

Physico-chemical and Antioxidant Properties of two Medicinal Wild Plants Grown in Moldova Region

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Abstract

The major objective of this study is to report physico-chemical (moisture, ash, protein, total phenolic compounds and ascorbic acid) and the antioxidant properties of methanol extracts of nettle (*Urtica dioica L.*) and wild garlic (*Allium ursinum*,) fresh and dried. The antioxidant properties of methanol extract of medicinal herbs were evaluated using free radical scavenging test. The phenols were extracted from the medicinal plants with methanol solvent and were quantified by the Folin-Ciocalteu method. The ascorbic acid content varied between 77.94 mg/100g in the fresh *Urtica dioica L.* and 39.55 from fresh *Allium ursinum*. The results showed that the total phenolic compounds in all medicinal plants decreased along processing. These results suggest that the medicinal plants sample extract with highest polyphenolic content will indicate the possibility of using them as ingredients in functional foods.

Keywords: antioxidant and antiradical activities, ascorbic acid, nettle, wild garlic.

1. Introduction

Plants were considered in ancient time's natural source of health beneficial, they had different uses, depending to the aim pursued. Plants are the basis for traditional medicines around the world for thousands of years, and today, the consumption of medicinal plants has increased due to their beneficial effects on the human body. The curative properties of plants are derived from a number of different compounds, among which phenolics, as secondary metabolites, are the most numerous [1].

Antioxidants are substances which inhibit the generation of free radicals and reduce which prevent or delay the oxidation reaction, such as lipid auto oxidation. Preventive antioxidants are able to absorb oxygen present as ascorbyl

palmitate, other antioxidants interrupt chain oxidation reactions. This includes substituted phenols, which are capable of reacting with a free radical hydrogen peroxide by releasing the OH group [2].

Fruits and vegetables rich in vitamins with antioxidant properties generally protect the body against oxidative stress (there is an imbalance between oxidizing agents and protective mechanisms of the cell) and associated diseases [3].

Recent studies indicate that vitamin A reduces the risk of cancer incidence initiated by free radicals or other strong oxidizing agents. Vitamin A (retinol) is not a powerful antioxidant, like its precursor β -carotene, which has a higher anti-cancer protective effect of retinol. The antioxidant effect of vitamin A is extended and amplified by vitamin C [4,5].

In fact, *Urtica dioica L.* has been used for a long time as a herbal tea, juice, hydroalcoholic extract or steamed leaves and roots. Nettle tea cure has been used as traditional medication for the

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treatment of diseases including inflammation of the urinary system, pathological urinary retention, abdominal pain, anemia, excessive menstruation, lower blood glucose and has a mild laxative effect. Nettle juice is indicated for arterial hypertension.

The *U. circularis* hydroalcoholic extract possesses sedative activity, anthelmintic activity and anti-inflammatory activity [6,7]. The plant is used traditionally as diuretic, emmenagogue, blood purifier and as anthelmintic. It is also used in nephritis, haematuria, jaundice and menorrhagia [8]. The antioxidant, hepatoprotective and anthelmintic activities of dried methanol extract of leaves of *U. dioica* was determined and the results revealed a dose dependent increase in anthelmintic activity of the extract at 25, 50, and 100 mg/mL concentrations [8].

It has been reported that the leaves of both cultivated and wild samples of *U. dioica* contain large amounts of caffeic acid derivatives, in particular, chlorogenic and 2-O-caffeoylmalic acid, which represent 71.5% in cultivated (C) and 76.5% in wild (W) of total phenolics [9].

Allium ursinum L., known also as wild garlic or bear's garlic is a wild growing *Allium* species found in the forests of Europe, as well in Romania and it's used as spice. Traditional folk medicine recommends the use of bear's garlic as an antiscorbutic, fever-fighting, also recommended in problems with intestines [10].

Wild garlic is rich in allyl sulfide, which prints the element characteristic taste and smell of plants of the genus *Allium* (most notably the onion and garlic). In addition, contains many valuable substances with therapeutic potential: vitamins A and C, B vitamins, minerals, calcium, iron, magnesium, phosphorus, essential oils, and carbohydrates and of course protein. Wild garlic has many therapeutic qualities, is a reliable aid in fighting diseases and also an excellent natural tonic.

It was demonstrated that consumption of *Allium ursinum* L., prevented respiratory disease, heart disease and contributes to improve digestion.

Recent studies revealed that *Allium ursinum* L., contain phenol carboxylic acids such as *p*-coumaric and ferulic acids in all five species (*Allium obliquum* L., *A. senescens* L. subsp. *montanum* (Fries) Holub, *A. schoenoprasum* L. subsp. *schoenoprasum*, *A. fistulosum* L. and *A. ursinum* L.) and analyses shows that it has antibacterial and antifungal effects [11]. *Allium*

senescens ssp. *montanum* ethanol extract contain 58.37 µg alliin/ml extract and 0.919 mg allicin/ml plant extract [12].

Results obtained by Štajner, D., & Popović, B. M., (2009) suggest that leaves possesses higher antioxidant and scavenging activities than bulbs in the majority of cultivated and wild *Allium* species examined [13].

Method of extraction (water infusion, decoction, 70% and 96% ethanol extracts and a methanol extract) affects the antioxidant activity, thus the extraction with 70% ethanol gives the highest DPPH [14].

Therefore, the aim of the present work was to analyze two medicinal plants in relation to their phenolic composition and antioxidant activity in raw and dried material. The vitamin C concentration, total phenolic compound content, total antioxidant capacity, in leaves from *Allium ursinum* L., and *Urtica dioica* L. plants growing wild were compared.

2. Materials and methods

2.1. Plant material

Fresh *Allium ursinum* L. and *Urtica dioica* L. leaves were collected from different points at Dragomirna forrest, from the Suceava plateau, an area of Romania, in April 2013 (table 1).

2.2. Sample preparation

The collected material was washed with distilled water to remove attached soil and other impurities, cleaned and some of the plants was shade oven-dried at 60°C until they reached a constant weight. All dried materials were ground to a fine powder using a porcelain mortar, after which they were stored in dark, sealed bottles at room temperature. To determine the total polyphenol content, ascorbic acid and the free radical scavenging activity, fresh samples were analyzed the day they were collected.

Extraction of Phenolics from samples

Raw (*Urtica dioica* L) and (*Allium ursinum* L.) leaves, were used for extraction with 80 % ethanol (Merck, Bucuresti, Romania) in Analytic chemistry Laboratory of Ștefan cel Mare University, Suceava, Romania [15].

Table 1. Information about the traditional knowledge of each plant studied

Plant species	Common name	Use	Diseases treated	Plant part	Condition of plant used
<i>Urtica dioica</i> L	Nettle	Medicinal and food	rheumatic diseases, gout, pathological urinary retention, diabetes, anemia.	Roots, stems and leaves	Fresh and dried
<i>Allium ursinum</i> L	Wild garlic	Medicinal and food	Diabetes, diseases of the nervous system, depurative role, gastrointestinal, cardiovascular diseases.	leaves	Fresh and dried

2.3. Preliminary physico-chemical characteristics

The determination of moisture and dry matter content in fresh leaves was effectuated according to the European Standard EN ISO 665/2000 by the drying process in a drying chamber at the temperature of 103°C.

The ash content was obtained by calcinations of 5g of sample at 600°C, until a homogeneous white ash without black points was obtained. The crude protein content ($N \times 6.25$) of the samples was determined following the macro-Kjeldahl method. Samples (about 1.0 g) were analyzed using a Mineral Six digester and an Auto Disteam semi-automatic distilling unit (International PBI). Fat content was determined by the Soxhlet method with petroleum ether solvent. The lipid content was determined gravimetrically (Extraction Unit 6, SER 148 model, VELP Scientifica, Italy).

Carbohydrate content was estimated by difference of the other components using the following formula: carbohydrate content = 100% - (% moisture + % protein + % fat + % ash). Total energy was calculated according to the following equation: Energy (kcal) = 4.0x (g protein + g carbohydrate) + 9.0 x (g lipid) [16].

2.4. Total phenolic compound content

The total polyphenol content (TPC) of the extracts was determined by spectrophotometer, using gallic acid as standard, according to the Folin-Ciocalteu method [17]. The results were expressed as mg of gallic acid equivalents (mg GAE). The correlation coefficient (r^2) for the calibration curve was 0.9954.

2.5. Total antioxidant capacity

The free radical scavenging activity was evaluated with the DPPH (1,1-diphenyl-2-picrylhydrazyl radical) assay directly on plant samples. The antiradical capacity of the samples was estimated.

The plants samples aliquot (0.5 mL) was added to freshly prepared DPPH reagent, and kept at room temperature. After incubating for 5 min, the absorbance of the resulting solutions was measured at 517 nm using a spectrophotometer. The control was conducted in the same manner, except that distilled water was used instead of the sample. Each day, the absorption of the DPPH solution was checked.

The percentage inhibition activity was calculated by the following formula:

PI_{50} % inhibition activity = $(A_{\text{blank}} - A_{\text{sample}}/A_{\text{blank}}) \times 100$, where A_{blank} is the absorption of the DPPH solution and A_{sample} is the absorption of the DPPH solution after the addition of the sample (nettle or wild garlic).

2.6. Vitamin C content

Extraction of ascorbic acid from samples

The extracts were obtained following the next protocol: 4 grams of leaves samples were extracted with 12 ml of acidified solutions (Perchloric acid and o- Phosphoric acid 1%) using a ceramic mortar and a pestle. The residue was re-extracted until the extraction solvents remained colorless (the total solvent volume was 50 ml). The extract was filtered through a Whatman no. 5 filter paper. The extracts were kept at -20°C until further analysis.

Ascorbic acid separation, identification and dosage

The ascorbic acid from the samples was separated, identified and dosed in a HPLC SHIMADZU system coupled with UV-VIS detector (DAD). ZORBAX - C18 column (5 μ m, 250x4.6) was used. The column was eluted in isocratic system with a mobile phase that consisted of phosphate buffer pH = 3.5 (TFA): solution 0.02 m/l of monopotassium phosphate and orthophosphoric acid 10%, adjusted to pH = 3.5. The pump flow

rate was set at 0.6 ml/min. The chromatograms were registered at 245 nm.

For ascorbic acid identification, standard L-ascorbic acid (Sigma 99% standard L ascorbic acid) was used. For dosage of ascorbic acid in the samples, a calibration curve was constructed using dilutions of standard L-ascorbic acid in distilled water. The correlation coefficient (r^2) for the calibration curve was 1.00.

2.7. Statistical Analyses. All the assays were carried out in triplicate. The results were expressed as mean values and standard error (SE) of the mean or standard deviation (SD) of the mean.

3. Results and discussion

Preliminary physicochemical characteristics

The results obtained from proximate composition value obtained for the *Allium ursinum* L., and *Urtica dioica* L. plants samples were shown in table 2.

According to scientific studies, moisture analysis were done to some nettle leaves which were collected from coastal part of Turkey (Aegean, Black Sea, Marmara, and Mediterranean region), average moisture content was 77.75% [18]. In our study, moisture content of nettles in leaves was 90.25 %.

Table 2. Initial physicochemical characteristics of nettle and wild garlic leaves

Characteristic*	Wild garlic (<i>Allium ursinum</i>)	Nettle (<i>Urtica dioica</i>)
Moisture %	90.25± 0.081	91.54± 0.029
Dry matter %	9.75± 0.025	8.46± 0.017
Crude protein %	1.8± 0.072	2.004± 0.007
Ash %	0.87± 0.011	0.92± 0.005
Fat content %	2.89± 0.022	3.21± 0.009
Carbohydrate %	4.19	2.326
Energy (kcal/100g)	51.436	47.603

Values represent mean ± SD and are expressed in 100 g fresh weight (FW) basis.

The crude protein content ranged for wild garlic from 0.8± 0.072 to 1.004± 0.007 % for nettle. It was observed that the protein content in wild garlic were comparable with wild greens

(asparagus, white bryony and black bryony) used in Iberian Peninsula traditional diet [19].

This result can be explained by the fact that nettle leaves are in general more fibrous than wild garlic. Fat content (3.21± 0.009 g/100 g) and ash (0.92± 0.005 g/100 g) content were higher in nettle (*Urtica dioica*). The value of protein found in this sample was lower than the concentration found in wild garlic, (3.3 g/100 g), [20] but similar to the one found in *Portulaca oleracea* leaves and flowers harvested in the rainy season (June to October of 2008) in region of Oaxaca (Mexico) (1.20±0.05), [21]. Other authors [22], reported that protein and lipid contents were equal to 4.34 ± 0.06 and 2.55 ± 0.05 g per 100 g of fresh material, respectively of wild asparagus spears *A. acutifolius* from Raviscanina, a small agricultural center near Caserta (Italy).

Total phenolic compound content

Phenolic compounds of wild medicinal plants can directly contribute to antioxidant properties. Wild medicinal plants are the main sources of antioxidant phytochemicals, that's why they should be included in the human diet, to prevent the chronic-degenerative diseases. Particular attention was given to *Allium ursinum* L., a wild medicinal plant traditionally gathered and consumed in some Romanian regions.

The evaluation of content of total polyphenolic compounds and losses after drying were show in figure 1.

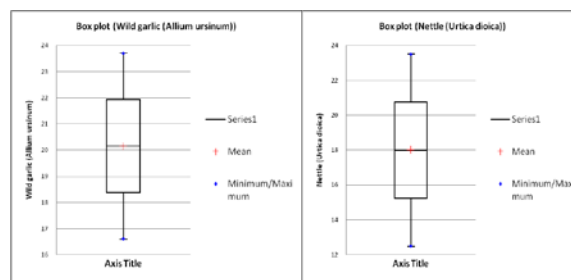


Figure 1. Total phenols concentrations mgGAE /100g of analyzed samples

The TPC of fresh nettle methanolic extract was approximately similar to that of fresh wild garlic methanolic extracts. According to a scientific research, total phenolic content of nettle could be ranged as leaves >root >stalk [18]. In this study, the results were given as fresh leaves 23.5 and dried leaves 12.5 mg GAE/100g.

Other previous conducted studies have stated the alcoholic extracts of *A. ursinum* L. ranged from 16.84 to 28.11 mg GAE/g plant weight in the samples of dry wild garlic [23].

Studies carried out on wild garlic in Serbia have shown that the leaves of *A. ursinum* L. have the lowest content of total phenolics (only 4.34 mg/g) [1]. Consumption of controlled diets rich in fruits and vegetables increased significantly the antioxidant capacity of plasma [8].

DPPH scavenging capacity assay

The lowest PI₅₀ value indicates the highest antioxidant activities against free radicals complex Methanolic extracts of fresh *Allium ursinum* L., had exceptionally high scavenging activity (figure 2). Free radicals are involved in lipid peroxidation and have a decisive role in the development of many chronic diseases such as cancer and cardiovascular diseases.

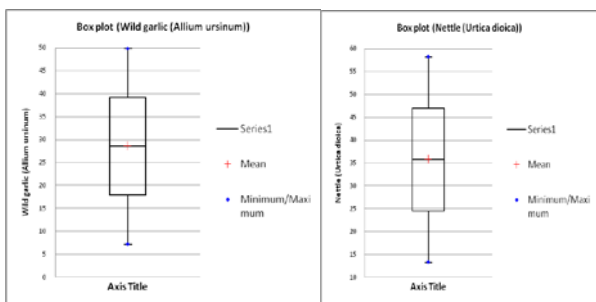


Figure 2. Scavenging percentage for radical DPPH in 2 species of *Allium ursinum* L. and *Urtica dioica* L. in two sample' extracts

Plants belonging to the *Urtica dioica* L were less active, using the DPPH method.

The ascorbic acid extracts of samples are presented in figure 3.

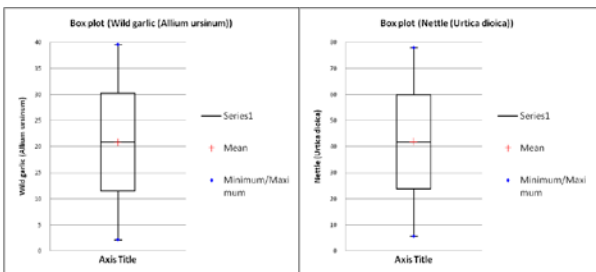


Figure 3. Ascorbic acid concentrations mg /100g of analyzed samples

Many studies on the antioxidant properties of *Allium ursinum* L., also revealed that they are good dietary sources of antioxidants [13,1].

As seen in the figure 3, during the processing period, the ascorbic acid has evidenced decreases in all analyzed wild vegetables samples. After dried, the ascorbic acid content has decreased by 92.71%, in dried nettle compared to the fresh nettle and in dried *Allium ursinum* L. decreased by 94.56%, compared to the fresh wild garlic.

Table 3. Descriptive statistics

Category	Freq. per cat.	Rel. freq. Cat. (%)	Cum. Rel. freq. (%)
PI dried	49.850	35.863	35.863
AA fresh	39.550	28.453	64.317
TP fresh	23.700	17.050	81.367
TP dried	16.600	11.942	93.309
PI fresh	7.220	5.194	98.504
AA dried	2.150	1.547	100.050

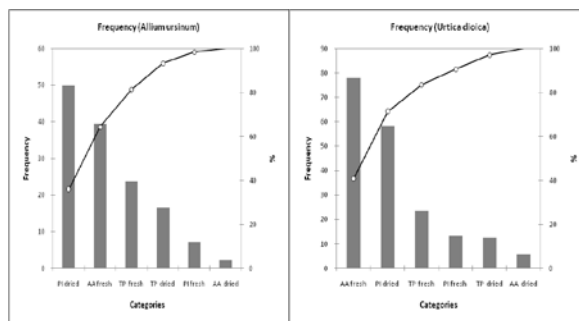


Figure 4. Pareto diagrams for the characteristics studied wild garlic and nettle

Pareto charts highlight the characteristics analyzed significantly positive values characterizing the two plants studied. Wild garlic has the highest antioxidant capacity compared with nettle and sample fresh nettle is high in ascorbic acid. To note is that polyphenol content remains constant and significantly high both fresh and dried as (81.36 % and 93.30 %).

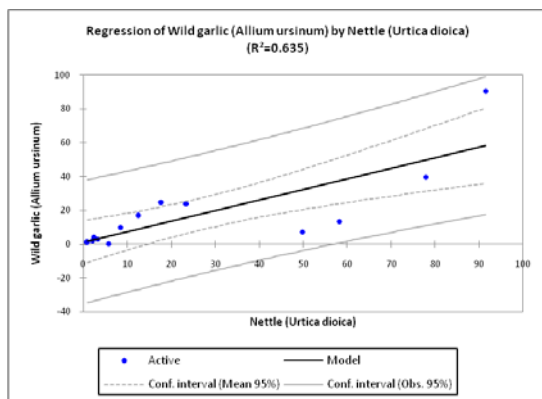


Figure 5. The regression curve for the results analyzed

The estimation of connections of the variables obtained by the determination at the two plants, the distribution of the grouped and a strong correlation between them. The correlation coefficient $r^2=0.635$ express this.

4. Conclusions

In this study, we have quantified the loss of phenolics, ascorbic acid and antioxidant capacity in *Allium ursinum L.* and *Urtica dioica* leaves that need to be dried. The present study showed that *Allium ursinum L.* and *Urtica dioica*, which are often included in some salad dishes as an ingredient, can be considered good sources of natural antioxidants for side dishes, commercial and medicinal uses.

Dried vegetables retained about 60 % of the phenolic values of raw vegetables. The biggest losses recorded after drying process was founded at ascorbic acid from wild garlic samples.

The present report contributes to quantifying the phenolic and ascorbic acid losses and to educate consumers to introduce aromatic herbs as a seasoning supplement in the diet of every age group.

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