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Evaluation of the antimicrobial effect of Badrashboo *(Dracocephalum moldavica)* essential oil and its interaction with some common antibiotics against some pathogenic bacteria

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ABSTRACT

Nowadays, due to the harmful effects of chemical preservatives in food products and antibiotic resistance too, the efforts of researchers to use natural and safe antimicrobial compounds, including plant essential oils, have increased. In the present study, after collecting the Badrashboo plant from the fields around Urmia city and drying it, extracting the essential oil from the plant was carried out using a Clevenger device, and the antimicrobial effects of this essential oil against some Gram-positive and Gram-negative food-borne pathogenic bacteria were determined by methods: Disk Diffusion Agar (DDA), Well Diffusion Agar (WDA), Minimum inhibitory concentration (MIC), Minimum bactericidal concentration (MBC) and interaction with four common broad-spectrum antibiotics including Vancomycin, Erythromycin, Chloramphenicol and Gentamicin were performed. The results of the DDA and WDA tests showed that the essential oil of Badreshbo had significant antimicrobial effects on all the tested bacteria in this study. The gram-positive bacteria were more sensitive than the gram-negative bacteria in front of this essential oil. The results of the MIC test of the essential oil for *Salmonella typhimurium*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Shigella dysentery*, *Staphylococcus aureus*, *Bacillus cereus*, and *Listeria monostogenes* were 2.5, 1.25, 1.25, 0.625, 0.312, 1.25 and 1.25 mg/ml. The MBC of the mentioned strains were 5, 5, 2.5, 5, 2.5, 2.5, and 2.5 mg/mL, respectively. Also, the results of the study of the interaction effect of Badreshbo essential oil with the mentioned antibiotics indicate synergistic effects of the essential oil with all four antibiotics tested. Therefore, considering the significant antimicrobial effects observed for Badrashbo essential oil in this study, it can be used in the food and pharmaceutical industries.

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1- Introduction

According to the statistics published by the World Health Organization, many people die every year due to food-borne diseases. In this regard, the excessive use of antibiotics has unfortunately led to the creation of resistant microbial strains [1]. Traditional antibiotics have gradually lost their effectiveness against many pathogenic bacteria due to the rapid growth of antibiotic resistance in microorganisms. Therefore, there is an urgent demand for new antimicrobial agents that can replace the antimicrobial activity of old antibiotics [2]. Medicinal and aromatic plants have been used in traditional medicine all over the world for thousands of years [3]. This growing interest in finding new and safe antimicrobial molecules of natural origin, especially from plants, has increased in the past decades. For this purpose, the scientific community has focused its attention on natural, safe and effective antimicrobial molecules, especially essential oils. The different molecules in essential oils can create a synergistic effect and provide higher protection than that obtained by individual molecules, as well as reduce the multidrug resistance that occurs in various infections and inhibit contamination by food pathogens [4].

Iran is considered one of the best regions in the world in terms of its geographical location and climate diversity in the field of growing different types of medicinal plants. It has been a source of production and consumption of medicinal plants for centuries. Of course, the medicinal and therapeutic properties of each plant depend on the type and amount of its effective compounds [5].

Badrashboo (Dracocephalum moldavica), also known as *Dragon head*, is a fragrant, herbaceous plant of the mint family, native to northwestern Iran and found in Tabriz, Urmia, Yazd, Mazandaran, and the Alborz mountains [6]. The fresh plant contains approximately 0.4% of volatile oils, of which 43% is Citral. This content may increase depending on the altitude of the cultivation site (for example, a 70% increase at 800 meters), while geraniol content decreases under the same conditions. The accumulation of volatile oils increases during the plant's reproductive period, reaching a maximum at the end of flowering. *Badrashboo* is typically planted in spring and harvested about 90 days later. Each hectare can yield eight to ten tons of green matter for volatile oil or dry matter production [7]. Due to its desirable sensory properties, this plant is often used as a food additive or infusion and possesses a wide range of medicinal effects including anti-inflammatory, antioxidant, heart protection, treatment of liver and stomach disorders, headache, and congestion [2, 8]. It finds applications in the pharmaceutical, cosmetic, food, and perfume industries [3]. Previous research has identified various main components of *Badrashboo* essential oil. These variations are attributed to factors like the plant's growth region, ecological and climatic conditions, and storage duration. Some of the most important compounds include geranial, neral, geraniol acetate, geranyl acetate, and hexadecanoic acid [9].

Considering the increasing consumer demand for natural preservatives, particularly plant essential oils as alternatives to chemical preservatives, and the growing body of research on the beneficial properties of *Badrashboo*, this study aims to investigate the antimicrobial activity of *Badrashboo* essential oil. This research will explore the oil's efficacy alone and in combination with antibiotics (Vancomycin, Erythromycin, Chloramphenicol, and Gentamicin) against a range of common foodborne pathogens: *Salmonella typhimurium*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Shigella dysentery*, *Staphylococcus aureus*, *Bacillus cereus*, and *Listeria monocytogenes*. The investigation into the combined effects of *Badrashboo* essential oil and antibiotics holds promise for potentially creating a synergistic effect, enhancing antimicrobial activity, and potentially overcoming emerging antibiotic resistance.

2- Materials and methods

2-1 - Preparation of materials

For the present study, culture mediums of Muller Hinton Agar and Muller Hinton Broth (QUE lab Company), blank discs and antibiotic discs including Vancomycin, Erythromycin, Chloramphenicol and Gentamicin (made by Padtan Teb Company), Dimethylsulfoxide (Uni Chem Company), syringe filter (0.22 Micron) and Triphenyl tetrazolium chloride (Solarbio Company) were used.

2-2- preparing *Badrashboo* and extraction of its essential oil

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The *Badrashboo* plant was purchased from the fields of the surrounding villages of Urmia city and was approved by the Faculty of Agriculture, Agricultural Sciences and Natural Resources University of Khuzestan with the scientific name *Dracocephalum moldavi L* and herbarium code KUAU653. The purchased plant was dried at room temperature and stored under refrigeration until essential oil extraction. Extraction of essential oil from *Badrashboo* was done with Clevenger. In this way, each time 100 grams of the powdered plant was poured with distilled water in the Clevenger and essential oil extraction was done within 3 hours. The collected essential oil was dewatered with sodium sulfate and filtered and kept in dark sterile containers at 4 degrees Celsius until the tests were performed, and the percentage of essential oil was obtained from equation (1):

Equation (1): percentage of essential oil = the weight of the essential oil obtained from the sample (grams) / the weight of the sample in grams × 100 [10].

2-3- Preparation of microbial strains and standard of 0.5 McFarland

Standard microbial suspensions were prepared from reserve strains of *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhymurium* ATCC 14028, *Escherichia coli* ATCC 25922, *Shigella dysentery* PTCC 188, *Staphylococcus aureus* ATCC 25923, *Listeria monocytogenes* ATCC 19115, *Bacillus cereus* ATCC 14579 available in the Food Microbiology Laboratory, Department of Science and Food Industry Engineering, Agricultural Sciences and Natural Resources University of Khuzestan. These strains were cultured and adjusted to a 0.5 McFarland turbidity using Mueller Hinton Agar [11].

2-4- The test of antimicrobial activity of *Badrashboo* essential oil by disk diffusion Agar (DDA) method

The method described by Yasin et al. (2022) was adapted for this study [11]. Briefly, all equipment and culture media were sterilized using an autoclave. Concentrations of 0.625, 1.25, 2.5, 5, 10, 20 and 40 mg/mL of *Badrashboo* essential oil were prepared in a solution containing 0.5% (w/v) Dimethylsulfoxide (DMSO) and 9.5% (w/v) distilled water. The solutions were then filtered and dispensed into sterile tubes. Mueller-Hinton agar plates were inoculated with 100 microliters of a 0.5 McFarland microbial suspension spread evenly over the surface. Sterile blank paper discs were placed on the inoculated agar at designated distances from each other and the plate's edge. 20 microliters of each essential oil concentration were pipetted onto the respective discs. The plates were incubated at 37°C for 24 hours in an incubator. Finally, the diameters of the clear zones (halos) surrounding the discs, indicative of inhibited microbial growth, were measured in millimeters [11].

2-5- The test of antimicrobial activity of *Badrashboo essential* oil by well diffusion Agar (WDA) method

Concentrations of 0.625, 1.25, 2.5, 5, 10, 20 and 40 mg/mL of *Badrashboo* essential oil were prepared in a solution containing 0.5% (w/v) Dimethylsulfoxide (DMSO) and 9.5% (w/v) distilled water. After filtration (for sterilization), the solutions were dispensed into sterile tubes. Mueller-Hinton agar plates were then inoculated with 100 microliters of a 0.5 McFarland microbial suspension spread evenly over the surface. To assess the antimicrobial effect, sterile wells were created in the agar using the tip of a sterile Pasteur pipette. These wells were made at a set distance from each other and the edge of the plate. 20 microliters of each essential oil concentration were then added to the respective wells. The inoculated plates were incubated at 37°C for 24 hours. Subsequently, the diameters of the inhibition zones (halos) surrounding the wells were measured in millimeters [12].

2-6- The test of the minimum inhibitory concentration (MIC) of *Badrashboo* essential oil

Concentrations of 0.078, 0.156, 0.312, 0.625, 1.25, 2.5, 5 from *Badrashboo* essential oil (mg/mL) were prepared in a solution containing 0.5% (w/v) Dimethylsulfoxide (DMSO) and 9.5% (w/v) distilled water to determine the minimum inhibitory concentration (MIC) [13]. A 96-well plate was used, where each well received 100 microliters of a different essential oil concentration and 20 microliters of a 0.5 McFarland microbial suspension. Two control wells were included in each row: one containing only the highest essential oil concentration (without microorganisms) and another containing Mueller-Hinton broth medium inoculated with the microbial suspension (without essential oil). The plate was incubated at 37°C for 24 hours. Then, 20 microliters of a 5

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mg/mL Triphenyl tetrazolium chloride (TTC) solution were added to each well. Viable microorganisms in the wells converted the TTC to a dark red or purple color. The MIC was recorded as the lowest concentration of essential oil that exhibited no color change and no microbial growth [14].

2-7- The test of the minimum bactricidal concentration (MBC) of *Badrashboo* essential oil

The minimum bactericidal concentration (MBC) of *Badrashboo* essential oil was determined using a modified version of the method by Carvalho et al. (2018) [15]. Briefly, 100 microliters of inoculum from wells showing no color change at the MIC (minimum inhibitory concentration) were transferred to fresh Mueller-Hinton agar plates. These plates were then incubated at 37°C. The MBC was identified as the lowest concentration that exhibited no bacterial growth after incubation.

2-8- The test of the interaction of *Badrashboo* essential oil in combination with some common therapeutic antibiotics

The Kirby-Boer method, as described by Zamanpour Borojeni et al. (2023), was used to assess the interaction between *Badrashboo* essential oil and four common antibiotics: Vancomycin, Erythromycin, Chloramphenicol, and Gentamicin. Briefly, the minimum inhibitory concentration (MIC) of the essential oil for each studied microbial strain was determined. Sub-inhibitory concentrations (half of the MIC for each organism) were then incorporated into Mueller-Hinton agar medium. Subsequently, 100 microliters of a 0.5 McFarland suspension of each microorganism were cultured onto the agar. Antibiotic discs were placed at appropriate distances from the edge of each plate. After incubation, the diameter of the inhibition zones around the discs was measured (in millimeters), following standard disk diffusion methods [16].

9-2- Statistical analysis

The data were analyzed using a completely randomized design with SPSS software (version 20). To assess the antimicrobial effect of different *Badrashboo* essential oil concentrations on various bacteria, one-way ANOVA was followed by Duncan's multiple range test for mean comparisons. The independent t-test was used to compare the antibiotic's antimicrobial activity and its interaction with the essential oil.

3-Results and Discussion

3-1-Efficiency of essential oil

The calculation of the efficiency of *Badrashboo* essential oil showed that after three hours, 0.5 ml of essential oil was extracted from 100 grams of *Badrashboo* plant powder, on the basis of which the volume-weight efficiency of *Badrashboo* essential oil was determined to be almost equal to 0.5%. This amount was consistent with the results of Yusufzadeh (2016), who investigated the changes in the percentage and components of the essential oil of *Badrashboo* plant in different regions of East and West Azerbaijan provinces. This researcher reported that the higher nitrogen and organic matter of the soil is probably one of the influential factors in increasing the percentage of essential oil in the plant population of a region, because essential oils are terpenoid compounds and their building units need elements such as nitrogen, and as a result, nitrogen is one of the most important factors. It has an effect on plant growth and the percentage of essential oil of medicinal plants [17]. A study by Amirian et al. (2023) demonstrated that several factors significantly influence the yield of *Badrashboo* essential oil, including the year of cultivation, the planting pattern used, and the type of fertilizer applied [18]. Murshidlou et al. (2020) further investigated the impact of drying methods on *Badrashboo* essential oil extraction, reporting a significant variation in yield from 0.45% to 1.85%. Their findings suggest that drying methods significantly affect the amount of essential oil extracted. They observed that lower drying temperatures minimize destructive forces and potentially increase essential oil content. Conversely, increasing the drying temperature from 40°C to 60°C led to a decrease in essential oil content. This is likely due to the damaging effects of higher temperatures on cell walls and membranes, causing the loss of volatile oils. Additionally, Murshidlou et al. (2020) propose that increased drying temperatures promote the decomposition of essential oil compounds through autoxidation and hydroperoxidation, ultimately reducing essential oil content by destroying storage cells [19].

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2-3- Disk diffusion agar and Well diffusion agar

Table No. 1 presents the antimicrobial effect of *Badrashboo* essential oil evaluated using the disc diffusion agar method. The investigation explored the influence of *Badrashboo* essential oil concentration on the inhibition of growth for various standard pathogenic bacteria. These bacteria included *Salmonella typhimurium*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Shigella dysentery*, *Staphylococcus aureus*, *Bacillus cereus*, and *Listeria monocytogenes*. The results showed that increasing the essential oil concentration from 0.625 to 40 mg/ml led to a significant upward trend ($p < 0.05$) in the diameter of the inhibition zone for all tested microorganisms. However, the lowest concentration (0.625 mg/ml) was ineffective against *Pseudomonas aeruginosa* and *Shigella dysentery*. According to Table number. 1, the investigation of the effect of each concentration of *Badrashboo* essential oil on the rate of inhibiting the growth of bacteria using the disc diffusion agar shows that at the lowest concentration of essential oil, the largest of bacterial growth with a diameter of 11.67 mm was related to the Gram-positive bacterium *Bacillus cereus* ($P<0.05$). All mentioned bacteria displayed inhibited growth in the disc diffusion agar method when treated with *Badrashboo* essential oil concentrations ranging from 1.25 to 40 mg/ml. The diameter of the inhibition zone exhibited a significant difference ($p<0.05$) depending on the concentration. Specifically, at concentrations of 1.25 and 2.5 mg/ml, *Bacillus cereus* displayed the best inhibition with halo diameters of 12.67 mm and 13 mm, respectively ($p<0.05$). For concentrations of 5, 10, and 40 mg/ml, *Staphylococcus aureus* and *Bacillus cereus* showed greater growth inhibition zone compared to other bacteria ($p<0.05$). Notably, the highest concentration (20 mg/ml) resulted in the most significant inhibition for *Staphylococcus aureus*, with a zone diameter of 21 mm ($p<0.05$).

Table 1. The mean inhibition zone diameter (mm) of *Badrashboo* essential oil on some pathogenic microorganisms (disk diffusion agar)

Microorganism	*Badrashboo* essential oil concentration (mg/mL)						
	0.625	1.25	2.5	5	10	20	40
S. typhimurium	8.00 ± 1.00^{eB}	8.33 ± 0.88^{deB}	10.33 ± 0.33^{cdBC}	11.33 ± 0.33^{cB}	14.00 ± 0.58^{bB}	16.00 ± 0.58^{bC}	31.67 ± 0.88^{aC}
E. coli	7.33 ± 0.33^{eBC}	8.33 ± 0.67^{eB}	8.67 ± 0.33^{eCD}	11.00 ± 0.58^{dB}	$13.,33\pm0.33^{cBC}$	16.33 ± 0.33^{bC}	32.00 ± 0.58^{aC}
P. aeruginosa	-	6.67 ± 0.33^{eB}	7.33 ± 0.33^{eD}	8.67 ± 0.33^{dC}	12.00 ± 0.58^{cC}	15.33 ± 0.33^{bC}	31.33 ± 0.33^{aC}
Sh. dysenteriae	-	7.67 ± 0.33^{dB}	9.33 ± 0.88^{cdBC}	10.67 ± 1.45^{cBC}	12.00 ± 0.58^{cC}	16.33 ± 0.33^{bC}	30.67 ± 0.88^{aC}
S. aureus	6.67 ± 0.33^{fBC}	7.33 ± 0.33^{fB}	11.00 ± 0.58^{eB}	14.33 ± 0.67^{dA}	18.67 ± 0.33^{cA}	21.00 ± 0.58^{bA}	41.00 ± 0.58^{aA}
B. cereus	11.67 ± 0.33^{dA}	12.67 ± 0.33^{cdA}	13.00 ± 0.58^{cdA}	14.33 ± 0.67^{cA}	18.00 ± 0.58^{bA}	19.33 ± 0.67^{bB}	43.33 ± 1.20^{aA}
L. moniocytogenes	6.33 ± 0.33^{eC}	7.67 ± 0.33^{eB}	10.33 ± 0.33^{dBC}	11.67 ± 0.33^{dB}	14.67 ± 0.33^{cB}	18.00 ± 0.58^{bB}	37.33 ± 0.88^{aB}

* Means (±SE) with different lowercase letters in each row and uppercase letters in each column show a significant difference at $P \leq 0.05$

Table No. 2 shows the antimicrobial effect of *Badrashboo* essential oil using the well diffusion agar method. This method involved increasing the concentration of *Badrashboo* essential oil from 0.625 to 40 mg/mL, which resulted in a significant increase ($P < 0.05$) in the diameter of the inhibition zone for non-growth of *Salmonella typhimurium*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Shigella dysentery*, *Staphylococcus aureus*, *Bacillus cereus*, and *Listeria monocytogenes*. The well diffusion agar method, unlike the disk diffusion agar method, inhibited the growth of *Pseudomonas aeruginosa* and *Shigella dysentery* bacteria even at a low dose of 0.625 mg/ml of essential oil ($P<0.05$). Evaluation of *Badrashboo* essential oil using the well diffusion agar method (Table 2) showed that at a concentration of 0.625 mg/ml, the diameter of the inhibition zone for *Listeria monocytogenes* (11.33 mm) and *Staphylococcus aureus* (10.67 mm) was significantly greater ($P < 0.05$) than that of *Escherichia coli* (8.67 mm). At a concentration of 1.25 mg/mL of essential oil, no statistically significant difference was observed in the diameter of the inhibition zone among the various bacteria. However, at 2.5 mg/mL, the

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inhibition zone for *Listeria monocytogenes* (19 mm) was significantly larger ($P < 0.05$) compared to *Salmonella typhimurium* (15.33 mm), *Escherichia coli* (14.33 mm), and *Shigella dysentery* (15.33 mm). At a concentration of 5 mg/mL, *Listeria monocytogenes* exhibited the largest inhibition zone (24 mm) among the tested bacteria ($P < 0.05$). *Badrashboo* essential oil exhibited greater inhibitory activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus cereus*, and *Listeria monocytogenes* at a concentration of 10 mg/mL compared to *Salmonella typhimurium* and *Shigella dysentey***.** At a concentration of 20 mg/mL, the essential oil showed the strongest inhibition against *Bacillus cereus*, with an inhibition zone diameter of 33 mm. Conversely, *Shigella dysentery* displayed the weakest inhibition at this concentration, with a zone diameter of only 25 mm. At a concentration of 40 mg/mL, the inhibition zone for *Staphylococcus aureus* (43.33 mm) and *Bacillus cereus* (42.33 mm) was significantly larger ($P < 0.05$) compared to *Salmonella typhimurium* (34 mm), *Pseudomonas aeruginosa* (34.33 mm), and *Shigella dysentery* (36.67 mm).

Table 2. The mean inhibition zone diameter (mm) of Badrashboo essential oil on some pathogenic microorganisms (well diffusion agar)

Microorganism	Badrashboo essential oil concentration (mg/mL)						
	0.625	1.25	2.5	5	10	20	40
S. typhimurium	10.00 ± 0.58^{fAB}	11.33 ± 0.67^{fA}	15.33 ± 0.88^{eBC}	18.33 ± 0.33^{dCD}	20.67 ± 0.67^{cB}	28.33 ± 0.33^{bC}	34.00 ± 1.00^{aE}
E. coli	8.67 ± 0.67^{fB}	11.33 ± 0.33^{fA}	14.33 ± 0.33^{eC}	20.67 ± 0.67^{dB}	24.67 ± 2.33^{cA}	30.33 ± 0.33^{bBC}	38.33 ± 0.33^{aBCD}
P. aeruginosa	9.67 ± 0.88^{dAB}	11.00 ± 0.58^{dA}	17.33 ± 1.45^{cABC}	19.67 ± 0.88^{cBC}	26.00 ± 1.00^{bA}	28.33 ± 0.88^{bC}	34.33 ± 0.33^{aDE}
Sh. dysenteriae	9.67 ± 0.33^{eAB}	10.67 ± 0.33^{eA}	15.33 ± 0.33^{dBC}	16.33 ± 0.67^{dD}	21.00 ± 0.58^{cB}	25.00 ± 1.00^{bD}	36.67 ± 0.88^{aCDE}
S. aureus	10.67 ± 0.33^{eA}	12.33 ± 0.33^{eA}	18.33 ± 0.88^{dAB}	20.33 ± 0.88^{dBC}	27.33 ± 0.67^{cA}	32.33 ± 0.67^{bAB}	43.33 ± 2.91^{aA}
B. cereus	9.67 ± 0.33^{fAB}	12.00 ± 0.58^{fA}	16.00 ± 1.15^{eABC}	19.33 ± 0.67^{dBC}	28.33 ± 0.33^{cA}	33.00 ± 1.00^{bA}	42.33 ± 1.20^{aAB}
L. moniocytogenes	11.33 ± 0.33^{fA}	12.00 ± 0.58^{fA}	19.00 ± 1.00^{eA}	24.00 ± 0.58^{dA}	28.33 ± 0.88^{cA}	31.00 ± 1.00^{bAB}	40.33 ± 0.33^{aABC}

* Means (±SE) with different lowercase letters in each row and uppercase letters in each column show a significant difference at $P \leq 0.05$

The results of this study suggest that *Badrashboo* essential oil exhibits specific antimicrobial activity against both Gram-positive and Gram-negative bacteria. This effect can likely be attributed to the presence of antimicrobial compounds like citral, geranial, and neral within the oil [20]. Asimovic et al. (2022) reported similar findings, attributing the antimicrobial effect to the presence of geranyl acetate, geranial, and neral, which are known for their antibacterial properties and thermal stability [21]. The antimicrobial effect of compounds such as geranyl acetate and geraniol has already been demonstrated in research by Selopi et al. (2022) [21] and Periradelira et al. (2020) [22]. These researchers propose that the mechanism of action for geraniol's antimicrobial activity (due to its lipophilic properties) involves binding to the lipids of the microorganism's cell membrane, interacting with cellular components, and increasing permeability, ultimately leading to structural degradation of essential intracellular sites. These findings support the link between the chemical composition of *Badrashboo* essential oil and its observed antimicrobial activity. Evaluation of *Badrashboo* essential oil's antimicrobial activity using disc diffusion and well diffusion agar methods revealed that, in most cases, the inhibition zone against Gram-positive bacteria was larger than that against Gram-negative bacteria. This observation aligns with the findings of Ehsani et al. (2014), who studied the antimicrobial properties of Iranian Badrashboo [23]. Sonbali et al. (2008) investigated the biological activity and chemical composition of Iranian Badrashboo essential oil. They found that *Badrashboo* essentialoil exhibited antimicrobial activity against all studied Gram-positive and Gram-negative bacteria. Notably, the strongest activity was observed against *Bacillus cereus*, *Staphylococcus aureus*, and *Staphylococcus epidermidis*, while *Klebsiella pneumoniae* bacteria remained unaffected [24]. Sima et al. (2023) investigated the antimicrobial properties

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of hydroalcoholic extracts from three *Badrashboo* species cultivated in Romania. The extracts were effective against *Staphylococcus aureus* but showed no effect on *Pseudomonas aeruginosa* [25]. However, Abdul Baki et al. (2007), who investigated the chemical and biological characteristics of Egyptian *Badrashboo* essential oil, announced that both groups of Gram-positive and Gram-negative bacteria showed the same sensitivity to *badrashboo* essential oil [26]. Further research by Rezaeikikhaei et al. (2018) explored the effects of an ethanolic extract of Badrashboo on antibiotic-resistant strains of Escherichia coli and Klebsiella pneumoniae. Their findings confirmed the extract's efficacy against both groups of bacteria [27].

Alizadeh et al. (2013) investigated the antimicrobial effects of aqueous and ethanolic eucalyptus extracts against various foodborne pathogens. Their findings showed that Gram-positive bacteria were more susceptible than Gram-negative bacteria. Notably, *Staphylococcus aureus* exhibited significantly higher sensitivity compared to *Escherichia coli* [28]. Tabatabai, Yazdi et al. (2015) investigated the antimicrobial activity of aqueous and alcoholic Bilhar plant extracts. Their research confirmed that Gram-positive bacteria were more susceptible than Gram-negative bacteria [29]. Heydari Sureshjani et al. (2014) further investigated the antimicrobial properties of celery extracts against foodborne bacteria. Their research showed that Gram-positive bacteria were more susceptible, particularly to the ethanolic extract [30]. Safari et al. (2020) investigated the antimicrobial properties of Shirazi thyme essential oil against various Gram-positive and Gram-negative bacteria. Interestingly, their findings revealed that Gram-negative bacteria were more susceptible [31]. It's worth noting that variations in plant habitat and chemical composition can influence these results. Notably, Gram-negative bacteria possess an additional outer membrane, doubling their defense against toxins and hydrophobic compounds. Conversely, the lack of this outer membrane in Gram-positive bacteria renders them more susceptible to external factors [32]. Figure 1 illustrates the antimicrobial effect of *Badrashboo* essential oil (20 mg/ml) on *Pseudomonas aeruginosa* and *Bacillus cereus*.

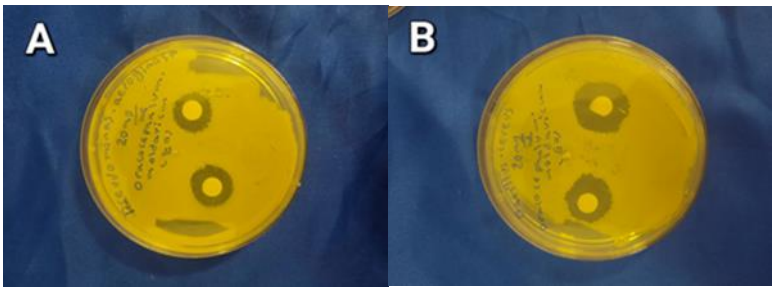

Fig. 1. Inhibition zone of Badrashboo essential oil (20 mg/mL) for *Pseudomonas aeruginosa* (A) and *Bacillus cereus* (B).

This study found that *Badrashboo* essential oil produced larger inhibition zones in well diffusion agar compared to disk diffusion agar. Similar results were reported by Rahmati and Alizadeh (2021) for antifungal activity of Shirazi thyme oil and Noshad and Alizadeh (2023) for the antibacterial activity of tatorre extract [33, 34]. This difference occurs because in well diffusion agar method, the oil directly contacts microorganisms, whereas in disk diffusion agar method, it needs to diffuse from the disk to the surrounding area to exert its effect [35].

3-3- MIC and MBC test results

Table 3 summarizes the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *Badrashboo* essential oil against various bacteria. The lowest concentrations (0.312 and 0.625 **mg/mL**) inhibited *Staphylococcus aureus* and *Shigella dysentery*, respectively. Consistent with the observed trend, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus cereus*, and *Listeria monocytogenes* showed inhibition at the concentration of 1.25 **mg/mL**. The inhibitory effect of the essential oil on *Salmonella Typhimurium* was obtained at the concentration of 2.5 **mg/mL**.

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Badrashboo essential oil exhibited a bactericidal effect against *Staphylococcus aureus, Bacillus cereus, Listeria monocytogenes,* and *Pseudomonas aeruginosa* at a 2.5 dilution. In contrast, a higher concentration (5 **mg/mL**) was necessary for the bactericidal effect against *Salmonella Typhimurium, Escherichia coli,* and *Shigella dysentery*.

Table 3. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the Badrashboo essential oil on some pathogenic microorganisms

Microorganism	MIC (mg/mL)	MBC (mg/mL)
S. typhimurium	2.5	5
E. coli	1.25	5
P. aeruginosa	1.25	2.5
Sh. dysenteriae	0.625	5
S. aureus	0.312	2.5
B. cereus	1.25	2.5
L. monocytogenesis	1.25	2.5

The results of the present study were consistent with the study of Ehsani et al. (2017) [36]. Noshad and Alizadeh (2021) investigated the antimicrobial activity of a methanol extract from sorrel against *Enterobacter aerogenes*, *Salmonella typhi*, *Staphylococcus aureus*, and *Streptococcus pyogenes* using a minimum inhibitory concentration (MIC) test. Their findings revealed that Staphylococcus aureus was the most susceptible bacteria to the extract, while Enterobacter aerogenes displayed the highest resistance [37]. Barzegar et al. (2021) also investigated the antibacterial properties of Henna extract. Their study evaluated the extract's efficacy against various Gram-positive and Gram-negative bacteria. They found that *Staphylococcus epidermidis* was the most susceptible bacteria, exhibiting the lowest inhibitory concentration (MIC). Conversely, *Escherichia coli* and *Salmonella typhimurium* displayed the highest resistance, as indicated by their higher MIC values. Furthermore, the minimum bactericidal concentration (MBC), the concentration required to kill the bacteria, was greater for *Escherichia coli* and *Salmonella typhimurium* compared to *Staphylococcus epidermidis* and *Listeria innocua* [38]. Previous studies on the antimicrobial activity of various plant essential oils have yielded mixed results regarding Gram-positive and Gram-negative bacteria susceptibility. For instance, Abdulbaki et al. (2007) reported no significant difference in MIC values between Gram-positive and Gram-negative bacteria for the essential oil of Egyptian *Badrashboo* [26]. Challenging the impermeability assumption, Plesiat et al. (1992) proposed that purine channels in the outer membrane might allow passage of some hydrophobic compounds [39]. Wendol et al. (2017) observed that some plant essential oil molecules are more effective against gram-positive bacteria, while others target gram-negative bacteria. However, the mechanism of action for these effects remains unknown [40]. The mode of action of essential oils depends on their chemical makeup, particularly the ratio of their active components. These components can interfere with bacterial proliferation in several ways, including: disintegration of the outer membrane of the phospholipid bilayer of bacteria, changes in the composition of fatty acids in the bacterial membrane, increased membrane fluidity, leading to leakage of potassium ions and protons, interference with glucose absorption.

Figure 2 shows a view of determining the minimum inhibitory concentration of *Badrashboo* essential oil on pathogenic bacteria.

[DOI: 10.22034/FSCT.21.156.150]

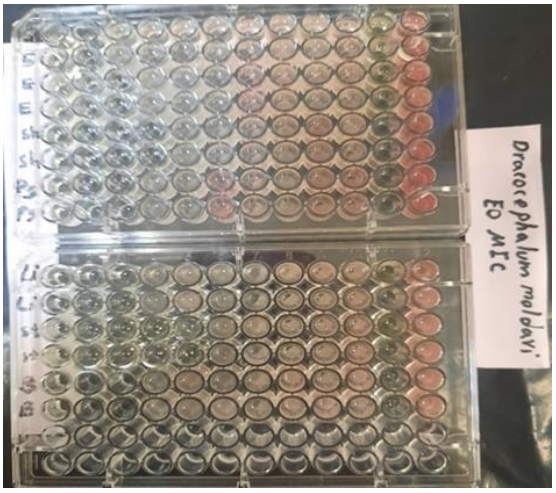

Fig. 2. The minimum inhibitory concentration (MIC) of Badrashboo essential oil on some pathogenic microorganisms.

4-3- The interaction of essential oil with common therapeutic antibiotics

Figures 3-6 evaluate the antimicrobial activity of vancomycin, erythromycin, chloramphenicol, and gentamicin against *Salmonella typhimurium*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Shigella dysentery*, *Staphylococcus aureus*, *Bacillus cereus*, and *Listeria monocytogenes* using the disk diffusion method. The results showed a synergistic effect between all tested antibiotics and *Badarshboo* essential oil. This synergy increased the diameter of inhibition zones against all tested bacteria compared to antibiotic treatment alone ($P < 0.05$).

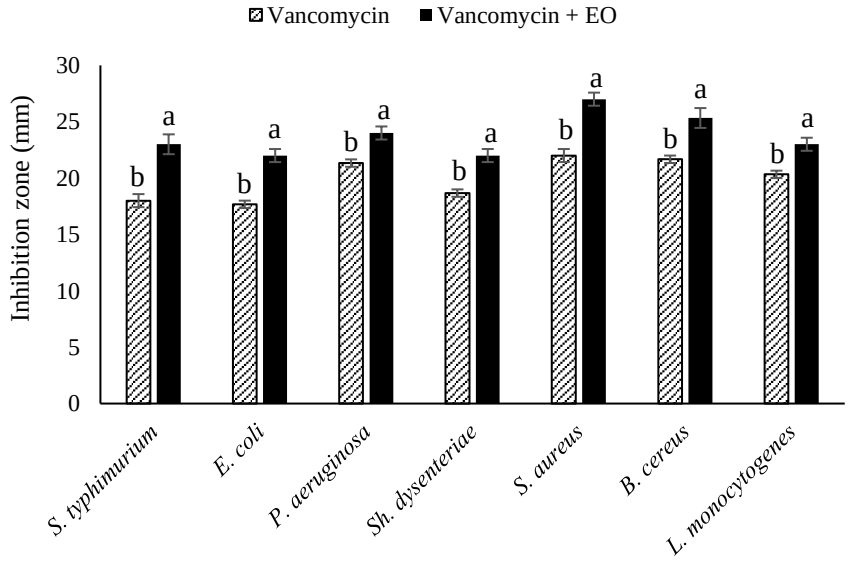

Fig 3. The interaction of Badrashboo essential oil with Vancomycin on bacterial pathogens. Means (±SE) with different letters in each microorganism show a significant difference at $P \leq 0.05$

[DOI: 10.22034/FSCT.21.156.150]

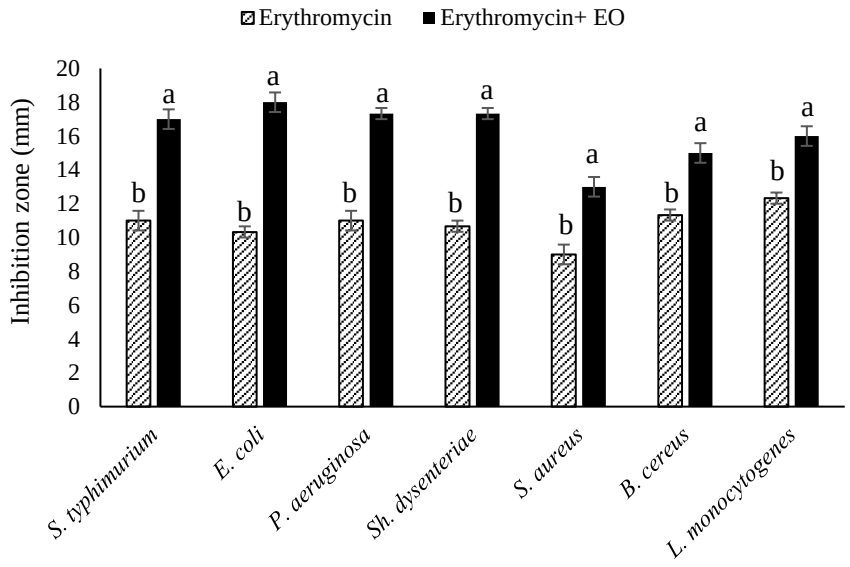

Fig 4. The interaction of Badrashboo essential oil with Erythromycin on bacterial pathogens. Means (±SE) with different letters in each microorganism show a significant difference at $P \leq 0.05$

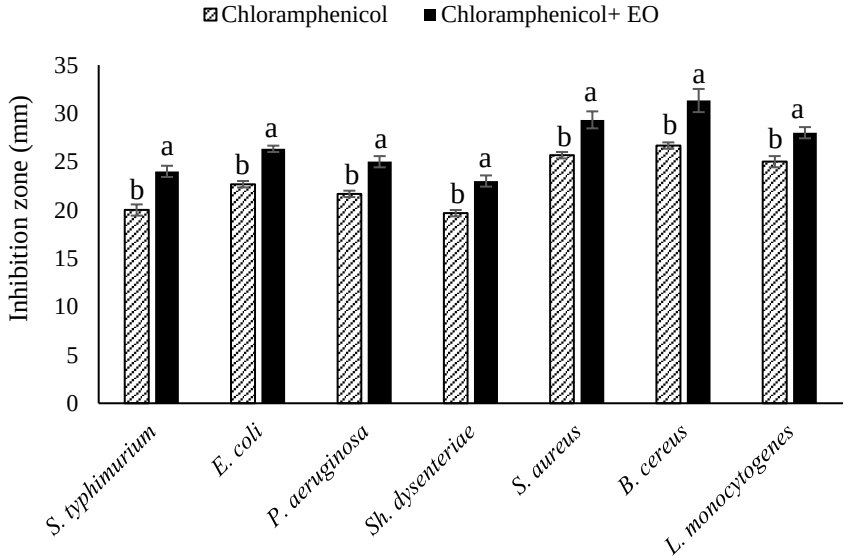

Fig 5. The interaction of Badrashboo essential oil with Vancomycin on bacterial pathogens. Means (±SE) with different letters in each microorganism show a significant difference at $P \leq 0.05$

[DOI: 10.22034/FSCT.21.156.150]

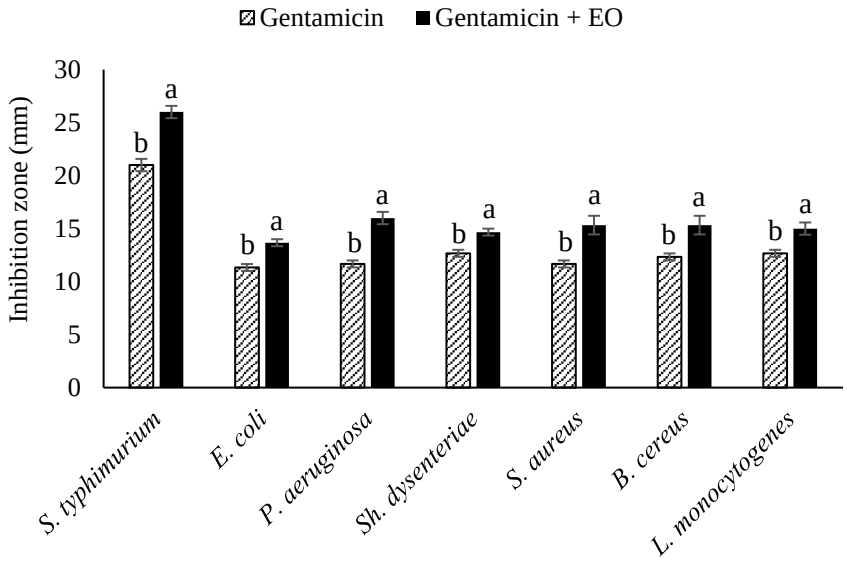

Fig 6. The interaction of Badrashboo essential oil with Gentamicin on bacterial pathogens. Means (±SE) with different letters in each microorganism show a significant difference at $P \leq 0.05$

The studied bacteria exhibited a synergistic effect when combined with all four commonly tested therapeutic antibiotics. Zamanpour et al. (2023) further supported this by investigating the interaction of black seed oil with Chloramphenicol on various bacteria. Their findings confirmed that black seed essential oil exhibited a synergistic effect against all the bacteria tested [16]. Alizadeh Behbahani et al. (2018) investigated the synergistic effects of fennel essential oil with Kanamycin against various pathogenic bacteria. Their findings demonstrated that fennel essential oil exhibited a synergistic effect when combined with kanamycin [42]. Tanavar et al. (2018) investigated the interaction of oregano essential oil with Gentamicin and chloramphenicol against food pathogens. Their research observed a synergistic effect between oregano essential oil and both antibiotics [43]. Ahmadnejad et al. (2024) investigated the interaction of the aqueous extract of Estbaraq [44] and Jalil Sarqeleh et al. (2024) [45] investigated the interaction of the aqueous extract of Jashir with the antibiotic nystatin against a number of pathogenic fungal species and found that both aqueous extracts of Estbaraq and Jashir improved the antimicrobial activity of nystatin. In the study by Safari et al. (2020) who investigated the interaction of Shiraz thyme essential oil with the antibiotics Gentamicin and Chloramphenicol against a number of bacteria, they reported that only in combination with Gentamicin did the zone of inhibition against *Listeria innocua* increase compared to the single treatment (thyme essential oil) [31].

4 – Conclusion

Given the significant antibacterial effect of *Badrashboo* essential oil against both Gram-positive and Gram-negative bacteria observed in this study, coupled with its pleasant aroma and sensory properties, suggests its potential as a desirable preservative in various food products. Additionally, clinical research may introduce *Badrashboo* essential oil as a treatment option for many diseases, offering low-cost and less side-effect treatment methods due to its natural antimicrobial properties.

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مقاله علمی_پژوهشی

بررسی فعالیت ضدمیکروبی اسانس بادرشبو (*Dracocephalum moldavica*) و برهمکنش آن با برخی آنتی‌بیوتیک‌های رایج درمانی علیه تعدادی از باکتری‌های بیماری‌زا

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چکیده

امروزه با توجه به اثر سوء نگهدارنده‌های شیمیایی در فراورده‌های غذایی و نیز مقاومت‌های آنتی بیوتیکی ایجاد شده، تلاش محققین برای استفاده از ترکیب‌های ضدمیکروبی طبیعی و بی‌خطر از جمله اسانس‌های گیاهی افزایش یافته است. در پژوهش حاضر پس از جمع آوری گیاه بادرشبو از مزارع اطراف شهر ارومیه و خشک کردن آن، اسانس‌گیری از گیاه به وسیله دستگاه کلونجر انجام شد و تاثیر ضدمیکروبی اسانس مذکور بر تعدادی از باکتری‌های گرم مثبت و گرم منفی بیماری‌زای ناشی از غذا با روش‌های انتشار در دیسک، انتشار در چاهک، حداقل غلظت بازدارندگی، حداقل غلظت کشندگی و برهمکنش با چهار آنتی‌بیوتیک رایج درمانی شامل: ونکومایسین، اریترومایسین، کلرامفنیکل و جنتامایسین انجام شد. نتایج آزمایش‌های دیسک و چاهک نشان داد که اسانس بادرشبو تاثیر ضدمیکروبی قابل ملاحظه‌ای بر همه باکتری‌های مورد مطالعه داشت و باکتری‌های گرم مثبت حساسیت بیشتری نسبت به اسانس داشتند. نتایج حداقل غلظت مهارکنندگی اسانس برای سویه‌های *سالمونلا تایفی‌موریوم، اشرشیا کلی، سودوموناس آئروژینوزا، شیگلا دیسانتری، استافیلوکوکوس اورئوس، باسیلوس سرئوس و لیستریا مونوسیتوژنز* به ترتیب ۲/۵، ۱/۲۵، ۱/۲۵، ۰/۶۲۵، ۰/۳۱۲، ۱/۲۵ و ۱/۲۵ میلی‌گرم بر میلی‌لیتر بود. حداقل غلظت کشندگی برای سویه‌های مذکور به ترتیب ۵، ۵، ۲/۵، ۵، ۲/۵، ۲/۵، و ۲/۵ میلی‌گرم بر میلی‌لیتر بود. همچنین نتایج حاصل از اثر ترکیبی اسانس بادرشبو با آنتی‌بیوتیک‌های ذکر شده نیز حاکی از تاثیر هم‌افزایی اسانس مذکور با هر چهار آنتی بیوتیک مورد آزمایش بود. با توجه به اثر قابل ملاحظه ضدمیکروبی مشاهده شده برای اسانس بادرشبو در پژوهش حاضر، می توان از آن در صنایع غذایی و دارویی استفاده کرد.

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