fenolna jedinjenja, prisutna u biljnim tkivima, nosioci antioksidantnih osobina.

Ključne reči: antioksidant, flavonoid, ekstrakt, fenol, *Tanacetum vulgare*

Introduction

Tanacetum is a genus of about 160 species of flowering plants in the Asteraceae family, native to many regions of the Northern Hemisphere. They are known commonly as tansies in english and povratič in serbian. *Tanacetum vulgare* L. (common tansy) is perennial, herbaceous plant species native to temperate Europe and Asia but invasive in other parts of world.

Tansy is a well-known herbal plant widely used in traditional medicine in south-eastern Serbia (Kojić et al., 1998). In recent times, preparing teas is contraindicated because of the toxic monoterpene, α-thujone, present in the plant tissue (Baranauskiene et al., 2014).

Tea prepared from *T. vulgare* is used as an antihelminthic, carminative, antispasmodic, stimulant to abdominal viscera, tonic, emmenagogue, antidiabetic, diuretic and antihypertensive (Lahlou et al., 2008). Various authors showed that the water and lipophilic extracts from *T. vulgare* possess antitumor, anti-inflammatory, antioxidant and antimicrobial activity (Lahlou et al., 2008).

To explore potential health benefits, dried parts of *T. vulgare* (flowers and leaves) were extracted with five different solvents (methanol, ethanol, acetone, water and isopropanol) and total phenols and flavonoids were determined. Series of in vitro antioxidant assays were performed (scavenging DPPH radical, scavenging ABTS radical, iron(III) to iron(II) reduction assay and cupric ion reducing antioxidant capacity assay).

Material and methods

Plant material and extract preparation

Areal parts of *Tanacetum vulgare* L. (Asteraceae) were collected in Mai 2012 from the mountain peak Babin zub (Latitude: 43.363 N; Longitude: 22.585 E), South-East Serbia. The plant material was air dried about two weeks, out of direct sunlight. Flowers and leaves were separated from the stems and were grinded into fine powder. Exact masses (0.25 g) of powdered flowers and leaves were extracted with five different solvents (methanol, ethanol, acetone, water and isopropanol) on ultrasonic bath. Every batch was extracted three times for 30 minutes, with 20+20+10 ml of appropriate solvent.

Reagents

1,1-Diphenyl-2-picrylhydrazyl radical (DPPH), 2,2'azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), potassium-persulfate and catechin were obtained from Sigma-Aldrich (Steinheim, Germany), methanol, ethanol and acetone were purchased from J. T. Baker (Deventer, Holland). Gallic acid was obtained from Carl Roth (Karlsruhe, Germany). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) were obtained from Acros Organics (Geel, Belgium). Folin-Ciocalteu reagent, Na_2HPO_4, NaH_2PO_4, Na_2CO_3, $NaNO_2$, NaOH, $Fe_SO_4 \cdot 7H_2O$, $K_3[Fe(CN)_6]$, trichloroacetic acid (TCA) and $AlCl_3$ were purchased from Merck (Darmstadt, Germany). All other chemicals were of analytical grade, and were used as received except that the solvents were distilled prior to use.

Determination of total phenolic (TP) content

Total phenolic content of the extracts were determined using Folin-Ciocalteau assay (Mitić et al., 2011). Briefly, 0.4 ml of extract were mixed with 2.0 ml of (20% w/v) Na_2CO_3 solution and 0.5 ml of FC reagent and made up to 10 ml with deionized water. The solution was mixed and, after aging for 120 min at 25°C, absorbance was measured at 760 nm, using Agilent 8453 UV-visible spectrophotometer (Agilent Technologies, USA). Results were expressed as mg of gallic acid equivalents (GAE) per g of the dry sample.

Determination of total flavonoid (TF) content

The total flavonoid content of *T. vulgare* extracts was determined by a colorimetric method according to Mitić et al. (2012). A known volume of the samples was mixed with 2 mL of distilled water and subsequently with 0.3 mL of a $NaNO_2$ solution (5%, w/w). After 5 min, 3 mL of $AlCl_3$ solution (1%, w/w) was added and the solution left for 5 min at room temperature. Then, 2 mL of NaOH solution (1 mol/L) was added to the mixture diluted with deionized water to the final volume of 10 mL. The mixture was thoroughly mixed and absorbance was immediately measured at 510 nm. Results were expressed as mg catechin equivalent (CE) per g of dry weight (d.w.).

DPPH free radical-scavenging assay

The antioxidant capacity of *T. vulgare* solvent extracts was studied through the evaluation of the extracts' free radical-scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical (Stojković et al., 2014). An aliquot (0.1 mL) of the different extracts was mixed with 2.5 mL of 100 µmol/L DPPH methanol solution. The mixture was thoroughly vortex-mixed, kept out of light for 30 min and absorption was measured at 515 nm. The absorption of the blank containing the same amount of methanol and DPPH solution was prepared and measured daily. The radical scavenging activity was calculated using the following formula:

$$\text{Scavenging effect (\%)} = [1-(\text{absorbance of the sample}/\text{absorbance of the blank})] \cdot 100$$

The results were expressed as mg of Trolox equivalents (TE) per 1 g of sample.

ABTS Radical-scavenging capacity assay

The ABTS radical cation ($ABTS^{•+}$) solution was prepared by the reaction of solutions of 7 µmol/L ABTS and 2.45 µmol/L of potassium persufate, at 23°C in the dark for 16 h. The $ABTS^{•+}$ solution was then diluted with 80% (v/v) aqueous ethanol to obtain a solution with the absorbance of 0.700 ± 0.020 at 734 nm (Veljković et al., 2013). The $ABTS^{•+}$ solution (3.9 mL) was added to 0.1 mL of the test sample and mixed thoroughly. The reaction mixture was left to stand at 23°C for 6 min and then the absorbance was measured at 734 nm. The total antioxidant activity of *T. vulgare* extracts was expressed as mg of TE per g of dry weight. Radical scavenging activity was calculated using the following formula:

$$\text{Scavenging effect (\%)} = [1-(\text{absorbance of sample}/\text{absorbance of blank})] \cdot 100$$

Iron(III) to iron(II) reduction assay (IRA)

Iron(III) to iron(II) reduction assay was performed according to Stojković et al. (2014). Different dilutions of the extracts (0.5 mL) were added to the mixture of 1.25 mL of the phosphate buffer (0.2 mol/L, pH 6.6) and 1.25 mL of potassium ferricyanide (1%, w/w). The resultant solution was incubated at 50°C for 20 min. After that, trichloroacetic acid solution (1.25 mL, 10%, w/w) was added, diluted with 4.25 mL of water and 0.85 mL of ferric chloride solution (0.1%, w/w) was added. After 30 min, the absorbance was measured at 700 nm. IRA of the extracts was expressed as mg gallic acid equivalents per g of dry weight of the sample.

Cupric ion reducing antioxidant capacity (CUPRAC)

The CUPRAC method was applied as described by Stojković et al. (2014). A mixture comprised of 1 mL of 10 mmol/L copper(II) chloride, 1 mL of 1mol/L ammonium acetate buffer at pH 7.0, and 1 mL of 7.5 mmol/L neocuproine solution was prepared, x mL sample solution and (1−x) mL distilled water were added, and well mixed (total volume: 4.0 mL). This final mixture in a stoppered test tube was left to stand at room temperature for 30 min. After that, the absorbance at 450 nm was measured against a blank. The total antioxidant activity of *T. vulgare* extracts was expressed as µmol of TE per g of dry weight.

Statistical analysis

All results were expressed as the mean ± standard deviation. Statistically significant differences were determined by one-way analysis of variance (ANOVA) followed by Tukey´s post hoc test for multiple comparison (Graph pad Prism version 5.03, San Diego, CA, USA). Probability values (p) less than 0.05 were considered to be statistically significant.

Results and discussion

Phenolic compounds (phenolic acids and flavonoids) are the natural constituents of plants. They are secondary metabolites and produced by plants as a response to different environmental factors (light, chilling, pollution, etc.) and to defend injured plants. Phenolic compounds are crucial for plant growth and reproduction. Also, they have various roles in plants (protective, signal molecules, gives colours of flowers, fruits, and leaves, etc.) but they are not understood completely (Ghasemzadeh & Ghasemzadeh, 2011). Polyphenols are well known for their antioxidant activity as radical scavengers and possible beneficial roles in human health, such as reducing the risk of cancer, cardiovascular and other diseases (Mitić et al., 2012).

The main carriers of antioxidant properties in plant tissues are phenolic compounds. Thus, total phenols and total flavonoids of *T. vulgare*’s flowers and leaves extracts were determined. The antioxidant capacity of the extracts were studied through four *in vitro* antioxidant assays: scavenging DPPH radical, scavenging ABTS radical, iron(III) to iron(II) reduction assay (IRA) and cupric ion reducing antioxidant capacity assay (CUPRAC). Obtained results are shown in **Tab. 1**.

Total phenols (TP) in various extracts are in the range from 62.7 to 12.2 mg of galic acid equivalents (GAE) per g of dried weight (dw) - for leaf extracts and from 42.1 to 7.2 mg GAE/g dw - for flower extracts. Methanol was observed as the best extraction medium for total phenolic compounds among the others solvents used (ethanol, water, isopropanol and acetone). The lowest amounts of phenolic compounds were extracted with acetone.

Total flavonoids found in mentioned extracts are in the range from 18.2 to 4.7 mg of catechin equivalents (CE)/g dw - for leaf extracts and from 10.5 to 4.0 mg CE/g dw - for flower extracts. The best solvent for flavonoid extraction was water, dissimilar to total phenols where methanol was the best one, but the observed difference was small

Table 1: Total phenols, total flavonoids and *in vitro* antioxidant tests

		TP	TF	ABTS	DPPH	IRA	CUPRAC
		mg GAE/g dw	mg CE/g dw	mg TE/g dw	mg TE/g dw	mg GAE/g dw	mg TE/g dw
Leaves	Ethanol	31.6 ± 0.5	9.3 ± 0.3	25.5 ± 0.5	12.3 ± 0.5	18.7 ± 0.5	115 ± 3
	Methanol	62.7 ± 0.5	15.3 ± 0.5	41.2 ± 0.5	28.1 ± 0.5	34.2 ± 0.5	214 ± 5
	Acetone	12.2 ± 0.5	4.7 ± 0.1	7.6 ± 0.2	4.9 ± 0.1	10.2 ± 0.5	53 ± 1
	Water	47.8 ± 0.5	18.2 ± 0.5	22.2 ± 0.5	25.9 ± 0.5	35.2 ± 0.5	129 ± 3
	Isopropanol	12.3 ± 0.5	6.3 ± 0.1	5.5 ± 0.2	4.2 ± 0.1	9.9 ± 0.5	34 ± 1
Flowers	Ethanol	19.0 ± 0.5	6.0 ± 0.1	12.2 ± 0.3	7.6 ± 0.3	13.9 ± 0.5	47 ± 1
	Methanol	42.1 ± 0.5	10.1 ± 0.3	27.6 ± 0.5	15.8 ± 0.5	27.0 ± 0.5	164 ± 3
	Acetone	7.2 ± 0.3	4.0 ± 0.1	4.5 ± 0.1	3.5 ± 0.1	9.2 ± 0.5	18.1 ± 0.5
	Water	42.3 ± 0.5	10.5 ± 0.3	19.9 ± 0.5	21.7 ± 0.5	25.8 ± 0.5	121 ± 3
	Isopropanol	10.3 ± 0.5	5.6 ± 0.1	4.8 ± 0.1	5.5 ± 0.2	10.0 ± 0.5	33 ± 1

Table 2: Correlation coefficient between total phenols, total flavonoids and different *in vitro* antioxidant assays

	TP	TF	ABTS	DPPH	IRA	CUPRAC
TP	1	0.9094	0.9474	0.9735	0.9737	0.9677
TF		1	0.7871	0.9470	0.9601	0.8158
ABTS			1	0.8613	0.8739	0.9790
DPPH				1	0.9802	0.8943
IRA					1	0.9118
CUPRAC						1

(leaf) or insignificant compared to the standard deviation (flower).

Phenol and flavonoid contents in various herbal teas (Veljković et al., 2013) are in the range from 26 to 241 mg GAE/g dw and from 12 to 85 mg CAE/g dw for phenols and flavonoids, respectively. According to this data, *T. vulgare* is a plant with small contents of phenolic compounds.

1,1-Diphenyl-2-picrylhydrazyl radical (DPPH) is a stable nitrogen-centered free radical whose color changes from violet to yellow upon reduction either by the process of hydrogen or electron-donation. Antioxidant capacity were in the range from 28.1 mg trolox equivalents (TE)/g dw (methanol extract of leaf) to 3.5 mg TE/g dw (ethanol extract of flower).

The ABTS assay method is based on the ability of antioxidant molecules to quench the long-lived ABTS cation radicals (which ethanol solution is blue-green with characteristic absorption at 734 nm). The addition of antioxidants to the solution of prepared radical cation reduces it to ABTS, causing decolorizing. Results were expressed as mg Trolox equivalent per g of dw. Antioxidant capacity, determined with this assay, was in the range from 41.2 mg trolox equivalents (TE)/g dw (methanol extract of leaf) to 4.5 mg TE/g dw (ethanol extract of flower).

IRA is based on reduction of ferricyanide ions to ferrocyanide in the presence of reductants (antioxidants) in the samples by donating an electron. The ferrocyanide ions then react with Fe^{3+} ions forming Prussian blue which strongly absorbs on 700 nm. Increasing absorbance at 700 nm indicates an increase in antioxidant ability of a sample. Leaf extracts of both water and methanol showed the highest values for reduction capacity (35.2 and 34.2 mg GAE/g dw, respectively). Acetone and isopropanol extracts of leaf and flower showed the lowest antioxidant capacity and there is no significant difference between the values according to ANOVA.

Another antioxidant assay used was CUPRAC. It is based on reduction of Cu^{2+} ion to Cu^{+} ion which reacts with neocuproine giving the complex with absorption maximum at 450 nm. The best antioxidant capacity showed methanol extract of leaf (214 mg TE/g dw) while isopropanol extracts of both leaf and flower had the lowest capacity (34 and 33 mg TE/g dw, respectively).

Correlation coefficient between total phenols, total flavonoids and different *in vitro* antioxidant assays are calculated and showed in the **Tab. 2**. The high values for the coefficients between TP and various *in vitro* tests suggest that phenolic compounds are the main carriers of antioxidant power in the plant tissues. Also, the high values for the coefficients among various *in vitro* tests proposed that the experimental errors were minor.

Conclusion

Methanol extracts showed the best antioxidant potential among the other solvents used (ethanol, water, acetone and isopropanol). The best extraction medium considering only phenols and flavonoids was also methanol (62.7 mg galic acid equivalents per gram of dry weight were found for leaves extract). Methanol is an extremely toxic solvent and it is not applicable for human usage, especially methanol extracts. On the other hand, water extracts are suitable for human intake and. Obtained results suggest that antioxidant potential of water extracts were as high as the appropriate methanol ones. In some cases water extracts showed higher antioxidant potential. It can be concluded that the water extraction is a good way to obtain phenolic compounds from *T. vulgare*. Comparing amounts of phenolic compounds found in the extracts and its antioxidant potential with other herbal teas and extracts, it may be concluded that *T. vulgare* is a plant species destitute with phenolic compounds. Obtained results suggest that phenolic compounds, presents in the plant tissues, are carriers of antioxidant properties.

Acknowledgements. This work was supported by the Ministry of Education and Science of Serbia (Project No 172061 and Project No 31060). The authors are grateful for the financial support provided by this Ministry.

References

Baranauskiene, R., Kazernaviciute, R., Pukalskiene, M., Mazdzieriene, R., Venskutonis, P., 2014: Agrorefinery of *Tanacetum vulgare* L. into valuable products andevaluation of their antioxidant properties and phytochemicalcomposition. *Industrial Crops and Products*, 60: 113–122.

Ghasemzadeh, A., Ghasemzadeh, N., 2011: Flavonoids and Phenolic Acids: Role and Biochemical Activity in Plants and Human. *Journal of Medicinal Plants Research*, 5: 6697–6708.

Kojić, M., Stamenković, V., Jovanović, D. 1998: Lekovite biljke jugoistočne Srbije. Zavod za udžbenike i nastavna sredstva, Beograd.

Lahlou, S., Tangi, K., Lyoussi, B., Morel, N., 2008: Vascular effects of *Tanacetum vulgare* L. leaf extract: *In vitro* pharmacological study. *Journal of Ethnopharmacology*, 120: 98–102.

Mitic, S., Stojkovic, M., Pavlovic, J., Mitic, M., Stojanovic, B., 2012: Antioxidant Activity, Phenolic and Mineral Content of *Stachys germanica* L. (*Lamiaceae*). *Oxidation Communications*, 35, (4): 1011–1020.

Stojkovic, N., Stojkovic, M., Marinkovic, M., Chopra, G., Kostic, D., Zarubica, A., 2014: Polyphenol Content and Antioxidant Activity of *Anthemis cretica* L. (*Asteraceae*). *Oxidation Communications*, 37, (1): 237–246.

Veljković, J,. Pavlović, A., Mitić, S., Tošić, S., Stojanović, G., Kaličanin, B., Stanković, D., Stojković, M., Mitić, M., Brcanović, J., 2013: Evaluation of individual phenolic compounds and antioxidant properties of black, green, herbal and fruit tea infusions consumed in Serbia: spectrophotometrical and electrochemical approaches. *Journal of Food and Nutrition Research*, 52: 12–24.