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Antibacterial Effect of Bitter and Sweet Almond Hydroalcoholic Extracts on *Streptococcus mutans*, *Lactobacillus acidophilus*, *Enterococcus faecalis*, *Streptococcus salivarius*, and *Streptococcus sobrinus*

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Abstract

Background and objectives: The use of antibacterial substances is necessary to reduce pathogens without damaging the normal flora of the oral cavity. Therefore, this study aimed to investigate the antibacterial effect of bitter and sweet almond extracts on *Streptococcus mutans*, *Lactobacillus acidophilus*, *Enterococcus faecalis*, *Streptococcus salivarius* and *Streptococcus sobrinus*.

Materials and methods: In this experimental study, laboratory samples were analyzed in three groups as follows: bitter almond extract (*Prunus Amygdalus amarus*) group, sweet almond extract (*Prunus Amygdalus dulcis*) group and 0.2% chlorhexidine. To determine the antibacterial effects of the extracts on the aforementioned bacteria, the disk agar diffusion method was performed according to the CLSI manufacturers. Data were collected and analyzed using SPSS26 software as well as Kruskal-Wallis and Mann-Whitney tests.

Results: Findings suggested that Chlorhexidine had the greatest antibacterial effect against the tested bacteria. Bitter and sweet almond extract had no effect on *E. faecalis* and despite the effect on other bacteria; this effect was not statistically significant ($P > 0.05$). A 12.33 mm and 10.6 mm inhibition zone diameter of the bitter and sweet almond extracts was observed against *S. sobrinus*, respectively.

Conclusion: The antibacterial effect of chlorhexidine on the tested oral bacteria was much higher than the bitter and sweet almond extracts. Also, the bitter and sweet almond extracts had no effect on *E. faecalis*. However, the bitter almond was more effective than the sweet almond.

Keywords: Bitter almond; Sweet almond; *Prunus Amygdalus*; Antibacterial; *Streptococcus; Enterococcus*; *Lactobacillus*.

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1. Introduction

The oral microorganisms dispersed in the dorsum of the tongue, mucosal surfaces, teeth and saliva have a diverse and distinct ecological habitat owing to the heterogeneous

properties of the oral tissues and structures. Over 700 cultivable bacterial species have been found in the oral cavity due to advances in molecular techniques allowing us to better comprehend the diversity of microbial ecosystems (1). Oral conditions, such as dental caries, periodontal diseases, and in some cases, bacterial endocarditis and pneumonia are caused by an imbalance in the oral microbiota. Therefore, reducing and eliminating pathogenic bacteria to maintain the normal range of oral flora is essential for preventing the oral diseases (2).

Some microorganisms, such as *Streptococcus Sanguis*, *Streptococcus salivarius*, *Streptococcus mutans*, *Streptococcus sobrinus* can attach to the dental structures and form the dental plaque (oral biofilm) (3, 4). Additionally, *Enterococcus faecalis* is a gram- positive facultative anaerobic coccus in the oral microflora that may contribute to persistent endodontic infections (5). Moreover, among the wide range of bacteria living in the oral cavity and creating acids from fermentable carbohydrates, *S. mutans* and Lactobacilli are the most well- known pathogens for dental caries (6).

Prevention of the infections caused by these organisms is difficult due to the resistant nature of the biofilms against antibiotics (7). Thus, scientists are looking for new solutions to deal with this problem. One of the ways to combat the biofilms is the use of the medicinal plants. The Rosaceae family of plants, such as almonds are the sufficient selection for their antimicrobial effect. *Prunus Amygdalus dulcis* refers to sweet almond and *Prunus Amygdalus amarus* refers to bitter almond. The latter are distinctive because they have amygdalin, a substance that gives them a bitter flavor (8). The phytochemical classes of macronutrients (fat, protein, and carbohydrate), micronutrients (minerals and vitamins), essential oils and different polyphenols have all been detected in almond. Probiotic, antimicrobial, antioxidant, anti- inflammatory, anti- cancer, hepatoprotective, cardiometabolic protection, nootropic, anxiolytic, sedative- hypnotic, and nervous system-improving properties are only a few of the biological benefits of this plant (9).

Due to the high prevalence of antibiotic-resistant bacteria, we need to search for new methods to reduce the pathogenic bacteria without damaging the normal flora. Almonds contain polyphenols, hydrocyanic acid and light sugars that give them antibacterial properties (10). Studies indicate that the polyphenolic compounds found in almond skin can inhibit the growth of oral pathogens such as *Staphylococcus aureus* and *Escherichia coli*, which are associated with periodontal disease and other oral infections. By reducing bacterial load in the oral cavity, almonds may help prevent gingivitis and promote overall gingival health. Furthermore, the anti- inflammatory effects of almond by- products can support healing processes in oral tissues, making them a promising natural adjunct in dental care. Continued research into these benefits could lead to innovative applications of almonds in maintaining oral hygiene and preventing dental diseases.(10, 11) Therefore, the present study hypothesized that the hydroalcoholic extracts of bitter and sweet almonds exhibit significant antibacterial effects against *Streptococcus mutans*, *Lactobacillus acidophilus*, *Enterococcus faecalis*, *Streptococcus salivarius* and *Streptococcus sobrinus*.

2. Materials and methods

The current experimental study was conducted in the Pharmacy and Medicine Faculties at Mazandaran University of Medical Sciences, Mazandaran, Iran. The sample size was calculated based on Tularat Sookto's study (12). Considering the significance level (α) of 0.05, the power of the test (1-β) is 95, the number of 2 samples is required for each group. The 200 gr of the bitter extract and 160 gr of the sweet extract seeds were powdered using a mortar. Using the maceration method, the powders were then soaked with 200 ml of 70% ethanol solvent (Merck, Germany) in a decantation funnel (Glassco, India) and were kept at room temperature for three days. The obtained extract was passed through filter paper and was concentrated by vacuum distillation using a rotary evaporator (IKA, Germany). Finally, it was dried with a freeze dryer (DorsataK, Iran) and kept in the refrigerator in a closed container away from light until use. For antimicrobial testing, the concentrations of the sweet and bitter almond powders dissolved in water were 770 mg/mL and 200 mg/mL, respectively (13).

The standard strains of the bacteria were purchased from the Iranian Research Organization for Science and Technology (IROST). The disk agar diffusion method was used to detect the antimicrobial effect of the extracts according to the Clinical and Laboratory Standards Institute (CLSI) manufacturers (14). Laboratory samples were analyzed in three groups as follows: the bitter almond hydroalcoholic extract in concentrations of 200, 100, and 50 mg/mL, the sweet almond hydroalcoholic extract in concentrations of 770, 385, and 192.5 mg/mL, and the chlorhexidine 0.2% . The antimicrobial effect of

these agents was determined against *S. mutans*, *S. salivarius* and *S. sobrinus* on Mueller Hinton (Condalab, Spain) blood agar containing 5% of sheep blood (15). However, the disk agar diffusion test for *L. acidophilus* was conducted on Man, Rogosa and Sharpe (MRS) agar (Condalab, Spain) containing 0.05% L-cysteine (Sigma, Germany) (16), and the Mueller Hinton agar (Condalab, Spain) was used for *E. faecalis* (17). All tests were done in three times and the means were reported in this study.

In the disk agar diffusion test, turbidity equal to 0.5 McFarland suspensions (1.5×10^8 cfu/mL) was prepared from each bacterium and was inoculated on culture media. Then, the blank disks were impregnated with 30 µL of different concentrations of the tested antimicrobial substances and were placed on the culture media of each bacterium. Then, the *L. acidophilus* was incubated under anaerobic conditions at 37˚C for 48 h, while the rest of the bacteria were incubated in the presence of 10% Co_2 at 37˚C for 48 h. Next, the plates were observed under the light and the inhibition zones of the bacterial growth were measured by a ruler. The no growth zone diameters were compared with the positive control (chlorhexidine 0.2%).

Data analysis was conducted at two descriptive and inferential levels. At the descriptive level, we used the frequency distribution table, mean, and standard deviation, and at the inferential level, the Kruskal-Wallis test was used to compare the results. The normality hypothesis was tested with the Shapiro-Wilks test. The analysis was done in SPSS v22 software and a P-value ≤ 0.05 was considered as the significance level.

3. Results

Findings of this study regarding the antibacterial properties of the hydroalcoholic extracts of the bitter and sweet extracts, along with the chlorhexidine showed that the 100, and 50 mg/mL concentrations of the bitter almond and the 385, and 192.5 mg/mL concentrations of the sweet almond did not have an antimicrobial effect against the tested bacteria. Therefore, only the antimicrobial results of 200 mg/mL for bitter extract and 770 mg/mL for sweet extract are presented in Table 1. The bitter almond had the better antimicrobial effect than the sweet almond. However, we found a considerable antimicrobial effect of the bitter almond against the *L. acidophilus* and *S. sobrinus.*

Descriptive information of bacteria was compared in three groups (Table 2). The average of *S. mutans*, *S. salivarius* and *S. sobrinus* was higher in the chlorhexidine group than in the other groups, but this difference was not statistically significant (P = 0.212, P = 0.069, and P = 0.069, respectively). Furthermore, the average in *E. faecalis* was zero in the study groups and no inhibition were observed. Although the chlorhexidine group had an average of 24, the difference between the groups was not statistically significant (P = 0.050). Additionally, the ave-

Table 1. Growth zone diameter of the bacteria caused by the medicinal extracts.

Bacteria	Indicator	Bitter almond extract	Sweet almond extract	Chlorhexidine
Streptococcus mutans	Mean (mm)	5.67	5	22.33
	SD	0.58	1	0.58
Enterococcus faecalis	Mean (mm)	0	0	25
	SD	0	0	1
Lactobacillus acidophilus	Mean (mm)	11.67	7	27.33
	SD	0.58	1	1.15
Streptococcus sobrinus	Mean (mm)	12.33	10.67	24.33
	SD	0.58	0.58	0.58
Streptococcus salivarius	Mean (mm)	7.33	5.67	20.67
	SD	0.58	0.58	0.58

Table 2. Descriptive information of different bacteria in three groups of antimicrobial agents

Bacteria	Group	Mean ± SD	Median	Average rank	P-value
Streptococcus mutans	*Prunus Amygdalus dulcis*	5.00 ± 1.00	5	2.83	0.212
	Prunus Amygdalus amara	5.67 ± 0.58	6	4.17	
	Chlorhexidine	22.33 ± 0.58	22	7.00	
Streptococcus salivarius	*Prunus Amygdalus dulcis*	5.67 ± 0.58	6	2.00	0.069
	Prunus Amygdalus amara	7.33 ± 0.58	7	5.00	
	Chlorhexidine	20.67 ± 0.58	21	7.00	
Streptococcus sobrinus	*Prunus Amygdalus dulcis*	10.67 ± 0.58	11	2.00	0.069
	Prunus Amygdalus amara	12.33 ± 0.58	12	5.00	
	Chlorhexidine	24.33 ± 0.58	24	7.00	
Enterococcus faecalis	*Prunus Amygdalus dulcis*	0.00 ± 0.00	0	3.50	0.050
	Prunus Amygdalus amara	0.00 ± 0.00	0	3.50	
	Chlorhexidine	25.00 ± 1.00	25	7.00	
Lactobacillus acidophilus	*Prunus Amygdalus dulcis*	7.00 ± 1.00	7	6.00	0.073
	Prunus Amygdalus amara	11.67 ± 0.58	12	15.00	
	Chlorhexidine	27.33 ± 1.15	28	7.00	

-rage of *L. acidophilus* was higher in the chlorhexidine group than in the other groups but the difference was not significant (P = 0.073).

4. Discussion

The primary aim of this study was to investigate the antibacterial effects of bitter and sweet almond extracts on several oral pathogens, including *Streptococcus mutans, Lactobacillus acidophilus, Enterococcus faecalis, Streptococcus salivarius* and *Streptococcus sobrinus*. The findings demonstrated that chlorhexidine exhibited the strongest antibacterial effect, while both almond extracts had no impact on *E. faecalis*. Although the bitter and sweet almond extracts showed some inhibitory effects on other bacteria, these were not statistically significant, with the bitter almond being slightly more effective than the sweet almond.

Due to the increased resistance rates of the bacteria caused by the inappropriate use of antibiotics, it seems necessary to find alternative drugs that have both antibacterial properties and the least side effects for humans. There are various bacteria in the oral cavity, some of which play an important role in maintaining health (normal flora) and some in causing oral and dental diseases (pathogens). Therefore, reducing and eliminating pathogenic bacteria and maintaining the normal flora is essential in preventing dental cavities and oral diseases. The present investigation assessed the antibacterial properties of the *Prunus Amygdalus dulcis* and *Prunus Amygdalus amarus* extracts on some oral bacteria including in dental caries, such as *S. mutans*, *S. salivarius*, *S. sobrinus*, *E. faecalis* and *L. acidophilus*. Topical use of the *Prunus Amygdalus dulcis* and *Prunus Amygdalus amarus* oil has antibacterial and antiparasitic properties, in addition to reducing itching in measles, scarlet fever

and eczema in children (18). Plants used in traditional medicine have a wide range of substances that can be used to treat chronic diseases. Some chemicals produced by plants have specific physiological effects on the human body. Alkaloids, flavonoids, tannins, and phenolic compounds are among the most vital bioactive plant compounds (19).

Based on the results of the present study, the largest diameter of the no growth zone of the investigated bacteria was related to chlorhexidine. This diameter in the *Prunus Amygdalus amarus* group was more for *S. sobrinus* (12.33 mm) followed by *L. acidophilus* (11.66 mm), *S. salivarius* (7.33 mm), *S. mutans* (5.66 mm), and finally *E. faecalis* (0 mm). In the *Prunus Amygdalus dulcis* group, the largest diameter belonged to *S. sobrinus* (10.66 mm) followed by *L. acidophilus* (7 mm), *S. salivarius* (5.66 mm), *S. mutans* (4.33 mm) and *E. faecalis* (0 mm). In all the investigated bacteria, except for *E. faecalis*, *Prunus Amygdalus amarus* was more effective than *Prunus Amygdalus dulcis*. In other words, *Prunus Amygdalus amarus* has more effective toxic substances and compounds compared to *Prunus Amygdalus dulcis*, however, the differences were not statistically significant.

In contrast to the present study, Musarra et al. reported significant antibacterial and antiviral effects in a combination of polyphenols found in the skin of almonds (10). This controversy can be due to using the almond kernels of different strains in our investigation. On the other hand, Pereira et al. suggested significant antibacterial effects of almond oil on *Staphylococcus aureus* and *Escherichia coli* which was also in contrast with the current findings (20). Using the hydroalcoholic extract and different strains can be attributed to these contrasts. Moreover, Bouaziz et al. revealed that solvent compounds of almond gum extract had significant antibacterial properties against *Enterobacter spp.*, *Salmonella typhimurium*, *Listeria monocytogenes*, *Micrococcus luteus* and *Bacillus subtilis* (21). They used different strains of bacteria which can be the reason for this controversy. Additionally, Abtahi et al. reported the significant antibacterial effects of *Prunus Amygdalus amarus* and *Tribulus terrestris* extracts on *S. aureus*, *Salmonella typhi*, *Pseudomonas aeruginosa* and *E. coli* (18).

Several parameters affect the antibacterial activity of the plants. Factors such as the amount of plant extract used the type of environment and materials, the concentration of the extract, and the extraction method as well as the used solvent. Extracts obtained by different methods and using different solvents from the same plant can show different antimicrobial effects on certain types of microorganisms. In addition, different concentrations of the extract also affect its antimicrobial activity. Previous studies showed that the antimicrobial effects of the plant were changed by different concentrations of the extract (22). The environment used in the antimicrobial tests also has a great effect on the antimicrobial properties of the extracts. Also, the use of different parts of the plant affects the antimicrobial property of the extract (18). It is suggested that the use of active ingredients of different fractions of the medicinal plants can increase the antimicrobial effect.

5. Limitations

A limitation of this study is the lack of replication for the chlorhexidine group, which prevented the calculation of standard deviation and limited our ability to assess variability within this group. Future studies should include adequate sample sizes for all groups to ensure more reliable statistical analyses and conclusions.

6. Conclusion

The antibacterial effect of chlorhexidine on oral bacteria including *Streptococcus mutans*, *Lactobacillus acidophilus*, *Enterococcus faecalis*, *Streptococcus salivarius* and *Streptococcus sobrinus* was much higher compared to the *Prunus Amygdalus amarus* and *Prunus Amygdalus dulcis* extracts. Moreover, these extracts had no effect on *E. faecalis*. However, the *Prunus Amygdalus amarus* extract was more effective than *Prunus Amygdalus dulcis* extract on other bacteria, but the difference was not statistically significant. These components may be the adjuvants helping the treatment of the oral infections.

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Ethics

The current experimental study was conducted in the Pharmacy and Medicine Faculties at Mazandaran University of Medical Sciences, Mazandaran, Iran. The study protocol was approved by the ethics committee of Mazandaran University of Medical Sciences with the ethical code of IR.MAZUMS.1401.528.

Author contributions

Leyli Sadri: Methodology
Mehdi Taghian: Conceptualization
Aboutaleb Rahimian: Investigation, Methodology
Abolfazl Hosseinnataj: Software
Melika Mollaei: Writing – Original draft
Hamid Reza Goli: Conceptualization, Writing – Review & editing

Conflict of interest

The authors declare no conflict of interest.

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