

## RESEARCH ARTICLE

Tana Sapunjieva<sup>1</sup>  
Iordanka Alexieva<sup>1</sup>  
Dasha Mihaylova<sup>2</sup>  
Aneta Popova<sup>1</sup>

## Antimicrobial and antioxidant activity of extracts of *Allium ursinum* L.

### ABSTRACT

The antioxidant and antimicrobial activity of extracts of *Allium ursinum* L. have been studied. Plant leaves were used in preparation of different water and alcohol (methanol and ethanol) extracts. The antioxidant activity was estimated with the use of DPPH and ABTS radical scavenging assays. Total polyphenolic content (TPhC), reduction capacity (RC) and antimicrobial activity against Gram (+) [*Listeria monocytogenes* and *Staphylococcus aureus*], and Gram (-) [*Escherichia coli*, *Salmonella enterica* subsp. *Enterica* serovar *Abony*.] bacteria were determined. We found that the method of extraction affects the antioxidant activity. The extraction with 70% ethanol gives the highest DPPH, ABTS, TPhC and RC values. *Allium ursinum* shows higher antimicrobial activity against Gram (+) than Gram (-) bacteria. The zones of inhibition growth are respectively 38/38/14/14 mm in diameter of 0.12 ml supernatant, applied to a selected agar medium inoculated beforehand with a 24 - hour liquid culture of the pathogen.

**Key words:** *Allium ursinum* L., radical scavenging activity, antimicrobial activity, Gram (+), Gram (-)

### Authors' addresses:

<sup>1</sup>Department of Catering and Tourism,  
University of Food Technologies,  
Plovdiv, Bulgaria.

<sup>2</sup>Department of Biotechnology,  
University of Food Technologies,  
Plovdiv, Bulgaria.

### Correspondence:

Aneta Popova  
Department of Catering and Tourism,  
University of Food Technologies,  
26 Maritsa Blvd.,  
4002 Plovdiv, Bulgaria.  
Tel.: +359 32 643037  
e-mail: popova\_aneta@yahoo.com

## Introduction

The aim of the present study was to evaluate the antioxidant and antimicrobial activity of the typical Bulgarian spice "levurda" (*Allium ursinum* L.). This plant has been used for centuries in folk medicine, food flavoring and as an ingredient for local dishes. The increased interest in using natural products as anti-aging, anti-inflammatory, anticancerogenic, antifungal and antimicrobial sources engendered the researchers' activity in studying the antioxidant and antimicrobial activity of *Allium ursinum* L.

## Materials and Methods

Wild "levurda" *Allium ursinum* L. (Krichim) collected in spring 2012 was analyzed fresh and dry. The methods of extraction were based on the traditional application of the studied plant: water infusion (HM /hydro module/- 20; a 30 min water extraction brought to its boiling point), dekoktion (HM-20, a 30 min extraction in boiling water), 70% and 96% ethanol extracts and a methanol extract (HM-20; a 3 times heat reflux extraction for 30 min with the relevant alcohol at

70°C), supernatant of fresh spices (the plant was mashed with quartz sand, then suspended in 0.1 mol/l K<sub>2</sub>HPO<sub>4</sub>, pH 7.0 and centrifuged 10 min at 4°C, 15000g) (Stajner et al., 2008). Antioxidant activity was estimated with the use of DPPH (modification of Brand- Williams et al., 1995) and ABTS (Re et al., 1999) radical scavenging methods and expressed as Trolox equivalent antioxidant capacity (TEAC, μM TE/g plant weight). A modified method of Kujala et al. (2010) with Folin-Ciocalteu's reagent was used for the determination of the total polyphenolic content (TPhC). Gallic acid was employed as a calibration standard and the results were expressed as gallic acid equivalents (GAE) per gram of plant weight. Reduction capacity (RC) was determined by the method of Oyaizu (1986) using L-ascorbic acid as a standard. The antimicrobial activity of the extracts was determined by a modified method of Tagg & McGiven (1971).

## Results and Discussion

### Antimicrobial activity

*Allium ursinum* exhibits antimicrobial activity against both Gram (+) and Gram (-) bacteria. The Gram (+)

## RESEARCH ARTICLE

inhibition zone was bigger compared to the Gram (-) zone (Table 1). Sulfide compounds possess an antifungal activity and plants containing them are a natural alternative for the prevention of mycosis. (Lazarevic et al., 2011). *Allium ursinum* is reported to have a significant content of compounds belonging to the same chemical group-thiosulfinate (Kyuing et al., 2012). Allicin is proven to have a high antifungal activity and one of the major constituents of *Allium ursinum* (Parvu et al., 2011).

**Table 1.** Antimicrobial activity of a supernatant of *Allium ursinum* plant leaves

Test- bacteria	Inhibition zone, mm
<i>Listeria monocytogenes</i> NCTC 11994	38
<i>Staphylococcus aureus</i> ATCC 25093	38
<i>Escherichia coli</i> ATCC 8739	14
<i>Salmonella enterica</i> subsp. <i>Enterica</i> serovar <i>Abony</i> NCTC 6017	14

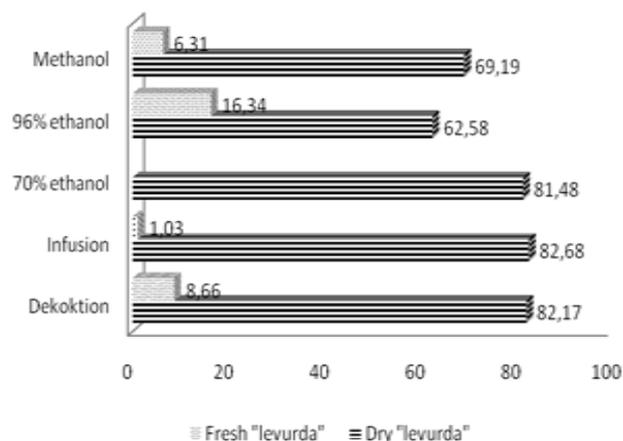
#### Antioxidant activity

The antioxidant activity was estimated using the ABTS<sup>+</sup> and DPPH<sup>•</sup> free radicals. It was proved that the extraction method affects the radical scavenging activity (Figure 1 and Figure 2) and the highest TEAC - values were determined by the heat reflux extraction with 70% ethanol toward the DPPH<sup>•</sup> free radicals (22.49  $\mu\text{M TE/g}$  plant weight). The highest TEAC – values concerning the ABTS<sup>+</sup> assay were achieved in the infusion extraction (82.68  $\mu\text{M TE/g}$  plant weight). The results correspond to the several studies conducted by Stajner et al. (2003, 2008). The leaves of *Allium ursinum* show a high antioxidant potential.

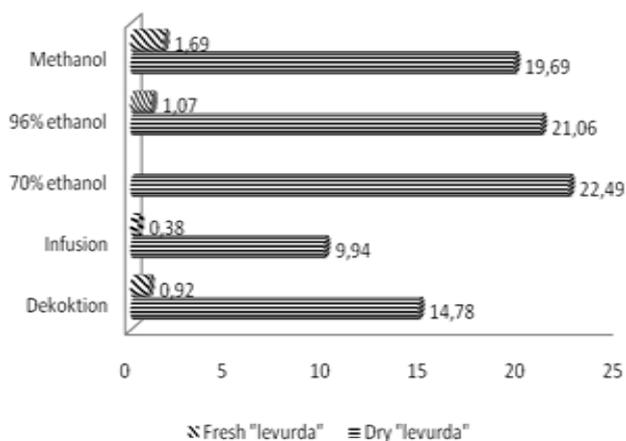
#### Total polyphenolic content

The results were expressed as gallic acid equivalents (Figure 3). Still, the 70% ethanol extract received the highest values.

The results ranged from 16.84 to 28.11 mgGAE/g plant weight in the samples of dry “levurda” and from 0.19 to 1.45 GAE/g plant weight in the samples of the fresh “levurda”. The highest total phenolic was found in the 70% ethanol extract of dry leaves (28.11 mgGAE/g dry plant weight) and the lowest in the infusion of fresh “levurda”leaves (0.19 mgGAE/g fresh plant weight).



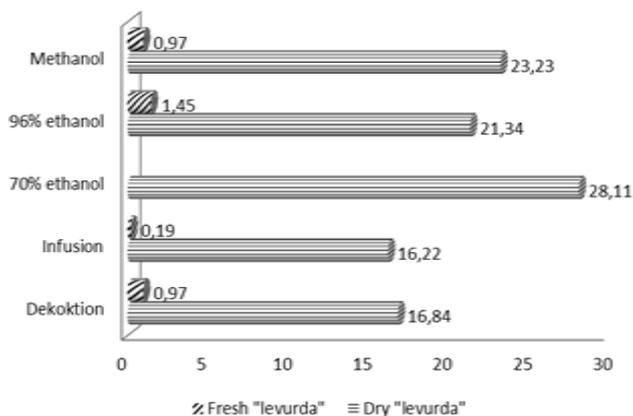
**Figure 1.** ABTS radical scavenging activity of *Allium ursinum*,  $\mu\text{M TE/g}$  plant weight



**Figure 2.** DPPH radical scavenging activity of *Allium ursinum*,  $\mu\text{M TE/g}$  plant weight

Depending on the data presented there can be found a possible correlation between the TPhC and the antioxidant activity measured by the two different methods (DPPH and ABTS radical scavenging assays). Due to the established relationships it can be conducted that the presence of the phenolic compounds seemed to be an important factor dictating free radical scavenging capacity of the extracts. Such correlation is being confirmed by other studies (Stajner et al., 2008).

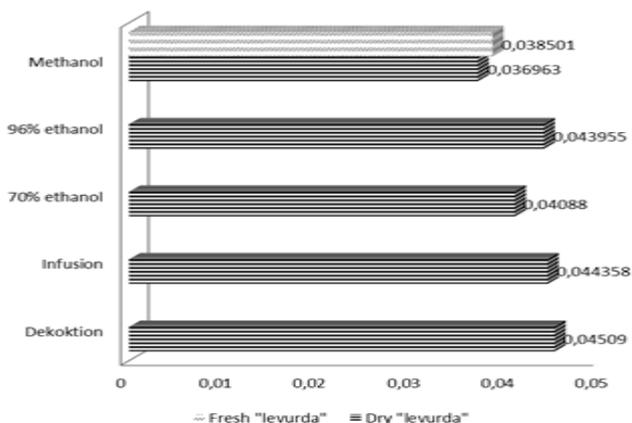
## RESEARCH ARTICLE



**Figure 3.** Total polyphenolic content of extracts of *Allium ursinum*, mgGAE/g plant weight.

### Reducing power

Reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity. Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipidperoxidation processes, so that they can act as primary and secondary antioxidants (Chanda et al., 2009). The results are expressed as mg equivalents ascorbic acid/l (Figure 4).



**Figure 4.** Reducing power of *Allium ursinum*, mg equivalents ascorbic acid/l

### Acknowledgement

The research leading to these results has received funding from the European Community's Seventh Framework Programme (FP7/2007-2013) under grant agreement n. 227118, project BaSeFood.

### References

- Bagiu R, Vlaicu B, Butnariu M. 2012. Chemical Composition and in Vitro Antifungal Activity Screening of the *Allium ursinum* L. (Liliaceae), Int. J. Mol. Sci., 13: 1426-1436.
- Brand-Williams, W, Cuvelier ME, Berset C. 1995. Use of a free radical method to evaluate antioxidant activity. Food Science and Technology, 28: 25-30.
- Chanda S, Dave R. 2009. In vitro models for antioxidant activity evaluation and some medicinal plants possessing antioxidant properties: An overview. African Journal of Microbiology Research, 3(13): 981-996.
- Kujala, T.S., Loponen, J.M., Klika, K.D., Pihlaja K. 2000. Phenolics and betacyanins in red beetroot (*Beta vulgaris*) root: distribution and effect of cold storage on the content of total phenolics and three individual compounds. J. Agric. Food Chem. 48: 5388-5342.
- Kyung K., Lee Y. 2001. Antimicrobial activities of sulfur compounds derived from S-alk(en)yl-L-cysteine sulfoxides in *Allium* and *Brassica*, Food Rev.Int. 17: 183-198.
- Lazarević JS et al. 2011. Chemical composition and antioxidant and antimicrobial activities of essential oil of *Allium sphaerocephalon* L. subsp. *sphaerocephalon* (Liliaceae) inflorescences, J. Sci. Food Agric. 91: 322-329.
- Oborny B, Botta-Dukat Z, Rudolf K, Morschhauser T. 2011, Population ecology of *Allium ursinum*, a space-monopolizing clonal plant, Acta Botanica Hungarica 53(3-4): 371-388.
- Oyaizu M. 1986. Studies of products of browning reaction: antioxidative activity of products of browning reaction prepared from glucosamine. Jpn. J. Nutr., 44: 307-315.
- Pârvu M, Pârvu A, Vlase L, Rosca-Casian O, Pârvu O. 2011. Antifungal properties of *Allium ursinum* L. Ethanol extract, Journal of Medicinal Plants Research, 5(10): 2041-2046.
- Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans CA. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biol. Med., 26: 1231-1237.
- Štajner D, Varga I. 2003. An evaluation of the antioxidant abilities of *Allium* species, Acta Biologica Szegediensis, 47(1-4): 103-106.
- Štajner D, Popović B, Čanadanović-Brunet J, Štajner M. 2008. Antioxidant and scavenger activities of *Allium ursinum*. Fitoterapia, 79: 303-305.
- Yen G, Duh P. 1994. Scavenging effect of methanolic extracts of peanut hulls on free radicals and active oxygen species. J. Agric. Food Chem. 42: 629-632.