



# Antimicrobial Effect of *Myrtus communis*. L. Essential Oils Against Oral Microorganism

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## Abstract

The aim of this study was to evaluate the antimicrobial effects of essential oils (EOs) isolated from *Myrtus communis* L. (myrtle) against *Streptococcus mutants*, *Streptococcus sanguis*, and *Streptococcus salivarius* through in vitro experiments. The EOs was isolated from myrtle leaves by the hydrodistillation method. The disc diffusion method was used to evaluate the zone of microbial growth inhibition by different concentrations of the EOs. The inhibition zone for all concentrations was measured in diameter (MM) after incubation at 37° for 18 hours. According to these measurements, the Minimal inhibitory concentration for each bacterium was reported. Tetracycline and erythromycin were used as positive control. The result showed that EOs of myrtle leaves had antimicrobial activity on disk-diffusions plates against all strains of Streptococcus, which were tested; however, *S. mutants* showed greater susceptibility than others. EOs of myrtle leaves could be recommended as a potential remedy for prevention the colonization of teeth by *Streptococcus* mutants and hinder the development of dental caries.

**Keywords:** Myrtus, Streptococcus, In Vitro, Dental Caries

## 1. Background

Oral infections are the most common dental diseases caused by bacteria. Periodontal disease and dental caries are known as chronic infections (1) and they can be linked with other serious chronic problems like cardiovascular disease (2). While we try to control pathogens, multi-drug resistant (MDR) strains are increasing and the limited numbers of antimicrobial agents are available for treatment of these infections (3). Except for antimicrobial resistance, side effects, high costs, and environmental problems led to novel antimicrobial agents or treatment strategies (4). Recently there is an increasing interest to use EOs for infections control (5). In oral medicine, EOs are used in many different ways, such as in oral hygiene, dental implants, as anxiolytic, and preservatives (6). EOs are plant's volatile secondary components and they are found particularly in plant's flowers and leaves (7). Secondary metabolisms are described as the "silent metabolism" due to their critical role in increasing plant fitness and defense (8). Until now, more than 3,000 essential oils have been described, of which about one-tenth are relevant for pharmaceutical, nutritional, or cosmetic industries. Several essential oils have a strong interest in research for their cytotoxic capacity. Great efforts are performed in order to inves-

tigate the potential therapeutic effects of oils against several diseases, especially those characterized by excessive cell growth and proliferation such as cancer or bacterial infections (9, 10). In fact, EOs have been used for over 5,000 years as health-promoting agents for the treatment of various diseases (7). Use of EOs in traditional Persian medicine (TPM) dates back prior to 637 AD. In addition, new clinical trials have highlighted specific positive effects of EOs on both the physiological and psychological of human subjects (11-15). Interestingly, some of the investigations evidenced that EOs can be useful against multidrug-resistant bacteria (16, 17). One of the plants that its EOs has been investigated is *Myrtus communis* L. (common name: myrtle). It is an evergreen shrub belonging to the *Myrtaceae* family (18).

a-Pinene, limonene, 1,8-cineole, and linalool have been reported as major compounds of Persian *M. communis* EOs (19). Limonene was found as an inhibitor of streptococcal biofilm formation in subrameniam study (20). In addition, Mitrakul reported that Citrus EOs contain L-limonene inhibited streptococcus mutants biofilm formation (21). Antimicrobial effects of the EOs of *Myrtus* against several bacterial and fungal strains such as *Staphylococcus aureus* and *Streptococcus pneumoniae* were also reported by Baharvand-Ahmadi et al. (22). Accordingly, the aim of this

study was to evaluate the in vitro antimicrobial effect of myrtle essential oils against different mutants of streptococcus that cause dental plaque and gum disease.

## 2. Methods

### 2.1. Plant Materials

*M. communis* leaves were collected from Izeh district of Khosestan-Iran in April 2011.

### 2.2. Microbial Cultures and Media

Mueller Hinton agar (MHA) and blood agar plates (BAPs) were prepared according to the (23) method.

*Streptococcus mutans* (PTCC 1683), *Streptococcus sanguinis* (PTCC 1449), and *Streptococcus salivarius* (PTCC 1448) obtained from Iranian research organization for science and technology (IROST). According to the kite protocol, microbial suspensions were obtained by diluting each bacterial vial in serialized serum. The microbial suspensions were cultured on MHA media at 37°C for 18 hours. Microbial suspensions were then made from the agar plates using the sterile serum at a concentration of approximately  $1.5 \times 10^8$  /mL (0.5 McFarland standards). The above suspension from each bacterium was used on BAP media (23).

### 2.3. Essential Oils Extraction

Essential oils extraction was carried out according to the method explained by Berka-Zougali et al. (24) with slight modifications. Leaves were washed and dried during 5 days. Hydrodistillation method was used. A quantity of 100 g of crushed leaves of myrtle was immersed in 500 mL of distilled water contained in a 2 L flask. Distillation was performed using a modified Clevenger's glass apparatus. The extraction process was carried out for 6 hours after the first drop of distillate until complete exhaustion of the plant. The distillation started after a heating time of 40 minutes. The condensation was carried out continuously with water chilled to 5°C. Trials were performed with three successive repetitions. The essential oils extracted were stored in dark glass bottles in 4°C until it was used.

### 2.4. Essential Oils Dilution

Various solvents such as ethanol, methanol, acetone, butanol, and diethyl ether were tested for their antimicrobial activities using the disc diffusion method. Methanol was selected as a diluting medium for the EOs due to the fact that it did not show any antimicrobial activity. Therefore, methanol was used as the control and 1/2, 1/4, 1/8, 1/16, 1/32, 1/64 dilutions of EOs were made with methanol. Undiluted oil was taken as dilution number one (100%) (25).

### 2.5. EOs Disc Preparation

Discs were bought from Padtan teb®. A 6 mm disc size was used (Whatman No.1). We put disks on the sterilized plates and loaded 20 µL of the EOs on each disc. Plates were put in 37°C for 30 minutes in order to evaporate the solvent.

### 2.6. Antimicrobial Analysis

The fresh oil we used was for its antimicrobial activities. Disc diffusion method was used. According to the method, sterile Mueller- Hinton agar and blood- agar medium was used for the antimicrobials assay of. *S. Mutans*, *S. salivaris*, and *S. Sanguis*.

The media in plates were allowed to solidify and then the microbial suspension was streak over the surface of the medium using a sterile cotton swab and under the aseptic conditions the EOs discs were placed on agar plates.

The plates were then incubated for 18 hours at 37°C in order to get reliable microbial growth. Microbial inhibition zone were measured using a ruler by millimeters (26). The mean of triplicate experiments of each treatment was determined and antibiotic and methanol were used as controls.

### 2.7. MIC Determination of the EOs

The minimal inhibitory concentration (MIC) was determined by the Etest method. Three different Bacterial suspensions in 0.5 McFarland concentration were spread on MHA petri dishes media. Eight discs of EOs from highest to the lowest concentration were put on the line on the surface of BAP media. Pure methanol disc was added as a control. Petri dishes were incubated for 18 hours at 37°C in order to get reliable microbial growth (23).

### 2.8. Statistical Analysis

Data were analyzed by SPSS version 16. ANOVA was used for comparing treatment groups means at  $P \leq 0.01$  in three replications.

## 3. Results and Discussion

Bacterial growth inhibition was noted for all streptococcus varieties in Tables 1 - 3. Inhibitory effect of all concentrations of EOs were evaluated by the positive control, which was antibiotic. The result showed that in some concentrations of EOs there weren't any significant changes compared to the commercial antibiotics. In Table 1, the EOs from *M. communis* inhibited *S. mutans* growth the same as tetracycline 30 µg, in 100%, 50%, 25%, and 12.5% of concentrations.

In the case of *S. salivaris*, inhibition zone of EOs 100% and tetracycline 30 µg were the same. Inhibition zone of

**Table 1.** Microbial Inhibition Zone of Different Dilution of Myrtle EOs, Antibiotic, Solvent Against *S. mutans*

Oil Dilutions, %	Rep1, mm	Rep2, mm	Rep3, mm	Mean, mm
100	31	29/2	30	30.5 <sup>a</sup>
50	25	26	25	25.3 <sup>a</sup>
25	21/5	22	21	21/5 <sup>a</sup>
12.5	18/6	18/5	18	18 <sup>a</sup>
6.25	17	18	16	17 <sup>b</sup>
3.125	11	11	10	10.7 <sup>b</sup>
1.562	0	0	0	0 <sup>b</sup>
<b>Control</b>				
Tetracycline 30 µg/disc	21	19	22	20.7
Methanol	0	0	0	0

<sup>a</sup>Non significant change compare to tetracycline.<sup>b</sup>Significant decrease compare to tetracycline.**Table 2.** Microbial Inhibition Zone of Different Dilution of Myrtle EOs, Antibiotic, Solvent Against *S. salivaris*

EOs Dilutions, %	Rep1, mm	Rep2, mm	Rep3, mm	Mean, mm
100	23	23	23.5	23.2 <sup>a</sup>
50	17	17	18.4	17.5 <sup>b</sup>
25	15	14	15	14.7 <sup>b</sup>
12.5	12	11	12	11.7 <sup>b</sup>
6.25	8	7	7	7.3 <sup>b</sup>
3.125	7	8	8	7.7 <sup>b</sup>
1.562	0	0	0	0 <sup>b</sup>
<b>Control</b>				
Tetracycline 30 µg/disc	26	26	26	26
Methanol	0	0	0	0

<sup>a</sup>Non significant change compare to tetracycline.<sup>b</sup>Significant decrease compare to tetracycline.**Table 3.** Microbial Inhibition Zone of Different Dilution of Myrtle EOs, Antibiotic, Solvent Against *S. sanguis*

EOs Dilutions, %	Rep1, mm	Rep2, mm	Rep3, mm	Mean, mm
100	26	26	24	25.3 <sup>a</sup>
50	24	23	24	23.7 <sup>a</sup>
25	20	21	20	20.3 <sup>b</sup>
12.5	15	14	14	14.3 <sup>b</sup>
6.25	7	7	8	7.3 <sup>a</sup>
3.125	0	0	0	0 <sup>a</sup>
1.562	0	0	0	0 <sup>a</sup>
<b>Control</b>				
Erythromycin 16 µg/disc	17	17	18	17.3
Methanol	0	0	0	0

<sup>a</sup>Significant change compare to tetracycline.<sup>b</sup>Non significant change compare to erythromycin.

other EOs concentrations was significantly lower than the tetracycline (Table 2).

*S. sanguis* growth was inhibited under EOs treatment even better than erythromycin (Table 3). When we used erythromycin 16 µg, the bacteria inhibited area was 17.3 mm. While by using 100% and 50% of EOs, we measured 25.3 mm and 23.7 mm of bacteria inhibited area, which was signifi-

cantly more than the area that was made by using 16 µg of erythromycin. The inhibitory area by 25% and 12.5% was the same as erythromycin. Lower concentrations of EOs had significantly lower inhibition than erythromycin (Table 3).

According to Tables 1 - 3, Myrtle oil has good antimicrobial activity against all three strains that were tested. Then, we determined the minimal inhibitory concentra-

tion (MIC) of EOs by disc diffusion method.

The MIC factor for the EOs of *M. communis* against bacteria is shown in Table 4. Essential oils MIC was 1.565% for *S. mutants* whereas it was 3.125% against *S. salivaris* and *S. sanguis*. It shows that *S. mutants* was more susceptible to *M. communis* oil than the others.

**Table 4.** Minimal Inhibitory Concentration of Myrtle EOs

Micro-Organisms	MIC, %v/v	PTCC
<i>S. mutants</i>	1.565	1683
<i>S. salivaris</i>	3.125	1448
<i>S. sanguis</i>	3.125	1449

Akin also searched about the antibacterial activity of the essential oils of *Eucalyptus camaldulensis* and *Myrtus communis* growing in Northern Cyprus. They reported that *M. communis* showed higher efficacy on Gram positive and Gram negative bacteria while *E. camaldulensis* was found to have a low activity (27). AlAnbori et al. found that the MIC of myrtle was 106.6  $\mu\text{g}/\text{mL}$  compared to 3.3  $\mu\text{g}/\text{mL}$  of chlorhexidine mouth rinse. They showed that the antibacterial effect of myrtle on Mutants of streptococci was due to its flavonoids content. Therefore, ethanolic extract of myrtle could be a potential remedy for the prevention of colonization by Mutants streptococci; thereby, preventing or hindering development of dental caries and single mouth rinse of myrtle extract could be significantly useful (28). Rasooli explained the antimicrobial effects of essential oils isolated from *M. communis* against nine different bacteria. They found that high monoterpenes hydrocarbons such as  $\alpha$ - Pinene and Limonene contribute to the strong antimicrobial activity of *M. communis* (25). Previous studies and our in vitro results proved that EOs extracted from myrtle has an inhibitory effect on various bacteria like streptococcus. According to our results, the myrtle EOs inhibitory was the same as tetracycline for *S. salivaris* and *S. mutants* in high concentrations and 100% of EOs was even better than erythromycin in the case of *S. sanguis*. On the other hand, MIC test showed that *S. mutants* is very sensitive to low concentration of the myrtle EOs.

Therefore, myrtle EOs could be a potential remedy for the prevention and development of dental caries. Using the EOs in mouthwash or gum could be a good suggestion for future studies.

### 3.1. Conclusions

The results from the myrtle EOs showed a good antibacterial activity towards *S. salivaris*, *S. sanguis*, and especially toward *S. Mutants*, which has been implicated in dental caries and plaque formation.

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