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Antibacterial Effects of the Hydroalcoholic Extract of Myrtus Communis Leaves on Streptococcus Mutans

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Objectives Although dental caries is the most common chronic oral disease globally, there is no comprehensive plan for preventing this microbial disease. Streptococcus mutans is one of the essential causes of dental caries. Owing to the adverse effects associated with antibiotics, researchers are focusing their efforts on the development of antimicrobial compounds derived from medicinal plants. This study aimed to investigate the antimicrobial effects of the ethanol extract derived from the leaves of Myrtus communis on *S.* mutans.
Methods In this in vitro study, the researchers initially procured the Myrtus communis extract. Subsequently, they determined its dry weight and evaluated its antimicrobial activity utilizing the well-dilution method. The antimicrobial efficacy was determined by measuring the minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and the inhibition zone diameter surrounding the extract. Ultimately, the data were examined using the Mann-Whitney test. Differences less than ($P<0.05$) was significant statistically.
Results The MIC and MBC of the extract for *S.* mutans were found to be 5 mg/mL and 10 mg/mL, respectively. Chlorhexidine, used as a positive control, exhibited the same values. Furthermore, the diameter of the inhibition zone around both the extract and chlorhexidine was measured to be 17±1 mm and 15±1 mm, respectively. The difference between these measurements was not statistically significant ($P=0.077$).
Conclusion The ethanol extract from the myrtle leaves was as efficient as chlorhexidine mouthwash against *S.* mutans. Further investigations are needed into the antibacterial effects of different medicinal plants on cariogenic microorganisms.
Keywords Streptococcus mutans; Myrtus, Herbal extract; Plant extract

Introduction

Dental plaque is a biofilm composed of microorganisms that adhere to the tooth surface and can lead to dental caries. Dental caries, often referred to as tooth decay, is one of the most prevalent oral diseases. It is primarily caused by the colonization of oral microorganisms, such as Streptococcus mutans (S. mutans) and Lactobacillus. [1, 2] Cariogenic microorganisms, such as S. mutans, are essential in initiating carious lesions in the enamel. [1]

Streptococcus mutans is a Gram-positive bacterium found in the mouth environment, which creates an acidic medium by metabolizing different carbohydrates. The primary virulence factor of this bacterium is its capacity for extracellular glucan synthesis, which contributes to the development of dental caries in both animals and humans. [3, 4] Today, more than 90% of schoolchildren and most adults have dental caries worldwide. This indicates the demand for improved diagnostic and therapeutic procedures in dentistry, especially in children. [5]

Mechanical plaque control techniques, such as tooth brushing and flossing, are widely adopted for dental plaque removal. [2, 6] However, some individuals, especially the handicapped or the elderly, may be unable to remove mechanical plaque sufficiently. Therefore, using antimicrobial mouthwashes may benefit such individuals. [6, 7] A study on irrigating solutions concluded that chlorhexidine leads to tooth discoloration, creates a burning sensation in the mouth, and causes loss of taste. [8]

The misuse or overuse of antibiotics is expanding worldwide. Synthetic drugs can have detrimental effects, such as liver complications, especially in children. [5] In relation to diseases caused by microorganisms, the growing resistance of many common pathogens to currently employed therapeutic agents, such as antiviral drugs and antibiotics, has reignited interest in the discovery of new anti-infective compounds. [9] In addition, not all people have access to synthetic drugs, and therefore, they may utilize herbal medicines as alternatives. [5]

Medicinal plants have a longstanding history in both the medical and dental fields, and their use is widespread globally. The anti-inflammatory, antibacterial, and antioxidant properties of these plants, coupled with their biocompatibility, account for the increasing interest in herbal medications. [5] Herbal medicines have seen a surge in popularity in recent years. This includes numerous therapeutic agents that have shown promising results in the treatment of periodontal diseases. [10]

So far, several studies have been conducted investigating the favorable antimicrobial properties of some natural plants on S. mutans. [2, 6, 9, 11, 12] Suitable antibiotics, including natural plant products, may decrease mortality and healthcare costs. [13] Myrtle (Myrtus communis), a member of the Myrtaceae family, is an evergreen, aromatic shrub characterized by its numerous stems and branches. This herb can be found in various locations, including the White Lab of Bakhtiari Valley, Khorasan River Valley, Sarab, Guilan-e-Gharb, Kerman, Maharlu in Shiraz,

Neyriz, Fasa, Mamasani, and Bandar Abbas. [14]

Verica Aleksic [15] proved that the myrtle herbal essence could have antimicrobial and antioxidant activities on Escherichia coli and S. aureus. In addition to its disinfectant properties, this species is utilized as a stomach strengthener and astringent. It is also employed in the treatment of respiratory and urinary tract diseases, both internally and externally. [15] Certain bumps, known as "plant galls", form on the stem of the myrtle tree. These bumps are rich in tannins. Some researches on extracted crystalline substances from the leaves of this herb, exhibited bacteriostatic properties. Moreover, these substances demonstrate germicidal effects at higher concentrations. This property is attributed to the phenolic and polyphenolic compounds present in the herb. [15]

The dried leaves of myrtle are known to contain compounds, such as terpinolene, cineol, linalool, terpineol, linalyl acetate, tannins, and flavonoids. Numerous reports have highlighted the anti-parasitic and anti-infective properties of this herbal extract. Furthermore, its antiviral effect has been harnessed in the production of a drug against the herpes simplex virus. This showcases the significant potential of medicinal plants in the development of new therapeutic agents. [14]

The World Health Organization (WHO) has introduced herbal medicines as plant‐derived substances that contain raw ingredients from one or multiple plants with therapeutic uses. [16] A few recent studies have investigated the antimicrobial activity of herbal sources against oral pathogens, and the use of herbal products has been one of the most successful strategies for detecting new drugs. However, to date, no antimicrobial herbal agent has been found to completely prevent dental caries. [1] Also, no prior study has evaluated the anti-caries effects of myrtle leaves. Therefore, the objective of this study was to assess the antimicrobial effects of the ethanol extract obtained from the leaves of Myrtus communis on S. mutans.

Methods and Materials

This in vitro study was carried out after obtaining the approval of the Ethics Committee of Zahedan University of Medical Sciences, Zahedan, Iran (IR.ZAUMS.Rec.1394.365). The bacterial community for this study was prepared using kits from the Bank of Microbes of Pasteur Institute in Tehran, with S. mutans bacteria present in the medium. The specific strain of S. mutans used was the Persian Type Culture Collection (PTCC) No. 1683.

Extract collection from the myrtle leaf:

The extract was produced by the macerating method. [2, 7] After the leaves were chopped, 50 g of each sample was macerated and stored in methanol (250 mL) in a dark location for 48 hours. Subsequently, the obtained extracts were filtered using a Whatman filter paper and concentrated at 50°C through rotary vacuum distillation. The concentrated extracts were then transferred to a sterile Petri dish and dried in an oven for two days.

Determination of the weight of dried extract

Initially, a test tube was weighed, and 1 mL of the extract was added. The content of the tube was then allowed to dry at room temperature. As the extract dried, its weight was measured. The difference between the initial and final weights was calculated to determine the weight of the dried extract.

Antibacterial examination:

The sensitivity of bacterial isolates against the myrtle extract was evaluated using the dilution method [2, 7] in an aqueous environment in round-bottom 96-well plates. Only the cultivation medium and bacterial suspension were added to the first row of wells on the plate. Additionally, 0.2% chlorhexidine mouthwash and a culture medium with a bacteria-free extract served as positive and negative controls, respectively. In the next row of the plate, 100 µL of Muller Hinton Broth was added to six wells. Following this, 100 µL of a diluted extract solution with a concentration of 10 mg/mL was added to the first well. Using a dilution method, concentrations of 10, 5, 2.5, and 1.25 mg/mL were prepared up to the sixth well, respectively. Finally, 20 µL of a bacterial suspension containing 10^7 units per milliliter, equivalent to 0.5 McFarland, was added to each well.

The contents of each well were mixed for two minutes using a plate reader equipped with a shocker. Blank discs were inoculated with 20 µL of each concentration of the extract and placed on Muller-Hinton and blood agar, which were previously cultivated with the bacterial strain. The spectroscopic activity was gauged at a wavelength of 630 nm at the initial time point. Subsequently, the plates were incubated at a temperature of 37°C for 24 hours. The first well that inhibited bacterial growth following incubation was identified as the Minimum Inhibitory Concentration (MIC).

To confirm the presence of transparent wells, 10 µL of the solution was extracted and transferred to a Mueller-Hinton Agar medium. After a 24-hour period, the first dilution that was capable of eliminating 99.9% of the bacteria was identified as the Minimum Bactericidal Concentration (MBC). The experiments were conducted in triplicate. [7, 14] The opacity or clarity of the wells was visually assessed. The bacterial inhibition zone was measured in millimeters (mm) and recorded using Vernier calipers. [7, 14] The various concentrations of chlorhexidine, including dilutions of 10, 5, 2.5, and 1.25 mg/mL, were considered as the MIC. A similar approach was applied for the extract of myrtle leaf herb.

At the end of the experiments, the data obtained from laboratory evaluations were described in SPSS Version 20, using the Spearman's correlation test and the Mann-Whitney test. Differences less than (P<0.05) was significant statistically.

Results

The results of this study revealed that the MIC and MBC of the myrtle leaf extract for S. mutans were 5.00 and 10.00 mg/mL, respectively. In comparison, the MIC and MBC of chlorhexidine were 50 and 100 mg/mL, respectively (as shown in Table 1). The Spearman's correlation test indicated a significant inverse relationship between the increase in the concentration of the experimental substances and the degree of opacity (r=0.986, P=0.000). Additionally, the diameters of the inhibitory growth zone around the herbal extract and chlorhexidine were 17±1 mm and 15±1 mm, respectively (as detailed in Table 2). However, this difference was not statistically significant (P=0.077).

Table 1- Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) for the Hydro-Alcoholic extract of Myrtus leaves and Chlorhexidine against S. mutans

Experimental substances	MIC (mg/mL)	MBC (mg/mL)
Hydro-Alcoholic extract of Myrtus leaves	5	10
Chlorhexidine	5	10

Table 2- Comparison of the halo diameter of the non-growth area between the Hydro-Alcoholic extract of Myrtus leaves and Chlorhexidine against S. mutans

Experimental substances	Halo diameter, mm (mean ± standard deviation)	Mann-Whitney P-Value*
Hydro-Alcoholic extract of Myrtus leaves	17± 1	0.077
Chlorhexidine	15± 1	

* The significance level is ≤ 0.05

Discussion

Despite being preventable, dental caries remains one of the most prevalent chronic diseases globally. Its microbial etiology is associated with factors that contribute to dental infections. Streptococcus mutans (S. mutans) is primarily responsible for dental caries. [2] Hence, in this study, the antimicrobial effect of the hydroalcoholic extract of myrtle leaves on S. mutans was evaluated by measuring the MIC, MBC, and inhibitory growth zone diameter.

Based on the laboratory findings, it can be stated that the MIC and MBC for both the myrtle leaf extract and chlorhexidine were 5 and 10 mg/mL on S. mutans, respectively. These results are in line with the findings of a study by Verica Aleksic et al. [15]. In their study, the myrtle herbal extract had antimicrobial effects on six Gram-positive bacteria (S. aureus, Micrococcus hiatus, S. pneumoniae, S. pyogenes, S. agalactiae, and Listeria monocytogenes) and four gram-negative bacteria (E. coli, Proteus vulgaris, Pseudomonas aeruginosa, and Campylobacter jejuni). [15]

Additionally, Taheri et al. [14] concluded that the extract of Myrtus communis leaves demonstrated a significant antibacterial effect against S. aureus and V. cholerae Ogawa serotype. However, it had no effects on Pseudomonas aeruginosa and only a minor impact on E. coli. The antimicrobial properties of the myrtle extract are attributed to compounds known as polyphenols, which primarily exhibit antibacterial activity. Additionally, two key substances, known as Myrtucommulone A and B, derived from this extract, have been found to exert antimicrobial effects, particularly on Gram-positive bacteria. [15]

Moreover, Rotstein related the antibacterial effect of myrtle herbal extract, especially on Gram-positive bacteria, to Mirtocomolon A. [17] Rotstein reported that the myrtle extract had no significant effects on Gram-negative bacteria. [17] However, this extract exhibited antimicrobial effects on Myrtucommulone, particularly against Gram-positive bacteria. This was evidenced by an inhibitory growth zone diameter of 35 mm in relation to S. aureus. [17]

In the current study, the diameter of the inhibitory growth zone around the myrtle herbal extract and chlorhexidine was found to be 17±1 mm and 15±1 mm, respectively; the observed difference was not statistically significant. This discrepancy can be attributed not only to the different bacteria used in the two studies, but also to the fact that Rotstein utilized the absolute composition of Myrtucommulone A. In contrast, our study did not employ the pure form of Myrtucommulone A. AL-Saimary et al. [18] explored the antibacterial effects of the methanol extract of myrtle herb on various bacteria, including S. aureus, P. aeruginosa, S. salivarius, S. sanguinis, mutans streptococci, and diphtheroids. The findings from these investigations

were consistent with the results of our study. It was suggested that the antimicrobial effect is triggered by an increase in oxygen free radicals and lipid peroxidation, which subsequently leads to damage to the microbial cell wall. [18]

Almas et al. [19] investigated the antimicrobial activities of eight different types of mouthwash and a 50% Miswak extract against several microorganisms, including S. faecalis, S. mutans and S. sanguis. They found that the antimicrobial effect of chlorhexidine surpassed that of the myrtle herbal extracts tested. They concluded that mouthwashes containing chlorhexidine demonstrated the highest antimicrobial activity. In contrast, the Miswak extract exhibited relatively low antimicrobial activity [19]; this conclusion is in contrast to this study. The main reason for this difference may be the high number of microorganisms in the study by Almas and colleagues.

However, the Hosseini Tabatabaei et al. [20] study showed that the ethanol extract of Juglandaceae (walnut tree roots) had desirable inhibitory effects on S. mutans, which agrees with the present study.

Moreover, in a study by Bhattacharyaa et al. [21], the plant extracts demonstrated varying levels of inhibitory effects on the growth of oral pathogens between the hexane and ethyl acetate extracts. The hexane extract of Piper betle showed maximum activity with an inhibition zone of 15 mm, while the ethyl acetate extract exhibited a slightly higher activity with an inhibition zone of 16 mm against S. mutans. [21] Also, the extract from white rice bran (Oryza sativa L.) exhibited an inhibitory effect on S. mutans. This effect was observed when both ethanol and distilled water were used as solvents. However, the antibacterial activity was more pronounced when distilled water was used as the solvent, compared to when ethanol was used. [22]

Zakavi et al. [7] revealed that high concentrations of both ethanolic and aqueous extracts from the Juglans regia bark demonstrated antimicrobial effects against S. sanguis, S. mutans, S. salivarius, and Staphylococcus species. However, the ethanolic extract was found to be more effective against S. mutans compared to the aqueous extract. Overall, the discrepancies observed among studies regarding the inhibitory growth zone diameter can be summarized by two main factors:

- The concentration of the effective herbal extract or antimicrobial substance varies across studies.
- The type of bacteria and herb examined differs from one study to another.

Conclusion

The ethanol extract derived from myrtle leaves demonstrated an inhibitory effect on S. mutans, akin to that of chlorhexidine. Given the increasing preference for herbal medicines due to their fewer side effects, it is crucial to conduct further research on the antibacterial effects of various medicinal plants against cariogenic microorganisms.

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Conflict of Interest

No Conflict of Interest Declared ■

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