QUANTITATIVE DETERMINATION OF POLYPHENOL COMPOUNDS FROM RAW EXTRACTS OF *ALLIUM, ALLIARA* AND *URTICA GENUS*. A COMPARATIVE STUDY

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Abstract: *Flavonoids are a large family of plant secondary metabolites, one of the largest groups of natural compounds known, principally recognized for their health-promoting properties in human diets. Most flavonoids outperform well-known antioxidants, such as ascorbate (vitamin C) and α-tocopherol (vitamin E), in vitro antioxidant assays because of their strong capacity to donate electrons or hydrogen atoms. Flavonoids have been demonstrated to accumulate with oxidative stress during abiotic and biotic environmental assaults. There are many foods that contain flavonoids and phenolic acids, besides these being dietary plants.*
This paper presents the spectroanalytical profile of the flavonoids and polyphenolic compouns from organs of Allium, Brassicaceae and Urticaceae genus raw hydroalcoholic extracts. The quantitative analysis of the examined chemical compounds showed that 70% ethanol solution was the best solvent used in order to obtain the highest polyphenolic content.
The content of phenol compounds was determined colourimetrically with the Folin–Ciocalteu (FC) reagent and was expressed in gallic acid equivalents (GAE).
The flavonoid contents was determined using a method based on the formation of complex flavonoid-aluminium and was expressed in quercetin equivalents (QE). The results show that the plants may be potent sources of natural antioxidants.

Keywords: *Allium ursinum, Alliaria petiolata, Urtica dioica, polyphenols, flavonoids*

Introduction

In recent years much attention has been devoted to natural antioxidant and their association with health benefits[1]. Plants are potential sources of natural antioxidants. It produces various antioxidative compounds to counteract reactive oxygen species (ROS) in order to survive[2]. Phenol compounds are responsible for major organoleptic characteristics of plant-particularly colour and taste properties. They are also reported to contribute to the health benefits associated with consumption of diets high in fruits and vegetables or plant-derived beverages. Innumerable studies have been devoted to polyphenols, their occurrence in plants and their effects on quality of life. However, plant polyphenol composition is still poorly understood. Furthermore, polyphenols are highly reactive compounds and good substrates for various enzymes, including polyphenoloxidases, peroxidases, glycosidases, and esterases. They undergo numerous enzymatic and chemical reactions during post harvest food storage and processing. Although the

occurrence of such reactions and their roles in the development or degradation of food quality is well documented, the structures of the resulting products are still poorly understood and their concentrations in food are usually unknown.

Plant polyphenols comprise a great diversity of compounds, among which flavonoids and several classes of nonflavonoids are usually distinguished[3]. The latter (Figure 1) are mostly rather simple molecules, such as phenolic acids (which are subdivided into benzoic acids and hydroxycinnamic acids, based on C1-C6 and C3-C6 skeletons, respectively) and stilbenes, but also include complex molecules derived from them (eg, stilbene oligomers, gallotannins, ellagitannins, and lignins). The flavonoids (figure 2) have a common nucleus consisting of 2 phenolic rings and an oxygenated heterocycle. They are divided into several groups differing in the oxidation state of the heterocyclic pyran ring (eg, anthocyanins, flavonols, and flavanols). More than 4000 flavonoids have been identified in plants, and the list is constantly growing[4]. This is because of the occurrence of numerous substitution patterns in which primary substitutes (eg, hydroxyl, methoxyl, or glycosyl groups) can themselves be substituted (eg, additionally glycosylated or acylated), sometimes yielding highly complex structures. Moreover, flavanols are also encountered as oligomers and polymers, referred to as condensed tannins or proanthocyanidins.

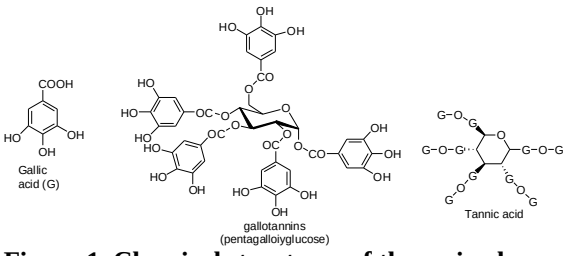

Figure 1. Chemical structures of the main classes of nonflavonoid polyphenols

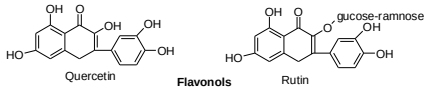

Figure 2. Chemical structures of the main classes of flavonoids

Plant polyphenol composition is highly variable both qualitatively and quantitatively; some of the compounds are ubiquitous, whereas others are restricted to specific families or species (eg, isoflavones in legumes). Polyphenol diversity in fruits[5] and in plant foods[6] has been described in excellent reviews. Within a single species, large variations may also occur, particularly because of genetic factors, environmental conditions, and growth or maturation stages.

Recently, there are numerous methods that have been developed to evaluate the polyphenol content of complex mixtures such as plant extracts[7]and to identify all possible mechanisms characterizing an antioxidant activity[8,9].

The aim of this study is to evaluate the total phenolic and flavonoid content of some Romanian plants which are in relationship with antioxidative activity and of the plants.

According to our knowledge, there are very few data regarding the potential antioxidant properties related to phenolic and flavonoid fractions of *Allium*, *Alliaria* and *Urtica* plants, which are widely used as salads and foods in most of the countries of the Balkan Peninsula. With respect to this, in the study we present our investigations on the total phenol and flavonoids content of the mentioned above plant extracts.

Materials and methods

Plant material. *The b*iological material analyzed in the present paper was collected from the North Dobrogea (Luncavita Forest) and is made from the following vegetal products: folium of *Allium ursinum* (wild garlic), *Alliaria petiolata* (garlic

mustard) and *Urtica dioica* (nettle). The harvesting was made when the leaves grew until maturity before the bloom of the plants and were macroscopic determined in Botanical Garden laboratory of Galati.

Chemicals. The chemicals were purchased from Sigma Co. Folin–Ciocalteu (FC) reagent was purchased from Merck (Germany). All other chemicals and reagents were of analytical grade.

Extracts preparation. The ground air-dried immature plants (5 g), were extracted using a method of extraction with ultrasounds with 70% ethanol for 24 h, at room temperature. After the extraction, the extracts were collected and filtered.
To remove chlorophylic pigments, ethanolic extract is subject to repeated extraction with petroleum ether until disappearance of its green colour.
Ethanolic phase obtained after extraction is used to determine flavonoids and polyphenols, the volume being adjusted to 100 mL with cold 70% ethanol.

Analysis of total phenolic content

The total polyphenol content (TPC) of the extracts was determined by spectrophotometry, using gallic acid as standard, according to the method described by the International Organization for Standardization (ISO) 14502-1[10,11]. Briefly, 1.0 mL of the diluted sample extract was transferred into triplicate to separate tubes containing 5.0 mL of a 1/10 dilution of Folin-Ciocalteu's reagent in water.
Then, 4.0 mL of a sodium carbonate solution (7.5% w/v) was added.
The tubes were then allowed to stand at room temperature for 60 min before absorbance at 765 nm (in a UV– Vis spectrophotometer) was measured against water. The TPC was expressed as gallic acid equivalents (GAE) in mg/100 g material.
The concentration of polyphenols in samples was derived from a standard curve of gallic acid ranging from 10 to 50 µg/mL (figure 1, Pearson's correlation coefficient: R^2: 0.9988).

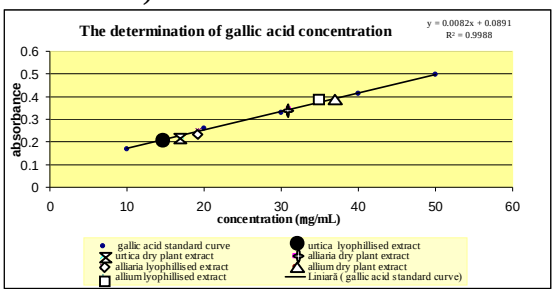

Figure 3 Standard curve of gallic acid and concentration in polyphenols of analyzed extracts

Estimation of total flavonoid content

Measurement of total flavonoid content in the investigated extracts was determined spectrophotometrically[12] using a method based on the formation of complex flavonoid-aluminium with the maximum absorbtivity at 430 nm. The aqueous dilutions of samples, in the amount of 1 ml, were separately mixed with 1 ml of 2% $AlCl_3$. After incubation at room temperature for 30 min, the absorbance of the reaction mixtures was measured at 430 nm. The flavonoids content was expressed as quercetin equivalents (QE) in mg/100 g material, by using a standard graph. (figure 4, Pearson's correlation coefficient: r^2 : 0.9732)

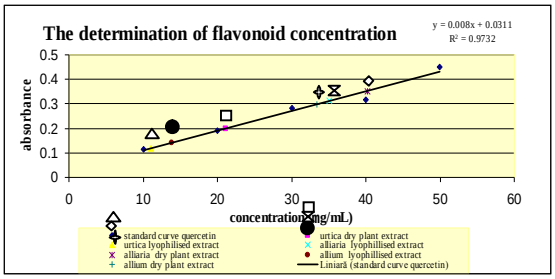

Figure 4 Standard curve of quercetin and concentration in flavonoid of analyzed extracts

Results and Discussion

Although most antioxidant activities from plant sources are derived from phenolic-type compounds, their effects are not always correlated with the presence of large quantities of phenolics. Therefore, both sets of data on phenolic and flavonoid compounds need to be examined together. With respect to this, the investigated plant extracts were analysed for total phenolic and flavonoid contents.

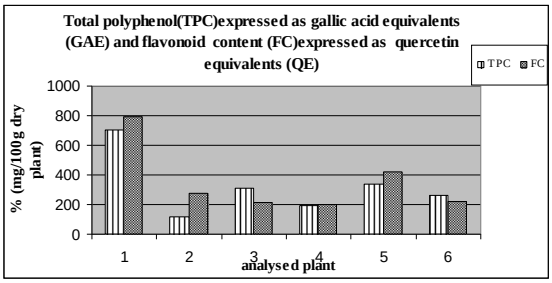

Figure 5-The total polyphenol content and flavonoid content of analyzed plants
Legend: *Allium ursinum* (1-dp, 2-lp), *Alliaria petiolata* (3-dp, 4-lp), *Urtica dioica* (5-dp,6-lp) dp-dry plant; lp-lyophilised plant

Polyphenols Content. The Folin-Ciocalteu assay is one of the oldest methods developed to determine the content of total phenols. In this work, the total polyphenol content of 3 samples of food plants, belonging to the Romanian autochthonous flora was analyzed.
The amount of total phenolics varied widely in plant materials and ranged from to 265 mg to 594 mg GAE/100g dried plant (Fig.5). The results are presented in Figures 3 . As shown in Figure 3, the total polyphenol content in plants was found higher in air dried plant than in lyophilized one. Our experiments show that the wild garlic is the richest in polyphenol compounds.

Total flavonoid contents. Furthermore, the results obtained from the evaluation of total flavonoid content also indicate great variations (Fig.4). In the wild garlic plant, the content of quercetin equivalents was notably higher than it was in nettle and mustard garlic.
Also, the total flavonoid content in plants was found higher in air dried plant than lyophilized plant.
The decreases of total phenolic and flavonoid contents in lyophilized plants are most probably caused by the solubility of compounds in the water removed by lyophilization .

Conclusion

In this work, a comparison between the total polyphenol and flavonoid content of 3 autochthonous plants was made (Fig.5).
The total polyphenol content and the flavonoid content are both parameters of quality in plants regarding their biological properties, and both assays should be applied for the antioxidant capacity studies.
In conclusion, of all the local plants extract analysed, the wild garlic (*Allium ursinum*) showed the highest yield of TPC and FC. The lyophilized plant extracts content of polyphenols are lower than air dried plant extracts. Our studies shows that Romanian plants may be potent sources of natural antioxidants because the total phenolic content had positive correlation with antioxidant capacity[12,13].
According to the results obtained, the plants from the autochthonous flora are of very good quality.

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