

Evaluation of the Bactericidal Effects of *Zingiber officinale*, *Aloysia citrodora* and *Artemisia dracunculus* on the Survival of Standard Gram-Positive and Gram-Negative Bacterial Strains

Mahdi Tajbakhsh¹, Neda Soleimani^{2*}

1. Bachelor of Plant Biology, Faculty of Life Sciences, Shahid Beheshti University, Tehran, Iran
2. Assistant Professor, Ph.D. in Medical Microbiology, Department of Microbiology and Microbial Biotechnology, Faculty of Life Science and Biotechnology, Shahid Beheshti University, Tehran, Iran

Abstract

Background and objectives: Historically, herbs and plants have been used for their therapeutic properties in the form of flavors and preservatives. Recently, the application of medicinal herbs has increased considering their numerous benefits and minimum side-effects. Treatment of bacterial infections is currently a major challenge in the healthcare systems across the world. The present study aimed to assess the bacterial effects of *Zingiber officinale*, *Aloysia citrodora* and *Artemisia dracunculus* essential oils on the survival of standard Gram-positive and Gram-negative bacterial strains.

Methods: In the present experimental study, we evaluated the effects of **Tarragon** (*A. dracunculus*), **Ginger** (*Z. officinale*) and **Lemon Beebrush** (*A. citrodora*) essential oils on 6 strains of *Staphylococcus aureus*, *Klebsiella* spp, *Salmonella typhimurium*, *Staphylococcus epidermidis*, *Proteus* spp and *Corynebacterium diphtheriae*. The well-diffusion method was applied to assess the antibacterial properties of the essential oils. Moreover, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) tests were used to determine the bacterial and inhibitory concentrations of the extracts.

Results: MIC and MBC results demonstrated that the ginger extract (0.125 mg/mL) had the most significant impact on *Staphylococcus aureus*, *Salmonella typhimurium*, and *Staphylococcus epidermidis*. Furthermore, tarragon extract (0.03125 mg/mL) had the most significant effect on *Staphylococcus aureus*, *Proteus* spp, and *Corynebacterium diphtheriae*. The antibacterial effects of these essential oils were not observed on other bacteria.

Conclusion: Medicinal plants have long been used for their therapeutic properties. According to the results, ginger and tarragon extracts are effective combinations for the treatment of the infections caused by Gram-negative and Gram-positive bacteria.

Keywords: Antibiotic resistance; Minimum bactericidal concentration; Minimum inhibitory concentration; *Zingiber officinale*; *Aloysia citrodora*; *Artemisia dracunculus*

Corresponding Author: Neda Soleymani

Address: Department of Microbiology and Microbial Biotechnology, Faculty of Science and Biotechnology, Shahid Beheshti University, Tehran, Iran

E-mail: N_Soleimani@sbu.ac.ir

Introduction

Historically, herbs and plants have been used as foods and medications. With the advancement of pharmaceutical sciences, which has led to the increased application of chemical compounds and the subsequent side-effects, special attention has been paid to the use of medicinal plants for healthcare purposes (1).

Extensive research has been conducted on new antibacterial medications with high effectiveness due to the increased drug resistance associated with the growing use of antibiotics against bacterial infections (2, 3). Antibiotic resistance in bacteria is a global phenomenon, encompassing a wide range of human pathogens and antibiotic groups (3, 4). In general, bacteria become resistant to antibiotics through several patterns. While some organisms are naturally resistant to some or all antibacterial agents, others gradually become resistant to antibacterial agents through molecular mechanisms (5, 6).

Currently, multidrug resistance of bacteria to antibiotics has been reported in many studies. Synthetic antibiotics play a pivotal role in the treatment of infectious diseases (7-9). However, the resistance of microorganisms to these agents and the associated complications is a major concern among researchers, encouraging the use of alternative herbal and organic compounds (6, 7).

Tarragon (*Artemisia dracunculus*) belongs to the flowering plant order Asterales and Asteraceae family. This perennial plant grows to an average height of 30 centimeters to one meter and has simple green leaves. The basal leaves of tarragon are cleft and three-lobed, and the middle lobe is larger than the side lobes, and the cluster-shaped capitols are small and green. Tarragon contains various chemical compounds, including estragole, ocimene, methyl chavicol, and a significantly small amount of tannin (10).

Ginger (*Zingiber officinale*) belongs to the Zingiberales order and Zingiberaceae family. With a height of 20-100 centimeters, this yellow plant has purple leaf-tints, and its main properties lie in the underground stem, known as the rhizome. Some of the main compounds found in ginger are camphene, phellandrene, zingiberene, cineole, citral and borneol (11). It is notable that gingerols and the corresponding shogaols are the major pungent compounds in ginger. This perennial herb has 70 species across the world and is originated in East Asia. In addition to its antifungal properties, ginger has been reported to exert sedative effects.

According to the literature, constant consumption of antibiotics has led to bacterial resistance to these agents. Therefore, further investigation has been focused on the use of natural antibacterial agents (11). For instance, lemon beebrush (*Aloysia citrodora*) is a medicinal herb with various properties, which could be considered as a proper alternative to synthetic antibiotics. Lemon verbena has narrow lanceolate leaves of approximately 7-10 centimeters and a knot-shaped base with triplets per node. The plant has 21 seeds and consists of an unbranched inflorescence of stalked flowers in the form of narrow clusters, with a small flower pot and two lips of pale purple.

Considering the wide application and numerous benefits of medicinal plants in clinical settings and in order to comprehend their effects, the present study aimed to evaluate the sensitivity of standard Gram-negative and Gram-positive bacterial strains to the herbal extracts of ginger, tarragon and lemon beebrush as new antibacterial compounds.

Materials and Methods

Preparation of Herbal Extracts

In this laboratory experiment, tarragon, lemon beebrush and ginger were purchased from FZ Biotech Company. The plants were completely washed, naturally dried, and powdered. The powder was placed in a one-liter flask with 300 milliliters of sterile, distilled water in order to extract the herbal essences using the Clevenger (Borosil, India) apparatus.

Standard Bacterial Strains

The standard strains of *Corynebacterium diphtheriae* (ATCC 13812), *Staphylococcus epidermidis* (PTCC 1114), *Salmonella typhimurium* (ATCC 14028), *Staphylococcus aureus* (ATCC 25923), *Proteus mirabilis*, and *Klebsiella pneumoniae* were purchased from Pasteur Institute of Iran and used to evaluate the antibacterial effects of the tarragon, lemon beebrush and ginger extracts. The identity of the evaluated strains was confirmed using differential, selective, dedicated media and biochemical tests, including catalase, oxidase, and glucose fermentation. Afterwards, the strains were cultured on tryptic soy broth (TSB) at the temperature of 37°C for 24 hours. Fresh bacterial culture was applied in the following stages of the study.

Antibacterial Effects of Herbal Extracts on Bacterial Strains

Bacterial Sensitivity Test Using the Disc-Diffusion Method

The well-diffusion method was applied to assess the antibacterial activities of tarragon, lemon beebrush and ginger (5). In addition, the bacterial inhibition zone was measured to determine the antibacterial effects. To do so, a bacterial suspension with the concentration of 1.5×10^8 cfu (equal to 0.5 McFarland Standard) was rubbed on Mueller-Hinton agar

(MHA) using a swap, and wells were formed on the agar surface using sterile pipettes (Pasteur Institute, Iran). Afterwards, 25 μ L of the extracts was injected into the wells, followed by the incubation of the plates in an inverted position at the temperature of 37°C for 18 hours. By the end of this stage, the diameter of the bacterial growth inhibition zone around the wells was measured in millimeters. In addition, physiological serum and gentamicin were used as negative and positive control, respectively. It is notable that three replications of the test were carried out (13).

Minimum Inhibitory Concentration (MIC)

The broth dilution method was applied to determine the minimum inhibitory concentration (MIC) in the bacterial strains (14). To this end, 300 μ L of MHA (Merck, Germany) was added to the 96-well microplate (dilution ratio: 1:2). After adding 300 μ L of the 1.2 concentration to the first well, 150 μ L of the mixture was collected from the first well and added to the second well, resulting in the dilution series in the wells.

In the next stage, 10 μ L of the bacterial suspension (10^8 CFU/mL) was separately added to the wells. Finally, the microplate was incubated at the temperature of 37°C for 18 hours. MIC was defined as the lowest concentration required to inhibit bacterial growth. In this process, the well containing no herbal essence and bacterial suspension was considered as the negative control (14). MIC was determined in triplications.

Minimum Bactericidal Concentration (MBC)

At this stage, 10 μ L of the contents of the wells was cultured on MHA (Merck, Germany) after 18 hours of incubation in order to determine the MBC level. Following that, the plates were incubated for 18 hours to

assess bacterial growth. MBC was defined as the lowest concentration of the herbal extracts without bacterial growth in 99.9% of the strains. It is notable that this process was repeated three times.

Statistical Analysis

Data analysis was performed in GraphPad software using one-way analysis of variance (ANOVA), and the data were expressed as mean and standard deviation. In all the statistical analyses, P -value of ≤ 0.001 was considered significant.

Results

Antibacterial Effects of Herbal Extracts on Bacterial Strains Using the Disc-Diffusion Method

In the current research, the antibacterial effects of the tarragon, lemon verbena, and ginger extracts were determined using the disc-diffusion method (5) and based on the measurement of the inhibition zone. According to the results of disc-diffusion in agar media, the largest inhibition zone was observed in the ginger extract for *Staphylococcus epidermidis* (diameter: 13 ± 1 mm). In addition, the largest inhibition zone in the tarragon extract was observed for *Salmonella typhimurium* (diameter: 5 ± 1 mm). On the other hand, the smallest inhibition zone (diameter: 1 ± 4 mm) belonged to *Corynebacterium* in the ginger extract (Diagram 1).]

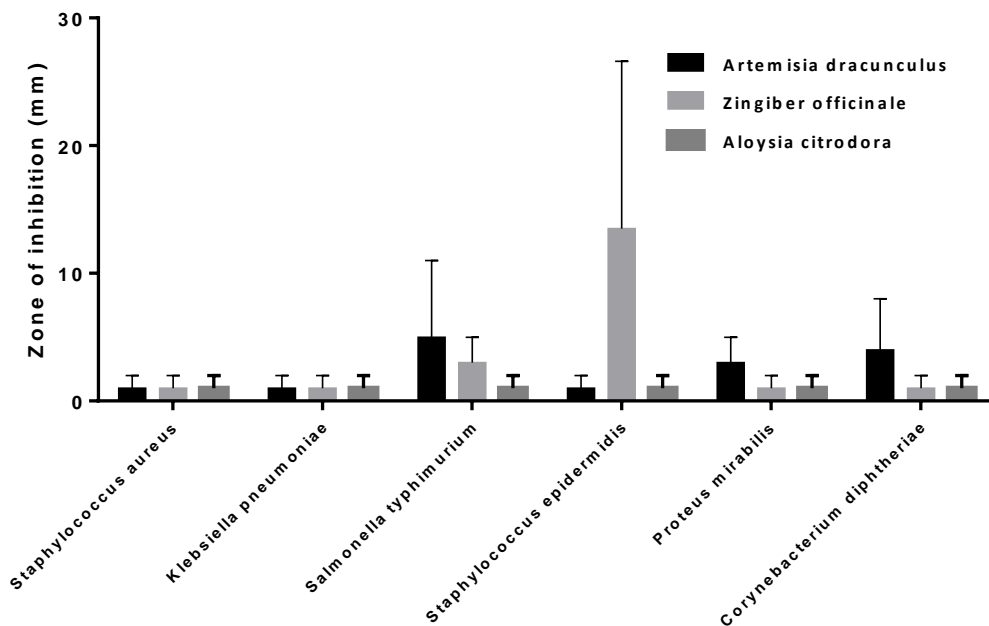


Diagram 1. Effects of Tarragon, Lemon beebrush and Ginger extracts on Inhibition Zone of Standard Bacterial Strains (Data as Means \pm Standard Deviation)

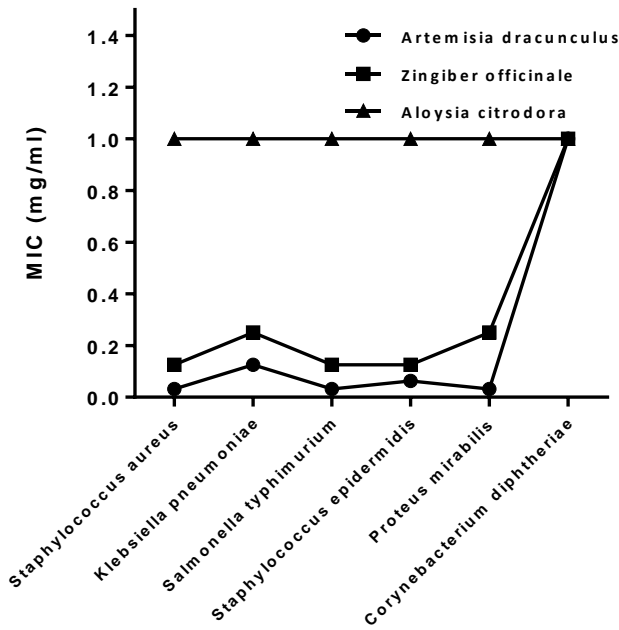


Diagram 2. MIC of Tarragon, Lemon beebrush and Ginger Extracts in Standard Bacterial Strains (Data as Means ± Standard Deviation)

According to the results, the ginger extract equally affected *Staphylococcus epidermidis*, *Staphylococcus aureus*, and *Salmonella typhimurium* with the concentration was 0.125 mg/mL in all the bacteria. However, the concentration was observed to be comparatively higher (0.25 mg/mL) for *Klebsiella* and *Proteus* spp.

Antibacterial Activities of Herbal Extracts

Evaluation of the MIC and MBC results indicated that the tarragon extract had the most significant inhibitory effect on *Proteus mirabilis*. In addition, the tarragon extracts exerted bactericidal effects on *Staphylococcus aureus* at the concentration of 0.03125 mg/mL, while the concentration was determined to be 0.0625 mg/mL for *Staphylococcus epidermidis*. Similar results were obtained regarding the bactericidal effects of this herbal extract on *Salmonella typhimurium* and *Staphylococcus aureus* (concentration: 0.03125 mg/mL). Moreover, the bactericidal effects of the tarragon extract were observed on *Klebsiella* and *Proteus* at the concentrations of 0.125 mg/mL and 0.03125 mg/mL, respectively.

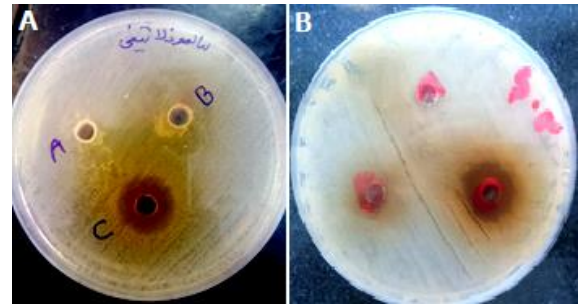


Figure 1. Effects of Tarragon, Lemon beebrush, and Ginger Extracts on Bacterial Strains in Disc-Diffusion (Inhibition Zone: A) *Salmonella*; B) *Staphylococcus aureus*)

Bacterial Strain	Inhibitory Effect in Liquid Culture (mg/mL)			
	Control (DW)	Ginger Extract (mg/mL MIC)	Tarragon Extract (mg/mL MIC)	Lemon beebrush Extract (mg/mL MIC)
<i>Corynebacterium diphtheriae</i>	-	-	-	-
<i>Staphylococcus aureus</i>	-	0.125	0.03125	-
<i>Staphylococcus epidermidis</i>	-	0.125	0.0625	-
<i>Klebsiella pneumoniae</i>	-	0.250	0.125	-
<i>Salmonella typhimurium</i>	-	0.125	0.03125	-
<i>Proteus mirabilis</i>	-	0.250	0.03125	-

*Stoke weight=0.95 mg/mL; DW= Distillated water

Table 2. The diameters of the growth inhibition (mm) zone in response to Tarragon, Lemon Beebrush and Ginger essential oils on among standard bacterial strains (Mean \pm SD)

Bacterial Strain	Lemon beebrush Extract	Tarragon Extract	Ginger Extract
<i>Corynebacterium diphtheriae</i>	1	4 \pm 1	1
<i>Staphylococcus aureus</i>	1	1	1
<i>Staphylococcus epidermidis</i>	1	1	13.5 \pm 1
<i>Klebsiella pneumoniae</i>	1	1	1
<i>Salmonella typhimurium</i>	1	5 \pm 1	3
<i>Proteus mirabilis</i>	1	3 \pm 1	1

Discussion

There are several causes for antibiotic resistance (6, 7). For instance, inherent bacterial resistance is caused by the permanent expression of β -lactamases (flocculation pumps), which pumps out antibiotics from cells to their peripheral environment. In addition to inherent resistance, bacteria may become resistant to the medications consumed during the treatment process. Acquired resistance following the receiving of resistance genes (e.g., β -lactamases and aminoglycosides-deactivating/modifying enzymes) leads to the excessive expression of efflux pumps, thereby reducing the expression of purines, and/or mutations in the target quinolone molecules. Since these mechanisms often occur simultaneously, bacterial strains may exhibit multidrug resistance (8).

Medicinal herbs are extensively used in traditional medicine and industries in the form of various flavors and preservatives. In the modern era, special attention has been paid to medicinal plants considering their wide application in disease treatment (7). Growing research in this regard has proposed new antimicrobial agents for the elimination of several microorganisms, including bacteria.

Over the recent decades, there has been an increasing need for such agents due to the

resistance of microorganisms to conventional medications in communities (8). With their special properties, medicinal herbs and their products are used as therapeutic supplements in the treatment of various disorders (11, 14). Furthermore, ongoing research has been focused on discovering effective antibiotic agents for the prevention and treatment of bacterial infections; the issue is highlighted with the emergence of new drug-resistant microorganisms (18).

Medicinal plants have been shown to produce various substances with antimicrobial activities, urging pharmaceutical companies to seek plant-based drug alternatives. For instance, several reports have been published on the antibacterial effects of cinnamon (19, 21). In a research by Suree et al., cinnamon extract exhibited acceptable antimicrobial effects on various strains of *Escherichia coli* (17). In addition, the antifungal effects of cinnamon extract and other herbs have been confirmed in a study by Ranasinghe et al. (16). In another study, the findings confirmed the antibacterial effects of cinnamon on *E. coli* and *Salmonella typhimurium* (4). On the other hand, Haghghi et al. assessed the antibacterial effects of *Thymus vulgaris*, *Petroselinum crispum*, *Cuminum cyminum*, and caraway on *Candida albicans*, comparing these effects with the antibacterial properties of fluconazole. According to the obtained results, the extracts of all the mentioned

plants had inhibitory effects on *Candida albicans*, while the most significant effects were observed in *Thymus vulgaris* and *Petroselinum crispum* (12).

According to the results of the present study, ginger extract had acceptable antibacterial properties, which is inconsistent with the previous studies in this regard. This discrepancy might be due to the difference in the species of indigenous plants and various levels of active ingredients in the studied herbs.

In a study, John M et al. demonstrated the positive effects of cinnamon extract on various species of *Candida albicans* and candidiasis (13). Moreover, Guddadaran et al. reported the antioxidant effects of cinnamon (9). Similar to the research by Ghasemi et al., several studies have confirmed the antibacterial effects of *Ferula gummosa*. In the current research, we assessed the antibacterial effects of tarragon and ginger on gram-positive bacteria (i.e., *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Salmonella*). In another study, the findings confirmed the antibacterial effects of ginger on pathogenic bacteria causing gastroenteritis, while the impact was observed to be comparatively more significant in *Pseudomonas* (8), and the least significant effect was denoted in *E. coli* in a research on the antibacterial effects of tarragon on several bacterial strains (15).

In another research in this regard, ginger extract was reported to exert antibacterial effects on *Helicobacter pylori*. According to the MIC test results in the present study, even small amounts of the tarragon extract (0.03125 mg/mL) had inhibitory effects on *Staphylococcus aureus*, *Salmonella typhimurium*, and *Proteus mirabilis*. Moreover, the herbal extract exhibited

acceptable inhibitory effects on other bacteria at certain dilutions.

In the current research, the lemon beebrush extract showed no inhibitory effects on the bacteria. According to the results of MIC, the ginger extract (concentration of up to 0.125 mg/mL) had inhibitory effects on *Staphylococcus epidermidis*, *Salmonella typhimurium*, and *Staphylococcus aureus*. Compared to the other studies in this regard, our findings revealed the effects of tarragon extract on gram-negative and gram-positive bacteria more distinctly. Such inconsistency in the obtained results could be attributed to the type of the indigenous plants, levels of the effective substances in the applied herbs, extract production technique, access to fresh herbal extracts, and differences in the examined bacterial strains (26).

The rate of bacterial resistance is on the rise, and such resistance could easily transfer from resistant bacteria to sensitive bacteria through a variety of patterns, thereby leading to antibiotic resistance (18, 19). According to the results of the present study, the herbal extracts of tarragon and ginger could be used as alternative or complementary combinations in the treatment of bacterial infections owing to their antibacterial properties. Currently, one of the main challenges in the treatment of infections and antibiotics therapy is resistance to antibiotics. Among the other therapeutic options in this regard are the herbal extracts of cinnamon and *Ferula gummosa*, which could be used independently or concomitantly along with other antibacterial agents for the treatment of bacterial infections (22-25). However, it is recommended that additional in-vivo tests be performed to evaluate the possible toxicity of these extracts, as well as their other properties and effects, so as to obtain a proper concentration for application in live samples.

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ارزیابی اثر کشندگی اسانس گیاهان *Artemisia dracunculus* و *Aloysia citrodora Zingiber officinale* بر میزان بقای سویه های استاندارد گرم مثبت و منفی

مهدی تاج بخش^۱، ندا سلیمانی^{۲*}

۱. کارشناسی زیست شناسی گیاهی، دانشکده علوم زیستی، دانشگاه شهید بهشتی، تهران، ایران.
۲. استادیار، دکتری باکتری شناسی پزشکی، گروه میکروبیولوژی و زیست فناوری میکروبی، دانشکده علوم و فناوری زیستی، دانشگاه شهید بهشتی، تهران، ایران

چکیده:

زمینه و هدف: گیاهان از گذشته جهت مصارف بهداشتی، درمانی و طعم دهنده کاربرد داشته و استفاده وسیع از آن ها و حداقل عوارض جانبی مشهود، ارزش این گونه فرآورده ها را بیشتر نموده است. امروزه درمان عفونت های باکتریایی از چالش های مهم در جهان محسوب می شود. هدف از این مطالعه، ارزیابی اثر کشندگی اسانس گیاهان *Aloysia citrodora Zingiber officinale* و *Artemisia dracunculus* بر میزان بقای سویه های استاندارد گرم مثبت و منفی می باشد.

روش بررسی: در این مطالعه تجربی، اثر اسانس گیاه ترخون (*A. dracunculus*) و زنجبیل (*Z. officinale*) و به لیمو (*A. citrodora*) بر روی ۶ سویه ی باکتریایی استافیلوکوکوس اورئوس، کلبسیلا، سالمونلا تیفی موریوم، استافیلوکوکوس اپیدرمیدیس، پروتئوس و کورینه باکتریوم دیفتریه بررسی گردید. برای بررسی خاصیت ضد باکتریایی اسانس ها، از روش های well diffusion و برای به دست آوردن غلظت مهار کنندگی و کشندگی از تست (MIC) minimum inhibitory concentration و Minimum Bactericidal Concentration (MBC) استفاده شد.

یافته ها: نتایج MIC و MBC نشان داد که بیشترین اثر اسانس زنجبیل با غلظت ۰/۱۲۵ میلی گرم در میلی لیتر بر روی باکتری های استافیلوکوکوس اورئوس و سالمونلا تیفی موریوم و استافیلوکوکوس اپیدرمیدیس مشاهده شد و در مورد اسانس ترخون بیشترین اثر بر روی باکتری های استافیلوکوکوس اورئوس، پروتئوس و کورینه باکتریوم دیفتریه و در غلظت ۰/۰۳۱۲۵ میلی گرم در میلی لیتر دیده شد. اثرات آنتی باکتریال این اسانس ها بر روی سایر باکتری ها مشاهده نشد.

نتیجه گیری: گیاهان دارویی از دیرباز مورد توجه بوده است. نتایج این بررسی نشان می دهد که اسانس های زنجبیل و ترخون می توانند به عنوان ترکیبی مناسب برای درمان برخی عفونت های ناشی از باکتری های گرم مثبت و منفی معرفی گردد.

کلمات کلیدی: مقاومت آنتی بیوتیکی، حداقل غلظت کشندگی، حداقل غلظت مهار کنندگی، *Zingiber officinal* و *Aloysia dracunculus* و *citrodora*

نویسنده مسئول: ندا سلیمانی

آدرس: گروه میکروبیولوژی و زیست فناوری میکروبی، دانشکده علوم و فناوری زیستی، دانشگاه شهید بهشتی، تهران، ایران.

ایمیل: N_Soleimani@sbu.ac.ir