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Original scientific paper

The potential of chives, wild garlic, and garlic plant extracts in suppression of bacterial growth

Abstract

Food spoilage is phenomenon associated with occurence of the microorganisms in agricultural products. Due to the ability of several microorganisms to colonize plant material, agrochemicals were proposed to suppress bacterial growth. However, overuse of agrochemicals has detrimental impact on environmental quality. Therefore, the objective of this research was to evaluate the potential of aqueous extracts of garlic (Allium sativum L.), wild garlic (Allium ursinum L.), and chives (Allium schoenoprasum L.) to suppress the growth of Enterococcus faecalis and Escherichia coli. The antimicrobial activity of these plant extracts was determined using the disc diffusion method. Mueller-Hinton agar was inoculated with bacterial inoculum; six filter paper disks, impregnated with 15 µl aqueous extract (1, 2 and 4%), were placed on each agar surface. The Petri dishes were incubated at 37 °C for 24 hours. The diameter of the zone of inhibition was measured and expressed in millimeters (mm). Almost all aqueous extracts showed a negligible effect on the growth of E. coli and E. faecalis. Significant suppression of bacterial growth compared with other treatments was observed with the aqueous extract of wild garlic at a concentration of 4%. This research confirms the potential of wild garlic extract in suppression of potential pathogens.

Key words: *antimicrobial activity, chives, garlic, inhibition zone, wild garlic.*

Introduction

Food spoilage is considered one of the most common causes of serious illness in developing countries (Pirbalouti et al., 2009; Sapkota et al., 2012). Gustavsson et al. (2011) estimated that 40% of global food production is lost annually due to various factors (including food spoilage caused by microorganisms). Microorganisms like bacteria, yeasts, and molds, are often involved in food spoilage (Lianou et al., 2016). They have the ability to utilize food products and produce metabolites responsible for food spoilage (Parlapani et al., 2017). Although food spoilage is associated with many Gram-positive bacteria such as *Enterococcus faecalis*, *Staphylococcus aureus*, and *Bacillus cereus* (Braga et al., 2005), most reports on food spoilage mention the presence of Gram-negative bacteria (Pandey and Singh, 2011). Some of the bacteria are associated with foodborne illness and food contamination. Enterococci are Gram-positive bacteria that are commonly found in aquatic and terrestrial environments as well as in plants because of their adaptability to various abiotic conditions (Badgley et al., 2010). Enterococci produce thermostable amines that can lead to poisoning (Giraffa, 2002). *Escherichia coli*, on the other hand, is a Gram-negative bacterium that is often isolated from fresh plant products because it is transmitted through manure-contaminated soil or contaminated irrigation water (Solomon et al., 2002). The possible bacterial plant infections can be controlled using agrochemicals (Shan et al., 2007).

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However, overuse of agrochemicals leads to the accumulation of residues, which then has impact on public health. In order to suppress the growth of pathogens and increase the shelf life of food (Gonelimali et al., 2018), environmentally friendly methods based on the use of natural additives have been developed (Mostafa et al., 2018). One of the methods is based on the use of aqueous or ethanol-based plant extracts from medicinal and aromatic plants, which have antimicrobial (Mathabe et al., 2005) and antioxidant properties (Talib and Mahasneh, 2010).

The genus *Allium* belongs to the *Alliaceae* family (Chehregani et al., 2007) and is characterized by antimicrobial activity against fungi and a variety of bacteria (Whitemore & Naidu, 2000). A variety of antimicrobial compounds have been isolated and determined from the species of this genus (Lanzotti et al., 2013). Although garlic (*Allium sativum* L.) is the most studied species in the genus Allium (Lanzotti et al., 2013), other plant species from the same genus: chives and wild garlic, also have antibacterial activity (Lupoae et al., 2013; Sihombing et al., 2018). Considering the reports by Safithri et al. (2011), Stupar et al. (2022), and Ghasemian et al. (2018) that aqueous extracts of Allium showed satisfactory effects on bacterial growth.

The objective of this research was to evaluate the potential of aqueous extracts of garlic (*Allium sativum* L.), wild garlic (*Allium ursinum* L.), and chives (*Allium schoenoprasum* L.) to suppress the growth of *Enterococcus faecalis* and *Escherichia coli*.

Material and methods

Pure cultures of *Escherichia coli* and *Enterococcus faecalis* belonging to the Laboratory of Microbiology (Faculty of Agricultural and Food Sciences Sarajevo, Bosnia, and Herzegovina) were used for this study. Plant material was collected from Vranica mountain (Central Bosnia and Herzegovina) and Novi Travnik municipality (Central Bosnian Canton, Bosnia and Herzegovina). Samples of wild garlic and chives were collected from Mount Vranica, while the plant material of garlic was obtained in Novi Travnik. Shoots and roots of wild garlic and chives, as well as the garlic clove were used for the preparation of the aqueous extracts. The preparation was performed according to the modified method of Norsworthy (2003); after drying and grinding, the plant materials (3, 6, and 12 g of the dried samples) were homogenized in 300 ml of distilled water. Thus, the final concentrations of plant material were 1, 2, and 4 % of dry weight.

The antimicrobial activity of these plant extracts was determined using the disc diffusion method. Mueller-Hinton agar, previously sterilized in an autoclave at 120°C for 20 min, was inoculated with bacterial inoculum adjusted to 0.5 McFarland standard turbidity (i.e., approximately $1x10^8$ CFU/ml). After 15 minutes of agar plate inoculation, 6 filter paper disks (6 mm diameter) impregnated with 15 µl aqueous extract were placed on each agar surface.

The Petri dishes were incubated at 37 °C in an incubator for 24 hours. The diameter of the zone of inhibition was measured and expressed in millimeters (mm). The interpretation of the obtained results was performed according to EUCAST (2017), where the effect of the extracts is classified as resistant (R), susceptible - increased exposure (I), and susceptible - standard dose (S).

After determining the zone of inhibition, statistical significance ($P<0.05$) between treatments was determined using SPSS software (v. 22, SPSS Inc., Chicago, IL, USA).

Results and discussion

The results of this study show that the zone of inhibition depends on the bacterial species, the type of plant extracts, and their concentration (Tables 1-4). Tables 1 and 3 show the average diameters of the zone of inhibition of *Escherichia coli* and *Enterococcus faecalis*, respectively.

Table 1. The average diameter of the inhibition zone of bacteria *Escherichia coli*
Tablica 1. Prosječni promjer zone inhibicije bakterije *Escherichia coli*

Plant extract	The concentration of aqueous extracts (%)	Inhibition zone diameter (mm)	EUCAST classification
garlic	1	6.0	R
	2	6.0	R
	4	6.0	R
wild garlic	1	6.0	R
	2	6.0	R
	4	7.5	I
chives	1	6.0	R
	2	6.0	R
	4	6.0	R

As shown in Table 1, the diameter of the inhibition zone of *E. coli* was not affected by the aqueous extract of chives and garlic. All concentrations of these extracts resulted in an inhibition zone of 6.0 mm for the same bacteria. Although several studies confirm the potential of aqueous chive extract in suppressing the growth of *E. coli* (Agustin et al., 2022; Sihombing et al., 2018), our results are not consistent with these findings. However, compared with this study, the presented authors used higher concentrations of chives extracts. Similar results were observed by Oyawoye et al. (2022) with garlic extracts. In our study, aqueous *Allium* extracts were used. Shirzadi Karamolah et al. (2017) reported that antimicrobial activity is often determined by the type of plant extracts. Moreover, Oyawoye et al. (2022) showed that ethanol *Allium* plant extracts exhibited a stronger inhibitory effect on the growth of various bacteria compared with aqueous extracts.

Table 2. Significance of obtained differences for *E. coli* growth using the Tukey test
Tablica 2. Značajnost dobijenih razlika za *E. coli* primjenom Tukey testa

Concentration (%)	Plant extracts			AIZD (mm) for concentrations
	garlic	wild garlic	chive	
1	6.000	6.000	6.000	6.000a
2	6.000	6.000	6.000	6.000a
4	6.000	7.500	6.000	6.500b
AIZD (mm) for plant extracts	6.000a	6.500b	6.000a	-

AIZD - Average inhibition zone diameter

Application of the highest concentration of the wild garlic extract resulted in a significant increase in the diameter of the *E. coli* inhibition zone compared with the lower concentrations (Table 2). However, the *E. coli* average inhibition zone at the highest and lowest extract concentrations was 7.5 and 6.0 mm, respectively. The weak antimicrobial potential of wild garlic extracts was previously described by Stupar et al. (2022). Therefore, according to the EUCAST criteria (2017), *E. coli* can be classified as resistant in all treatments except the 4% wild garlic treatment (Table 1).

Table 3. The average diameter of the inhibition zone of the bacteria *Enterococcus faecalis*
Tablica 3. Prosječni promjer zone inhibicije bakterije *Enterococcus faecalis*

Plant extract	The concentration of aqueous extracts (%)	Inhibition zone diameter (mm)	EUCAST classification
garlic	1	6.0	R
	2	6.0	R
	4	6.0	R
wild garlic	1	6.0	R
	2	6.0	R
	4	12.0	I
chives	1	6.0	R
	2	6.0	R
	4	6.0	R

Table 4. Significance of obtained differences for *E. faecalis* growth using the Tukey test
Tablica 4. Značajnost dobivenih razlika za *E. faecalis* primjenom Tukey testa

Concentration (%)	Plant extracts			AIZD (mm) for concentrations
	garlic	wild garlic	chive	
1	6.000	6.000	6.000	6.000a
2	6.000	6.000	6.000	6.000a
4	6.000	12.000	6.000	8.000b
AIZD (mm) for plant extracts	6.000a	8.000b	6.000a	-

AIZD - Average inhibition zone diameter

From the data presented in Table 3, it can be seen that the aqueous garlic and chives extracts showed no significant effect on suppressing the growth of *E. faecalis*. Although different concentrations of garlic extract showed a reduction in the viability of *E. faecalis*, no significant differences were found between the concentrations used (Octavia et al., 2019). In contrast, Birring et al. (2015) found that garlic extract was able to disrupt *E. faecalis* biofilm formation. Moreover, the best results were obtained with a garlic concentration of 70%, which is a significantly higher concentration than in this study. The significantly highest inhibition zone diameter was observed when 4% garlic extract was used compared with other plant extracts and concentrations (Table 4). Although Stupar et al. (2022) reported the moderate effect of wild garlic extract on the growth of *E. faecalis*, Pavlović et al. (2012) showed that *Allium ursinum* extract exhibited strong antimicrobial performance against the same bacteria. However, previous reports have shown contradictory results regarding the effects of wild garlic extracts against Gram-positive and Gram-negative bacteria (Ivanova et al., 2009; Lupoae et al., 2013; Putnoky et al., 2013). Stupar et al. (2022) suggest that conflict results can be explained by differences in extraction, solvents and plant parts used for the extract production. As in the experiment with *E. coli*, *E. faecalis* can be considered resistant in all treatments, except for the treatment with the highest wild garlic extract concentration (Table 3).

Conclusion

According to the results of this study, almost all aqueous extracts showed a negligible effect on the growth of *E. coli* and *E. faecalis*. Significant suppression of bacterial growth compared with other treatments was observed with the aqueous extract of garlic at a concentration of 4%. Further research will focus on the chemical characterization of this extract and the effects of higher concentrations against other bacterial genera.

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Izvorni znanstveni rad

Potencijal biljnih ekstrakata vlasca, medvjeđeg luka i češnjaka u supresiji bakterijskog rasta

Sažetak

Kvarenje hrane je pojava povezana sa prisustvom mikroorganizama u poljoprivrednim proizvodima. Zbog sposobnosti nekih mikroorganizama da koloniziraju biljni materijal, predložena je upotreba agrokemikalija za suzbijanje rasta bakterija. Međutim, prekomjerna uporaba agrokemikalija ima štetan utjecaj na kvalitetu okoliša. Stoga je cilj ovog istraživanja bio procijeniti potencijal vodenih ekstrakata češnjaka (Allium sativum L.), medvjeđeg luka (Allium ursinum L.) i vlasca (Allium schoenoprasum L.) u suzbijanju rasta Enterococcus faecalis i Escherichia coli. Antimikrobna aktivnost ovih biljnih ekstrakata određena je disk difuzijskom metodom. Mueller-Hinton agar inokuliran je bakterijskim inokulantom; šest diskova filter papira, impregniranih sa 15 µl vodenog ekstrakta (1, 2 i 4%), postavljeno je na svaku površinu agara. Petrijeve zdjelice inkubirane su na 37 °C u trajanju od 24 h. Promjer zone inhibicije izmjeren je i izražen u milimetrima (mm). Svi vodeni ekstrakti pokazali su inhibitorni učinak na rast E. coli i E. faecalis Vodeni ekstrakt medvjeđeg luka u koncentraciji od 4% značajno je suzbio rast bakterija u usporedbi sa drugim tretmanima. Ovo istraživanje potvrđuje potencijal ekstrakta medvjeđeg luka u suzbijanju potencijalnih patogena.

Ključne riječi: *antimikrobna aktivnost, vlasac, češnjak, zona inhibicije, medvjeđi luk*