Biotechnological Journal of Environmental Microorganisms (BJEM)

Biotechnological Journal of Environmental Microorganisms(BJEM) 1(1) 2022 45-51

Antimicrobial activity of Ethanolic extracts of (*Tragopogon collinus* L. and *Allium eriophyllum* L.) on some selected environmental and pathogenic bacteria

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Received: 21 june 2022 / Revised: 24 July 2022 /Accepted: 26 July 2022

Abstract

Due to the increase in the incidence of infectious diseases and the prevalence of antibiotic resistance in bacteria in different environments, there is an urgent need to discover new antimicrobial compounds. The present study aimed to determine the antibacterial activity of the ethanolic extracts of the edible plants *Tragopogon Collinus* L. and *Allium eriophyllum* L. against some environmental and pathogenic bacteria such as Staphylococcus aureus, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli*, and *Enterococcus faecalis*. The extraction of medicinal plants was conducted using ethanol and the percolation method. The well diffusion method was used to determine the antimicrobial effects of the extracts, and the minimum inhibitory concentration (MIC) of microbial growth. The ethanol extract of *T. collinus* showed an inhibitory effect on all bacteria in 250 µg/ml concentration, and the greatest effect was observed on *S. aureus* and *E. faecalis*, with 20 and 18 mm diameter of zone, respectively. In the 125 µg/ml concentration, with a 21 mm diameter of zone, it had the highest antimicrobial effect on *K. pneumoniae*, compared with other bacteria. Regarding the ethanolic extract of *A. eriophyllum* L., the highest antibacterial effect at 250 µg/ml concentration was 20 and 22 mm zone diameters. The effect of this plant extract on *P. aeruginosa* was lower than its effect on other bacteria. The MIC of the extract of *Tragopogon collinus* L.was found at a 5.75 µ/ml concentration on *S. aureus* and *E. faecalis*. The MIC of the ethanolic extract of *A. eriophyllum* L. was found at 11.5 µ/ml concentration on *S. aureus*, *P. aeruginosa*, and *K. pneumoniae*. The results of this study indicate that *T. collinus* and *A. eriophyllum* have antibacterial properties.

Key words: Antibacterial activity, Ethanolic extract, *Tragopogon collinus* L., *Allium eriophyllum* L

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Biotechnological Journal of Environmental Microorganisms(BJEM) 1(1) 2022 45-51

1. Introduction

Antimicrobial resistance is a global public health crisis (Pierce et al., 2021). Plants contain many biologically active natural materials. Although these bioactive compounds are not nutritional, they have important medicinal properties (Ivanišová et al., 2021). Plants are important natural materials used in complementary medicine (Saleh Mohammed et al., 2022). Indeed, plants have been used as medicines for more than 5000 years, as a source of antibiotics, antineoplastic, analgesics, cardio protective. (Newman and Cragg., 2016). About 70–90% of the population in developing countries continues to use ancient medicines based on plant extracts (Anand et al., 2019).

Tragopogon collinus is an herbaceous plant from the family of chicory (Maleki et al., 2021) The compounds identified in these plants are flavonoids, terpenes, saponins, benzyl and hydroisocoumarin, phenolic compounds, and sterols, in which, many of them have been identified during chemotaxonomic studies of these plants. Some plants in the TPE genus, by their strong antioxidant effect, prevent inflammatory damage to tissues and in some concentrations prevent DNA damage (Farzaei et al., 2014). This plant has chemical compounds such as carbon hydrate and inulin, which are used in traditional medicine as an appetize.

Allium eriophyllum is from the garlic family. It is actually a type of mountain garlic (Zhao et al., 2022). It has chemical compounds composed of sulfur and polysulfuronyl polyvinyl sulfate and a type of unstable aldehyde, and its essential oil contains sulfur compounds. The therapeutic properties of *Allium eriophyllum* essential oil are the same as those of garlic essential oil since its compounds are similar to those of garlic. Because of the presence of large amounts of cysteine sulfoxides and sulfoxide amino acids in it, this plant also has anti-diabetic and antioxidant properties (Mosavi et al., 2019). Garlic was shown to have anti-diabetic, antihypertensive, antihyperlipidemic, antiplatelet, anticoagulant, antiatherosclerotic, and anti-oxidative stress properties (Mozafari et al., 2017).

Many studies by different researchers have reported that plants have many important biological activities such as antioxidant, antimicrobial, anti-aging, anticancer, antitumor, DNA protective, antiallergic (Nisa et al., 2019; Zazharskyi et al., 2019; Nguyen et al., 2020; Dias et al., 2021; Akgül et al., 2022). *Tragopogon collinus* and *Allium eriophyllum* grow naturally in the western regions of Iran and are edible. The nomads and people of the western region of Iran, especially Kermanshah province, have used these medicinal plants without knowing enough about their antimicrobial properties. Since these plants have been used in society for centuries, this study aimed to investigate the antibacterial effects of their ethanolic extract on some indicator and environmental bacteria.

2. Materials and Methods

2.1. Microbial Strains

In this study, five standard strains, including *E.coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 10031, and *Enterococcus faecalis* ATCC 9854 were purchased from the Persian Type Culture Collection (PTCC, IROST, Tehran/Iran). Lyophilized vials were cultured under sterile conditions according to the manufacturer's instructions. In addition, five environmental isolates of the same strains kept in the microbiology laboratory of the Lahijan Branch, Islamic Azad University were used in this study.

2.2. Collection of Plants and Preparation of Ethanolic Extract

Plants were collected from their natural habitat (Dalahoo mountains Kermanshah province, Iran), dried in the air, and then kept in shade for a week. Identification of collected plants was performed in the herbarium of botany in the Gorgan Branch, Islamic Azad University, Gorgan, Iran.

For preparing the extracts, the leaves of the dried plants were powdered by an electric mill, then 200 grams of powder was carefully weighed and used to extract by the percolation method. Then, ethanol was eliminated using a rotary evaporator and vacuum distillation method. This extract was considered a pure extract, of *Tragopogon collinus* and *Allium eriophyllum* plants. Then, the concentrations of 250, 125, 62.5, 31.25,

Biotechnological Journal of Environmental Microorganisms(BJEM) 1(1) 2022 45-51

15.62, 7.81, 3.9, 1.95, 0.97, and 0.48 µg/ml using 100% Dimethyl Sulfoxide (DMSO) were prepared by serial dilution.

2.3. Examining the Antibacterial Effect

After preparing the extracts, the agar well diffusion method was used to determine antibacterial activities, and serial dilution was used to determine the MIC of the extracts.

2.3.1. Well Diffusion Method

In the agar well diffusion method, a microbial suspension the turbidity equivalent to McFarland 0.5 was prepared from the 24-hour bacterial cultivation in Mueller Hinton broth and then cultured on the Mueller Hinton agar plates. Using a cork borer, wells with a diameter of 7 mm were made. 100 microliters of different concentrations of the extracts were poured into the wells and then incubated for 24 hours at 37 °C. By measuring the diameter of inhibition zones, the sensitivity or resistance of the bacteria to the extracts was determined. In this study, gentamicin and 100% DMSO were used as positive control and negative controls, respectively

2.3.2. Minimum inhibitory concentration determination

A serial dilution was prepared from the plant extracts used in the 100% DMSO solution. For this purpose, 5 ml of primary extract of plants (500 µg/ml concentration) was added to the tubes containing 100% DMSO until it was completely dissolved, and a uniform solution was prepared. Plants were dissolved in 15 ml of 100% DMSO solution. For this reason, the initial concentration of the extracts of *Tragopogon collinus* and *Allium eriophyllum* was determined to be from 0.48 µg/ml to 250 µg/ml.After autoclaving, the Erlenmeyer flasks containing Mueller Hinton broth were cooled to 50°C, and 5mL of different concentrations of the prepared extracts were added to each Erlenmeyer flask. Then shaken gently so that the extract was completely distributed in the medium. In this way, the final concentrations of extracts in Mueller Hinton Agar medium were estimated to be 23, 11.5, 5.75, 2.87, 1.43, 0.71, 0.35, 0.17, 0.08, and 0.04 µg/ml. Then, suspensions from each bacterial equal to 0.5 McFarland standards were prepared. Twenty microliters of each concentration were inoculated on the surface of each plate. Plates were incubated for 24 hours at 37°C, and bacterial growth was checked for each concentration. The lowest concentration of the extract inhibited the growth considered MIC. All experiments were performed in triplicates.

3. Results

The ethanolic extract of the *Tragopogon collinus* showed an inhibitory effect on all bacteria at a concentration of 250 µg/ml. This effect on *S.aureus* and *E. faecalis* was the greatest among the bacteria, with inhibition zone diameters of 20 and 15 mm, respectively. The least amount of concentration of the extract that had a significant inhibitory effect (≥ 12mm) was 3.9 µg/ml and 0.97 µg/ml against *S. aureus* and *E. faecalis* and were 1.95, 62.5, and 31.25 µg/ml on *P. aeruginosa*, *E. coli*, and *K. pneumoniae*, respectively (Table 1).

Table 1. Diameter of Inhibition zone of ethanolic extract of Tragopogon collinus against bacteria in comparison with gentamicin using agar well diffusion method

	concentrations (µg/ml)											
Diameter of inhibition zone Bacteria	250	125	62.5	31.25	15.62	7.81	3.9	1. 95	0.97	0.48	Positive control 1.25 µg/ml	Negative control
staphylococcus aureus	20	18	19	19	16	13	13	10	10	9	27	-
pseudomonas aeruginosa	15	14	13	12	11	10	-	-	-	-	18	-
Escherichia coli	14	15	13	10	10	10	8	-	-	-	28	-
Klebsiella pneumonaie	15	21	20	17	11	-	-	-	-	-	28	-
Enterococcus faecalis	18	18	18	18	15	13	13	12	12	10	28	-

Note. (-) indicates neutral; Negative control: DMSO 100%; Positive control: Gentamicin

Biotechnological Journal of Environmental Microorganisms(BJEM) 1(1) 2022 45-51

K. pneumoniae and *S. aureus* showed the greatest inhibition zone when treated with ethanolic extracts of *Allium eriophyllum*; however, the diameter of this zone was the same as thezone caused by gentamicin. The zone diameter ≥ 12mm was not observed in any case at a concentration lower than 7.8 µg/ml (Table 2).

Table 2. Inhibition zone diameter of the Ethanolic extract of Allium eriophyllum against bacteria in comparison with gentamicin using agar gel diffusion method.

Zone diameter of the bacterium	Concentration (µg/ml)										Positive control 1.25 µg/ml	Negative control
	250	125	62.5	31.25	15.62	7.81	3.9	1. 95	0.97	0.48		
Staphylococcus aureus	20	19	17	15	13	12	10	-	-	-	27	-
pseudomonas aeruginosa	16	15	15	13	12	11	10	8	-	-	18	-
Escherichia coli	17	16	15	12	10	8	7	-	-	-	28	-
Klebsiella pneumoniae	22	20	18	14	11	9	8	-	-	-	28	-
Enterococcus faecalis	18	17	15	14	13	12	10	-	-	-	28	-

Note. (-) indicates neutral; Negative control: DMSO 100%; Positive control: Gentamicin

The determined MIC concentrations of the ethanolic extracts of Tragopogon collinus and Allium eriophyllum against E. faecalis and S. aureus, P. aeruginosa, E. coli and K. pneumoniae are shown in Table 3.

Table 3. The MIC concentrations of ethanolic extracts of *Tragopogon collinus* and *Allium eriophyllum* against bacterial strains

Type of bacteria	Plant Extract MIC µg/ml	
	Tragopogon collinus	*Allium eriophyllum*
S. aureus	11.5	5.75
P. aeruginosa	11.5	11.5
E. coli	23	11.5
K. pneumoniae	11.5	11.5
E. faecalis	23	5.75

4. Discussion

The therapeutic agents derived from plants are justified by the emergence of diseases and the growth of scientific knowledge about herbal medicines as important alternatives or complementary treatments for diseases (Sasidharan et al., 2011). The discovery of new antibacterial agents capable of dealing with antibiotic-resistant bacteria is a priority in the field of scientific research (Martinez et al., 2021 a). In this field, certain natural compounds of plant origin have emerged as powerful potential therapeutic tools (Martinez et al., 2021 b). Many studies have shown that medicinal plants contain coumarins, flavonoids, phenolics, alkaloids, terpenoids, tannins, essential oils, lectins, polypeptides, and polyacetylenes. These bioactive compounds are used as a starting point for antibiotics synthesis to treat infectious diseases (Parvin et al., 2014). Plants produce an invaluable source of secondary metabolites in response to environmental factors such as attack by herbivores, abiotic stress, or interspecific interactions (Yang et al., 2018). Since ancient times, humans have used these secondary metabolites of plants in various fields, including medicine and gastronomy. The vast chemical diversity of plant secondary metabolites and their long history of traditional use make plants very attractive natural reservoirs for research into the discovery of new antimicrobial compounds. Rapid technological advancement and the application of new, increasingly efficient methodologies have allowed the identification and characterization of numerous antibacterial agents in recent years (Katz and Baltz, 2016).

In Iran, medicinal plants have not been exploited and used in the same way as they are in developed countries, so most people do not know

Biotechnological Journal of Environmental Microorganisms(BJEM) 1(1) 2022 45-51

about the valuable plants scattered throughout Iran or they are not familiar with their healing properties. Although edible plants may have therapeutic effects, they may not be beneficial to the body when consumed in such a way. The lack of knowledge of their existence in Iran or the lack of recognition of these plants is still the biggest obstacle to their use (Zargari, 1996).

In this study, two plant extracts were used because the properties of these plants remain unknown, and they are widely used for medicinal and edible purposes, especially in the west of Iran. Because there are currently no scientific studies that examine the antimicrobial properties of these plants' extracts, this research is of great significance. Examining the antimicrobial effects of the ethanolic extract of these plants on bacterial strains is important.

The results of the present study showed that the ethanol extracts of plants at different concentrations had antibacterial effects on all bacteria tested.

According to the results of this research, ethanol extracts from these plants worked differently against different types of bacteria depending on the concentration used. The extract of the *Tragopogon collinus* had the most significant antibacterial effect on *S. aureus* in 250 µg/ml concentration with a 20 mm zone diameter. Moreover, in the 125 µg/ml concentration, it had the most significant antibacterial effect on *K. pneumoniae* with a 21 mm inhibitory growth zone diameter. The ethanol extract of the *Allium eriophyllum* had the most significant antibacterial effect on *K. pneumoniae* at 250 µg/ml concentration with a 22 mm inhibitory growth zone diameter. The results of the antibacterial effect of the *Tragopogon collinus* and *Allium eriophyllum* on *S. aureus* were also significant, whereby the most significant effects of the two plants were obtained in 250 µg/ml concentration with 20 mm inhibitory growth zone diameter. In determining the MIC of these plants on *S. aureus*, *Tragopogon collinus* extract at a concentration of 5.75 µg/ml showed an inhibitory effect on S. aureus, while the MIC of *Allium eriophyllum* extracts on this bacterium at a concentration of 11.5 µg/ml was obtained. Hence, an extract of *Tragopogon collinus* with a lower concentration was more effective against this bacterium. Therefore, it can be used as a candidate drug for treating *S. aureus*, one of the most important infectious agents.

Regarding *E. coli*, the antibacterial effect of the ethanolic extract of *Allium eriophyllum* was greater than that of *Tragopogon collinus* in 250 µg/ml concentration, with a 17 mm zone diameter compared with that of 14 mm in *Tragopogon collinus*. But in determining the MIC, the extract of *Tragopogon collinus* at a concentration of 11.5 µg/ml showed a greater inhibitory effect on E. coli compared with the extract of Allium eriophyllum (at a concentration of 23 µg/ml).

The antimicrobial effects of the ethanolic extracts of *Tragopogon collinus* and *Allium eriophyllum* on *E. faecalis* were also significant. *Tragopogon collinus* extracts had similar effects in concentrations of 250 to 3.25 µg/ml with a zone diameter of 18 mm, and the extract of this plant had antimicrobial activity on *E. faecalis* up to 0.97 µg/ml concentration. In contrast, *Allium eriophyllum* extract had the greatest effect only at a concentration of 250 µg/ml with a zone diameter of 18 mm, and with decreasing concentrations, the diameter of the growth zone decreased, and in other concentrations, it had weaker effects compared to the *Tragopogon collinus* extract. Examining the obtained concentrations of the ethanol extracts of *Tragopogon collinus* and *Allium eriophyllum* to determine their MICs showed that the MIC of *Tragopogon collinus* extract had significant inhibitory effects on *E. faecalis* at a concentration of 5.75 µg/ml. On the other hand, the MIC of *Allium eriophyllum* extract on *E. faecalis* was observed at a concentration of 23 µg/ml, which had a weaker inhibitory effect on this bacterium compared to the *Tragopogon collinus* extract. Investigating the antibacterial effect of the ethanolic extract of *Tragopogon collinus* and *Allium eriophyllum* on P .aeruginosa, it was found that the highest effect was related to the *Allium eriophyllum* extract at a concentration of 250 µg/ml with a zone diameter of 16 mm, and as the concentration decreased, its effect on the bacteria decreased, and the diameter of the non-growth zone diameter decreased. The highest effect related to the extract of the *Tragopogon collinus* was observed on *P. aeruginosa* at a concentration of 250 µg/ml, and the non-growth zone diameter was 15 mm. The lowest concentration of this extract had antibacterial properties on

P. aeruginosa was 31.25 µg/ml, which showed weaker effects compared to the lowest concentration of *Allium eriophyllum* extract, at a concentration of 15.62 µg/ml. The MIC of both plant extracts on *P. aeruginosa* was observed to be the same at a concentration of 11.5 µg/ml.

The results of the investigation into the antibacterial effects of the extract of *Tragopogon collinus* and *Allium eriophyllum* on *K. pneumoniae* showed that the most antibacterial effect of the ethanolic extract of *Tragopogon collinus* was at a concentration of 125 µg/ml with a growth inhibition zone of 11 mm. On the other hand, the greatest antibacterial effect of the ethanolic extract of *Allium eriophyllum* was at a concentration of 250 µg/ml with a growth inhibition zone of 22 mm. Moreover, the decrease in the concentration of the extract of this plant reduced the non-growth zone diameter of this bacterium. The MIC of the ethanol extract of *Tragopogon collinus* and *Allium eriophyllum* on *K.pneumoniae* was the same and was observed at a concentration of 11.5 µg/ml. In a study conducted abroad by Zurideh et al. 2008, it was shown that the methanolic extract of Nigella sativa plant had an inhibitory effect in different concentrations on two pathogenic bacteria *S. aureus* and *P. aeruginosa*, but had no antimicrobial effect on two pathogenic bacteria, *E. coli* and *K. pneumoniae* (Zuridah et al., 2008). In another study by Abere et al. (2007), it was found that the methanolic extract of the leaves of the *Mitracarpus scaber* plant had an inhibitory effect on the bacteria *Staphylococcus aureus*, *K.pneumoniae*, and *E. coli* at a concentration of 75 µg/ml, while no antimicrobial effect was observed on *P. aeruginosa* at the same concentration. Our results showed that the ethanolic extracts of *Tragopogon collinus* and *Allium eriophyllum* had more antibacterial effects than their methanolic extracts. It should be noted that the results obtained from the investigation of the antibacterial effects of the extracts of *Tragopogon collinus* and *Allium eriophyllum* on the bacteria used in the present study showed lower effects compared to the antibiotic gentamicin, which was used as a positive control in this research with a concentration of 1.25 µg/ml. This is due to the use of pure and complete extracts of these plants, which contain countless mixtures of substances that are less effective than the antibiotic gentamicin, and if the combination of substances with the antimicrobial effect found in these extracts is isolated and purified, the effects will be more comparable to gentamicin.

In a general conclusion, it can be stated that the ethanolic extracts of *Tragopogon collinus* and *Allium eriophyllum* have demonstrated antibacterial activity on the studied strains in vitro, and it is necessary to conduct a wider and more extensive study in vitro so that the effective doses of these extracts on these bacteria and clinical strains, as well as their precise formulation to achieve maximum bioavailability, can be determined, and, finally, these extracts can be introduced as a new and potentially effective antimicrobial drug to the world of medicine and microbiology. This research clearly shows that medicinal plants have an antibacterial effect, which validates their use in traditional medicine.

Acknowledgments

We are grateful to Dr. Masoumeh Mazandarani, for helpful assistance in Gorgan Branch the Islamic Azad University and head of the microbiology research laboratory in the Lahijan Branch, Islamic Azad University, as well as to my dear friend Farshad Ejraee for kind helps in this project.

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