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## Research Article

# Evaluation of Antimicrobial Activities of Melaleuca alternifolia (Tea tree) Oil Mouthwash on Candida albicans and Streptococcus mutans: In Vitro Pilot Study

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# Evaluation of Antimicrobial Activities of Melaleuca alternifolia (Tea tree) Oil Mouthwash on Candida albicans and Streptococcus mutans: In Vitro Pilot Study

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## Abstract

**Statement of the problem:** Herbal mouthwashes have attracted attention recently due to their antimicrobial and anti-inflammatory effects and fewer side effects than chemical preparations. To choose the most suitable mouthwash component among these herbal preparations, in vivo and, in vitro research should be done, respectively.

**Objective:** The effects of the mouthwashes containing Melaleuca alternifolia (tea tree oil, TTO) on the infectious agents Streptococcus mutans and Candida albicans were evaluated. The results were compared with the effect of chlorhexidine (CHX), the accepted gold standard.

**Material-Method:** A 2% concentration of TTO was prepared, and suspensions of microorganisms Candida albicans and Streptococcus mutans by Mc Farland 0.5 standard were obtained. The macrodilution tube method was used to determine the lowest concentration inhibiting growth, and the disk diffusion method was used to determine the growth inhibition zones. Each method was applied for both TTO and CHX.

**Results:** It was determined that TTO did not inhibit the growth of S. mutans and C.albicans at 2% concentration and MIC value could not be obtained. On the other hand, it was observed that after the treatment with CHX, in C.albicans and,S.mutans samples inhibition zones with the diameters of 10 and 20 mm were formed, respectively.

**Conclusion:** TTO cannot be a successful alternative to CHX for its 2% concentration; It has been concluded that it can increase its antimicrobial effect with different concentrations and other essential oil combinations.

**Keywords:** Tea tree oil, Candida albicans, Streptococcus mutans, Chlorhexidine, Mouthwash

**Introduction**

In recent years, many herbal products utilised in integrative and alternative medicine have attracted the attention of both researchers and individuals. One of these products is the Australian-origin Melaleuca alternifolia (Tea tree) plant, and the essential oil obtained from this plant "tea tree oil (TTO)". With its antimicrobial properties, TTO is included as an active ingredient in many topical formulations used to treat skin infections. This vegetable oil, which is widely used in Australia, Europe, and North America, has been frequently involved in vitro and in vivo studies.1

TTO consists of terpene hydrocarbons, mainly monoterpenes, sesquiterpenes, and their associated alcohols. [2] Terpenes are volatile, aromatic hydrocarbons and are considered isoprene polymers with the formula $C_5H_8$.

Of all the claimed benefits of TTO, its antimicrobial activity has undoubtedly received the most attention. The earliest reported utilization of the plant M. alternifolia was by the Bundjalung Aborigines of northern New South Wales as inhaling the crushed leaves of "tea trees" for the treatment of coughs and colds or applying on wounds in mush form.[3]

Due to the antibacterial and antifungal activity of TTO, its use as a mouthwash is also recommended. In this study, the sensitivity of Streptococcus mutans and Candida albicans, which cause caries, gingivitis, and other oral infections, to mouthwashes containing TTO was investigated and it was aimed to compare with the effect of chlorhexidine (CHX), which is considered the gold standard in routine use.

**Materials & Methods**

Ethical approval of the study was given by Nevsehir Haci Bektas University's "Non-Invasive Clinical Research Publication Ethics Committee" (Decision no: 366/25.10.2021). Microbiological analyzes of the study were carried out in the laboratories of Nevsehir Haci Bektas University Science Technology Research and Application Center (BTUAM) and the Microbiology laboratory of the Faculty of Engineering and Architecture.

In the study, the ATCC 25175 strain of S.mutans and standard strains of C.albicans ATCC 90028 were used. To obtain the mouthwash form, it was decided to prepare pure TTO (Biovitals®, Turkey) at a concentration of 2% based on the literature review and homogenized to 2 ccs of pure oil in 100 ml of deionized water.[1] The macrodilution tube method and agar diffusion technique were used for the antifungal and antibacterial activity study.

**Candida albicans Macrodilution Tube Method**

The suspension obtained from a 24-hour culture of C. albicans grown on Saboraud Dextrose Agar (SDA) was mixed in 0.85% saline, which corresponds to a wavelength of 530 nm in a UV spectrophotometer (Tetra PG, T60). It was prepared to contain $10^6$ CFU/ml colonies by the McFarland 0,5 standard. A doubled serial dilution of ½ was created by adding 1 ml of yeast concentration to liquid RPMI 1640 (Sigma, Germany) medium. Then, 1 cc of 2% TTO was added to all tubes except the negative control tube and incubated for 24-48 hours at 37°C.

After incubation, the passage was taken from each tube to SDA and the first tube without growth was left under the same incubation conditions to be evaluated as the minimal inhibitory concentration (MIC) value. The same procedures were applied for 0.2% chlorhexidine digluconate (Klorhex®).

**Candida albicans Agar Diffusion Technique**

For the agar diffusion technique, paper points with a diameter of 5 mm were sterilized in an autoclave and impregnated with 2% TTO. The suspension of *C. albicans* conforming to the 0.5 Mc Farland standard was inoculated with glucose-added Müller Hinton Agar smear technique and left in the center of a TTO-impregnated paper point medium; The zone of inhibition was measured after 24-48 hours of incubation. In the same way, the disc diffusion technique was applied with sterile paper points impregnated with CHX with the same standard dimensions.

**Streptococcus mutans Macrodilution Tube Method**

The amount of colonies of *S.mutans* grown on 5% Columbia Blood Agar (BD, GmbH) suspended in 0.85% saline was prepared according to the McFarland tube no. 0.5 standard and measured in a UV Spectrophotometer (Tetra PG), T60) was prepared at 600 nm wavelength as $1.5x10^8$ CFU/ml. 1 ml of this suspension was added to Müller Hinton Broth medium and diluted to form serial multiples of ½. TTO was not added to the negative control tube, bacteria were inoculated for growth control. The same procedures were applied for 0.2% chlorhexidine digluconate (Klorhex®).

**Streptococcus mutans Agar Diffusion Technique**

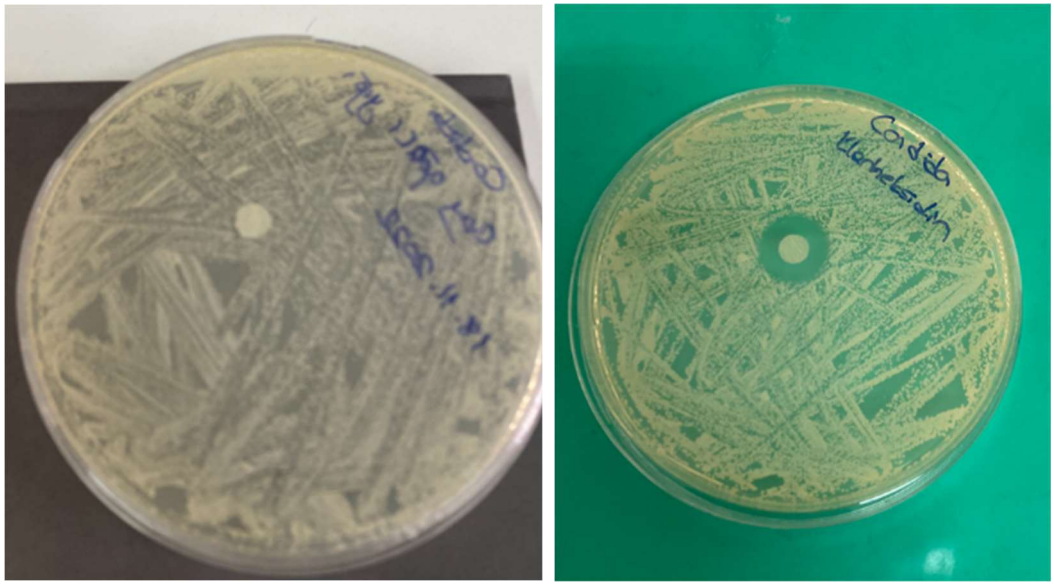
For the agar diffusion technique, paper points with a diameter of 5 mm were sterilized in an autoclave and impregnated with 2% TTO. The suspension of *S.mutans* conforming to the 0.5 Mc Farland standard was inoculated with the Müller Hinton Agar smear technique and left in the center of a TTO-impregnated paper point medium; The zone of inhibition was measured

after 24-48 hours of incubation. In the same way, the disc diffusion technique with sterile paper points impregnated with CHX was applied with standard dimensions.

**Results**

In the study, to determine the sensitivity of *Candida albicans* to 2% TTO, seeding was done from each dilution value to SDA and growth occurred in each dilution layer. The growth-inhibiting MIC value could not be determined at this concentration of TTO and was considered resistant. As the inhibition zone of *C. albicans* did not develop as a result of the agar dilution technique, *C. albicans* was found to be resistant to 2% TTO with both techniques. In contrast, an inhibition diameter of 10 mm was detected in CHX-impregnated paper points (Figure 1).

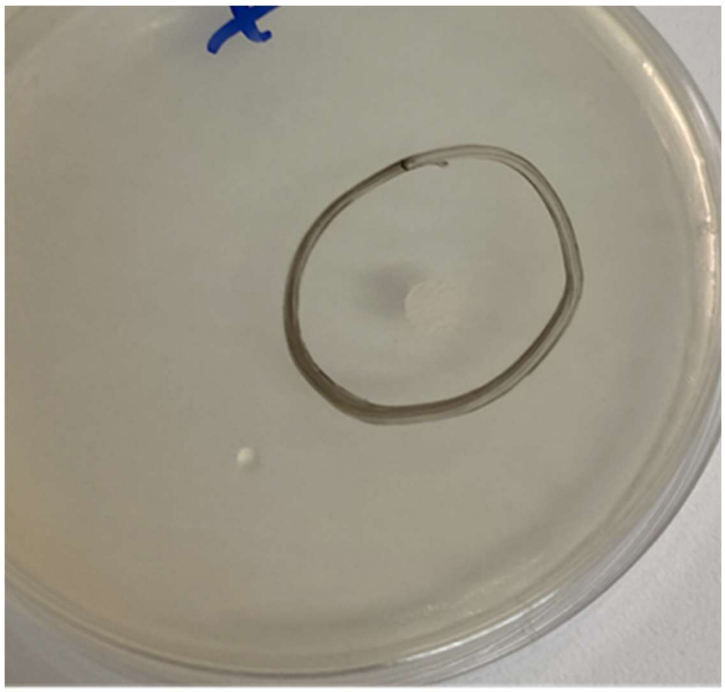
**Figure 1**. *C.albicans* is resistant to TTO by agar diffusion method (left), and it forms an inhibition zone with a diameter of 10 mm against CHX (right).



The MIC value of *Candida albicans* against CHX was determined as 0.125 mg/ml as a result of the passages taken from the dilution tubes.

*Streptococcus mutans* was also found to be resistant to 2% TTO and its MIC value could not be obtained. It was measured that *S. mutans* formed a 20 mm zone diameter around the paper points containing CHX, and the MIC value was determined at the level of 0.0625 mg/ml with the macro dilution technique (Figure 2).

**Figure 2**. *S. mutans* forms an inhibition zone with a diameter of 20 mm against CHX



The antibacterial and antifungal effects of tea tree oil mouthwash and mouthwash containing chlorhexidine on *S.mutans* and *C.albicans* are summarized in Table 1.

**Table 1**. Antimicrobial activities of both types of mouthwash on *S. mutans* and *C.albicans*

| Mouthwash | *S.mutans* | *C. albicans* |
|---|---|---|
| %2 TTO | R | R |
| %0.2 CHX | 20 mm inhibition diameter (MIC: 0.0625 mg/ml) | 10 mm inhibition diameter (MIC:0.125 mg/ml) |

R: Resistant

MIC: Minimum Inhibitory Concentration

**Discussion**

Among the microorganisms in the oral microflora, *Streptococcus mutans* and *Candida albicans* play an important role in the development of periodontal diseases and caries etiology.

Shortly after application to the oral cavity, the surfaces of hard biomaterials are colonized by microorganisms that form structured communities called biofilms. The opportunistic yeast Candida albicans has been shown to colonize on a wide variety of dental materials, including resin-based ones.[4,5] While mostly harmless to healthy individuals, it can cause oral and systemic candidiasis in immunocompromised hosts such as patients with AIDS or receiving chemotherapy or radiation.

Mouthwashes are often used to reduce the microbial load in the oral cavity or to control or eliminate bad breath, in addition to mechanical cleaning. It is recommended that these preparations, which contain active substances with antimicrobial activity, should be used before oral and periodontal surgery, including tooth extraction and implant placement.[8] The compound accepted as the gold standard for mouthwashes is chlorhexidine gluconate and other bisbiguanide derivatives. Chlorhexidine can prevent the formation of gingivitis to a certain extent with its plaque inhibition effect.[9] However, mouthwashes containing chlorhexidine also have some side effects. Staining of teeth and other oral surfaces, temporary impairment of taste, desquamation, and allergic reactions – even anaphylaxis can be listed among these effects.[10]

For this reason, mouthwashes containing essential oils and herbal extracts have recently been used as an alternative to chlorhexidine. The most popular of the herbal essential oils and distilled pure hydrosols used for their anti-inflammatory and antiseptic properties are eucalypti, sage, clove, aloe vera, thyme, sanguinarine, tea tree, and many other natural ingredients that can be included in the list due to their same qualities.[11]

*Melaleuca alternifolia* (tea tree) is used as an adjunct agent for local irrigation in the control of microbial dental plaque and gingivitis, and in periodontitis.[12]

In our study, the in vitro antimicrobial effect of the preparation prepared from a 2% concentration of tea tree oil, which is considered an alternative to chlorhexidine, was investigated against *Streptococcus mutans* and *Candida albicans.* It was determined that *S.mutans* and *C. albicans* were resistant to the tea tree preparation prepared at this concentration, and the MIC value could not be obtained. On the other hand, 0.2% chlorhexidine digluconate was found to have antimicrobial activity for both microorganisms.

In an in vivo study by Ripari et al. investigating the effects of tea tree oil mouthwash in the treatment of gingivitis, the effects of tea tree on parameters such as gingival index (GI), plaque index (PI), pocket depth, and bleeding index on probing (BI) were compared with chlorhexidine and index scores were investigated. It has been concluded that it can be an alternative to chlorhexidine due to its properties such as reducing the feeling of numbness in the mouth, and not causing darkening in the tooth color.[13]

In another in vivo study comparing garlic, 2% tea tree oil, and CHX, it was found that all three preparations inhibited the growth of oral microorganisms, but TTO produced undesirable side effects such as nausea and a burning sensation in the mouth.[14].

In the in vivo study of Salvatori et al.[15], 1.5% concentration of TTO was studied and its effects were compared with 0.12% chlorhexidine digluconate. It has been reported that TTO leads to a decrease in the signs of gingivitis, but its antimicrobial and anti-inflammatory effects are so low that they cannot be compared with CHX. Oral toxicity studies of Melaleuca alternifolia have been performed with animal and human experiments, and muscle weakness and central nervous system depression findings have been observed at higher concentrations.[15] In our study, it was concluded that 2% TTO was not sufficient for the inhibition of *S. mutans* and *C.albicans*.

It is noteworthy that the studies in which CHX and herbal mouthwashes are mostly compared are in vivo studies and that the number of in vitro studies in the literature is low. Based on the results obtained from in vitro studies investigating the effects of reproductive inhibition, turning to clinical studies will undoubtedly be the most controlled and guaranteed method. In this study, it was concluded that the colony numbers of S. *mutans* and *C. albicans* were evaluated quantitatively after TTO exposure and TTO could not be an alternative to CHX with a 2% concentration.

In our study, the lowest concentration (MIC) at which CHX inhibited the growth of C. *albican*s was determined as 0.125 mg/ml. In a similar study comparing CHX with other mouthwashes containing essential oil, *C. albicans* MIC value was determined as 0.625 mg/ml, similar to our results.[16]

Values similar to the inhibition effect of S.mutans against CHX were found by Motamedifer et al. However, it was also stated in the mentioned study that when the concentration of herbal mouthwashes is increased, an antimicrobial effect close to CHX is achieved.[17]

In this study, a 2% solution of TTO was evaluated in vitro, the effects of different concentrations were not examined. This situation, which can be considered as a limitation of the study, can be ignored as TTO may cause difficulties in clinical studies due to its bitter and pungent taste at higher concentrations.

Topical use of 5% concentration of TTO has shown antimicrobial effect even on methicillin-resistant Staphylococcus aureus (MRSA).[18] However, oral use is not recommended at these concentrations in toxicity studies.[19] In our study, in vitro efficacy could not be determined for *S.mutans* and *C.albicans*, since TTO which was used at 2% concentration presented any effect, however, higher concentrations could not be tolerated as a mouthwash.

Studies on the formation of synergism as a result of the combined use of TTO with different herbal ingredients may increase the potential for a stronger in vitro antimicrobial activity. This pilot study and its obtained results may contribute to further synergism-antagonism research on TTO and other vegetable essential oils and hydrosols.

Expanding the researches using different ingredients in in vivo studies may allow the generation of new preparations to be used as alternative to chemical mouthwashes.

**Conclusion**

Based on our study, any antibacterial or antifungal effects of 2% TTO concentration on *S.mutans* and *C.albicans* were observed and it was determined that TTO could not be an alternative to the superior antimicrobial activity of CHX. However, further studies evaluating the effects of different concentrations of miscellaneous herbal essential oils on various microorganisms is needed.

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