PHYTOCHEMICAL AND PHARMACOLOGICAL PROPERTIES OF ALLIUM URSINUM

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FITOHEMIJSKE I FARMAKOLOŠKE KARAKTERISTIKE *ALLIUM URSINUM* (SREMUŠA)

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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, lecitins, 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, nesanice 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 consummation 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

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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

Previosuly 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-b eta-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 → 2)-[3-acetyl]-β-d-glucopyranoside and 3-*O*-α-l-rhamnopyranosyl-(1 → 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-cpumaric 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 phenoics 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→4)-α-l-rhamnopyranosyl-(1→4)-[α-l-rhamnopyranosyl-(1→2)]-β-d-glucopyranoside and (25*R*)-spirost-5,25(27)-dien-3β-ol 3-*O*-α-l-rhamnopyranosyl-(1→4)-α-l-rhamnopyranosyl-(1→4)-[α-l-rhamnopyranosyl-(1→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→4)-α-l-rhamnopyranosyl-(1→4)-[α-l-rhamnopyranosyl-(1→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 liophylisate-supplementation to 25 mg/kg sildenafil orally administred 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 tiosulfinates, 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 exhibits 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*, *Saccharomycopsis 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.

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CONFLICT OF INTEREST

The authors declare there is no actual conflict of interest.

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