

ANTIBACTERIAL ACTIVITY OF *Allium ursinum* L.

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Abstract: *Allium ursinum* L. is popularly known as sremush, krijemush, wild or bear onion. It is used in the diet as a vegetable (salad and spice) and in human medicine as an aid. It is important for its nutritional and medicinal value. Since it is mostly used in the diet seasonally at the time of arrival and in a fresh state, as a salad, it can be a significant source of certain nutrients and minerals that are of great importance for human health. Resistance of bacteria to antimicrobial substances (antibiotics and chemotherapeutics) is one of the most significant problems in public health. The problem of antimicrobial resistance is related to pathogenic bacteria and zoonotic agents, but this resistance also burdens apathogenic bacteria that transmit resistance genes through the food chain. The aim of this study was to examine the antimicrobial activity of *Allium ursinum* L. on the growth of apathogenic bacteria isolated from food of animal origin and determine the type of action on the tested isolates. The plant material used in this paper is a fresh leaf of *Allium ursinum* L. harvested on the slopes of the Kruševo Brdo mountain, Kotor Varoš municipality, Republic of Srpska, Bosnia and Herzegovina. Sremush leaves were chopped, sunflower oil was added and a thick mass of sremush was diluted with distilled water in a ratio of sremush:water 80:20%. The agar diffusion method was used to examine the antibacterial activity. Antibacterial activity was tested on 10 bacterial coagulase strains of negative staphylococci isolated from food of animal origin, from the collection of the Laboratory for Microbiology of food, feed, and water of the Public Institution Veterinary Institute of Republic of Srpska "Dr Vaso Butozan" Banja Luka. The results of the analysis indicate good inhibitory properties of *Allium ursinum* L. on 8 out of 10 tested apathogenic bacteria with an inhibition zone from 9.67 mm to 45.00 mm. Bactericidal activity was confirmed in four isolates in all three replicates, and bacteriostatic in two isolates. Given that the contribution of apathogenic micropores from food of animal origin in the transmission of antimicrobial resistance through the food chain is not fully known, the results indicate that *Allium ursinum* L. has good antibacterial properties, future studies should focus on more bacteria.

Keywords: *Allium ursinum* L., apathogenic bacteria, antibacterial activity, resistance

Introduction

Infections caused by various microorganisms require adequate and timely therapy, so as not to lead to more serious organic exacerbations. Therapy, in today's practice, usually involves the use of antibiotics. Antibiotics are effective only in

bacterial infections, and their excessive use in the most significant factor leads to the development of resistant bacterial strains. In addition to resistance, the problem of excessive use of antibiotics is also reflected in the disruption of normal bacterial flora and the body's immune system. Therefore, it is necessary to popularize natural medicinal substances as an alternative approach to the prevention and treatment of various types of infections. The reason for this is their proven pharmacological action, minimal or no side effects, and the reduction of resistance.

Allium ursinum L. – wild onion, forest onion, or beat onion is a medicinal plant from the Amaryllidaceae family. Wildflower is an edible plant related to onion and garlic. It belongs to the *Asparagales* order, the Amaryllidaceae family, the *Allioideae* subfamily, and the *Allium* genus. The species name "ursinum" is of Latin origin, derived from the word "Ursus" (bear) [1]. The name of the bear's onion plant is associated with a folk tale according to which bears, after waking up from their winter sleep, consume this plant in order to get rid of toxins from the body and restore energy [2].

In traditional medicine, *Allium ursinum* L. has been used since ancient times, however, studies of its composition and pharmacological action are more recent. In addition to water, proteins, carbohydrates, fats, organic acids, fiber, and ash, onion contains vitamin A, β carotene, vitamins B1, B2, and B4, niacin, folic acid, vitamin C, potassium, calcium, sodium, silicon, magnesium, sulfur, phosphorus, chlorine, manganese, iron, aluminum, iodine, copper and zinc [2, 3]. Thanks to its rich chemical composition, in addition to cooking, this plant can also be used for medical purposes as an antimicrobial agent, for body detoxification, and in the prevention and treatment of cardiovascular diseases [4]. Numerous *in vitro* and *in vivo* studies have confirmed a wide range of pharmacological activities, including anti-inflammatory, antimicrobial, anticancer, cardioprotective and neuroprotective, antidiabetic, antiallergic, and antiasthmatic effects of *Allium ursinum* L. [2, 4, 5, 6, 7, 8]. Bear onion is one of the ingredients in products that have been used therapeutically for a long period of time as detoxification and antiatherogenic drugs at the University Hospital in Bucharest [9].

The aim of this work is to examine the antimicrobial activity of fresh spring onion leaves extract on the growth of apathogenic bacteria isolated from food of animal origin and determine its type of action.

Material and method

Material

The plant material used in this work is a fresh leaves of *Allium ursinum* L. harvested on the slopes of the Kruševo Brdo mountain, municipality of Kotor Varoš, Republic of Srpska, Bosnia and Herzegovina. The leaves of spring onion were chopped in a blender with the addition of sunflower oil and salt, and for the purposes of the experiment, the thick mass of spring onion was diluted with distilled water in a ratio of spring onion: water 80:20%.

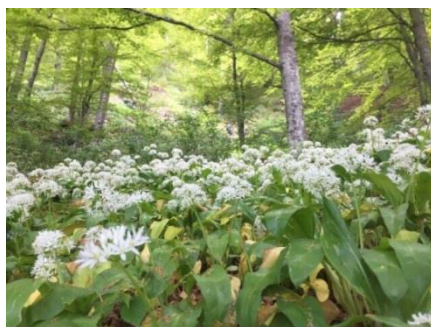


Figure 1. *Allium ursinum* L. (Photo: Kalaba V.)

Test method

To test the antibacterial activity of *Allium ursinum* L., the agar diffusion method [10] was used, by placing cylinders with a diameter of 9 mm on a solid sterile substrate (Müller-Hinton agar - MHA) into which the prepared plant material was instilled. 100 µl of distilled water was added to the cylinders as a control. In order to speed up the diffusion of the onion into the agar, the plates were left for 30 minutes at a temperature of 4°C, and incubated for 18 hours in aerobic conditions at 37°C±1°C. The diameter of the zone of inhibition was measured.

Test microorganisms

The antibacterial activity of *Allium ursinum* L. was tested on 10 bacterial strains of coagulase-negative staphylococci (KNS isolated from food of animal origin) from the collection of the Laboratory for Microbiology of Food, Animal Feed, and Water, Veterinary Institute of the Republic of Srpska "Dr Vaso Butozan" Banja Luka, Republic of Srpska. Cultures of the test microorganism were transferred under aseptic conditions, using a microbiological sieve, to a test tube with sterile saline solution and suspended by intensive shaking on a vortex. The density of the suspension was adjusted using a densitometer to correspond to the value of 0.5 McFarland standard. The results for the examined parameters were obtained by measuring in three repetitions and expressed as mean ± standard deviation.

Action type

In order to determine whether the bear figure had a bactericidal or bacteriostatic effect, the type of action was also determined. A small piece of agar was taken from the zone of inhibition and added to the nutrient broth. Incubation was carried out for 24 hours at 37°C. If the broth becomes cloudy after incubation, it is considered that the effect of the oil is bacteriostatic, that is, if the broth remains clear, the effect of the oil is bactericidal.

Results and discussion

The results of testing the antimicrobial effect of *Allium ursinum* L. on apathogenic bacteria isolated from food of animal origin are shown in Table 1.

Table 1. Antimicrobial effect of *Allium ursinum* L. on KNS*

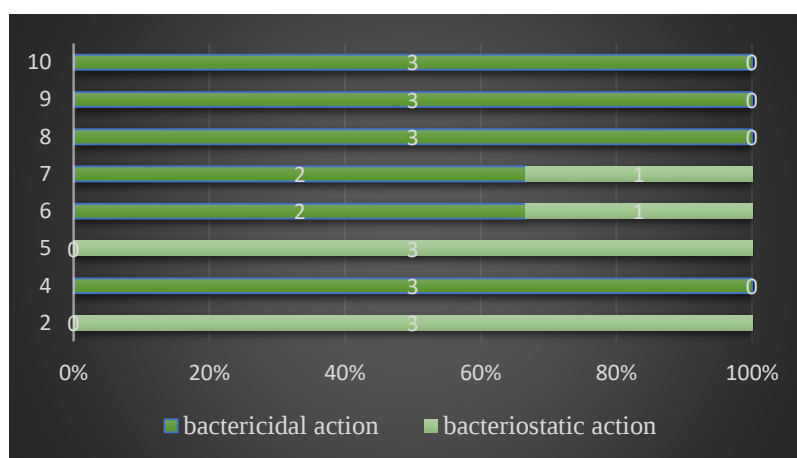
KNS*	Zones of inhibition (mm)	KNS*	Zones of inhibition (mm)
	Bear's onion: water (80:20%)		Bear's onion: water (80:20%)
1	0	6	26.67±4.714
2	11.67±0.471	7	18.00±1.633
3	0	8	24.33±1.247
4	20.67±0.471	9	45.00±0.00
5	9.67±0.471	10	42.33±2.055

*coagulase-negative staphylococcus

Allium ursinum L. had an inhibitory effect on 8 out of 10 KNS isolates tested. The zones of inhibition ranged from 9.67 mm to 45.00 mm. *Allium ursinum* L. did not have an inhibitory effect on the two isolates in any repetition. The agar-diffusion method, which tests the antibacterial activity on selected bacterial strains, is evaluated according to the size of the growth inhibition zone, which depends not only on the antibacterial activity but also on the rate of diffusion of the antibacterial components through the agar. The antibacterial potential of the genus *Allium* is generally related to the content of sulfur components, however, other compounds may also contribute to the antibacterial effect of the onion [2, 11]. Also, the results and interpretation of the results of the disc-diffusion method can be influenced by numerous factors, including the bacterial species and strain, growth conditions (time, temperature), and pH of the substrate [12].

The results of this work are in agreement with the results of other researchers who studied the antimicrobial activity of *Allium ursinum* L. The inhibitory effect of water and methanol extracts from the leaves of the bear onion plant was proven on the following bacteria: *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Proteus mirabilis*, *Salmonella enteritidis* as well as on the mold *Cladosporium spp.*, *Aspergillus niger*, *Rhizopus nigricans*, *Geotrichum candidum*, *Penicillium expansum*, *Candida lipolytica*, *Mycoderma*, and *Saccharomycopsis fibuligera* [2, 13]. Analyzing the ethanol extract of the flowers and leaves of the bear's onion, it was determined that the flower extract has a better effect on *Aspergillus niger*, *Botrytis cinerea*, *Botrytis paeonies*, *Fusarium oxysporum*, *Penicillium gladioli*, and *Sclerotinia sclerotiorum*, and the reason for the stronger effect may be the higher content of allicin in the flowers than in the leaves of the bear's onion [14]. Tests of the antimicrobial activity of different extracts (acetone, chloroform, n-butanol, and water) obtained from fresh flowers and fresh leaves of wild onion showed that the acetone extract of flowers and leaves, as well as the chloroform extract of the leaves, have an inhibitory effect on *Staphylococcus aureus* while the chloroform extract of the leaves showed an inhibitory effect on *Candida albicans* [15].

The antibacterial activity of certain plant species can vary from partial to complete inhibition of bacterial growth. Graph 1 shows the type of action of *Allium ursinum* L.



Graph 1. Bactericidal and bacteriostatic effect of *Allium ursinum* L.

Allium ursinum L. had a bactericidal effect in all three repetitions on KNS isolates marked with numbers 4, 8, 9, and 10, and on isolates numbers 6 and 7 in two repetitions. The bacteriostatic effect of *Allium ursinum* L. in all three repetitions was confirmed in isolates number 2 and 5 and in isolates number 6 and 7 in one repetition each.

Although *Allium ursinum* L. (bear's onion) has been known for centuries as a substitute for *Allium sativum* L. (garlic), its study, that is, pharmacological studies, started quite late, only 20 years ago. The presence of chemical compounds with high therapeutic potential makes this plant species a candidate for an effective medicinal product. Problems that may arise are related to the production of uniform plant material because the composition of *Allium ursinum* L. is very sensitive to changes in growing conditions, which could hinder planned production and standardization.

Conclusion

Allium ursinum L., a wild species of the genus *Allium* L., has been very popular since ancient times. It is widely used in alternative medicine, has antimicrobial properties, and has definitely been recognized as a valuable herb in recent years.

In this paper, the antibacterial activity of scallion leaves originating from the territory of the municipality of Kotor Varoš, Republic of Srpska was investigated. The results proved the inhibitory action of 8 out of 10 tested apathogenic bacteria (KNS) with an inhibition zone from 9.67 mm to 45.00 mm. Bactericidal activity was confirmed in four isolates in all three repetitions, and bacteriostatic in two isolates in all three repetitions. The obtained results show good antibacterial properties of the leaves of *Allium ursinum* L., so future studies should be focused on examining a larger number of different bacteria.

Natural substances with antimicrobial activity, such as *Allium ursinum* L. are very effective in the treatment, and especially the prevention, of various infections. For this reason, their use should be preferred, all with the aim of rationalizing antibiotic therapy and reducing resistance to different antibiotics.

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ANTIBAKTERIJSKA AKTIVNOST *Allium ursinum* L.

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Sažetak: *Allium ursinum* L. je u narodu poznatiji kao sremuš, crijemuš, divlji ili medvjedi luk. U ishrani se koristi kao povrće (salata i začín), a u humanoj medicini kao pomoćno sredstvo. Značajan je zbog svoje nutritivne i ljekovite vrijednosti. Pošto se u ishrani najvećim dijelom koristi sezonski u vrijeme prispjeća i u svježem stanju, kao salata, može da bude značajan izvor pojedinih hranljivih materija, mineralnih materija, koje su od velikog značaja za zdravlje ljudi. Otpornost bakterija na antimikrobne supstance (antibiotike i hemoterapeutike) jedan je od najznačajnijih problema u javnom zdravstvu. Problem antimikrobne rezistencije se veže uz patogene bakterije i uzročnike zoonoza, ali ta otpornost opterećuje i apatogene bakterije koje prenose gene rezistencije kroz prehrambeni lanac. Cilj ovoga rada je da se ispita antimikrobna aktivnost *Allium ursinum* L. na rast apatogenih bakterija izolovanih iz hrane životinjskog porijekla i da se odredi tip djelovanja na ispitivane izolate. Biljni material korišten u ovom radu je svježi list *Allium ursinum* L. ubran na padinama planine Kruševo Brdo, opština Kotor Varoš, Republika Srpska, Bosna i Hercegovina. Listovi sremuša su usitnjeni, dodato je suncokretovo ulje, te je gusta masa sremuša razrijeđena destilovanom vodom u omjeru sremuš:voda 80:20%. Za ispitivanje antibakterijske aktivnosti korišćena je agar difuziona metoda. Antibakterijska aktivnost je ispitana na 10 bakterijskih sojeva koagulaza negativnih stafilokoka izolovanih iz hrane životinjskog porijekla, iz kolekcije Laboratorije za mikrobiologiju hrane, hrane za životinje i vodu Javne ustanove Veterinarski institut Republike Srpske "Dr Vaso Butozan" Banja Luka. Rezultati analiza ukazuju na dobra inhibitorna svojstva *Allium ursinum* L. na 8 od 10 testiranih apatogenih bakterija sa zonom inhibicije od 9,67mm do 45,00mm. Baktericidno djelovanje je potvrđeno kod četiri izolata u sva tri ponavljanja, a bakteriostatsko kod dva izolata. Imajući u vidu da doprinos apatogene mikrofore iz hrane životinjskog porijekla u prenosu antimikrobne rezistencije kroz prehrambeni lanac nije u potpunosti poznat, te da dobijeni rezultati ukazuju da list *Allium ursinum* L. ima dobra antibakterijska svojstva, buduće studije je potrebno usmjeriti ka ispitivanju većeg broja bakterija.

Ključne riječi: *Allium ursinum* L., apatogene bakterije, antibakterijska aktivnost, rezistencija