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# ANTIOXIDANT ACTIVITY AND TOTAL PHENOLIC CONTENT IN *ALLIUM URSINUM* AND *RANUNCULUS FICARIA*

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**Abstract:** *Allium ursinum and Ranunculus ficaria, some perennial herbs that are consumed in domestic regions in Romania especially during springtime, are considered efficient spring tonic, giving a boost to our immune system. Ethanol extracts from their leaves were tested in vitro in order to evaluate their antioxidant potential by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay and the total phenolic content was also determined. A. ursinum leaves extract exhibit antioxidant activity over 77% with an $EC_{50}$ value of 322 μg/ml while R. ficaria leaves extract exhibit antioxidant activity over 80% with an $EC_{50}$ value of 88.6 μg/ml respectively, however higher than that of epigallocatechingallate ($EC_{50}$ value of 1.2 μg/ml) and gallic acid ($EC_{50}$ value of 0.8 μg/ml) which were used as positive control in this experiment. The amount of total phenolic content was 1.42±0.09 g GAE/100g extract in the case of A. ursinum leaves extract and 1.79±0.11 g GAE/100g extract in the case of R. ficaria leaves extract. These results indicate that A. ursinum and R. ficaria possess an important potential to be used as functional food ingredient or nutraceutical.*

**Keywords:** *antioxidant activity, total polyphenol, Allium Ursinum, Ranunculus Ficaria*

## 1. Introduction

Many antioxidants compounds are vital substances, which possess anti-inflammatory, antiathero-screlotic, antiploriferative, antitumor, antimutagenic, anticarcinogenic, antibacterial or antiviral activities to a grater or lesser extent [1- 2]. There is an increasing interest in the efficacy and use of naturally derived antioxidants, therefore functional foods and nutraceuticals has received much attention in the recent years. Polyphenols present in medicinal and dietary plants possess an ideal chemical structure for free radical scavenging activity some have been shown to be more effective than tocopherols and ascorbate when tested *in vivo*. Polyphenols possess a high reactivity as hydrogen or electron donors, and from the ability of the polyphenol derived radical to stabilize and delocalize the unpaired electron and from their ability chelate transition metal ions [3- 4]. Dietary antioxidants protect against free radicals such as reactive oxygen species, in the human body, which are continuously produced *in vivo* results in cell death and tissues damage [5]. Also, those reactive oxygen species can damage DNA, which causes mutations and chromosomal damage, the production of excessive free radicals stimulate the oxidative damage and such situation led to more than one hundred disorders in humans including atherosclerosis, coronary heart disease,

neurodegenerative disorders, cancer and they also play a major role in aging process [6]. Due to detection of many bioactive compounds in food with possible antioxidant activity, there has been increased interest in the relationship between antioxidants and disease risks [7]. Therefore, the present study was undertaken to evaluate the antioxidant capacity of leaves extracts of *A. ursinum* and *R. ficaria,* some perennial herbs as compared with some compounds with high antioxidant capacity, and the total phenolic content in these extracts was measured.

*Allium ursinum* L., known also as wild garlic or bear's garlic is a wild growing *Allium* species found in the forests of Europeas well as in Romania. The species belong to *Allium* family have been reported, to be used as a remedy for the prevention and treatment of certain diseases [8]. *A. ursinum* has not yet been cultivated and it did not gained any particular importance. The fresh leaves or dried herb is used in local cuisines especially in Eastern Europe. The leaves are edible and can be used as a salad or as spice, or they can be boiled as vegetable. The bear's garlic is also a common "wild" vegetable in Ukraine and Russia. It is sold on local markets as fresh, pickled, or salted and is becoming increasingly popular in the Czech Republic and Germany [9].

*A. ursinum* is widely used as spice and traditional folk medicine recommends the use of bear's garlic asan antiscorbutic, fever-fighting, also recommended in problems with intestines. In medieval medicine, the leaves of *A. ursinum* were used as a therapy for cardiovascular diseases [10]. A "*in vitro"* cardioprotective action of *A. ursinum* was first described back in 1993 [11]. It has been reported that wild garlic has a greater effect on lowering the blood pressure of rats than regular garlic [12]. Several biological activities of *A. ursinum* plants, such as antioxidative, [13] cytostatic, and antimicrobial, [14] and antifungal properties [15], were also reported. The potential health benefits of bear's garlic have been attributed mainly to the sulfur-containing compounds. High amounts of volatile compounds, such as sulfides and disulfides, which had been identified in bear's garlic, have a direct impact on the quality of *A. ursinum* as a medicinal plant and as a spice [16]. Recently, Xu *et al.*, confirmed that *A. ursinum* water extract can inhibit proliferation and induce apoptosis in AGS (gastric cancer cell line) cells, indicating that in most of the investigated cases, diallyl sulfites are responsible for this effect [17]. Because of the content of allin, allicin, and other sulfuric compounds, the plant possesses parasite-killing, fungicidal, and antibacterial properties. Other components, such as lectins and flavonoids, have been found in *A. ursinum* [18 - 19]. Flavonoids were found to be responsible for the inhibition of platelet aggregation in humans and to have antioxidant activity [20]. *A. ursinum* is found to be more beneficial than *A. sativum* in *in vivo* and *in vitro* studies. Thus, *A. ursinum* showed a higher effect in increasing high-density lipoprotein (HDL) and decreasing total cholesterol, as well as lowering the systemic blood pressure [21]. While all portions of *A. ursinum* were foundto exhibit antioxidant property, the leaves were found to have the highest activity [20]. This activity could be caused by the high content of flavonoids. However, the chemical profile of flavonoids in the leaves, stalks, and seeds of *A. ursinum* has not been fully studied. There are only five flavonoids isolated from *A. ursinum* by Carotenuto 1996 [18].

Traditionally, early leaves of *R. ficaria* are edible fresh or preapared as ingredient of different cusine. However, is not recommended to use more than 50-100 g of fresh leaves. The plant is used to treat

haemorrhoids, early leaves are high in vitamin C can prevent scurvy. The plant contains protoanemonina mild toxin, however, the process of heating or drying turns the toxin to anemonin, which is non-toxic and has antispasmotic and analgesic properties [22]. There are few scientific reports to date about *R. ficaria* leaves extract.

## 2. Materials and methods

### *Chemicals*

1,1-Diphenil-2-picryl-hydrazyl (DPPH) was purchased from Sigma-Aldrich. Gallic acid and Epigallocatechinegallate were from Sigma-Aldrich.

### *Preparation of vegetable samples*

Fresh *A. ursinum* and *R. ficaria* were purchased from the local market in Suceava city. The vegetables were washed with tap water measured and suspended into a 35% v/v ethanolic solution then extracted at 80°C for 2 hours. After evaporating to dryness and freeze drying the crude extract was obtained. The dry powder extract was used to assay the antioxidant activity and the total polyphenol content.

### *DPPH radical scavenging activity assay*

Radical scavenging activity against the 1,1-Diphenil-2-picryl-hydrazyl (DPPH) was measured as previously described by Barla 2009 [23]. Briefly the sample was dissolved in ethanol and mixed with a solution of 0.4 mg/ml DPPH, 0.5 ml. After incubation at room temperature for 30 min. the absorbance was measured at 520 nm. The $EC_{50}$ value, denoting the effective concentration of sample required to scavenge 50% of DPPH free radicals, was calculated by graphical regression analysis, and expressed as µg/ml.

### *Total phenolic content*

The total phenolic content of the aqueous ethanolic extract of each sample was obtained using the Folin–Ciocalteau method as previously described by Velioglu 1998 [24], with some modifications. One hundred microliters of sample extracts were mixed with 100 µl of the Folin–Ciocalteu reagent, vortexed for 5 min. Following this, 200 µl of sodium carbonate solution (9%) and 1600µl of deionized water was added and the reactants were mixed and left at room temperature for 30 min. Absorbance was determined at 760 nm against the blank, and a gallic acid calibration curve (0–500 mg/l) was constructed and used to determine the total phenolic content of the samples, expressed as Gallic Acid Equivalents (GAE).

## 3. Results and discussions

The antioxidant property of ethanol leaves extract of *A. ursinum* and *R. ficaria* were evaluated. DPPH radical is a commonly used substrate for easy evaluation of antioxidant activity based on its stability in the radical form and simplicity of the assay. The principle of this assay consists in the color change of DPPH solution from purple to yellow as the antioxidant compound quenches the radical found in the extract. The color changes can be measured quantitatively by spectrophotometer absorbance at 520 nm. The *A. ursinum* and *R. ficaria* ethanolic extracts showed good antioxidant capacity. For the same extract amount *R. ficaria* showed a slight stronger antioxidant activity (Figure 1), *R. ficaria* extract showed an $EC_{50}$ lower than that of *A. ursinum* extract this was in accord with the fact that the total phenolic content was higher in *R. ficaria* extract, as shown in the Table 1. However, the antioxidant power of *R. ficaria* extract was

over 3 times stronger than that of *A. ursinum* extract.

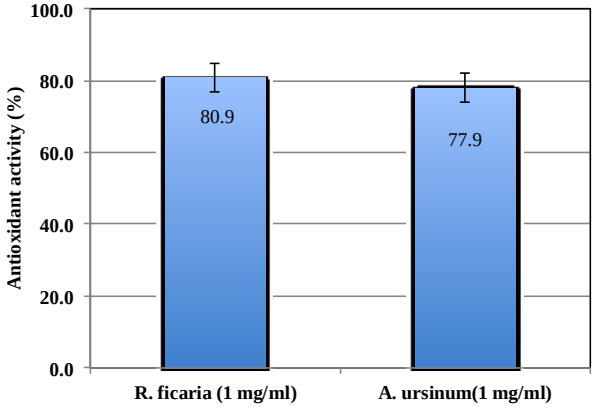


**Fig. 1. The antioxidant activity of *A. ursinum* and *R. ficaria* crude extracts (Concentration of 1 mg/ml).**

This result indicates that this effect can be correlated with the polyphenols content in *R. ficaria*. However, the $EC_{50}$ of *A. ursinum* and *R. ficaria* extracts were higher than that of gallic acid and epigallocatechinegallate, which were used as positive controls in this experiment.
Results suggest, in the case of *A. ursinum* extract, that the observed antioxidant activity might be given by the various kinds of flavonols present in the extracts and possible mainly kaempferol derivatives as reported by Oszmiaski on 2013 [25].

**Table 1.**

**Antioxidant activity and total phenolic content of *A. ursinum* and *R. ficaria***

| | **Antioxidant activity $EC_{50}$ (µg/ml)** | **Total phenolics (g GAE/100g extract)** |
|---|---|---|
| ***Allium ursinum*** | 322 | 1.42 ± 0.09 |
| ***Ranunculus ficaria*** | 88.6 | 1.97 ± 0.11 |
| **EGCG*** | 1.2 | – |
| **Galic Acid** | 0.8 | – |

**EGCG-epigallocatechinegallate*

Plant phenolic constituents are one of the major groups of compounds acting as antioxidants. Phenolics are able to scavenge reactive oxygen species due to their electron donating properties. The total phenolic content was determined using Folin-Ciocalteu method, reported as gallic acid equivalents by reference to standard curve. Total phenolic content in *A. ursinum* extract was 1.42± 0.09 g GAE/100g of extract and 1.97± 0.11g GAE/100g of extract in the case of *R. ficaria* extract. The total phenolic content did not differ significantly in these pants extract.

## 4. Conclusion

In conclusion *A. ursinum* and *R. ficaria* exhibit antioxidant capacity and its extracts contain substantial amounts of polyphenolic compounds. Generally, high levels of phenolic compounds are responsible for strong antioxidant capacity. Consumption of these vegetables especially during springtime can provide a good source of antioxidants. Therefore, these plants extract seams to possess an important potential to be used as functional food ingredient or nutraceutical. Finally, this report showed that despite the fact that *R. ficariais* is even less common than *A. ursinum*, exhibited interesting properties and further investigation are to be done concerning some particular biological activities of *R. ficaria* extracts.

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