PHYTOCHEMICAL AND PHARMACOLOGICAL PROPERTIES OF ALLIUM URSINUM

Milos Krivokapic¹, Jovana Bradic², Anica Petkovic², Marina Popovic²

¹Agency of Medicines and Medical Devices of Montenegro, Podgorica, Montenegro

²University of Kragujevac, Faculty of Medical Sciences, Department of Pharmacy, Kragujevac, Serbia

FITOHEMIJSKE I FARMAKOLOŠKE KARAKTERISTIKE ALLIUM URSINUM (SREMUŠA)

Miloš Krivokapić¹, Jovana Bradic², Anica Petković², Marina Popović²

¹Agencija za lijekove i medicinska sredstva Crne Gore, Podgorica, Crna Gora

²Univerzitet u Kragujevcu, Fakultet medicinskih nauka, Katedra za farmaciju, Kragujevac, Srbija

Received / Priljen: 19. 02. 2018.

Accepted / Prihvaćen: 19. 02. 2018.

ABSTRACT

Allium ursinum L. is a perennial herbaceous plant belonging to the Alliaceae family. Several classes of bioactive compounds have been isolated from *A. ursinum* so far, among them sulfur and phenolic compounds as quantitatively the most distributed constituents, responsible for pharmaceutical effects of the plant. Small amounts of steroidal glucosides, lecithins, fatty acids and several amino acids, as well as the essential oil are also present in *A. ursinum*. This plant species is characterized by a long history of use in traditional medicine in the prevention and treatment of cardiovascular disease, as digestive stimulant, antimicrobial agent, as a remedy in respiratory problems, insomnia and fainting. Despite its widespread use for medicinal purposes since the ancient time, studies referring to its pharmacological activity are still lacking. In this review, we summarized the current knowledge related to the phytochemical and pharmacological properties of *Allium ursinum*. This study may be a starting point for future researches in this field, which would fully clarify therapeutic potential of *A. ursinum* and make it a possible candidate for medicinal product.

Keywords: *Allium ursinum*, wild garlic, ramson, biological activity, chemical composition

SAŽETAK

Sremuš je višegodišnja zeljasta biljka koja pripada porodici Alliaceae. Do sada je iz sremuša izolovano nekoliko klasa biološki aktivnih jedinjenja, među kojima su fenolna i jedinjenja sumporna kvantitativno najzastupljenija i odgovorna za farmakološke efekte ove biljne vrste. Male količine steroidnih glikozida, lecitina, masnih kiselina i aminokiselina, kao i etarskog ulja su takođe prisutna u sremušu. Ova biljna vrsta se karakteriše dugom istorijom upotrebe u tradicionalnoj medicini u prevenciji i lečenju kardiovaskularnih bolesti, kao lek kod respiratornih tegoba, nesаницe i nesvestice. I pored njene široke primene u medicinske svrhe od davnih vremena, i dalje nedostaju istraživanja koja se odnose na njenu farmakološku aktivnost. U ovom preglednom radu sumirana su trenutna saznanja vezana za fitohemijske i farmakološke karakteristike sremuša. Ova studija može biti osnova za dalja istraživanja u ovoj oblasti, koja bi u potpunosti razjasnila terapijski potencijal sremuša i učinila ga mogućim kandidatom za medicinski proizvod.

Ključne reči: Sremuš, divlji luk, medveđi luk, biološka aktivnost, hemijski sastav

INTRODUCTION

Allium ursinum L. (synonyms: wild garlic, ramson or bear garlic) is a perennial plant species which grows in fens and river woods of Europe and Asia. However it isn't distributed in different areas in Russia, but it can occur in Mediterranean region (1, 2). The Latin species name "ursinum" has been derived from "ursus" (bear) since there is a belief that the first thing bears eat after awakening from winter hibernation to remove toxins is exactly this herb (3). *A. ursinum* L. has been used for centuries in traditional

medicine both as prophylactic and therapeutic agents. In the past two decades consumption of this plant species as a dietary supplement and food has gained attention of modern medicine due to its various health benefits (3, 4). Published papers referring to its chemical constituents and protective effects on health place his positive activity even above *Allium sativum* (garlic), the most famous plant from Alliaceae family (5). Numerous biologically active compounds are presented in this herb such as: sulphur

UDK: 616.72-002-053.2
Ser J Exp Clin Res 2021; 22 (4): 357-362
DOI: 10.2478/sjcr-2018-0003

Corresponding author:

Assistant Professor Marina Tomovic, Ph.D.
Department of Pharmacy, Faculty of Medical Sciences, University of Kragujevac,
Svetozara Markovica 69, 34 000 Kragujevac, Serbia
Phone: +381 64 14 14 910, Fax: +381 34 306800/ext 112;
E-mail: marinapop@gmail.com

compounds, phenolics, steroidal glycosides etc., however sulphur compounds mostly contribute to its pharmacological activity (3, 6, 7). Despite the history of traditional applications of *Allium* species as both condiments and phytopharmaceuticals, researches based on its composition and pharmacological activity are still lacking. The aim of our study was to summarize the current knowledge referring to the chemical constituents and biological activity of *A. ursinum* and to highlight its therapeutic potential that has been recognized in traditional medicine from ancient times.

Description

There are two subspecies of *A. ursinum* which differs in pedicel morphology: *A. ursinum* ssp. *ursinum* and *A. ursinum* ssp. *Ucrainicum*. First one is distributed in western and middle Europe, while the second one is distributed in eastern and southern Europe (8-10). Both of them grow up to the height of 50 cm in deciduous woodlands with nutrient-rich and moist soils. It is characterized by elongated narrow bulbs (1.5-6 cm in size), dark green leaves with a distinct garlic-like scent (up to 50 cm long) and white flowers (11, 12). The whole herb is edible and curative. Active growth of *A. Ursinum* begins in late February and early March, when there is enough of light. Afterwards the leaves are collected on April and May, while the blooming period lasts from April until the first half of the May (3, 13). Restricting factors for their distribution are the presence of aluminum in high concentration in soil (14).

Chemical constituents

Previously conducted studies revealed the presence of sulfur and phenolic compounds, steroidal glycosides etc in *A. ursinum* (3, 6, 7).

Sulfur compounds

The most important chemical constituents present in *A. ursinum* that has been considered to mostly contribute to therapeutic benefits of the plant are sulfur compounds (6). Characteristic smell and taste of this plant derives from alliin and γ -glutamylcysteines, representatives of a special class of pharmacologically active organo-sulfuric compounds *S*-alk(en)yl-L-cysteine-sulfoxides. Plant disruption leads to a hydrolysis of those non-volatile secondary metabolites and formation of volatile alk(en)yl-thiosulfinates, such as allicin and lipid-soluble allyl sulfur compounds such as diallyl disulfide (DADS) and diallyl trisulfide (DATS) (15, 16). Allicin and methyl-allyl- or dimethyl thiosulfinates are the major thiosulfinates that can be detected after hydrolysis of cysteine sulfoxides. Nevertheless thiosulfinates are unstable and decomposes rapidly to (poly) sulfides, dithiins, ajoenes etc (11, 17).

The presence of methiin ((+)-*S*-methyl-L-cysteine-sulfoxide), alliin ((+)-*S*-2-propenyl-L-cysteine-sulfoxide),

isoallin ((+)-*S*-(1-propenyl)-L-cysteine-sulfoxide), propiin ((+)-*S*-propyl-L-cysteine-sulfoxide) and ethiin (*S*-ethyl-cysteine-sulfoxide) has been proved in wild garlic. Since methiin and alliin has been found in the highest quantities *A. ursinum* is considered to belong to a methiin/alliin-type *Allium* species (3, 18).

Time of harvest affects the amount of cysteine-sulfoxides in this herb, thus suggesting that the highest concentration would be achieved if it was collected in March and April (12, 19). Sulfur-containing compounds, with the highest amount of allyl polysulfides, have also been identified in the essential oil of *A. ursinum* (12, 20-22).

Phenolic compounds

The presence of phenolic compounds, predominantly kaempferol derivatives, has also been proved in *A. ursinum*. Phytochemical investigation of ethanol extract of wild garlic leaves has led to the isolation of : kaempferol 3-O-beta-neohesperidoside-7-O-[2-O-(trans-p-coumaroyl)]-beta-D-glucopyranoside, kaempferol 3-O-beta-neohesperidoside-7-O-[2-O-(trans-feruloyl)]-beta-D glucopyranoside, kaempferol 3-O-beta-neohesperidoside-7-O-[2-O-(trans-p-coumaroyl)]-3-O-beta-D-glucopyranosyl]-beta-D-glucopyranoside, kaempferol 3-O-beta-glucopyranoside and kaempferol 3-O-beta-neohesperidoside (23). Furthermore, extract prepared from dry leaves of *A. ursinum* (fresh leaves were freeze dried) contained 3-O- β -D-glucopyranoside, 3-O- β -D-glucopyranosyl-7-O- β -D-glucopyranoside, 3-O- α -L-rhamnopyranosyl-(1 $\rightarrow$ 2)-[3-acetyl]- β -D-glucopyranoside and 3-O- α -L-rhamnopyranosyl-(1 $\rightarrow$ 2)-[6-acetyl]- β -D-glucopyranoside in addition to several of the previously mentioned compounds (24). Another study has reported that fresh flowers of wild garlic contain the following constituents: thymidine, adenosine, astragalin (kaempferol-3-O-beta-D-glucopyranoside, kaempferol-3-O-beta-D-glucopyranosyl-7-O-beta-D-glucopyranoside, kaempferol-3-O-beta-D-neohesperoside and kaempferol-3-O-beta-D-neohesperoside-7-O-beta-D-glucopyranoside (21).

It has been confirmed that the yield of phenolic compounds depends on extraction method, extraction solvent and part of the plant used for extraction. A previously conducted research indicate that total polyphenol content in the leaf extract was higher when the extract was prepared by maceration with 70 % ethanol in comparison with the ultrasound-assisted extraction (25). Qualitative differences in the phenolic fractions between acidified methanol extracts of green and yellow leaves, stalks, and seeds of wild garlic exist, suggesting that the content of the kaempferol derivatives and compounds acylated with p-coumaric acid were the highest in yellow leaves followed by green leaves. Generally viewed, seeds contained the minimal amount of phenolic compounds, however more non-acetylated in comparison to acetylated flavonoid glycosides with p-coumaric acid compounds were found in stalks and seeds compared to leaves (26). Additionally other group of authors has revealed that total free phenolics content in bulbs

was lower than in leaves, however the amount of bound phenolics were similar (27). The highest content of gallic acid was found in 96% methanol extract of *A. ursinum*, followed by 80% methanol extract, while the lowest was noticed in 96 % ethanol extract (28). Furthermore the ecotypes of *A. ursinum* differ in chemical composition (29). The possible discrepancy in the amount of phenolics found by different authors may be the consequence of different methods used for quantification.

Steroidal glycosides

Phytochemical researches have reported the common presence of steroidal glycosides in *A. ursinum*. Diosgenin 3-O- α -l-rhamnopyranosyl-(1 $\rightarrow$ 4)- α -l-rhamnopyranosyl-(1 $\rightarrow$ 4)-[α -l-rhamnopyranosyl-(1 $\rightarrow$ 2)]- β -d-glucopyranoside and (25*R*)-spirost-5,25(27)-dien-3 β -ol 3-O- α -l-rhamnopyranosyl-(1 $\rightarrow$ 4)- α -l-rhamnopyranosyl-(1 $\rightarrow$ 4)-[α -l-rhamnopyranosyl-(1 $\rightarrow$ 2)]- β -d-glucopyranoside has been isolated from the bulbs of wild garlic. Furthermore, a pregnane glycoside such as 3-hydroxy-pregna-5,16-dien-20-on 3-O- α -l-rhamnopyranosyl-(1 $\rightarrow$ 4)- α -l-rhamnopyranosyl-(1 $\rightarrow$ 4)-[α -l-rhamnopyranosyl-(1 $\rightarrow$ 2)]- β -d-glucopyranoside were found in the underground parts of wild garlic (30).

Other compounds

The major *predominant* biologically active substances in *A. ursinum* responsible for the effects of *A. ursinum* on maintaining good health are undoubtedly sulfur and phenolic compounds. However there are other chemical constituents presented in this herb: lectins, polysaccharides, fatty acids (palmitic, linoleic, oleic, palmitoleic, stearic, α -linolenic, and myristic acid), many amino acids (asparagine, glutamine, aspartic acid, glutamic acid, arginine, alanine, glycine, threonine), pigments etc (3, 31, 32).

A. ursinum usage

There is an evidence that *A. ursinum* has been extensively used worldwide in the treatment of many disease and conditions even since the Mesolithic period (33). All parts of the plant are edible either raw or cooked and becoming increasingly popular in local cuisines of Europe. Leaves may be used as a salad, spice, boiled as a vegetable. Therapeutic potential of *A. ursinum* is attributed to leaves, herb and bulbs, which are at the same time the most commonly used in traditional medicine (3, 26).

Pharmacological effects of *A. ursinum*

The main application of wild garlic is in the prevention and treatment of cardiovascular diseases and as antimicrobial agent.

Effects of A. ursinum on cardiovascular diseases

A. ursinum and its preparations play one of the most important role in prevention and treatment of cardiovascular diseases. The relevant literature showed that consumption of ramson significantly decreased blood pressure, serum cholesterol and triglyceride. Furthermore, this wild plant inhibits platelet aggregation due to the presence of flavonoids and prevents progression of atherosclerosis. Experimental as well as clinical studies demonstrated these favorable cardiovascular effects when various preparation of *A. ursinum* was used (2, 3, 27).

Groundbreaking in vitro investigation conducted in 1992 brought novel information about potential effect of wild garlic aqueous extract on blood pressure. This group of authors showed that leaf water extract administered in concentration of 0.3 mg/kg inhibited angiotensin 1-converting enzyme (ACE) almost two times higher than garlic leaf extract. Assumption for ACE inhibition may be due to gamma-glutamyl peptides which are presented in wild garlic two times more than in cultivated garlic (34). One year later Rietz and co-workers examined plasma ACE activity and size of ischemic zone and ischemia/reperfusion induced arrhythmias. In wild garlic group ACE activity was significantly reduced in comparison to control group. In this study rats were on standard diet with 2 % of pulverized *A. ursinum* leaves for 8 weeks (35). Hypertensive effect of *A. ursinum* was noticed in experiments conducted on Spontaneously Hypertensive Rats. In this investigation rats were divided into following groups: control group fed with standard diet, group fed with diet containing 1% w/w wild garlic and group fed with diet containing 1% w/w garlic for 45 days when SBP were measured. Reduction of final mean systolic blood pressure (SBP) in wild garlic group was 173 ± 0.7 mmHg in comparison to control group 189 ± 1.2 mmHg. Despite the fact that wild garlic was used in lower concentration 0,1% w/w, SBP was significantly lower compared to rats which consumed garlic in the same concentration (5). It has been suggested that hypotensive effect of wild garlic may be due to activation of nitric oxide system and decreased activation of RAS (36).

It has been reported that *A. ursinum* essential oil increased fluidity of deep layers of the artificial liposome membrane. Also they claimed that ramson essential oil may be involved in the regulation of membrane functions in hypertension, as well as an antioxidant (37). In addition one group of researchers conducted investigation on monocrotaline (MCT) induced pulmonary arterial hypertension rat model by comparing 2% wild garlic liophyllisate-supplementation to 25 mg/kg sildenafil orally administered for 8 weeks. Findings from this study suggest beneficial effect of wild garlic demonstrated by echocardiographic and isolated heart functions, or histological analyses (38).

As mentioned above, allicin (allyl 2-propenethiosulfinate or diallyl thiosulfinate) belongs to thiosulfates, main compounds of *A. ursinum* produced by influence of allinase on allin. Some researchers described hypotensive ef-

fect of allicin in Spontaneously Hypertensive Rats as well as in CKD chronic kidney disease rat model. Allicin has been proven to abolish oxidative stress by preventing the generation of free radicals and exert cardioprotective effects likely through downregulation of angiotensin II receptor type 1 (AT1R) and Keap1 expression (39).

Antimicrobial activity

Numerous number of studies support the fact that *A. ursinum* may be used both internally and externally as an antimicrobial agent. Extracts prepared from fresh flowers and leaves exert antifungal and antibacterial activity, predominantly oriented on Gram (+) and less on Gram (-) bacteria (40, 41). Antimicrobial potential of this plant is attributed to sulfide compounds, especially allicin with proven antifungal effect (42).

Flowers and leaves of *A. ursinum* possess different antifungal potential, thus suggesting that flower extract is more *efficient* antifungal agent (41). Additionally, antibacterial, antifungal and antiparasitic activity of bulb juice and water extract from bulbs of *A. ursinum* has also been revealed (3, 43, 44).

Antibacterial and antifungal activity of the plant extract depends on the extraction solvent, thus suggesting that higher effect is expected from methanol compared to water extract. Methanol extract has been shown to exhibit antimicrobial activity against both Gram (+) and Gram (-) bacteria including: *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Proteus mirabilis*, *Salmonella enteritidis* and fungi such as: *Cladosporium* sp., *Aspergillus niger*, *Rhizopus nigricans*, *Geotrichum candidum*, *Penicillium expansum*, *Candida lipolytica*, *Mycoderma*, *Saccharomyces fibuligera*. On the other hand water extract was efficient only against *B. Subtilis*. (3).

Other pharmacological effects

A. ursinum is efficient in treatment of respiratory tract problems (cold, fever, bronchitis), as digestive stimulant, vulnerary antidiarrheal, antiphlogistic, antimicrobial agent (3, 45). Furthermore, its effect in removal of toxic substances from the organism has been also recognized since the ancient time (24) Researches suggested that *A. ursinum* may inhibit the activity of 5-lipoxygenase and cyclooxygenase (46). The chloroform extract from flower stems showed the most promising cytotoxic effect against murine cancer cell lines melanoma B16 and sarcoma XC (Trypan Blue Exclusion Test of Cell Viability) (47).

This plant possess antioxidant activity due to the presence of phenolic compounds and high antioxidant enzymes activities, including catalase, glutathione peroxidase and superoxide dismutase. In that sense, consummation of *A. ursinum* protects cell proteins and membranes against oxidation and consequential damage (2, 32, 45, 48).

CONCLUSION

The results of the mentioned studies clearly demonstrate that wild garlic exert myriad beneficial effects mainly attributed to sulfur compounds, thus absolutely justifying its widespread traditional use. However further studies in this field are necessary in order to fully clarify its therapeutic potential and make it become a source for pharmaceutical exploitation.

ACKNOWLEDGMENT

The authors would like to express gratitude to the Faculty of Medical Sciences, University of Kragujevac for Grant No. JP 06/17.

CONFLICT OF INTEREST

The authors declare there is no actual conflict of interest.

REFERENCES

1. Oborny B, Botta-Dukát Z, Rudolf et al. (2011). Population ecology of *Allium ursinum*, a space-monopolizing clonal plant. *Acta Botanica Hungarica*, 53, 371-388.
2. Pavlović DR, Veljković M, Stojanović NM et al. (2017). Influence of different wild-garlic (*Allium ursinum*) extracts on the gastrointestinal system: spasmolytic, antimicrobial and antioxidant properties. *J Pharm Pharmacol*, 69, 1208-1218.
3. Sobolewska D, Podolak I, Makowska-Wąs J. (2015). *Allium ursinum*: botanical, phytochemical and pharmacological overview. *Phytochem Rev*, 14, 81-97.
4. Leporatti ML, Ivancheva S. (2003). Preliminary comparative analysis of medicinal plants used in the traditional medicine of Bulgaria and Italy. *J Ethnopharmacol*, 87, 123-42.
5. Preuss HG, Clouatre D, Mohamadi A et al. (2001). Wild garlic has a greater effect than regular garlic on blood pressure and blood chemistries of rats. *Int Urol Nephrol*, 32, 525-30.
6. Carotenuto A, De Feo V, Fattorusso E et al. (1996). The flavonoids of *Allium ursinum*. *Phytochemistry*, 41, 531-6.
7. Xu XY, Song GQ, Yu YQ et al. (2013). Apoptosis and G2/M arrest induced by *Allium ursinum* (ramson) watery extract in an AGS gastric cancer cell line. *Oncotargets Ther*, 6, 779-83.
8. Karpaviciene B. (2006). Distribution of *Allium ursinum* L. in Lithuania. *Acta Biol Univ Daugavp*, 6, 117-122.
9. Rola K. (2012). Taxonomy and distribution of *Allium ursinum* (Liliaceae) in Poland and adjacent countries. *Biologia*, 67, 1080-1087.

10. Oborny B, Botta-Dukat Z, Rudolf K et al. (2011). Population ecology of *Allium ursinum*, a space-monopolizing clonal plant. *Acta Bot Hung.* 53, 371–388.
11. Sendl A. (1995). *Allium sativum* and *Allium ursinum*: Part 1 Chemistry, analysis, history, botany. *Phytomedicine.* 1, 323–39.
12. Schmitt B, Schulz H, Storsberg J. (2005). Chemical characterization of *Allium ursinum* L. depending on harvesting time. *J. Agric. Food Chem.* 53, 7288–7294.
13. Jandl R, Kopeszki H, Glatzel G. (1997). Effect of dense *Allium ursinum* (L.) ground cover on nutrient dynamics and mesofauna of a *Fagus sylvatica* (L.) woodland. *Plant Soil.* 189, 245–255.
14. Andersson ME. (1993). Aluminium toxicity as a factor limiting the distribution of *Allium ursinum* L. *Ann Bot.* 72, 607–611.
15. Boscher J, Auger J, Mandon N, et al. (1995). Qualitative and quantitative comparison of volatile sulphides and flavour precursors in different organs of some wild and cultivated garlics. *Biochem Syst Ecol.* 23, 787–791.
16. Yoo KS, Pike LM. (1998). Determination of flavor precursor compound S-alk(en)yl-L-cysteine sulfoxides by an HPLC method and their distribution in *Allium* species. *Sci Hortic.* 75, 1–10.
17. Bagiu RV, Vlaicu B, Butnariu M. (2012). Chemical composition and in vitro antifungal activity screening of the *Allium ursinum* L. (Liliaceae). *Int J Mol Sci.* 13, 1426–36.
18. Kubec R, Svobodová M, Velíšek J. (2000). Distribution of S-Alk(en)ylcysteine sulfoxides in some *Allium* species. Identification Of a new flavor precursor: S-ethylcysteine sulfoxide (Ethiin). *J Agric Food Chem.* 48, 428–33.
19. Fritsch RM, Keusgen M. (2006). Occurrence and taxonomic significance of cysteine sulfoxides in the genus *Allium* L. (Alliaceae). *Phytochemistry.* 67, 1127–35.
20. Copra-Janicijevic A, Muradic S, Huseinovic S et al. (2008). Isolation of essential oils of *Allium ursinum* L. from Bosnia. *Planta Med.* 74, PI42.
21. Ivanova A, Mikhova B, Najdenski H et al. (2009). Chemical composition and antimicrobial activity of wild garlic *Allium ursinum* of Bulgarian origin. *Nat Prod Commun.* 4, 1059–62.
22. Błażewicz-Woźniak M, Michowska A. (2011). The growth, flowering and chemical composition of leaves of three ecotypes of *Allium ursinum* L. *Acta Agrobot.* 64, 171–180.
23. Carotenuto A, De Feo V, Fattorusso E et al. (1996). The flavonoids of *Allium ursinum*. *Phytochemistry.* 41, 531–6.
24. Wu H, Dushenkov S, Ho CT et al. (2009). Novel acetylated flavonoid glycosides from the leaves of *Allium ursinum*. *Food Chem.* 115, 592–595.
25. Gitin L, Dinică R, Parnavel R. (2012). The influence of extraction method on the apparent content of bioactive compounds in Romanian *Allium* spp. leaves. *Not Bot Horti Agrobot Cluj Napoca.* 40(1), 93–97.
26. Oszmiański J, Kolniak-Ostek J, Wojdyło A. (2013). Characterization and content of flavonol derivatives of *Allium ursinum* L. plant. *J Agric Food Chem.* 61, 176–84.
27. Djurdjevic L, Dinic A, Pavlovic P et al. (2004). Allelopathic potential of *Allium ursinum* L. *Biochem Syst Ecol.* 32, 533–544.
28. Condrat D, Mosoarca C, Zamfir AD et al. (2010). Qualitative and quantitative analysis of gallic acid in *Alchemilla vulgaris*, *Allium ursinum*, *Acorus calamus* and *Solidago virga-aurea* by chip-electrospray ionization mass spectrometry and high performance liquid chromatography. *Cent Eur J Chem.* 8, 530–535.
29. Błażewicz-Woźniak M, Michowska A. (2011). The growth, flowering and chemical composition of leaves of three ecotypes of *Allium ursinum* L. *Acta Agrobot.* 64, 171–180.
30. Sobolewska D, Janeczko Z, Kisiel W et al. (2006). Steroidal glycosides from the underground parts of *Allium ursinum* L. and their cytostatic and antimicrobial activity. *Acta Pol Pharm Drug Res.* 63, 219–223.
31. Smeets K, Van Damme EJ, Van Leuven F et al. (1997). Isolation, characterization and molecular cloning of a leaf-specific lectin from ramsons (*Allium ursinum* L.). *Plant Mol Biol.* 35, 531–535.
32. Štajner D, Szölloosi Varga I. (2003). An evaluation of the antioxidant abilities of *Allium* species. *Acta Biol Szeged.* 47, 103–106.
33. Kubiak-Martens L. (2002). New evidence for the use of root foods in pre-agrarian subsistence recovered from the late Mesolithic site at Halsskov, Denmark. *Veg Hist Archaeobot.* 11, 23–31.
34. Cloutre D. (1995). *European Wild Garlic. The Better Garlic.* San Francisco CA: Pax Publishing. pp. 1–38.
35. Rietz B, Isensee H, Strobach H et al. (1993). Cardio-protective actions of wild garlic (*Allium ursinum*) in ischemia and reperfusion. *Mol Cell Biochem.* 119, 143–50.
36. Mohamadi A, Jarrell ST, Shi SJ et al. (2000). Effects of wild versus cultivated garlic on blood pressure and other parameters in hypertensive rats. *Heart Dis.* 2, 3–9.
37. Godevac D, Vujisic L, Mojovic M et al. (2008). Evaluation of antioxidant capacity of *Allium ursinum* L. volatile oil and its effect on membrane fluidity. *Food Chem.* 107, 1692–1700.
38. Bombicz M, Priksz D, Varga B et al. (2017). A Novel Therapeutic Approach in the Treatment of Pulmonary Arterial Hypertension: *Allium ursinum* Liophyllisate Alleviates Symptoms Comparably to Sildenafil. *Int J Mol Sci.* 18(7), pii: E1436.
39. García-Trejo EM, Arellano-Buendía AS, Argüello-García R et al. (2016). Effects of Allicin on Hypertension and Cardiac Function in Chronic Kidney Disease. *Oxid Med Cell Longev.* 2016:3850402.
40. Mihaylova DS, Lante A, Tinello F, Krastanov AI. (2014). Study on the antioxidant and antimicrobial activities of *Allium ursinum* L. pressurised-liquid extract. *Nat Prod Res.* 28(22), 2000–5.
41. Parvu M, Parvu AE, Vlase L et al. (2011). Antifungal properties of *Allium ursinum* L. ethanol extract. *J Med Plants Res.* 5, 2041–2046.

42. Ankri S, Mirelman D. (1999). Antimicrobial properties of allicin from garlic. *Microbes Infect.* 1, 125-9.
43. Tynecka Z, Szcześniak Z, Głowniak K. (1993). The effect of various environmental conditions on the antimicrobial activity of *Allium ursinum*. *Planta Med.* 59(7 SUPPL), A701.
44. Chudzik B, Malm A, Rajtar B et al. (2010). The fresh extracts of *Allium* species as potential in vitro agents against planktonic and adherent cells of *Candida* spp. *Ann Univ Mariae Curie-Skłodowska, DDD Pharm.* 23, 73–78
45. Pejatović T, Samardžić D, Krivokapić S. (2017). Antioxidative properties of a traditional tincture and several leaf extracts of *Allium ursinum* L. (collected in Montenegro and Bosnia and Herzegovina). *JMES.* 8, 1929-34
46. Sendl A, Elbl G, Steinke B et al. (1992). Comparative pharmacological investigations of *Allium ursinum* and *Allium sativum*. *Planta Med.* 58, 1-7.
47. Sobolewska D, Galanty A, Michalik M. (2012). Preliminary evaluation of cytotoxic activity of *Allium ursinum* extracts. *Czas Aptek.* 12, 41–44.
48. Stajner D, Popović BM, Canadanović-Brunet J et al. (2008). Antioxidant and scavenger activities of *Allium ursinum*. *Fitoterapia.* 79, 303-5.