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Antimicrobial Effects of Aqueous and Alcoholic Extracts of *Allium schoenoprasum* on Some Bacterial Pathogens

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ABSTRACT

Aims Many infectious diseases had traditionally been cured with herbal medicines. Antimicrobial agents are often produced synthetically to increase the food durability and quality. The purpose of this study was to determine the antimicrobial properties of the aqueous and alcoholic extracts of *Allium schoenoprasum*.

Materials & Methods In this experimental study, after preparation *Allium schoenoprasum* samples, aqueous and alcoholic extracts were prepared and their minimum inhibitory concentration (MIC) was determined against *Staphylococcus aureus, Bacillus cereus, Escherichia coli and Vibrio cholerae* by micro broth dilution method. Erythromycin was used as the control.

Findings The MIC of alcoholic and aqueous extracts of A. *schoenoprasum* was 16-256 and 32->256μg/ml, respectively and MBC of them were 32-256 and 64->256ug/ml, respectively. The A. *schoenoprasum* exhibited higher activity against *S. aureus and B. cereus* strains.

Conclusion The extracts of A. *schoenoprasum* have antimicrobial effect on *S. aureus, B. cereus, E. coli* and *V. cholerae* strains in micro broth dilution method.

Keywords Antimicrobial Agents; *Allium Schoenoprasum*; *Escherichia coli*; *Vibrio cholerae*; *Staphyloco ccus aureus*

CITATION LINKS

[1] Antimicrobial activity of some medicinal plants of the island Soqotra [2] Anti-inflammatory and antimicrobial effects of heat-clearing Chinese herbs: A current review [3] Immunomodulatory and antimicrobial effects of some traditional Chinese medicinal herbs: A review [4] Assessment of the antibacterial activity of some traditional medicinal plants on some food-borne pathogens [5] Association between drugs and herbal products: In vitro enhancement of the antibiotic activity by fractions from leaves of Croton campestris A, (Euphorbiaceae) [6] Evaluation of antimicrobial activity of Curcuma longa rhizome extract against Staphylococcus aureus [7] The association between drugs and herbal products: In vitro enhancement of the antibiotic activity by extracts of dry floral buttons of Egletes viscosa L, (macela) [8] Anti-inflammatory effects of Allium schoenoprasum L. leaves [9] Allium schoenoprasum L.: A review of phytochemistry, pharmacology and future directions [10] Structure and cytotoxicity of steroidal glycosides from Allium schoenoprasum [11] Inhibition of Listeria monocytogenes by pomegranate (Punica granatum) peel extract in meat paté at different temperatures [12] Textbook of diagnostic microbiology-e-book [13] Bailey & Scott's diagnostic microbiology [14] Edible flowers: A review of the nutritional, antioxidant, antimicrobial properties and effects on human health [15] Chemical composition, antibacterial and antioxidant activities of six essentials oils from the Alliaceae family [16] Compounds from Allium species with cytotoxic and antimicrobial activity [17] Evaluation of fermentation conditions triggering increased antibacterial activity from a near-shore marine intertidal environment-associated Streptomyces species [18] Antibacterial effects of curcumin: An in vitro minimum inhibitory concentration study [19] Antibacterial effects of cinnamon: From farm to food, cosmetic and pharmaceutical industries

Introduction

The use of medicinal plants over the centuries has been the only source of treatment for diseases, and despite the advances of science and technology in developing the industrial medicines, these plants are still widely used [1]. Many infectious diseases had traditionally been cured with herbal medicines and by technological progresses, efforts are being made to identify and extract those effective antimicrobial herbal compounds [2].

Raw and processed foods may easily be contaminated by different microorganisms in inappropriate conditions of transport and storage that leads to the growth of bacteria, causing food destroy, and related human infections [3]. Antimicrobial agents are often produced synthetically to increase the food durability and quality [4]. However, consumers are more aware of side effects of chemical preservatives nowadays and demand for fresh and more natural foods. Hence, with the aim of replacing the synthetic chemicals in food storage, the extraction of natural antimicrobial compounds from essential oils of various plant species are progressed in various laboratory methods [5]. The excessive use of antibiotics has led to an increase in drug resistance among bacterial species. Therefore, finding new antimicrobial agents with the least side effects seems essential and important [6].

According to the World Health Organization reports, more than 80% of the world population (nearly 5 billion people), still uses herbal medicines to treat diseases. Approximately a quarter of the worldwide used drugs are of plant origin, either directly extracted from plants or synthesized on the basis of plant compounds. Studies have demonstrated that the high tendency to use herbal medicines to eradicate infections is due to the lower side effects of these drugs compared to chemical drugs. Along with this growing trend, the resistance to commonly used antibiotics is a matter of the day, so new and low-risk antibiotics are needed [7].

Chives (*Allium schoenoprasum*) is one of the potential antioxidant and antibacterial plants, which belongs to the Alliaceae family. It is very fragrant and has no prominent head with cylindrical leaves, similar to the grass, which is used as food flavor [8, 9]. It contains thiamin, phosphorus and zinc and is a very good source of fiber, vitamin A, vitamin C, riboflavin, vitamin B6, folate, calcium, iron, magnesium, potassium and copper. Allyl sulfide compounds are known as the main antimicrobial agent of chives.

The purpose of this study was to determine the antimicrobial properties of the aqueous and alcoholic extracts of *Allium schoenoprasum*.

Materials and Methods

In this experimental study, *Allium schoenoprasum* samples were collected from mountainous areas of Hamadan City, Iran. After transferring to the laboratory and confirming the correctness of the species [10], samples were dried at room temperature and away from direct light, during 14 days. The sieved (mesh 40) powders were stored until the extraction process in darkness at 4°C.

To obtain the water-soluble extract of *A. schoenoprasum*, 95ml of sterilized distilled water was added to 5 grams of the prepared powder and mixed by a magnetic stirrer for an hour and kept at 60°C for 16 hours. The resulting mixture was sterilized using a vacuum pump (Busch R5; Germany) and sterilized with a sterile filter paper twice. The solution was sterilized by passing through a 0.45µ sterile filter and kept in a dark glass at 4°C [10].

Heparatian method was used to obtain the alcoholic extract of *A. schoenoprasum* [11]. The appropriate amount of the plant powder was transferred into an opaque glass container and mixed 1:10 with an initial alcohol solvent and placed on a magnetic shaker for 4 hours at 40°C. The extract was filtered and the pellet was diluted again with a second alcohol solvent under the same conditions on a magnetic shaker. The both extracts were placed at 40°C vacuum to concentrate the final extract. The extract was kept at 4°C until use.

Four bacterial ATCC strains, *Escherichia coli, Staphylococcus aureus*, *Bacillus cereus*, and *Vibrio cholerae,* were selected to study the antimicrobial properties of the extract. The strains were confirmed by phenotypic tests [12]. They had been prepared from Pasteur institute of Iran and Iran genetic research center.

The bacterial strains were cultured in Tryptic Soy Broth (TSB). The turbidity of the bacterial suspensions was reached to 0.5McFarland (approximately 1.5×10^{8} CFU/ml) for testing. One milliliter of the standard inoculum suspension (containing 1×10^{6} organisms) was inoculated to 10 tubes containing equal volumes of the extract dilutions (0.25, 0.5, 1, 5, 10, 25, 50, 75, 100 and 150ug/ml). Erythromycin was used as the positive control and 1ml of microbial suspension added to 1ml of distilled water was used as the negative control. After bacterial culture on the Mueller Hinton Agar and 18-22h of incubation, the MIC was measured by observation of any dilution without bacterial growth [13].

Accordingly, after adding the concentrations of the extract to the test tubes and placing them in a 37°C incubator for 24 hours, the first tube in which the growth was not observed was considered as the minimum inhibitor concentration (MIC) and the first tube in which no growth was observed after culture of 20μl on a MHA medium was considered as the minimum bactericidal concentration (MBC). The means were analyzed by Duncan's test using SPSS 19. The differences were determined based on a completely randomized factorial design using ANOVA.

Findings

A. schoenoprasum extracts had significant effects on the growth of all 4 bacteria ($p<0.05$). The alcoholic extract had higher inhibitory effect against all 4 bacterial strains than the aqueous extract. *S. aureus* and *B cereus* were more susceptible to *A. schoenoprasum* extract compared to *E. coli* and *V. cholera* (Table 1).

Table1) The MIC (μg/ml) and MBC (μg/ml) of *A. schoenoprasum* on *B. cereus, S. aureus, E. coli* and *V. cholerae* at 1.5×10^6 opacity

Isolates	MIC	MBC
Water extract		
B. cereus	32	64
S. aureus	32	64
V. cholerae	128	256
E. coli	256	>256
Alcoholic extract		
B. cereus	16	32
S. aureus	16	32
V. cholerae	64	128
E. coli	128	256
Erythromycin		
B. cereus	2	4
S. aureus	8	16
V. cholerae	4	8
E. coli	16	32

Discussion

As the purpose of this study was to determine the antimicrobial properties of the aqueous and alcoholic extracts of *Allium schoenoprasum*, it was demonstrated that *S. aureus* and *B cereus* were more susceptible to *A. schoenoperasom* extract compared to *E. coli* and *V. cholera*, which is possibly due to the presence of a different cell wall in two types of Gram-positive and -negative bacteria and lack of an external membrane in those Gram-positive species. In general, Gram-negative bacteria exhibit higher resistance to germicides than Gram-positive ones [14].

It seems, however, that the cause of higher Gram-negative spp. resistance to plant extracts is likely to be the complexity of the dual cell membrane of these organisms in comparison with the single-glycoprotein/Teichoic acid layers. It also seems that the resistance of microbial cells depends on the rate and degree of dissolve or permeability of the antimicrobial agents in the cell membrane. However, this cannot be a complete explanation for the difference in the sensitivity of Gram-positive and -negative bacteria, the difference in hydrophobicity of the cell membrane surface was also suggested as an effective factor. The compounds of the penetrating extract tend to swell and affect the activity and ultimately lead to cell death. Extract compounds also have different antibacterial effects, so that the hydroxyl group in molecules, such as carvacrol, thymol, and menthol, are very important for their antibacterial properties. The chives extract demonstrated a significant effect on the sensitivity of *B. cereus, E. coli* and *V. cholera* and the alcoholic extract had higher inhibitory effects against *B. cereus, E. coli* and *V. cholerae* than the aqueous extract. It can be due to the phenolic compounds content in the alcoholic extract that is mainly Carvacrol, eugenol and thymol [15].

Investigations on the mechanism of action of the extracts have shown that these compounds increase the permeability of the membrane.

A. shoenoprasum can be found in most areas of Iran and thus is available as an edible food and even effective herbal drug. Because of low toxic effects even so many levels higher than MIC and MBC levels (against bacterial strains) on eucaryotic cells (although cell culture studies are needed in this regards for a better understanding), this edible herb is suitable for further antimicrobial studies [16].

It should be noted that the composition of the extract and the severity of the antimicrobial effects of a species in different regional conditions may be different, and these differences can be due to the difference in harvesting season, extraction time, and geographical status, even in different parts of the plant. Another factor influencing the antibacterial effects of an extract or essential oil of a plant is the culture medium used for antibacterial testing. Differences in the antibacterial effects of a substance in a variety of media have been demonstrated [17].

On the other hand, the extracts are a mixture of esters, aldehydes, alcohols, ketones and terpenes, which are classified into two groups of main and secondary compounds. Their quantity can vary with respect to the climate, soil and vegetation composition. Furthermore, the time and stage of planting, the used part of the plant and extraction method can also affect the composition of the extract and its antimicrobial effect. According to the results of this study, although the *A. schoenoperasum* extract had significant bacteriostatic effects on all species, it has a greater effect on Gram-positive bacteria. By comparing the results to previous publications [18, 19] and other herbal extracts, the extract of *A. schoenoprasum* exhibited a suitable antimicrobial properties.

The limitations of this study were lack of enough *in vitro* and *in vivo* studies and molecular tests for a better understanding of *A. shoenoprasum* against bacterial species.

It is suggested that *A. shoenoprasum* as a suitable antimicrobial herbal drug to be tested against bacterial pathogens and proper molecular studies are needed in this regard.

Conclusion

The alcoholic extract of *A. schoenoperasum* had more antimicrobial properties than the aqueous extract. The *A. schoenoperasum* extracts have higher inhibitory effects against *S. aureus* and *B. cereus*.

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Authors' Contribution: Farshad Nojoomi designed the study, Mahtab Vafaei, Abdolmajid Ghasemian and Seyyed Khalil Shokouhi Mostafavi performed the laboratory work, writing, edition and data analysis.

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