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Scientific Research

Antimicrobial effect of *Prangos ferulacea* aqueous extract on some pathogenic fungal species and its interaction with nystatin antibiotic

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ABSTRACT

The plant *Russian knapweed* is known as a blood sugar lowering drug, and the antimicrobial properties of its extracts and essential oils have been proven; Therefore, this study was conducted with the aim of extracting the aqueous extract of *R. knapweed* and investigating its antimicrobial activity. The aqueous extract of *R. knapweed* was extracted by the maceration method. The antibacterial activity of the extract against Escherichia coli, *Salmonella typhi*, *Listeria monocytogenes*, and *Bacillus cereus* was investigated using disk diffusion agar, well diffusion agar, minimum inhibitory concentration, and minimum bactericidal concentration methods. The results of this research showed that the antimicrobial activity of the extract was dependent on the concentration and the highest diameter of the inhibition zone was observed at the concentration of 100 mg/ml of the extract. Based on disk diffusion agar and well diffusion agar tests, *Listeria monocytogenes* bacteria had the highest and *Escherichia coli*, *Salmonella typhi*, and *Bacillus cereus* had the lowest inhibition zones, respectively. The minimum inhibitory concentration for *Escherichia coli*, *Salmonella typhi*, *Listeria monocytogenes*, and *Bacillus cereus* bacteria was obtained as 64, 64, 16, and 32 mg/ml, respectively, and the minimum bactericidal concentration values for these bacteria were 512, 512, 256, and 256 mg/ml, respectively. In generally, the Gram-positive bacteria were more sensitive to the extract compared to the Gram-negative types. The growth of pathogenic bacteria and the diseases transmitted by these bacteria can be controlled by using natural antimicrobial compounds such as *R. knapweed* aqueous extract.

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1- Introduction

In recent years, diseases caused by infections and food poisoning have increased not only in poor and underdeveloped countries, but also in developed countries despite high health standards [1]. The connection between food consumption and human diseases was recognized very early, and Hippocrates (460 BC) reported that there was a strong connection between food consumed and human disease. Food-borne pathogens such as viruses, parasites, and bacteria*Listeria monocytogenes* ،*Escherichia coli*, different types of salmonella, etc. are biological agents that can cause foodborne illness. The outbreak of food-borne diseases is defined as the occurrence of two or more similar diseases as a result of consuming a common food [2]. Microorganisms that play a role in the occurrence of food poisoning and infections become resistant to antibiotics and antimicrobial drugs using different mechanisms, and this resistance can be observed against two or more antibiotics and antimicrobial drugs. This is considered a serious threat to human health, hence the efforts to discover new antimicrobial agents have increased [3]. In recent years, due to the harmful effects of chemical compounds and environmental issues, the tendency to use natural antimicrobial compounds such as essential oils and plant extracts has increased [4].

Plant extracts contain phenolic compounds such as phenolic acids, tannins, flavonoids, etc., which can be used against a large number of microorganisms. The antimicrobial effects of plant extracts against a wide range of microorganisms have been proven in various studies [5, 6]. For example, the antimicrobial activity of Mocha extract on a number of pathogenic and spoilage microorganisms*salmonella typhi, Escherichia coli* ،*Staphylococcus epidermidis* ،*Listeria Inuqua* And*Candida albicans* reported [7]. Also, the antimicrobial activity of red bell pepper extract [8], fennel plant extract [9], parsnip extract [10], hyssop extract [11], oregano extract, Shirazi thyme and rosemary extract [12] has also been proven.

Bitter plant is a medicinal plant that lowers blood sugar, which grows as a weed in the gardens and fields of Khuzestan province, especially in the cities of Ramhormoz and Dezful. Bitter with a scientific name*Russian knapweed* Or*Acroptilon repens* It is a herbaceous and hairless plant with a height of 10-50 cm and it belongs to the pea family [13]. Bitter is a multi-year plant and grows through seeds and rhizomes in wheat and corn fields and causes the flour of these crops to become bitter [14]. The bitter plant seeds contain glucoside, alkaloids and saponins and are rich in flavonoids. The extract of this plant plays a role in the treatment of epilepsy, chronotropic and hypertension [13]. It has also been reported that bitter plant seeds are effective in inhibiting gastric mucosal damage and have gastric protective and anti-secretory effects on gastric mucus [15]. Antimicrobial effect of bitter plant ethanol extract on pathogens such as*Staphylococcus aureus*، *Bacillus subtilis*، *Pseudomonas aeruginosa* And*Pastorala multocida* has been investigated [16].

Due to the medicinal and antimicrobial properties of the bitter plant, the purpose of this research is to investigate the antimicrobial properties of the aqueous extract of this plant on a number of pathogenic bacteria such as*Escherichia coli* ، *Salmonella typhi* ،*Listeria monocytogenes*And *Bacillus cereus* Was.

2- Materials and methods

1-2- bitter plant extract

After washing, bitter plant was completely dried at room temperature. Then the dried parts were powdered using a mill. Extraction from powdered plant was done using cold soaking method. Thus, 200 grams of the powdered plant was soaked in 500 ml of water and kept for 24 hours at room temperature under stirring conditions. After heating the sample for 20 minutes, the solution was centrifuged for 10 minutes at a speed of 300 rpm and filtered with filter paper. At the end, the prepared extract was stored in a closed container in the refrigerator [17].

2-2- Preparation of microbial suspension

After obtaining bacterial strains from the Faculty of Food Science and Technology, Khuzestan University of Agricultural Sciences and Natural Resources, the lyophilized bacterial cultures were cultured again under sterile conditions in Mueller Hinton broth for 24 hours at 37°C. In order to prepare fresh microbial suspension, stock culture was given in slant culture nutrient agar and after washing with sterile Ringer's solution, thick microbial suspension was prepared. This suspension was used to adjust the turbidity of the experimental suspension. The turbidity at 630 nm of each sample was adjusted using a spectrophotometer. The turbidity of the microbial suspension based on 0.5 McFarland or 10 CFU/ml^8 × 1.5 was prepared [18].

3-2- Agar diffusion disc

To perform the agar disk diffusion test, concentrations of 25, 50, 75 and 100 mg/ml extract were prepared. Then the blank disks were immersed in each of the prepared extract concentrations for 15 minutes. After the surface culture of each of the pathogens on Mueller Hinton agar culture medium, discs stained with the extract were fixed on the surface of the culture medium. At the end, the Petri dishes were kept in a greenhouse at 37°C for 24 hours, and the antimicrobial effect was determined by the diameter of the growth halo around the discs [19].

2-4- Agar well

In the agar well method, after creating wells on the surface of the Mueller Hinton agar culture medium and the bacterial suspension curd on the culture medium, 20 microliters of each of the prepared concentrations (25, 50, 75 and 100 mg/ml) were poured into the wells. and after being kept in a greenhouse for 24 hours at 37°C, the diameter of the growth halo was measured in millimeters [20].

2-5- minimum inhibitory concentration

The minimum inhibitory concentration of bitter plant aqueous extract was evaluated using microdilution method. From each of the concentrations of 16, 32, 64, 128, 256 and 512 mg/ml of the extract along with the microbial suspension was poured into a 96-well plate. Then the plate was kept at 37°C for 24 hours. After incubation, 20 microliters of 5% 2,3,5-triphenyltetrazolium chloride was added to each well of the plate. The first concentration in which no red color was observed was considered as the minimum inhibitory concentration of the extract [21].

2-6- Minimum lethal concentration

In order to determine the minimum lethality concentration of the extract, 100 microliters of the concentration of the wells without red color change in the minimum inhibitory concentration test were cultured on Mueller Hinton agar culture medium. The first concentration at which no bacterial growth was observed was reported as the minimum lethal concentration of the extract [22].

7-2- Statistical analysis

All the experiments of this research were done in three replicates. SPSS software (version 26) was used to analyze the data. Data evaluation was done using one-way analysis of variance and Duncan's multiple range test to determine significant differences ($p < 0.05$) was used.

3. Results and Discussion

In Table 1, the results of the antibacterial effect of bitter plant aqueous extract against each of the pathogenic bacteria used by the agar disk diffusion method are shown. It can be seen that increasing the concentration of the extract causes a significant increase in the diameter of the growth halo. However, bacteria*Escherichia coli, Salmonella typhi and Bacillus cereus* The smallest diameter of the aura of no growth and bacteria*Listeria monocytogenes* They attributed the greatest aura of lack of growth. The concentration of 25 mg/ml extract has an antimicrobial effect on bacteria*Escherichia coli* ،*Salmonella typhi* And*Bacillus cereus* did not show. Also in bacteria*Escherichia coli* No significant difference was observed between the diameter of growth halos in concentrations of 75 and 100 mg/ml extract. in bacteria*Listeria monocytogenes* Among all concentrations, a significant difference was found between the diameter of the growth halo. in bacteria*Salmonella typhi and Bacillus cereus*Similar results were found. In general, the diameter of the halo of non-growth in gram-positive bacteria*Listeria monocytogenes* And*Bacillus cereus* Larger than Gram-negative bacteria*Salmonella typhi* And*Escherichia coli* This indicates the greater sensitivity of gram-positive bacteria to bitter plant extract.

Table 1- Antimicrobial activity of *Russian knapweed* extract based on agar disk diffusion method (mm)

Concentration	25 mg/ml	50 mg/ml	75 mg/ml	100 mg/ml
Microorganism				
Escherichia coli	-	7.40 ±0.29^{b}	9.70 ±0.36^{a}	10.10 ±0.52^{a}
Salmonella typhi	-	7.20 ±0.32^{c}	9.30 ±0.24^{b}	11.50 ±0.42^{a}
Listeria monocytogenes	7.00 ±0.13^{d}	8.90 ±0.17^{c}	11.00 ±0.24^{b}	13.90 ±0.46^{a}
Bacillus cereus	-	7.60 ±0.14^{c}	9.90 ±0.11^{b}	12.30 ±0.27^{a}

Values are expressed as mean ±standard deviations, $n = 3$; different letters (a, b, c and d) in each row show significant difference at $p \leq 0.05$.

The results of the antimicrobial effect of bitter water extract based on the agar well method are reported in Table 2. In this method, similar to the agar disk diffusion method, the diameter of non-growth halos increased significantly with increasing extract concentration. The smallest halo diameter in bacteria*Escherichia coli* And*Salmonella typhi*and the largest halo diameter in bacteria*Listeria monocytogenes* was observed. The concentration of 25 mg/ml extract has antimicrobial effect on bacteria*Escherichia coli* And*Salmonella typhi*did not show. But in these bacteria, the diameter of the halo of non-growth in the concentrations of 50, 70 and 100 mg/ml were significantly different from each other. in bacteria*Listeria monocytogenes* However, a significant difference was observed in the diameter of the non-growth halo in all concentrations. in bacteria*Bacillus cereus* In concentrations of 25 and 50 mg/ml extract, there was no significant difference in the diameter of the growth halo, while there was a significant difference in other concentrations. As can be seen, at a constant concentration of the extract, the diameter of the halo of non-growth in the agar well method was larger than that of the agar disk

diffusion method. In this way tooDiameter of non-growth halo in bacteria*Listeria monocytogenes* And*Bacillus cereus* (Gram positive) larger than bacteria*Salmonella typhi* And*Escherichia coli* (gram negative) was.

Table 2- Antimicrobial activity of *Russian knapweed* extract based on agar well diffusion method (mm)

Concentration / **Microorganism**	25 mg/ml	50 mg/ml	75 mg/ml	100 mg/ml
Escherichia coli	-	7.80 ±0.14^{c}	10.30 ±0.40^{b}	12.20 ±0.26^{a}
Salmonella typhi	-	7.60 ±0.16^{c}	10.00 ±0.28^{b}	12.10 ±0.13^{a}
Listeria monocytogenes	7.30 ±0.18^{d}	9.60 ±0.12^{c}	11.50 ±0.17^{b}	14.80 ±0.38^{a}
Bacillus cereus	7.10 ±0.35^{c}	7.70 ±0.48^{c}	10.60 ±0.43^{b}	14.00 ±0.47^{a}

Values are expressed as mean ±standard deviations, $n = 3$; different letters (a, b, c and d) in each row show significant difference at $p \leq 0.05$.

The results of tests of the minimum inhibitory concentration and the minimum lethal concentration of bitter water extract against pathogenic bacteria are presented in Table 3. Minimum inhibitory concentration for bacteria *Listeria monocytogenes 16*mg/ml, for bacteria *Bacillus cereus* 32 mg/ml and for bacteria*Escherichia coli* And*Salmonella typhi* equal to 64 mg/ml,*Staphylococcus aureus* which indicates the greater sensitivity of Gram-positive bacteria compared to Gram-negative strains to the extract. The minimum lethal concentration for these bacteria*Escherichia coli* ،*Salmonella typhi*، *Listeria monocytogenes* And*Bacillus cereus* It was 512, 512, 256 and 256 mg/ml, respectively.

Table 3- Antimicrobial activity of *Russian knapweed* extract based on minimum inhibitory/bactericidal concentration method (mg/mL)

Microorganism	**MIC (mg/mL)**	**MBC (mg/mL)**
Escherichia coli	64	512
Salmonella typhi	64	512
Listeria monocytogenes	16	256
Bacillus cereus	32	256

Plants contain secondary metabolites that are stored as inactive precursors such as phenol, flavonoid and flavone in different parts of the plant and can show antimicrobial activity in vitro and in vivo [23-26]. The results of this research showed that the water extract of the bitter plant has a greater antimicrobial effect against gram-positive bacteria compared to gram-negative bacteria. This is due to the presence of a single layer of mucopeptide in the cell membrane of Gram-positive bacteria, which makes them more sensitive to antimicrobial agents. While in the cell membrane of gram-negative bacteria, there is a more complex layer of lipopolysaccharide and phospholipid with a lower diffusion rate, which is hydrophobic to antimicrobial compounds [27-29]. It was also found that the diameter of non-growth halos in the agar well method is larger compared to the agar disk diffusion method. The reason for this is the direct contact of bacterial species with

the extract in the agar well method, while the diffusion speed of the extract after passing through the disk surfaces in the agar disk diffusion method determines the inhibitory effect in this method [30]. In the investigation of the antimicrobial effect of bitter extract on a number of gram positive and negative bacteria by agar disk diffusion method, it was found that the prepared extract with a halo diameter of about 13 mm on gram positive bacteria.*Staphylococcus aureus* And*Bacillus subtilis* It has the most antimicrobial effect [16]. Antimicrobial effect of bitter plant essential oil on 3 gram positive bacteria*Staphylococcus aureus* ، *Staphylococcus epidermidis* And*Staphylococcus saprophyticus* and 3 gram-negative bacteria*Escherichia coli*، *Salmonella typhi* And*Shigella flexneri* It was measured by the agar well method by measuring the growth inhibition zone. has been reported that*Staphylococcus saprophyticus* And*Staphylococcus epidermidis* showed strong inhibition zones while*Staphylococcus aureus* showed low inhibition and Gram-negative bacteria showed resistance to the essential oil [31]. Antimicrobial property of extracts prepared with ethanol, chloroform, ethyl acetate and water from bitter plant by 4 methods of agar disk diffusion, agar well, minimum inhibitory and lethal concentration on bacteria*Pseudomonas aeruginosa* It was investigated and reported that the prepared extracts significantly prevent the growth of this bacterium [32]. Based on the results obtained in this study, the aqueous extract of bitter plant can be used as an antimicrobial compound to prevent food pathogens.

4 - Conclusion

The results of this study showed that by increasing the concentration of bitter water extract, its antibacterial activity increased and the type of bacteria was effective on the incidence of antimicrobial properties. Therefore, due to the natural nature of bitter plant extract, it can be used as a natural antibacterial compound against a wide range of microorganisms that cause infection and food poisoning. However, additional tests are needed to discover the mechanism of antimicrobial activity of the extract and increase its use in many food and pharmaceutical products.

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6- Resources

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چکیده

گیاه تلخه به عنوان یک داروی پایین آورنده قند خون شناخته می‌شود و خواص ضدمیکروبی عصاره‌ها و اسانس‌های تولیدی از آن به اثبات رسیده است؛ بنابراین، این مطالعه با هدف استخراج عصاره آبی گیاه تلخه و بررسی فعالیت ضدمیکروبی آن انجام شد. استخراج عصاره آبی تلخه با استفاده از روش خیساندن صورت گرفت. فعالیت ضد باکتریایی عصاره آبی تلخه در برابر باکتری‌های *اشرشیا کلی*، *سالمونلا تیفی*، *لیستریا مونوسیتوژنز* و *باسیلوس سرئوس* با استفاده از روش‌های دیسک دیفیوژن آگار، چاهک آگار، حداقل غلظت مهارکنندگی و حداقل غلظت کشندگی بررسی گردید. نتایج این پژوهش نشان داد که فعالیت ضدمیکروبی عصاره وابسته به غلظت بوده و بالاترین قطر هاله عدم رشد در غلظت ۱۰۰ میلی‌گرم در میلی‌لیتر عصاره مشاهده گردید. بر اساس آزمون‌های دیسک دیفیوژن آگار و چاهک آگار، باکتری *لیستریا مونوسیتوژنز* بالاترین و *اشرشیا کلی*، *سالمونلا تیفی* و *باسیلوس سرئوس* کمترین قطر هاله عدم رشد را به خود اختصاص دادند. حداقل غلظت مهارکنندگی برای باکتری‌های *اشرشیا کلی*، *سالمونلا تیفی*، *لیستریا مونوسیتوژنز* و *باسیلوس سرئوس* به ترتیب برابر با ۶۴، ۶۴، ۱۶ و ۳۲ میلی‌گرم در میلی‌لیتر به دست آمد و مقادیر حداقل غلظت کشندگی برای این باکتری‌ها به ترتیب ۵۱۲، ۵۱۲، ۲۵۶ و ۲۵۶ میلی‌گرم در میلی‌لیتر بود. بطور کلی، باکتری‌های گرم مثبت در مقایسه با انواع گرم منفی در برابر عصاره حساس‌تر بودند. رشد باکتری‌های پاتوژن و بیماری‌های منتقله از این باکتری‌ها را می‌توان با استفاده از ترکیبات ضد میکروب طبیعی مانند عصاره آبی گیاه تلخه کنترل نمود.