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Antimicrobial and Interactive Effects of the Aqueous Extract of *Bistorta officinalis* (Anjbar) on Selected Pathogenic Microorganisms *In vitro*

Behrooz Alizadeh Behbahani^{*1}, Mohammad Golbashy², Alaa G. Al-Hashimi³

1-Associate Professor, Department of Food Science and Technology, Faculty of Animal Science and Food Technology, Agricultural Sciences and Natural Resources University of Khuzestan, Mollasani, Iran.

2-Assistant Professor, Department of Plant Production and Genetics, Faculty of Agriculture, Agricultural Sciences and Natural Resources University of Khuzestan, Mollasani, Iran.

3-Food Science Department, College of Agriculture, University of Basrah, 61004 Basrah, Iraq.

ARTICLE INFO	ABSTRACT
Article History: Received:2025/4/17 Accepted:2025/5/28 Keywords: Bistorta officinalis, Antimicrobial activity, Foodborne pathogens, Natural preservatives, Minimum inhibitory concentration, Synergistic effect. DOI: 10.22034/FSCT.22.166.278. *Corresponding Author E- B.alizadeh@asnrukh.ac.ir	Foodborne diseases caused by pathogenic microorganisms pose a significant global health challenge, with an estimated 600 million cases and 420,000 deaths annually. This study investigates the antimicrobial potential of <i>Bistorta officinalis</i> (Anjbar) aqueous extract against foodborne pathogens, including <i>Listeria monocytogenes</i>, <i>Staphylococcus epidermidis</i>, <i>Erwinia amylovora</i>, <i>Pseudomonas syringae</i>, <i>Xanthomonas campestris</i>, and <i>Salmonella typhi</i>. The extract was prepared by soaking dried root powder in distilled water, followed by filtration and centrifugation. Antimicrobial activity was evaluated using disk diffusion agar, well diffusion agar, and minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) methods. Results showed that <i>L. monocytogenes</i> and <i>S. epidermidis</i> were the most susceptible, with inhibition zones of 12.06 mm and 11.95 mm, respectively, at 100 mg/mL extract concentration. The MIC values ranged from 4 mg/mL for <i>L. monocytogenes</i> to 16 mg/mL for <i>E. amylovora</i> and <i>S. typhi</i>. The MBC values were lowest for <i>L. monocytogenes</i> (128 mg/mL) and highest for <i>E. amylovora</i> and <i>S. typhi</i>. A synergistic effect was observed when the extract was combined with tetracycline. The study highlights the concentration-dependent antimicrobial efficacy of <i>B. officinalis</i> extract, particularly against Gram-positive bacteria, due to their permeable cell wall structure. These findings suggest that <i>B. officinalis</i> extract can serve as a natural preservative, offering a safe and eco-friendly alternative to synthetic additives in food preservation.

1-Introduction

Foodborne diseases caused by pathogenic microorganisms represent a significant global public health issue, impacting millions annually and placing a considerable economic strain on healthcare systems and the food industry. The World Health Organization estimates that 600 million individuals become ill each year from consuming contaminated food, resulting in approximately 420,000 deaths related to foodborne illnesses [1]. Common pathogens like strains of *Salmonella*, *Escherichia coli*, *Listeria monocytogenes*, and *Staphylococcus aureus* are frequently responsible for severe infections, such as gastroenteritis, septicemia, and even fatalities [2, 3]. The ongoing presence of these pathogens in food systems highlights an urgent need for effective strategies to inhibit their growth and ensure food safety. While traditional preservation methods like refrigeration and heat treatment are often inadequate for completely eradicating these microorganisms, there is a pressing need to investigate alternative preservation techniques [4, 5].

Although synthetic preservatives are commonly used to prolong the shelf life of food products and curb microbial growth, concerns regarding their long-term safety and health impacts have grown significantly. Long-term use of these substances has been associated with adverse health effects, including allergic reactions and potential carcinogenicity [6, 7]. This has spurred consumers and regulatory bodies to seek safer, natural alternatives to synthetic preservatives, prompting research into plant-based options [8-14].

Botanical preservatives, especially plant extracts, have gained attention as promising alternatives due to their antimicrobial, antioxidant, and anti-inflammatory properties. Plants produce various

secondary metabolites, including phenolics, flavonoids, alkaloids, and essential oils, which have demonstrated strong antimicrobial effects against foodborne pathogens. These natural compounds not only improve food safety but also cater to the increasing consumer demand for clean-label products that do not contain synthetic additives [15–25]. Among the many plants studied for their preservative qualities, Anjbar (*Bistorta officinalis*) has been noted for its bioactive properties.

B. officinalis, a perennial herb indigenous to Europe and Asia, has been used traditionally in folk medicine for its anti-inflammatory, antioxidant, and antimicrobial effects. It is rich in bioactive compounds, including tannins, flavonoids, and phenolic acids, which contribute to its therapeutic benefits [26]. Recent research has underscored the potential of extracts and essential oils from *B. officinalis* as natural preservatives in food applications. For instance, its methanolic extract has demonstrated significant antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli*, indicating its viability for food preservation [27]. Additionally, the antioxidant properties of the plant can help mitigate oxidative spoilage, thereby expanding its usability in food applications.

This study seeks to explore the antimicrobial effects of *B. officinalis* aqueous extract by employing methods such as disk diffusion agar, well diffusion agar, and determining the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against pathogenic microorganisms.

2- Materials and Methods

2.1. Extract preparation

Fresh roots of Anjbar were gathered from the Sahand highlands in Azerbaijan. Initially, the roots were chopped into smaller segments and subsequently dried in

the shade. Following this, 300 g of the dried root powder was soaked in 1200 ml of distilled water for 72 hours at room temperature, after which it was filtered and centrifuged for 20 minutes. The resulting supernatant was dried at 37 °C, and the resulting semi-solid mass was then stored in the refrigerator for later use [26].

2.2. Antimicrobial activity

The antimicrobial effectiveness of the aqueous extract of Anjbar was assessed against *Erwinia amylovora*, *Pseudomonas syringae*, *Xanthomonas campestris*, *Salmonella typhi*, *Staphylococcus epidermidis*, and *Listeria monocytogenes* using disk diffusion agar, well diffusion agar, and minimum inhibitory and bactericidal concentration methods.

2.2.1. Disk diffusion agar

Various concentrations of the extract (25, 50, 75, and 100 mg/ml) were sterilized with a 0.45-micron syringe microfilter. Blank discs were subsequently soaked in the extract solutions for 15 minutes before being positioned on the Mueller Hinton agar surface. The culture medium was incubated at 37°C for 24 hours, and the antimicrobial effect was evaluated by measuring the diameter of the inhibition zone surrounding the discs [28].

2.6.2. Well diffusion agar

In this procedure, a microbial suspension was applied to Mueller Hinton agar plates in Petri dishes using an L-shaped spreader. Subsequently, wells with a diameter of 6 mm were formed on the surface of the culture medium, into which extracts at varying concentrations (25, 50, 75, and 100 mg/ml) were introduced. The Petri dishes were then incubated at 37°C for 24 hours, after which the diameter of the inhibition zones surrounding the wells was measured [24].

2.2.3. Minimum inhibitory and bactericidal concentrations ((MIC & MBC)

The minimum inhibitory concentration (MIC) was determined employing the broth microdilution technique. Serial dilutions of the aqueous extract of Anjbar (512, 256, 128, 64, 32, 16, 8, 4, 2, and 1 mg/mL) were prepared in Mueller-Hinton broth on microplates, to which microbial suspensions (1.5×10^8 CFU/mL) were added. The plates were then incubated at 37°C for 24 hours. After this period, triphenyltetrazolium chloride (5 mg/mL) was added, followed by a 30-minute incubation, with a red coloration indicating microbial activity in the wells. The MIC was identified as the lowest concentration of the extract that inhibited visible growth. For the minimum bactericidal concentration (MBC) test, 100 µl of culture from each well that showed no red color was spread onto Mueller Hinton agar. These plates were incubated at 37°C for 18 to 24 hours, and the lowest dilution exhibiting complete growth inhibition was recorded as the MBC of the extract [29–31].

2.2.4. Interaction of Anjbar extract with tetracycline antibiotic

The study examined the interaction between Anjbar extract and tetracycline antibiotic by using concentrations equal to half of the minimum inhibitory concentration. In this process, a standard microbial culture was prepared as a lawn on Mueller Hinton agar containing the extract. A disk of tetracycline antibiotic was placed on the surface of the microbial culture using sterile forceps. The samples were then incubated at 37°C for 24 hours, after which the zone of inhibition was measured and recorded in millimeters [17].

2.3. Statistical analysis

All experiments were conducted in triplicate. The results were analyzed using Minitab software (version 16) and Tukey's test at a significance level of 5%.

3-Results and Discussion

The findings illustrated in Figure 1 demonstrate the antimicrobial properties of

the aqueous extract of Anjbar against various foodborne pathogens, assessed through the disk diffusion agar method by measuring the zone of inhibition. Analysis revealed significant differences in the inhibition zones across different bacterial species. Notably, *Listeria monocytogenes* (12.06 mm) and *Staphylococcus epidermidis* (11.95 mm) exhibited the largest zones of inhibition, suggesting they were the most susceptible to the plant extract ($p < 0.05$). In contrast, *Pseudomonas syringae*, *Xanthomonas campestris*, *Erwinia amylovora*, and *Salmonella typhi* displayed smaller inhibition diameters, with mean values ranging from 10.37 to 9.38 mm, indicating a moderate sensitivity to the aqueous extract of Anjbar.

Moreover, the concentration of the extract significantly influenced the diameter of inhibition. The highest concentration (100 mg/ml) resulted in the most extensive inhibition zones (13.25 mm); as the concentration decreased, the inhibition zones also significantly diminished,

highlighting a concentration-dependent antimicrobial effect where higher concentrations of the extract were more effective in suppressing bacterial growth. The interaction between bacterial type and extract concentration further revealed that at the top concentration (100 mg/ml), *Listeria monocytogenes* (15.90 mm) and *Staphylococcus epidermidis* (14.70 mm) exhibited the largest inhibition zones ($p < 0.05$). As the extract concentration decreased, the inhibition diameters for these bacteria also declined, though they remained larger compared to other bacteria at the same concentrations.

Overall, the antimicrobial efficacy of the aqueous plant extract, as determined by the disk diffusion agar method, was significant against a broad spectrum of foodborne pathogens, with its effectiveness influenced by both bacterial species and extract concentration. These results suggest that the plant extract could serve as a natural antimicrobial agent for controlling foodborne pathogens, particularly at higher concentrations.

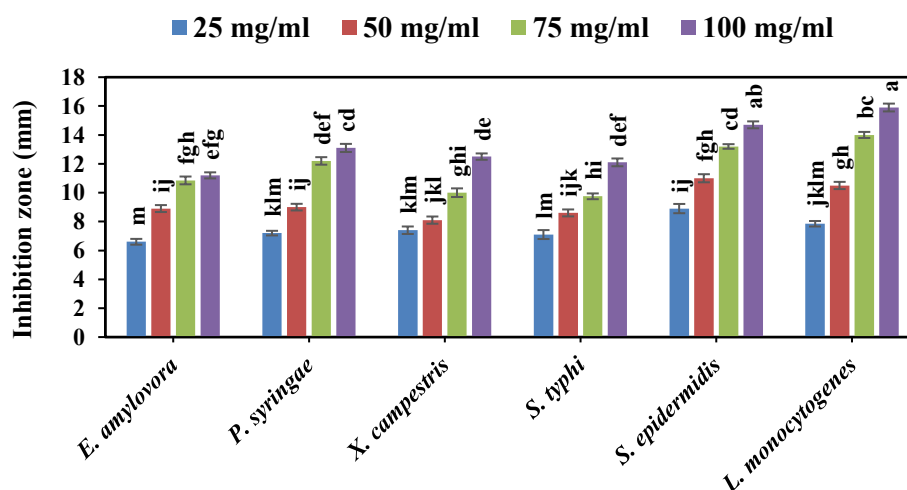


Figure 1. The antimicrobial activity of *Bistorta officinalis* aqueous extract based on disk diffusion agar method. Treatments labeled with different letters show significant differences at $p < 0.05$.

Figure 2 illustrates the antimicrobial effects of the aqueous extract of Anjbar on various foodborne pathogens assessed through the well diffusion agar method. The results indicate that *Listeria monocytogenes* and

Staphylococcus epidermidis exhibited the highest mean inhibition diameters (12.67 mm and 12.65 mm, respectively), demonstrating a greater sensitivity of these bacteria to the extract. The concentration of the extract significantly influenced the

inhibition diameter, with the highest concentration (100 mg/ml) resulting in the largest zone of inhibition (14.15 mm), reflecting the most potent antimicrobial activity. Additionally, the interaction results confirmed that *Listeria monocytogenes* and *Staphylococcus epidermidis* were the most susceptible strains to the aqueous extract of Anjbar.

Furthermore, the extract's effectiveness was found to be concentration-dependent, as larger concentrations yielded wider zones of inhibition. A comparison of the no-growth diameters between the disk diffusion agar and well diffusion agar methods revealed that the agar well method produced larger zones of no growth.

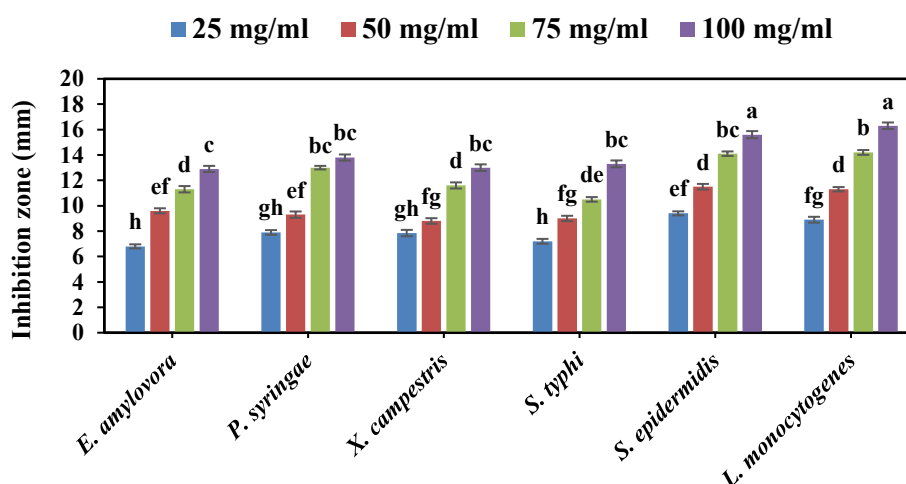


Figure 2. The antimicrobial activity of *Bistorta officinalis* aqueous extract based on well diffusion agar method. Treatments labeled with different letters show significant differences at $p < 0.05$.

Figure 3 presents the minimum inhibitory concentration (MIC) values, expressed in mg/mL, for the evaluated aqueous extract of Anjbar against various foodborne pathogens. A lower MIC value signifies greater efficacy of the plant extract in suppressing the growth of these pathogens.

The most notable MIC of 4 mg/mL was recorded for *Listeria monocytogenes*, suggesting that the extract is highly effective and may fully inhibit the pathogen's growth at minimal concentrations. In contrast, the highest MIC values of 16 mg/mL were found for *Erwinia amylovora* and *Salmonella typhi*.

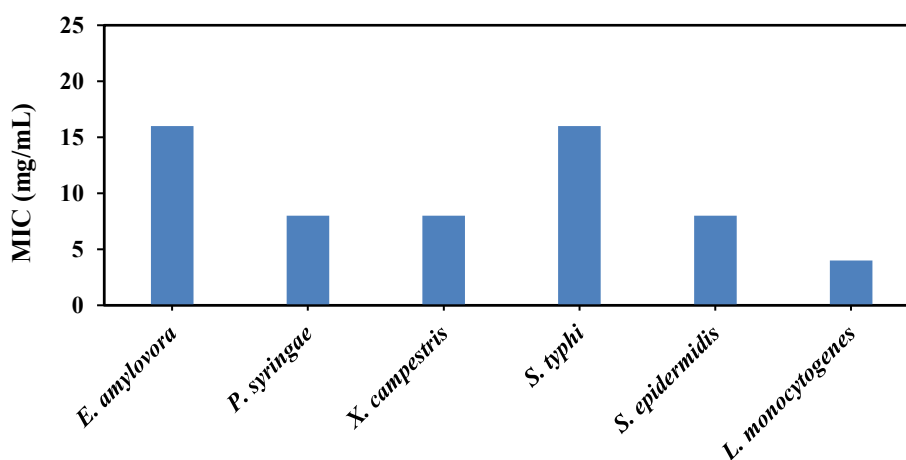


Figure 3. The antibacterial activity of *Bistorta officinalis* aqueous extract based on minimum inhibitory concentration (MIC) method.

Figure 4 illustrates the findings regarding the minimum bactericidal concentration (MBC) of the aqueous extract of Anjbar. The results indicate that the lowest MBC

recorded was 128 mg/ml for *Listeria monocytogenes*, while the highest MBC was observed for *Erwinia amylovora* and *Salmonella typhi*.

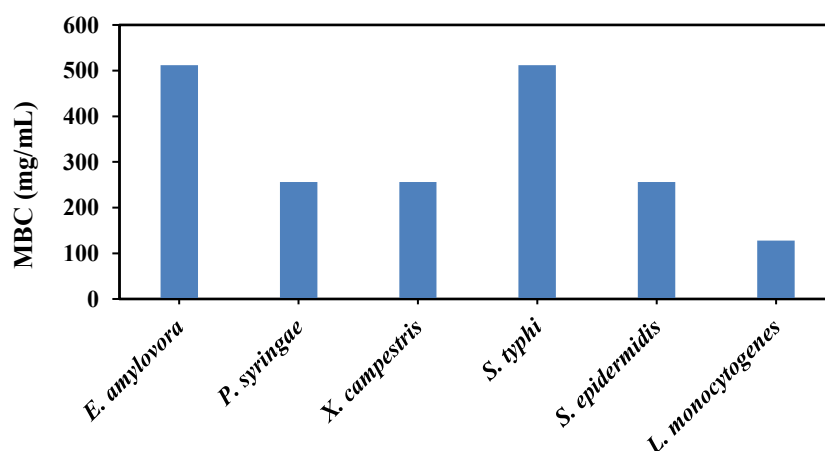


Figure 4. The antibacterial activity of *Bistorta officinalis* aqueous extract based on minimum bactericidal concentration (MBC) method.

The findings illustrated in Figure 5 demonstrate the combined antimicrobial effects of Anjbar's aqueous extract and tetracycline against various foodborne pathogens. *Listeria monocytogenes* and *Staphylococcus epidermidis* exhibited the highest mean diameter of the inhibition zone, while other bacteria displayed moderate sensitivity to the Anjbar extract. Additionally, the interaction between the herbal extract and tetracycline yielded a zone of inhibition measuring 16.99 mm,

which signifies a notable increase in effectiveness compared to tetracycline alone (13.70 mm). These results suggest that the combination of the herbal extract and tetracycline produces a synergistic effect, significantly enhancing antimicrobial activity against a broad spectrum of foodborne pathogens. This integrated approach presents a promising strategy for managing foodborne pathogens, potentially reducing reliance solely on antibiotics and the associated risk of antibiotic resistance.

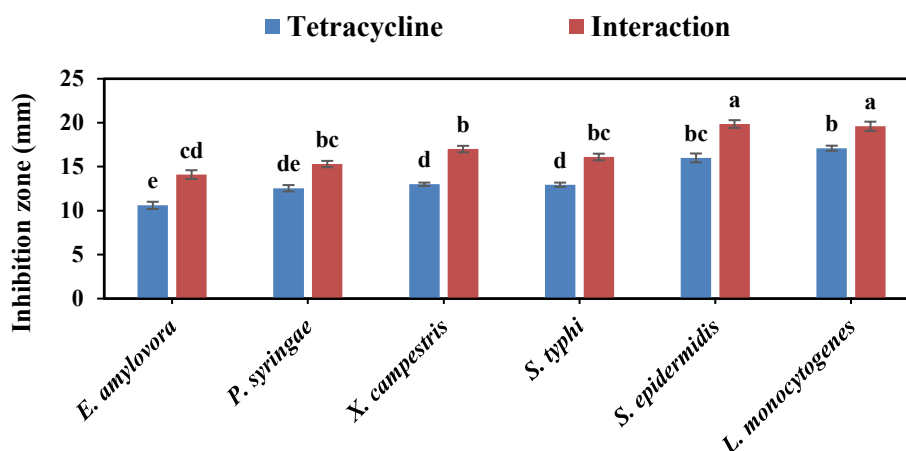


Figure 5. The antibacterial activity of *Bistorta officinalis* aqueous extract based on interaction method (tetracycline+extract). Treatments labeled with different letters show significant differences at $p < 0.05$.

In their 2020 study, Pawlowska et al. examined the antibacterial and anti-inflammatory effects of the aqueous extract of *Polygonum bistorta*. Their findings revealed that the rhizome extract is rich in notable phytochemicals, particularly flavan-3-ols and galloyl glucose, which are significant in modulating neutrophil activity crucial for inflammation and wound healing. These results endorse the traditional application of *Polygonum bistorta* as an effective topical remedy for skin infections and inflammatory conditions. Furthermore, the aqueous extract demonstrated considerable antibacterial activity against prevalent skin pathogens, including *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Helicobacter pylori* [32].

In a separate study, the antibacterial properties of the ethanolic extract of *Polygonum bistorta* root were assessed, comparing its efficacy to common therapeutic antibiotics. The broth microdilution method was employed to analyze antibacterial effects, determining MIC and MBC values. Additionally, the disk diffusion agar method was used to evaluate the extract's impact on the growth of *Enterobacter aerogenes*, *Klebsiella pneumoniae*, *Proteus vulgaris*, and *Salmonella typhimurium* relative to antibiotics like erythromycin, penicillin, and amoxicillin. The results indicated that the hydroalcoholic extract of the root exerted a significant inhibitory effect on the tested bacteria, with the inhibition zone diameter increasing alongside extract concentration. At high concentrations, the antibacterial efficacy of the extract was comparable to, or superior than, that of the tested antibiotics. The broth microdilution method further confirmed the inhibitory and bactericidal actions of the extract, identifying *Proteus vulgaris* as the most resistant and *Klebsiella pneumoniae* as the most sensitive to the plant extract [33].

Qilich et al. (2014) similarly explored the antibacterial properties of the methanolic

extract of *Polygonum bistorta* against *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Staphylococcus aureus* using various antimicrobial methods, including disk diffusion agar and minimum inhibitory concentration tests. Their results further validated the significant antimicrobial effects of the extract against the studied pathogens [27].

The antimicrobial properties of plant extracts are attributed to the presence of phenolic compounds, which, due to their hydrophobic nature and ability to bind to amine and hydroxylamine groups in proteins, effectively disrupt cell membranes and mitochondrial lipids, altering membrane permeability and leading to bacterial cell death [34, 35].

Notably, the study findings indicated that Gram-positive bacteria are more susceptible to the aqueous extract of Anjbar than Gram-negative bacteria. This observation aligns with reports suggesting that Gram-positive bacteria are generally more sensitive to essential oils or plant extracts due to differences in cell wall structure and permeability. Gram-positive bacteria, characterized by a thick peptidoglycan layer in their cell wall, are more susceptible to the penetration of hydrophobic compounds, as they lack an outer membrane. In contrast, Gram-negative bacteria possess a thinner peptidoglycan layer and an additional outer membrane made up of lipopolysaccharides, which creates a barrier that hinders the access of hydrophobic compounds to the inner membrane [12, 17, 29, 31, 36, 37].

Overall, the aqueous extract of the Anjbar plant contains bioactive compounds such as polyphenols, terpenes, and alkaloids that inhibit the growth of pathogenic and spoilage microorganisms, potentially extending the shelf life of food products. This extract presents a natural alternative to synthetic preservatives, effectively catering

to consumer preferences for eco-friendly products.

4-Conclusion

The study emphasizes the notable antimicrobial potential of the aqueous extract of *Bistorta officinalis* (Anjbar) against various foodborne pathogens, demonstrating its effectiveness as a natural preservative in the realm of food science. The extract exhibited antimicrobial activity that was dependent on concentration, with higher levels resulting in larger zones of inhibition, particularly against Gram-positive bacteria such as *Listeria monocytogenes* and *Staphylococcus epidermidis*. The determined MIC and MBC values further validated the extract's efficacy, identifying *Listeria monocytogenes* as the most sensitive pathogen. Moreover, the observed synergistic effect between the Anjbar extract and tetracycline points to a promising approach to enhance antimicrobial effectiveness while decreasing dependency on antibiotics, thereby lessening the risk of antibiotic resistance. These findings are consistent with prior research on the antimicrobial properties of plant extracts, which attribute their effectiveness to bioactive compounds like polyphenols, terpenes, and alkaloids that disrupt microbial cell membranes and inhibit growth. Overall, the study highlights the potential of *B. officinalis* extract as a natural, eco-friendly alternative to synthetic preservatives, which meets consumer demand for clean-label products and contributes to enhanced food safety and shelf life. Further research is needed to investigate its applications across various food systems and evaluate its long-term stability and safety.

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مقاله علمی-پژوهشی

اثر ضد میکروبی و برهمکنش عصاره آبی انجبار بر تعدادی از میکروارگانیسم های بیماری زا در شرایط آزمایشگاهی

بهروز عزیزاده بهبهانی^{۱*}، محمد گلباشی^۲، Alaa G. Al-Hashimi^۳

۱-دانشیار، گروه علوم و مهندسی صنایع غذایی، دانشکده علوم دامی و صنایع غذایی، دانشگاه علوم کشاورزی و منابع طبیعی خوزستان، ملاتانی، ایران

۲-استادیار، گروه مهندسی تولید و ژنتیک گیاهی، دانشکده کشاورزی، دانشگاه علوم کشاورزی و منابع طبیعی خوزستان، ملاتانی، ایران

3-Food Science Department, College of Agriculture, University of Basrah, 61004 Basrah, Iraq

اطلاعات مقاله	چکیده
تاریخ های مقاله : تاریخ دریافت: ۱۴۰۴/۱/۲۸ تاریخ پذیرش: ۱۴۰۴/۳/۷	بیماری های ناشی از غذا که توسط میکروارگانیسم های بیماری زا ایجاد می شوند، با حدود ۶۰۰ میلیون مورد و ۴۲۰،۰۰۰ مرگ سالانه، یک چالش بهداشت جهانی مهم است. در مطالعه حاضر، پتانسیل ضد میکروبی عصاره آبی انجبار (<i>Bistorta officinalis</i>) در برابر عوامل بیماری زای غذایی از جمله لیستریا مونوسیتوژنز، استافیلوکوکوس اپیدرمیدیس، اروینیا امیلوورا، سودوموناس سیرینگه، زانتوموناس کمپستریس و سالمونلا تیفی مورد بررسی قرار گرفت. عصاره با خیساندن پودر ریشه خشک شده در آب مقطر و سپس فیلتراسیون و سانتریفیوژ تهیه شد. فعالیت ضد میکروبی با استفاده از روش های دیسک دیفیوژن آگار، چاهک آگار و حداقل غلظت مهارکننده (MIC) و حداقل غلظت باکتری کشی (MBC) ارزیابی شد. نتایج نشان داد که لیستریا مونوسیتوژنز و استافیلوکوکوس اپیدرمیدیس با مناطق بازدارندگی به ترتیب ۱۲/۰۶ و ۱۱/۹۵ میلی متر در غلظت ۱۰۰ میلی گرم بر میلی لیتر حساس ترین حساسیت ها بودند. مقادیر MIC از ۴ میلی گرم در میلی لیتر برای لیستریا مونوسیتوژنز تا ۱۶ میلی گرم در میلی لیتر برای اروینیا امیلوورا و سالمونلا تیفی متغیر بود. مقادیر MBC برای لیستریا مونوسیتوژنز کمترین (۱۲۸ میلی گرم در میلی لیتر) و برای اروینیا امیلوورا و سالمونلا تیفی بالاترین بود. هنگامی که عصاره با تتراسایکلین ترکیب شد، یک اثر هم افزایی مشاهده شد. این مطالعه اثر ضد میکروبی وابسته به غلظت عصاره انجبار، به ویژه در برابر باکتری های گرم مثبت، به دلیل ساختار دیواره سلولی نفوذپذیر آنها را برجسته می کند. این یافته ها نشان می دهد که عصاره انجبار می تواند به عنوان یک نگهدارنده طبیعی عمل کند و جایگزینی امن و سازگار با محیط زیست برای افزودنی های مصنوعی در نگهداری مواد غذایی باشد.
کلمات کلیدی: انجبار، فعالیت ضد میکروبی، پاتوژن های غذایی، نگهدارنده های طبیعی، حداقل غلظت بازدارنده، اثر هم افزایی.	
DOI: 10.22034/FSCT.22.166.278. * مسئول مکاتبات: B.alizadeh@asnruk.ac.ir	