

COMPARISON OF THE ANTIBACTERIAL AND ANTI-BIOFILM EFFECTS OF CORIANDER AND GARLIC ESSENTIAL OILS WITH CHLORHEXIDINE ON STREPTOCOCCUS MUTANS AND LACTOBACILLUS ACIDOPHILUS

Saeed Khoshnood¹, Amir Reza Rostami², Fahimeh Feili^{*3}

¹Assistant Professor of Medical Bacteriology, Department of Microbiology, School of Medicine, Clinical Microbiology Research Center, Ilam University of Medical sciences, Ilam, Iran.

²Oral and dental health research center, Ilam University of Medical sciences, Ilam, Iran.

³Assistant Professor of Restorative Dentistry, Department of Restorative Dentistry, School of Dentistry, Ilam University of Medical sciences, Ilam, Iran.

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*Corresponding Author: Fahimeh Feili

Assistant Professor of Medical Bacteriology, Department of Microbiology, School of Medicine, Clinical Microbiology Research Center, Ilam University of Medical sciences, Ilam, Iran. DOI: <https://doi.org/10.5281/zenodo.18184708>

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ABSTRACT

Dental caries and periodontal diseases are among the most prevalent infectious diseases in humans. Therefore, this study was conducted with the aim of comparing the antibacterial and anti-biofilm effects of coriander and garlic essential oils with 0.2% chlorhexidine against *Streptococcus mutans* and *Lactobacillus acidophilus*. In this research, two pathogenic bacteria, *Streptococcus mutans* and *Lactobacillus acidophilus*, were used. These bacteria were purchased from the Iranian Genetic Resource Bank. To prepare fresh cultures of the microorganisms, surface inoculation was performed on Blood Agar medium for each microbial strain one day prior to the microbiological tests. The suspension of the microbial strains was standardized based on the 0.5 McFarland turbidity standard (1.5×10^8 colony forming unit/mL). To quantify the biofilm-forming capacity in the isolates of the two aforementioned bacteria, the Microtiter Plate (MTP) method was employed. 180 of TSB medium containing 1% glucose was poured into the wells of the ELISA plate, and then 20 of the suspension of the studied bacteria, equivalent to the 0.5 McFarland standard, was added to each well (To reduce error, each isolate was considered in three repetitions and in three wells). After 48 hours of incubation, to remove unattached bacteria, they were washed with PBS buffer solution, and 120 of methanol was used to fix the attached bacteria. A significant difference was observed in the diameter of the inhibition zone for the bacterium *Streptococcus mutans* among the three essential oils of garlic, coriander, and 0.2% chlorhexidine. Furthermore, for the bacterium *Lactobacillus acidophilus*, a significant difference was observed among all three groups (garlic essential oil, coriander essential oil, and 0.2% chlorhexidine) with three replicates for each essential oil ($\chi^2 = 6$ and $P = 0.02$). Both *Streptococcus mutans* and *Lactobacillus acidophilus* exhibited moderate biofilm formation intensity. Garlic essential oil at a concentration of 5 uL/mL coriander at a concentration of 0.31uL/mL and 0.2% chlorhexidine at a concentration of 1.25 uL/m caused a reduction in biofilm formation by 25%, 100%, and 50%, respectively. Based on the results obtained in our study, coriander can be introduced as one of the selected options for subsequent studies as a mouthwash candidate.

KEYWORDS: Antibacterial effect, Coriander and Garlic essential oils, Chlorhexidine, *Streptococcus mutans* and *Lactobacillus acidophilus*.

INTRODUCTION

Medicinal plants encompass a significant portion of the existing biodiversity in many regions of the world. The presence of biologically active compounds derived from plants in 25% to 50% of prescribed drugs worldwide attests to the considerable economic value of these plants in human societies.^[1] Medicinal plants hold a special value and importance in maintaining the health and hygiene of contemporary societies, as they can play an effective role in the treatment and prevention of infectious diseases and poisoning. Medicinal plants can be considered a potentially useful chemical resource, only a fraction of which has been utilized.^[2, 3]

Coriandrum sativum L. is a medicinal herb belonging to the Apiaceae family with a growth period of 100 to 120 days. Its fruit and vegetative organs contain an essential oil with medicinal properties. The medicinal properties of this plant include being an appetite stimulant, an anti-flatulent, a digestive aid, a soothing agent for nerves and rheumatic pain, and a blood glucose reducer.^[4,5] Fresh coriander shoots are consumed in salads and soups, and its seeds are used in the food industry as a seasoning. Due to the presence of the active compound, essential oil, this plant is also of significant importance in the pharmaceutical, food, cosmetic, and health industries. Coriander fruit powder is a popular spice in food, and it has been shown that its essential oil and extract possess antibacterial, antioxidant, anti-diabetic, and anticancer properties.^[6,7] Coriander has been widely used in traditional medicine for the treatment of digestive disorders, respiratory and urinary system diseases, relief of anxiety and insomnia, allergies, bloody diarrhea, burns, cough, cystitis, vertigo, edema, fever, headache, hemorrhoids, acne, inflammation, urinary tract infections, and vomiting.^[8,9]

Garlic (*Allium sativum*) is recognized as one of the oldest and most famous medicinal plants for treating most diseases (10). This plant possesses properties such as antiviral, antifungal, antibacterial, and antioxidant effects. *Garlic* has been identified as a potent antibacterial agent, and its inhibitory property has been proven against both Gram-negative and Gram-positive bacteria (11–13). Among the effects associated with the use of *Garlic*, a reduction in blood lipids, systolic blood pressure, glucose levels, and inflammatory biomarkers can be mentioned.^[12,14,15]

Another agent effective in treating patients is Chlorhexidine mouthwash. Mouthwash plays a significant role, as an adjunct tool alongside mechanical plaque inhibition (i.e., brushing and flossing), in controlling supragingival plaque and gingivitis. An ideal mouthwash, in addition to having a broad antimicrobial spectrum, must also possess low drug resistance.^[16] Chlorhexidine is highly effective as a positive control in eliminating pathogenic Gram-positive and Gram-negative microorganisms, including *Streptococcus mutans*. In fact, Chlorhexidine mouthwash has been introduced as an effective drug.^[17,18]

Given the importance of the subject, this study was conducted with the aim of comparing the antibacterial and anti-biofilm effects of garlic and coriander essential oils with chlorhexidine against *Streptococcus mutans* and *Lactobacillus acidophilus*

METHODS

This research was conducted as a descriptive-analytical study on microbial samples (*Streptococcus* and *Lactobacillus*) under the influence of three extracts: garlic, coriander, and 0.2% chlorhexidine. In this laboratory study, three replicates were performed for each sample (*Streptococcus* and *Lactobacillus*) under each extract (garlic, coriander, and 0.2% chlorhexidine).

The method of sample collection and data collection tools involved gathering data in the form of the zone of inhibition report, MIC (Minimum Inhibitory Concentration), MBC (Minimum Bactericidal Concentration), and an anti-biofilm effect assessment. After obtaining permission from the University's Ethics Committee, the *in vitro* laboratory work commenced. The essential oils were purchased, and the bacteria were acquired in lyophilized form from the Iranian Biological Resource Center (IBRC). The antibacterial and anti-biofilm effects of chlorhexidine, coriander essential oil, and garlic essential oil were evaluated using the well diffusion plate method and broth microdilution method.

Preparation of Pathogenic Microorganisms

Two pathogenic bacteria were used in this study: *Streptococcus mutans* and *Lactobacillus acidophilus*. These bacteria were purchased from the Iranian Biological Resource Center (IBRC). To prepare fresh cultures for the microbiological tests, a surface culture was performed on Blood Agar medium one day prior to the tests for each microbial strain. The bacterial strain suspension was adjusted based on the turbidity of 1.5×10^8 Colony Forming Units/mL using the 0.5McFarland standard.

Well Diffusion Plate Method:

To initially evaluate and compare the effect of different essential oils on *Streptococcus mutans* and *Lactobacillus acidophilus*, the well diffusion plate method was utilized. First, the bacteria were cultured on Mueller Hinton Agar medium. Then, using a Pasteur pipette, three wells or holes were created in the medium. Subsequently, 50 μ L of each essential oil was dispensed into the wells, and the plates were incubated at 37°C for 24 hours. After this period, the diameter of the zone of inhibition around each well was measured. This procedure was repeated three times, and the average diameter of the zone of inhibition for each essential oil was determined.

MIC of Chlorhexidine

To compare the antimicrobial effects of the essential oils with a commercial antimicrobial agent, 0.2% chlorhexidine was used. Serial dilutions of 0.2% chlorhexidine (10, 5, 2.5, 1.25, 0.62, 0.31, 0.07, 0.03, 0.01 mg/mL) were prepared in broth.

MIC for Coriander

To determine the MIC of coriander essential oil, the bacterial suspension was first standardized to the 0.5McFarland standard concentration. The essential oil was then dissolved in 10%DMSO, and ten serial dilutions (ranging from 10 to 0.01 μ L/mL) were prepared in broth. To each well of the microplate, broth, standardized bacteria, and subsequently the diluted essential oil solutions were added. After 24 hours of incubation and consideration of positive and negative controls, the Minimum Inhibitory Concentration (MIC) was calculated in μ L/mL.^[19]

MIC for Garlic

First, the bacterial culture was prepared to the 0.5McFarland standard. The garlic essential oil was dissolved in 10%DMSO and serially twofold diluted to concentrations ranging from 0.01 to 10 μ L/.

These dilutions were added to the microplates containing broth and bacteria. After 24 hours of incubation, the Minimum Inhibitory Concentration (MIC) of this essential oil against the pathogenic bacteria was determined.

Anti-biofilm Activity of Essential Oils:

The anti-biofilm activity of the essential oils was evaluated by inoculating sub-inhibitory concentrations in Mueller Hinton Broth supplemented with 1% glucose. A mixture of the essential oil and the bacterial suspension was placed in the wells, with a positive control (no essential oil) and a negative control (only culture medium) considered. Following 72 hours of incubation at 37°C, the formed biofilms were washed with ethanol and stained with Crystal Violet. Finally, glacial acetic acid was added to each well to extract the dye, and the optical density was read at a wavelength of 595 nm using an ELISA reader.

Determination of Minimum Bactericidal Concentration (MBC)

To determine the MBC of the coriander seed and garlic essential oils, 100 µl from each well where no color change was observed was transferred to Mueller Hinton Agar medium and cultured. The lowest concentration of the essential oil that showed no bacterial colony growth was reported as the MBC.^[20,21]

Results of the MIC Method

For MIC determination, dilutions of the essential oil were prepared in Mueller Hinton Broth and inoculated with the microbial suspension (final concentration of 5×10^5) in triplicate. After 24 hours of incubation, the lowest inhibitory dilution was recorded as the MIC. The anti-biofilm activity of the essential oils (0.03 to 0.31 µl) against the biofilm (comprising *Streptococcus mutans* and *Lactobacillus acidophilus*) was investigated using Mueller Hinton medium supplemented with 1% glucose. After 48 hours of incubation, the biofilms were stained with Crystal Violet, and their optical density was read at 595 nm. Finally, the percentage of biofilm reduction was calculated using a formula based on the optical density readings of the positive control (C), negative control (B), and treated samples (T).^[13]

RESULT

Analysis of the results of the parametric test for repeated measures showed that a significant difference was observed between the amount of growth inhibition zone for *Streptococcus mutans* bacteria for the three essential oils of garlic, coriander and chlorhexidine 0.2%, and $p=0.028$ and $x^2=6$. Also, for *Lactobacillus acidophilus* bacteria with three repetitions for each essential oil of garlic, coriander and chlorhexidine 0.2%, a significant difference was also observed between all three groups. ($X^2=6$ and $p\text{-value}=0.028$)(Table1).

Table 1: Plate well results of the effect of different essential oils on *Streptococcus mutans* and *Lactobacillus acidophilus*.

Bacteria	Zone of no growth (mm) repeated three times			P-value
	Garlic	Coriander	Chlorhexidine 0.2%	
<i>Streptococcus mutans</i>	44, 5,	11,12, 13	10,11,10	0.028
Mean \pm SD	4,33 \pm 0.58	12 \pm 1	10,33 \pm 0.58	
<i>Lactobacillus acidophilus</i>	6,7,6	18,17,19	12,11,13	0.027
Mean \pm SD	6,33 \pm 0.58	18 \pm 1	12 \pm 1	

Both *Streptococcus mutans* and *Lactobacillus acidophilus* bacteria had moderate biofilm formation intensity. Garlic essential oil at a concentration of 5 µl/ml, coriander at a concentration of 0.31 µl/ml, and 0.2% chlorhexidine at a concentration of 1.25 µl/ml caused a 25%, 100%, and 50% reduction in biofilm formation, respectively (Table 2).

Table 2: MIC and MBC results of the effect of different essential oils on *Streptococcus mutans* and *Lactobacillus acidophilus*.

Bacteria	Minimum Inhibitory Concentration Range (µl/ml)		
	Garlic	Coriander	Chlorhexidine 0.2%
<i>Streptococcus mutans</i>	10 - 2.5	10 - 0.03	10 - 0.31
Lowest concentration	2.5	0.03	0.31
<i>Lactobacillus acidophilus</i>	1.25 10 -	10 - 0.01	10 - 0.15
Lowest concentration	1.25	0.01	0.15
MBC (µl/ml)			
<i>Streptococcus mutans</i>	5	0.07	0.62
<i>Lactobacillus acidophilus</i>	2.5	0.02	

DISCUSSION

This study was conducted with the aim of comparing the antibacterial and anti-biofilm effects of Coriander and Garlic essential oils with chlorhexidine against *Streptococcus mutans* and *Lactobacillus acidophilus*, given the importance of the topic. Various studies have investigated the effectiveness of chlorhexidine mouthwash on patient improvement. A study conducted by Ajagannanavar et al. compared the effectiveness of chlorhexidine with aqueous and alcoholic extracts of the Stevia plant. The results of this research showed that the alcoholic extract of Stevia has greater inhibitory power against the cariogenic bacteria, namely *Streptococcus mutans* and *Lactobacillus acidophilus*, compared to its aqueous extract. Despite this slight superiority over the aqueous form, the antimicrobial effect of the alcoholic Stevia extract was ultimately assessed as weaker compared to the standard substance, chlorhexidine.^[22] In the study by Evans et al., it was also shown that the dominance of chlorhexidine over other tested mouthwashes was clearly evident, as it created the highest growth inhibition rate for both *S. sanguinis* and *S. mutans* strains.^[23]

In the study by Hegde and colleagues, 75 children aged 8 to 12 years who had dental caries were examined, and the effect of using green tea extract compared to chlorhexidine was investigated. In accordance with the findings, although the green tea extract mouthwash caused a reduction in children's dental caries, the reduction rate in the group that received the chlorhexidine mouthwash was greater.^[24] Puri et al. used a mouthwash containing coriander, clove, and mint. In accordance with the findings, this mouthwash demonstrates a high potential for use as an adjunctive agent alongside conventional (mechanical) methods for treating and preventing gum inflammation (gingivitis).^[25]

Regarding studies that examined the effect of Garlic on the oral status of patients, the study by Sasi et al. can be mentioned. In accordance with the findings, while in vitro and intervention studies have confirmed the antimicrobial activities of garlic against oral pathogens (such as *S. mutans* and *L. acidophilus*), as well as its beneficial antioxidant, anti-inflammatory, and anti-carcinogenic effects against periodontitis and caries, conflicting results have been reported regarding the unique properties of its bioactive compounds and how to increase their bioavailability; considering these contradictions and discussions related to the safety of oral consumption, the potential of this extract as a therapeutic solution for oral and dental diseases remains significant.^[26] In fact, it can be said that garlic serves as a suitable alternative to antibiotics and has broad antibacterial effects against various types of bacteria.^[27] Given the potential mentioned for garlic extract, investigating its effects in combination with common dental antibiotics, especially at low doses, can be a promising research strategy. It is expected that garlic can create a Synergism effect with these antimicrobial agents.^[28,29] Houshmand et al. conducted a study on the effect of garlic on dental plaque, and it was shown that all microorganisms studied were susceptible to garlic extract, and no resistance was observed. These findings suggest that garlic extract can be an effective active component in oral hygiene formulations such as toothpaste

and mouthwash to combat caries and gum diseases, although further studies are necessary to definitively prove this efficacy in the clinical setting.^[28] Also, the study conducted by Groppo et al. showed that the use of a mouthwash containing 2.5% garlic extract significantly reduced the number of *Streptococcus mutans* bacteria—one of the main causes of dental caries—in the mouths of participants.^[30]

CONCLUSION

The results of the comparative study between the three mouthwashes (Coriander, Garlic, and Chlorhexidine) showed that Coriander essential oil has high potential. For this reason, the Coriander mouthwash is specifically introduced as the option of choice for the next stages of research. This is to develop a new generation of more effective mouthwashes.

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Conflict of Interest

No Conflict of Interest

Ethical Considerations

In order to comply with ethical standards in research, a consent form was nevertheless prepared for all study participants and the intervention was fully explained. The present study was conducted after obtaining the creative code (IR.MEDILAM.REC.1402.190) from the Ethics Committee of Ilam University of Medical Sciences.

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