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ANTIBACTERIAL ACTIVITY OF ALLIUM URSINUM TINCTURE ON PATHOGENS ISOLATED FROM FOOD

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Abstract*. Allium ursinum - wild garlic is a plant used not only in culinary applications but also valued for its medicinal properties. Due to its chemical composition, it can be used as an antimicrobial agent, for detoxification, and in the prevention and treatment of cardiovascular diseases. The leaves of this plant contain sulfur-based compounds, phenols, vitamin C, chlorophyll, and carotenoids. Allium ursinum L is a rich source of iron and adenosine and exhibits potent bactericidal, antiparasitic, and antimicrobial effects. As a natural antibiotic containing high levels of vitamin C, it is beneficial in therapies for atherosclerosis and scurvy. Additionally, due to its high sulfur content, it possesses the ability to protect cells from infection and inflammation. In recent years, the antibacterial activity of Allium ursinum extract has been studied. However, reports on the antibacterial activity of Allium ursinum tincture prepared in a traditional manner in the Republic of Srpska against certain food-isolated pathogens are still limited. The aim of this study was to assess the antibacterial activity of traditionally prepared Allium ursinum tp.entericaincture against two isolates of Salmonella enterica subsp.enterica one isolate of Salmonella spp., and three isolates of Staphylococcus aureus, pathogens commonly transmitted through food. The antibacterial activity against selected food-isolated pathogens was evaluated using the disk diffusion method. The study results showed that the traditionally prepared Allium ursinum tincture exhibits certain antibacterial properties against the tested pathogens.*

Keywords*: Allium ursinum, antimicrobial activity, pathogenic bacteria*

Introduction

Every day, we are bombarded with offers of alternative methods for treating specific ailments such as high blood pressure, elevated cholesterol levels, certain cardiovascular issues, and more. The use of medicinal herbs to create traditional preparations in alternative medicine has a long-standing tradition. However, their efficacy from a scientific perspective remains insufficiently explored.

The production of functional food has become a global trend in the food industry in recent years. There is an increasing demand for food that possesses not only nutritional but also medicinal properties. Biological, chemical, and pharmacological research results have indicated that the application of medicinal plant extracts can enhance the quality and nutritional value of food [1,2,3]. Both the food and pharmaceutical industries are actively seeking alternative preservatives that can improve the safety and quality of products. Consequently, there is a growing trend in the use of phytonutrients as substitutes for chemical additives, offering antioxidative and antibacterial properties [3,4].

"*Allium ursinum*," commonly known as bear's garlic or wild garlic, typically grows in higher mountainous regions. This plant is characterized by a strong aroma and a mildly spicy taste. The traditional use of *Allium ursinum* has been confirmed through numerous pharmacological studies. Both in vivo and in vitro research have confirmed a broad spectrum of pharmacological activities, including anti-inflammatory, antimicrobial, anticarcinogenic, cardioprotective, neuroprotective, antidiabetic, antiallergic, and anti-asthmatic effects [5,6,7,8].

Allium ursinum is esteemed in traditional medicine as an antifungal agent, utilized both internally and externally. Several scientific studies have explored the antimicrobial activity of various extracts prepared from different parts of the plant, tested in vitro against a wide range of bacterial and fungal strains. However, to our knowledge, there are no available literature sources regarding the antibacterial activities of tinctures prepared in a traditional manner under household conditions.

The aim of this study was to investigate the antibacterial properties of *Allium ursinum* tincture prepared in a traditional manner under household conditions against selected foodborne pathogens and to determine whether the extract acts bactericidally or bacteriostatic on the tested strains.

Materials and methodes

In this study, the material we used was a tincture of *Allium ursinum* prepared traditionally under household conditions. The plant material used in this study was fresh leaves of *Allium ursinum L*, gathered from the slopes of Mount Kruševo Brdo in the municipality of Kotor Varoš (Republic of Srpska). Immediately after harvesting, the bear's garlic leaves were chopped and placed in a clean, sterile jar, into which alcohol 40% concentration was poured. The prepared jar was then placed in a warm, sunlit area. After 20 days, with occasional shaking, the contents of the jar were strained into a clean, sterile jar and start to use.

For testing the antibacterial activity of the *Allium ursinum* tincture, three isolates of Gram-negative and three isolates of Gram-positive bacteria were used (Table 1). All isolates were of food origin and part of the collection of isolates from the Laboratory for Food, Feed, and Water Microbiology of the Veterinary Institute "Dr. Vaso Butozan" in Banja Luka.

Table 1. Bacterial strain isolated from food.

Isolate code	Strain
Isolate 1 G(-)	*Salmonella spp.*
Isolate 2 G(-)	*Salmonella enterica subsp.enterica*
Isolate 3 G(-)	*Salmonella enterica subsp.enterica*
Isolate 4 G(+)	*Staphylococcus aureus*
Isolate 5 G(+)	*Staphylococcus aureus*
Isolate 6 G(+)	*Staphylococcus aureus*

The test microorganism cultures were transferred into tubes containing nutrient broth aseptically using a microbiological loop and suspended by vortex (IKA VORTEX). The density of the suspension was determined using a densitometer (DENSILA METER – "Erba") and adjusted to match the value of 0.5 McFarland standard (1.5×10^8 CFU/ml).

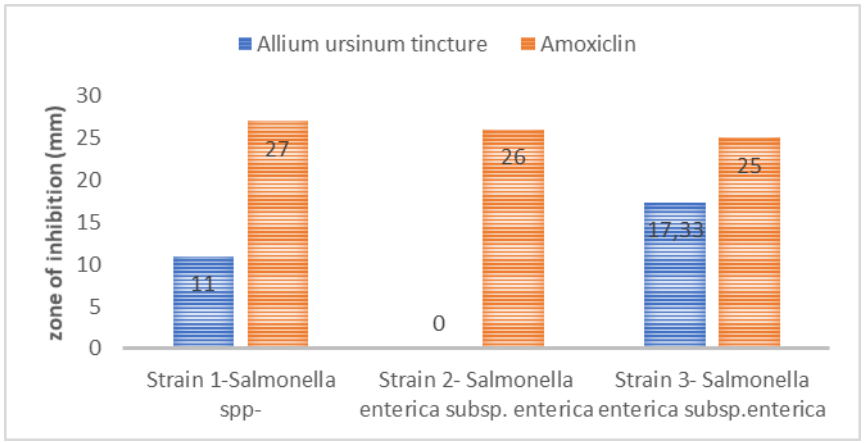
The sensitivity of the isolated strains to *Allium ursinum* tincture was conducted using the disk diffusion method [9]. Using a micropipette, 0.1 ml of the prepared culture suspension was spread onto the surface of Mueller Hinton agar plates (Conda Co, Spain). This process was repeated for each strain. Metal cylinders with a diameter of 9 mm were placed on the surface of the solidified inoculated media, and 100 µl of *Allium ursinum* tincture was pipetted into the cylinders. Amoxicillin was used as the positive control, while 40% alcohol served as the negative control. The place was incubated for 18 hours under aerobic conditions at 37°C±1°C.

Following the measurement of the inhibition zones on surfaces, the tested strains were classified according to the Clinical and Laboratory Standards Institute (CLSI) criteria [10] into three categories: sensitive, moderately sensitive, and resistant. Results for the tested parameters were obtained through measurements in triplicate and expressed as the mean value ± standard deviation.

However, since growth inhibition doesn't necessarily imply bacterial death, and the disk diffusion method cannot differentiate between bactericidal and bacteriostatic effects, to determine whether *Allium ursinum* has bactericidal or bacteriostatic activity, a small piece of agar from the inhibition zone was transferred into nutrient broth. Incubation was carried out for 24 hours at 37°C. If the broth became cloudy after incubation, it was considered that *Allium ursinum* acted bacteriostatically. Conversely, if the broth remained clear after incubation, the effect of *Allium ursinum* was considered bactericidal.

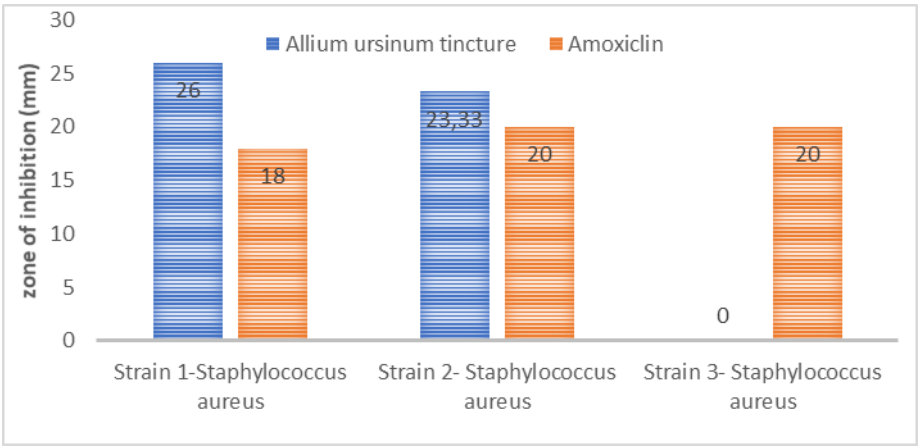
Results and discussion

The antibacterial activity of *Allium ursinum* tincture, prepared traditionally, was examined against three isolates of *Salmonella spp.* and three isolates of *Staphylococcus spp.* and the results are presented in Graphs 1 and 2.



Graph 1. Antibacterial activity of *Allium ursinum* tincture compared to Amoxicillin against *Salmonella spp.*

From the provided graph (Graph 1), we observe that two isolates of **Salmonella** showed moderate sensitivity to the antibacterial action of *Allium ursinum* tincture, with inhibition zone diameters measuring 11.66 mm for *Salmonella spp* and 17.33 mm for one isolate of *Salmonella enterica subsp.enterica*. One isolate of *S. Enteritidis* exhibited resistance to the tested tincture. *Salmonella* isolates were significantly more sensitive to amoxicillin, used as the positive control.



Graph 2. Antibacterial activity of *Allium ursinum* tincture compared to Amoxicillin on *S. aureus.*

When it comes to Gram-positive bacteria, specifically *Staphylococcus aureus*, used in this study, one strain was resistant to the tincture's action, while the other two strains were inhibited, with inhibition zone diameters measuring 23.33mm and 26 mm. As seen from the attached graph (Graph 2), the *Allium ursinum* tincture exhibited

stronger antibacterial activity against these two *S. aureus* isolates compared to amoxicillin, which was used as the positive control.

Comparing the results of *Allium ursinum* tincture prepared traditionally in this study with those of other research studies is challenging due to the lack of a standard test for interpreting the obtained results. Most studies have been conducted using microdilution methods rather than measuring the inhibition zone. Available literature describes several methods for testing antimicrobial potential, but unfortunately, their sensitivity is not consistent and comparable. There is no standardized protocol for screening antimicrobial drugs, pure substances, or plant extracts. The lack of standardized tests and criteria for interpreting bacterial sensitivity testing complicates comparing results between different researchers [11,12,13].

The disk diffusion method is a simple technique used in routine work to determine bacterial sensitivity to antimicrobial drugs, pure substances, or plant extracts. The results of the disk diffusion method depend on various factors such as temperature, bacterial incubation period, bacterial type, growth and survival rates, agar thickness, pH of the medium, time elapsed since the extracts were produced or obtained, extraction methods, and others [14,15]. Additionally, different parts of *Allium ursinum* (bulb, leaves, and flower) used for extraction (and making medicinal preparations) contain different active components that influence the antimicrobial activity of the extract.

Numerous scientific studies support the fact that *Allium ursinum* can be used as a natural antimicrobial agent. However, some studies have shown contradictory results regarding the antibacterial activity of *Allium ursinum* extracts against different Gram-positive and Gram-negative bacteria [16,17,18,19,20]. Different results in testing antibacterial activity can be explained by isolating different active compounds using different solvents during extraction, different extraction methods, the plant's origin and part, extraction temperature [21,22,23]. Błażewicz-Woźniak and Michowska [24] through comparative research on biometric characteristics and the chemical composition of leaves of three *Allium ursinum* species, found significant differences between ecotypes in terms of the quantity of selected components and the number of components in their essential oils.

Sapunjieva et al. [25] investigated the antimicrobial activity of a 70% ethanol extract of *Allium ursinum* against Gram-positive bacteria (*L. monocytogenes, S. aureus*) compared to Gram-negative bacteria (*E. coli, Salmonella enterica subsp. Enterica serovar Abony*) and reported stronger antibacterial action against the tested Gram-positive bacteria.

Pavlović et al. [26] examined the antibacterial activity of five different extracts of *Allium ursinum* (70% ethanol; 96% ethanol; distilled water; 80% aqueous methanol; absolute methanol) against five Gram-negative and two Gram-positive bacterial strains and found the most significant antibacterial activity in the 96% extract against *S. enterica* and *Staphylococcus aureus*.

Lupoae et al. [19] tested the antimicrobial activity of nine Allium ursinum extracts prepared with different solvents (water, 9% acetic acid, 70% ethanol v/v) against 10 microorganisms (pure bacterial cultures, yeasts, and fungi) isolated from food, wound secretions, throat swabs, urine, and oral mucosa. The ethanolic extract of *Allium*

ursinum exhibited a high inhibition zone against *S.aureus* and *Streptococcus pyogenes*.

The antibacterial action of the *Allium* genus is often attributed to sulfur compounds, but scientific research has shown that other compounds, such as phenols, especially flavonoids, contribute to the antimicrobial effect [27]. Flavonoids exert their antibacterial activity by interacting with microbial cells through protein complex formation involving hydrogen or hydrophobic bonds or forming covalent bonds, thereby inactivating microbial adhesion, enzymes, and transport proteins [28].

The research results support the views of researchers that it is more important to study the antimicrobial action of plant extracts than their individual components [29].

The antimicrobial activity of plant extracts and their components can vary from partial to complete inhibition of bacterial growth, where plant oil extracts exhibit bacteriostatic or bactericidal activity. To determine whether *Allium ursinum* has bactericidal or bacteriostatic activity, a small piece of agar from the inhibition zone was transferred into nutrient broth. In all three repetitions, *Allium ursinum* tincture acted bacteriostatically on *Salmonella spp*. and bactericidally in all three repetitions on one isolate of *S. Enteritidis*. The *Allium ursinum* tincture exhibited bactericidal effects on two strains of *S. aureus* in all three repetitions.

Conclusion

The antibacterial activity of *Allium ursinum* tincture prepared traditionally was tested against six food-isolated pathogens (two isolates of *Salmonella Enterica*, one isolate of *Salmonella spp*, and three isolates of *Staphylococcus aureus*). The results of this study confirmed that the *Allium ursinum* tincture, prepared traditionally, possesses certain antibacterial activity against both Gram-positive and Gram-negative bacteria and exhibited bactericidal effects on Gram-positive bacteria in all repetitions.

This study serves as an introductory exploration into future laboratory investigations and encourages the reintroduction of herbal preparations into the food industry as food additives and effective preservatives. Additionally, it highlights their potential use in preventive and therapeutic approaches for various conditions caused by the tested pathogens.

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