

Composition and Antioxidant Activity of Aqueous Extracts Obtained from Herb of Tansy (*Tanacetum Vulgare L.*)

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*The aim of this study was to determine the phenolic content and antioxidant activity of extracts obtained from herb of *Tanacetum vulgare L.* (tansy) harvested in two different locations from the western part of Romania. Aqueous extracts of tansy presented high antioxidant activity that ranged between 295.77 and 653.65 mg GAE/L, and remarkable percentage of inhibition (31.82-65.05 %). The compounds quantified in the plant extracts that were determined by UHPLC are: ascorbic acid, riboflavin, pyrocatechol, rutin, quercetin, and kaempferol. Extracts obtained from tansy flowers showed higher content of phenols compared to that obtained from leaves. Similarly, the flower extracts present a high antioxidant activity that could suggest their use as natural additives in food preservation and also in pharmaceutical industry and veterinary medicine.*

Keywords: Tansy, *Tanacetum vulgare L.*, antioxidant activity, polyphenols

Tansy (*Tanacetum vulgare L.*) is an aromatic perennial plant from *Asteraceae* family that is native from Eurasia and is established also as invasive plant in North America. Traditionally, tansy has been used as an anthelmintic, tonic, emmenagogue, antihypertensive, carminative, anti-spasmodic, antidiabetic, diuretic, and as anti-inflammatory plant. Plants are also cultivated in gardens, and used as medicines, aromatics, spices, cosmetics, insecticides and herbal acaricides. Various extracts obtained from herb of tansy exhibit biological properties such as antioxidant, antibacterial, antifungal properties as well as antiradical and repellent activity [1-4].

Tansy contains into the herbal parts essential oils, non-volatile compounds (such as lactones), and phenolic derivatives (e.g. phenolic acids, flavonoids). Numerous studies focused on obtaining and characterizing the essential oils obtained from tansy. Most alcoholic extracts of *Tanacetum vulgare* are reported to exhibit biological effects, including anti-inflammatory, antioxidant, and antibacterial properties. Acetone and chloroform extracts obtained from tansy flowers and leaves presented anti-inflammatory activity. Lahlou et al. showed that tansy leaves water extract has a good diuretic effect on male Wistar rats after oral administration, presenting no renal toxicity or any other adverse effects during the study period [5].

Studies on rats and mice have found that tansy extracts presented anti-inflammatory properties [3,4]. For example, the vasodilatory properties of tansy extracts were demonstrated in rat aorta, in a concentration-dependent mode [3]. Extracts obtained from tansy have had antioxidant activity [1]. The antibacterial properties of ethanolic extracts have also been reported in studies using *Escherichia coli* and *Staphylococcus aureus* [2].

The variation in volatile compounds from tansy were intensively studied [6-10], but the lack of information about the constituents found both in flower and in leaves imposed the study of the chemical composition and properties of extracts obtained from it.

The content in active substance of the plant is primarily influenced by soil composition and quality [11-14]. There is often the problem of pollution through inappropriate discharges of household waste, animal husbandry [15], industry's (pharmaceutical and medical [16,17], food, chemical and oil [18-21] etc.) waste and, of course, waste water, etc. [22-26].

Meteorological conditions are another factor that influences the content in active substances of any plant [11, 27-29].

Very numerous papers of us and other authors are focused to determine the composition and biologic activities of essential oils and extracts obtained from diverse parts of plants harvested in Romania [27-33]. In the present study, extracts from aerial parts - flowers and leaves from tansy collected in the western part of Romania, were obtained with water and analysed using HPLC. The composition in phenolic acids, total phenol content, as well as their antioxidant activity was determined.

The chemical composition of tansy essential oils is well-known to differ with regional variability, and more than 30 chemotypes have already been classified according to the most dominant constituent in the oil. In our study the phenolic acids, total phenol content, and antioxidant activity of plant extracts obtained from different aerial parts of *Tanacetum vulgare L.* (tansy) from two different areas (Arad and Bihor County) were assessed.

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All the authors have equal contribution at this original article.

Experimental part

Materials and methods

Plant material and aqueous extract preparation

The aerial parts (flowers and leaves) of *Tanacetum vulgare* plants were collected in 2016 from two localities situated in the western part of Romania: Julita (46° 2' 0" North, 22° 8' 0" East) and Nadas (46° 13' 0" North, 21° 57' 0" East). The samples were dried at room temperature (20–25°C). 20 g of plant material was grinded and extracted with 500 mL distilled water for 2 h, at 100°C. The extracts were filtered through PTFE (0.45 µm) before further analysis.

Chemicals and reagents

HPLC standards (ascorbic acid, riboflavin, pyrocatechol, rutin, quercetin and kaempferol) were purchased from Sigma Aldrich (Fluka, Switzerland), while acetonitrile (HPLC grade) was purchased from Merck (Germany). All of the other reagents and solvents used in the experiments were of adequate analytical grade, used without further purification and were obtained from Sigma Aldrich (Fluka, Switzerland), and Merck (Darmstadt, Germany).

Total phenolic content

Total phenolic content of the extracts was determined by using Folin-Ciocalteu reagent in a slightly modified method based on previous reports [34–36]. Briefly, the aqueous extracts obtained were diluted with distilled water (1:25). To 1 mL sample were added 0.5 mL Folin-Ciocalteu reagent, 2 mL Na₂CO₃ (20%) and 5 mL distilled water. The mixture was kept in the dark for 90 min, thereafter the absorbance was recorded against a blank prepared in the same conditions, at 765 nm, by using a UV-Vis double beam spectrophotometer (Specord 200, Analytik Jena Inc., Jena, Germany). A calibration curve for gallic acid as reference was obtained (20, 40, 100, 160, 200 mg/L), then the regression equation and the correlation coefficient were calculated and the results were expressed in mg GAE/L. Total phenolic content of the extracts was thereafter expressed as gallic acid equivalents (mg GAE/L). All experiments were performed in triplicates.

DPPH radical scavenging activity

In order to determine the antioxidant activity of the extracts the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical-scavenging capacity was assessed using a spectrophotometric method [35]. The DPPH was dissolved in ethanol (0.2 mM) and 3 mL of the resulting solution were mixed with 0.1 mL sample (20 mg/mL). Absorbance was recorded at 517 nm after 1 h incubation in the dark. Positive controls containing 2.5 to 50 mg/L gallic acid in ethanol were used as reference. Inhibition of the DPPH stable free radical was calculated with equation (1):

$$\% \text{Inhibition} = \frac{Abs_{\text{control}} - Abs_{\text{sample}}}{Abs_{\text{control}}} \times 100 \quad (1)$$

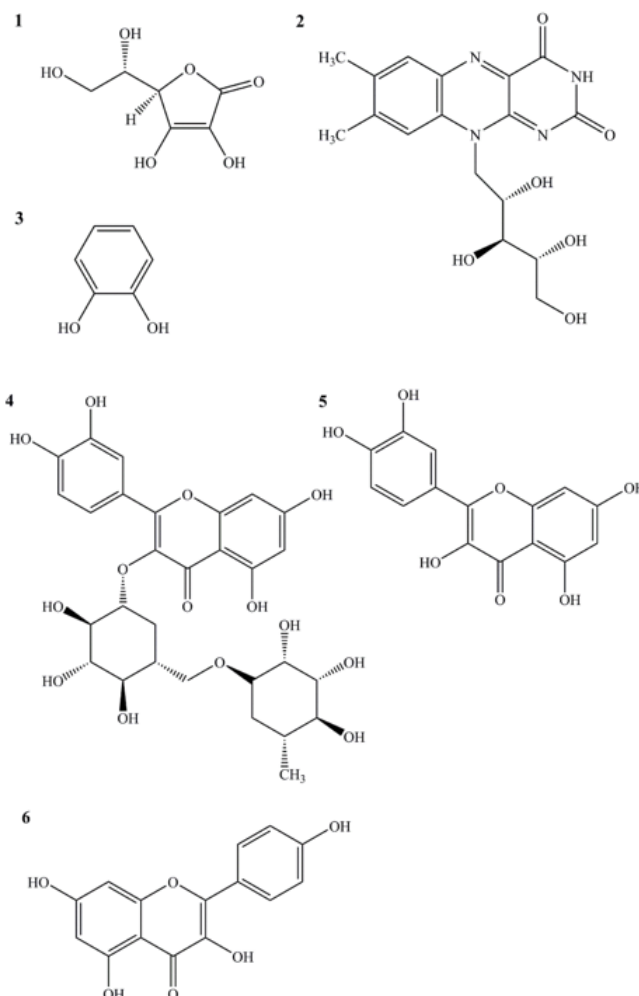
where:

Abs_{control} - absorbance of 0.2 mM DPPH in ethanol;
Abs_{sample} - absorbance of 0.2 mM DPPH + extract.

Analysis of organic acids, riboflavin and flavonoids by HPLC

Ascorbic acid, riboflavin, pyrocatechol, rutin, quercetin and kaempferol (scheme 1) concentrations were determined by using an HPLC method.

Briefly, a high performance liquid chromatograph (Nexera X2, Shimadzu, Tokyo, Japan) equipped with a diode array detector (M30A, Shimadzu, Tokyo, Japan) and a Nucleosil 100-3-C18 reversed-phase column (4.0 mm



Scheme 1. Organic derivatives analysed by HPLC: 1. ascorbic acid, 2. riboflavin, 3. pyrocatechol, 4. rutin, 5. quercetin and 6. kaempferol

i.d. × 125 mm column length, 3 µm particle size, Macherey-Nagel GmbH, Duren, Germany) were used. The column temperature was maintained at 30°C and the flow rate at 1 mL · min⁻¹. The solvents used for the chromatographic elution consisted of ultra-pure water with 0.1% TFA (A) and acetonitrile (B). The chromatographic elution program used was as follows: 95% A and 5% B that was changed linear gradient to 42% B for 5 min, followed by a linear gradient to 35% B in 30 min. Thereafter, the eluent was changed to the initial composition of 95% A and 5% B linear gradient for 5 min. The measurements have been done at λ = 350 nm. All experiments were performed in triplicates.

Results and discussions

Tanacetum vulgare L. plants harvested in two locations from the western part of Romania, namely Julita and Nadas, were collected in 2016. Aqueous extracts of leaves and flowers, respectively were obtained by hydro distillation. The total phenolic content and antioxidant activity of the extracts were determined. In table 1 are presented total phenol content (mg GAE/L) and antioxidant activity (Inhibition %) of tansy extracts obtained from leaves and flowers. Total phenolic content of the extracts was determined by using Folin-Ciocalteu method. In this regard, we determined by spectrophotometry the absorbance of aqueous extracts that reacted with Folin-Ciocalteu reagent and compared them with the standard solutions of gallic acid equivalents (GAE). The total phenolic content is increasing in the following order: tansy leaves extract-Nadas < tansy leaves extract-Julita < tansy flowers extract-Julita < tansy flowers extract-Nadas.

Table 1
TOTAL PHENOLIC CONTENT (GAE) AND ANTIOXIDANT ACTIVITY (INHIBITION) OF TANSY EXTRACTS OBTAINED FROM LEAVES AND FLOWERS COLLECTED IN JULITA AND Nadas

Sample of Tansy extract		GAE [mg /L]	Inhibition %
Leaves	Julita	335.77	33.48
	Nadas	295.77	31.82
Flowers	Julita	621.54	61.85
	Nadas	653.65	65.05

As it is shown in table 1, total phenol content varied in obtained extracts in the range from 295.77 to 653.65 mg GAE/L. Extracts obtained from tansy flowers showed the highest content of phenols (653.65 mg GAE/L). The lowest amounts of phenolic compounds were observed in extracts obtained from leaves of tansy (295.77 mg GAE/L). The total phenolic content values obtained in the present study for the aqueous extracts of tansy herb are different than that reported for methanol extracts of the tansy herb obtained in the presence of ultrasounds [8], namely 96.2 mg GAE/g for flowers and 112.6 mg GAE/g for leaves. These data demonstrate, once again the importance of the solvent and conditions used to prevail the extracts from plants.

In order to determine the antioxidant properties of the extracts we used the DPPH (1,1-diphenyl-2-picrylhydrazyl)

colorimetric method. The single electron transfer reaction that undergo in the DPPH assay in the presence of free radical species is related to the decrease of absorbance compared with that of negative control. As it is depicted in table 1 the inhibition varied between 31.82% for tansy leave extract and 65.05% for tansy flower extract. Our results are consistent with the results reported previously for tansy extracts by Devrnja et al. [8]. For example the methanolic extracts obtained by Devrnja et al. from tansy plants presented the following values: IC₅₀ 58.3 µg/mL for flowers and IC₅₀ 77 µg/mL for leaves.

In table 2 are presented the concentration in ascorbic acid, riboflavin, pyrocatechol, rutin, quercetin and kaempferol (mg/L) of tansy extracts obtained from leaves and flowers, respectively determined through HPLC method.

In figure 1 are presented the chromatograms of the *Tanacetum vulgare* aqueous extracts obtained from leaves (a) and flowers (b) harvested in Julita, in 2016. The highest quantity of ascorbic acid was found in tansy leaves extracts: 10.89 mg/L and 9.75 mg/L for plants harvested in Julita and Nadas, respectively. Smaller quantities of ascorbic acids were determined in the extracts of tansy flowers, with the lowest value, 7.08 mg/L for the extract obtained from tansy flowers from plants harvested in Nadas. In the tansy flowers extracts were determined more than double quantity of riboflavin (329.55 mg/L - Julita

Substance [mg /L]	Leaves		Flowers	
	Julita	Nadas	Julita	Nadas
Ascorbic acid	10.89	9.75	9.43	7.08
Riboflavin	158.55	127.71	329.55	325.43
Pyrocatechol	452.41	430.83	618.93	614.10
Rutin	0	0	16.58	18.25
Quercetin	0.82	0.72	1.18	1.23
Kaempferol	0.001	0.001	0.15	0.08

Table 2
ASCORBIC ACID, RIBOFLAVIN, PYROCATECHOL, RUTIN, QUERCETIN AND KAEMPFEROL CONCENTRATIONS OF TANSY EXTRACTS OBTAINED FROM LEAVES AND FLOWERS

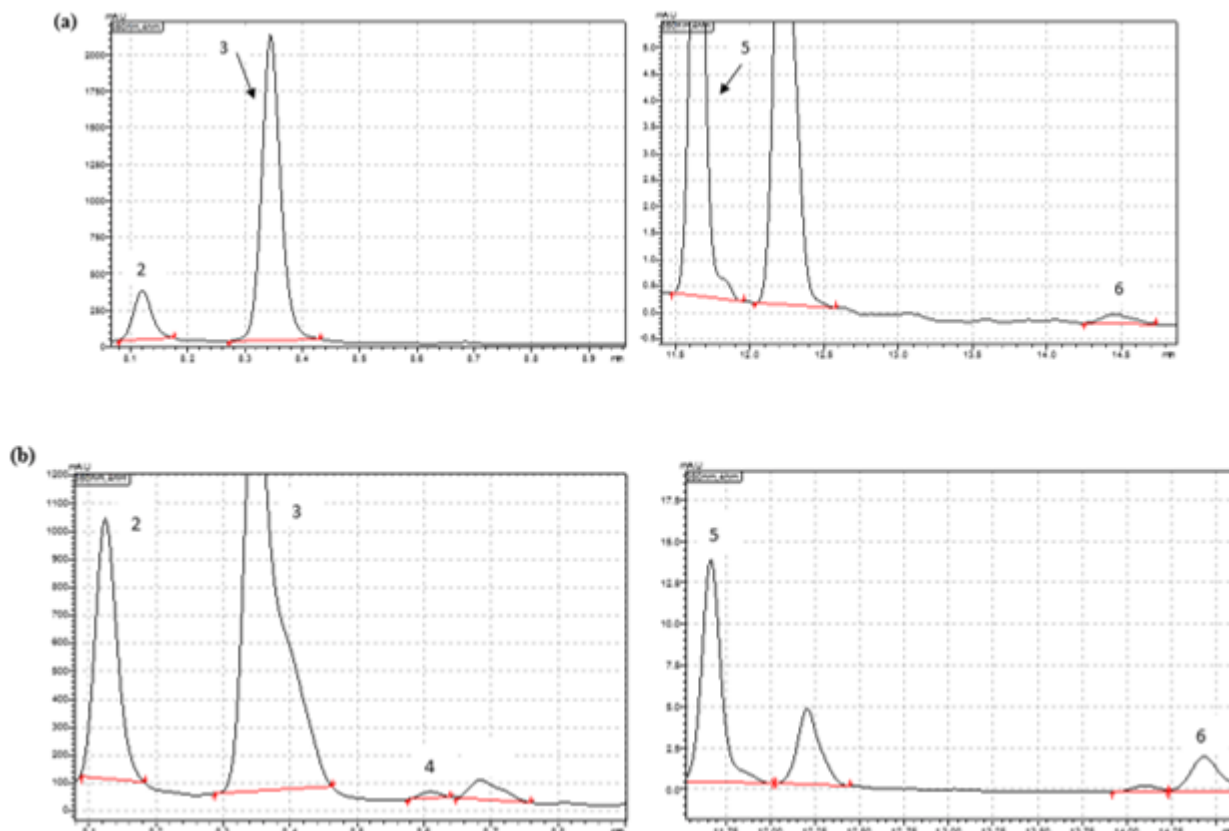


Fig. 3. Chromatograms of *Tanacetum vulgare* aqueous extracts obtained from leaves (a) and flowers (b) harvested in Julita. The compounds identified are: 2 - riboflavin, 3 - pyrocatechol, 4 - rutin, 5 - quercetin, and 6 - kaempferol

and 325.43 mg/L - Nadas), compared to that from tansy leaves extracts.

Pyrocatechol was observed to be in higher concentration in the flower extracts (618.93 mg/L-Julita, 614.10 mg/L - Nadas) compared to that found in leaves extracts (452.41 mg/L-Julita, 430.83 mg/L - Nadas).

From the flavonoid family we determined the content of rutin, quercetin, and kaempferol from the tansy extracts. The highest concentration in flavonoids was established for the flowers extracts. The highest quantity of rutin (18.25 mg/L) and quercitin (1.23 mg/L) was found in tansy flowers extract collected from Nadas. Tansy leaves extract (Nadas) have had the smallest amount of compounds determined in the present study.

We determined also significant amount of ascorbic acid, riboflavin, pyrocatechol, rutin, quercitin and kaempferol in all investigated extracts, our results being in good correlation with earlier reported studies [1,2]. The antioxidant activity and the total concentration of phenols are quite high, suggesting further research into the use of these plants to prevent and treat diseases [37,38].

Conclusions

The aqueous extracts obtained from herb of *Tanacetum vulgare* presents high phenolic content that lead to an important antioxidant activity that indicate tansy as a valuable source of biologically active compounds. Extracts obtained from tansy flowers exhibited higher content of phenols compared to that obtained from leaves, which lead to the same trend also for the antioxidant activity. Similarly, the concentrations of riboflavin, pyrocatechol, rutin, quercetin and kaempferol were determined to be higher in extracts obtained from flowers compared with that determined in the leaves extracts. These results suggest that aqueous extracts obtained from flowers of *Tanacetum vulgare* may be used as natural additives in food preservation and also in pharmaceutical industry and veterinary medicine.

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