The effect of tea tree oil in combination with antibiotics and toothpastes against *Staphylococcus epidermidis*

Abubaker Elrotob¹*, Idris Kabalci ²,³

¹Department of Biomedical Engineering, Engineering Faculty, Karabuk University, Karabuk, Türkiye
²Department of Electric-Electronic Engineering, Engineering and Natural Science Faculty, Uşak University, Uşak, Türkiye
³Department of Electric-Electronic Engineering, Engineering Faculty, Şırnak University, Şırnak, Türkiye.

Abstract

The mouth, where the digestive process initiates, is a crucial organ in the body, necessitating a healthy set of teeth for effective food mastication. However, certain bacteria contribute to biofilm and periodontal pocket formation, leading to an increase in oral and Dental issues. This is especially prominent in areas with bacterial plaque, where germs are becoming more resistant to common antimicrobials and treatments. Natural products such as plant essential oils (EOs), renowned for their antibacterial properties, are utilized to control various microorganisms. This study aims to evaluate the combined impact of antimicrobials and treatments. Natural products such as plant essential oils (EOs), renowned for their antibacterial properties, have been found effective against *Staphylococcus epidermidis* isolated from the oral environment. The experimental approach involved the disc diffusion method, utilizing 6 mm filter paper discs on Muller Hinton agar. Petri dishes were divided into three sections: one for the antibiotic or toothpaste alone, another for tea tree oil alone, and a third for the combination of tea tree oil with the antibiotic or toothpaste. Following incubation at 37°C for 24 hours, the inhibition zone diameters were measured. The results demonstrated a significant synergy between tea tree oil and antibiotics/toothpastes, as indicated by the increased average diameter of the inhibition zone after combination. Notably, Metronidazole with oil exhibited an inhibition zone slightly equal to the oil alone, suggesting its ineffectiveness against this bacterium and emphasizing the antimicrobial properties of tea tree oil alone confirming that, the oil is not synergistic with Metronidazole.

Keywords: Tea tree oil, *Staphylococcus epidermidis*, Antibiotics, Toothpastes, Combined effects.

1. Introduction

The oral cavity is a primary gateway for bacteria to enter the human digestive system, and it harbors a diverse array of microbial species within the oral biofilm. With over 750 bacterial species identified in the oral cavity, half of which remain unidentified, some are associated with various oral disorders [1]. Dental caries and associated illnesses such as gingivitis and periodontitis are the most common oral diseases worldwide, affecting individuals of all ages in both developed and developing countries [2]. Periodontal disease ranks among the most prevalent Dental issues. Periodontitis, an advanced condition causing alveolar bone loss, leads to significant tooth loss, especially in developing and impoverished regions [3]. The biofilm enables bacteria to adhere to and remain in foreign materials [4]. These sticky substances comprise both the original bacterial layers and subsequent bacterial colonists, which combine to form Dental plaque. The end products of the numerous plaque bacteria, called organic acids, can puncture the enamel and cause the many caries bacteria to begin eroding the dentin [5]. Seven species of Staphylococcus were identified in the oral cavities of patients with periodontal disease and those in good health; the most prevalent strains were *Streptococcus aureus* and *Staphylococcus epidermidis* [6]. *S. epidermidis* is one of the most popular in Dental caries or Dental pulp and is determined both in sufferers with Dental infection as well as commensally in humans at different sites such as the skin, nasal cavity, groin, etc., [7]. Due to its propensity for causing hospital-acquired infections, particularly through medical device contamination, *S. epidermidis* poses a significant challenge in healthcare settings. Certain bacterial strains further complicate treatment approaches by exhibiting resistance to antibiotics [8-9]. The spread of drug-resistant bacteria is one of the biggest threats to the successful treatment of microbiological disorders [10].

Numerous plant extracts have been found to have antibacterial properties, and these properties have been found to intensify when coupled with antibiotics. This phenomenon promotes the development of novel antimicrobial products or
strategies against these microorganisms, particularly drug-resistant bacteria [11]. Tea tree oil (TTO), an essential oil derived from melaleuca alternifolia, has shown promise in vitro as a broad-spectrum antimicrobial agent against a variety of microorganisms [12]. TTO consists of terpene hydrocarbons, mainly monoterpenes, sesquiterpenes, and their alcohols attached to them. Terpenes are volatile aromatic hydrocarbons and are associated with isoprene polymers [13]. Research indicates that the essential oil of melaleuca alternifolia has the capacity to suppress strong oral bacterial growth [14]. However, one promising approach to antibiotic resistance is combination therapy, in which traditional drugs are mixed with natural products [15]. Previous studies have also looked at the antimicrobial activity of TTO in combination with other antimicrobials. A hot gel containing itraconazole and TTO showed synergistic benefits in the treatment of cervical candidiasis [16]. In addition, essential oils have been shown to have synergistic effects with pristinamycin, ciprofloxacin, gentamicin, and cefixime [17]. When TTO and amphotericin B were added to C. albicans, C. glabrata, C. tropicalis, C. krusei, C. guilliermondii, and C. parapsilosis, the areas of growth inhibition around impregnated discs larger than that surrounding discs containing only TTO [18]. A study conducted on Staphylococcus aureus found that discs impregnated with TTO and other essential oils had larger zones of growth inhibition surrounding them than discs impregnated with TTO alone [16,19]. Nonetheless, most antimicrobial mouthwashes contain a blend of multiple essential oils. This allows for the combination of desired antibacterial activity and a pleasant aroma [14]. Consequently, Tea Tree Oil (TTO) activities are related to the synergistic effects among the essential oil's constituents or combinations of essential oils from various sources [20]. The study by Wei et al. illustrated that the combination of tea tree oil (TTO) and Amoxicillin had a positive impact. TTO demonstrated a reduction in bacterial adhesion and invasion, coupled with the inhibition of gene expression, ultimately disrupting bacterial activities [21]. According to a study conducted by Iseppi et al., the synergistic combination of essential oils and antibiotics emerges as a viable therapeutic approach against methicillin-resistant Staphylococcus aureus (MRSA) bacteria. This approach enables a reduction in the necessary antibiotic concentration for effective therapeutic use [22]. This study evaluates the synergistic effects of four antibiotics, four toothpaste types, and crude commercial tea tree oil against oral cavity-isolated S. epidermidis bacteria.

2. Materials and Methods

2.1. Materials

Raw commercial tea tree leaf oil from Perfumes Land Company in Libya, primarily sourced from India, was used at a concentration of 200 μL/ml, as determined in previous experiments. Additionally, four types of toothpaste were employed: Sensodyne and Denta, manufactured in Turkey; Iбанa, manufactured in Germany; and Cariax Gingival Kin, manufactured in Spain. The study also included four antibiotics Augmentin (30mg), Amoxicillin (25mg), Azithromycin (15mg), and Metronidazole (5mg) manufactured by the UK company Oxoid. French-sourced dimethyl sulfoxide (DMSO) and Tunisian-sourced distilled water were also utilized in the experiments.

2.2. Bacteria and Preparation of Inoculums

A clinically isolated strain of S. epidermidis that we used in our research was previously isolated from the mouth and kept. It was obtained from the Microbiology and Immunity Department of the Misurata Oncology Centre in Libya. The bacteria were grown and activated on blood agar for 24 hours, and then they were transferred into a test tube containing peptone water to create a bacterial suspension that met the 0.5 McFarland opacity standard. Mueller-Hinton (MH) agar was fully covered in bacterial streaks after a sterile cotton swab was dipped into the test tube [9].

2.3. Study Design

By using the agar disc diffusion method, Bauer et al. assessed the combination effect's antimicrobial activities in vitro [23]. The suspension of S. epidermidis was equally spread on Mueller Hinton Agar (Merck, Darmstadt, Germany) using a sterile cotton swab, and the plates were left to dry for fifteen minutes. The experiments were carried out in triplicate using 6 mm filter paper discs [10,24].

2.4. Synergism effect between Tea tree oil with Antibiotics and Toothpastes

The synergistic effects of tea tree oil with antibiotics were evaluated using the method outlined by [11,25]. Petri dishes with S. epidermidis on Mueller-Hinton agar were partitioned into three sections. The first section exclusively received an antibiotic disc on its surface, while the second section had filter paper saturated with 5 μL of plant oil (concentration: 200 μL/ml). In the third segment, a second antibiotic disc was impregnated with five microliters of the same established oil concentration (200 μL/ml) [3]. To assess the incorporation of toothpastes, a similar method was employed as for antibiotics, with a slight modification. A filter paper disc was immersed in the toothpaste solution (10 g/10 ml) for 30–40 minutes [26] before being placed in the first section of the Petri dish. Only the oil was applied using filter paper in the second section. In the third section, a filter paper disc soaked in toothpaste solution and another disc impregnated with 20 μL of oil were used together. After a 24-hour incubation at 37°C, the diameters of the cleared zones were measured and compared with those of the antibiotic alone. Triplicate tests were conducted for each oil [11].

2.5. Statistical Analysis

The three sets of observations were analyzed in triplicate, and the results were expressed as mean ± standard deviation (SD) values. Data processing was conducted using Microsoft Excel 2010.

3. Results and Discussion

The combination of tea tree oil with antibiotics and toothpaste demonstrated a pronounced synergy, leading to a notable increase in the average diameter of the inhibition zone against S. epidermidis bacteria.

3.1. Combination effect of Tea tree oil with Antibiotics

In three experimental trials involving of combination effect of tea tree oil with antibiotics, the results demonstrated a significant increase in the diameter of the augmentin, amoxicillin, and azithromycin inhibition zones. In the case of metronidazole, the oil had an equal effect before and after the combination, with no effect observed for this antibiotic alone.
Elrotob and Kabalci, 2024

Figure 1 and 2. The combination of tea tree oil with augmentin resulted in an expansion of the average inhibition zone diameter from 18.8mm to 23.13mm, while amoxicillin exhibited an increase from 19.6mm to 24.3mm. Additionally, the inhibition zone diameter for azithromycin increased from 24.3mm to 27.6mm. However, no synergistic effect was observed when combining tea tree oil with metronidazole. The average diameter of the inhibition zone after this combination remained consistent with the average diameter observed for the oil alone, indicating no discernible impact on the antibiotic's efficacy. This underscores the ineffectiveness of metronidazole against this bacterium, as highlighted in Table 1 and Figure 2.

3.2. Combination effect of Tea tree oil with Toothpastes

The results showed a considerable increase in the diameter of the inhibitory zone in three experimental trials involving the sensodyne, ipana, denta, and cariax gingival kin against *S. epidermidis*, with no impact detected for sensodyne alone, Figure 3 and 4. When four different toothpaste brands (sensodyne, ipana, denta, and cariax gingival kin) were mixed with tea tree oil, the study found substantial synergistic effects that changed the effects on *S. epidermidis* bacteria. In particular, the average inhibition zone diameter improved dramatically from 0 to 21.27mm when sensodyne and tea tree oil were combined. The average inhibition zone diameter increased noticeably from 14.5mm to 23.47mm when ipana and tea tree oil were combined. According to this, the average inhibition zone diameter increased significantly from 17.6mm to 28.43mm when tea tree oil and denta were combined. The average inhibition zone diameter rose dramatically from 16.1 mm to 29.6mm when cariax gingival kin and tea tree oil were combined, as shown in Table 2 and Figure 4. The widespread use of antibiotics and antimicrobials, along with the proliferation of oral antimicrobial and hygiene products, has led to the emergence of bacterial strains resistant to certain antimicrobial classes. This global concern limits effective treatment options for infected patients. Ongoing research aims to understand and address the spread of antimicrobial resistance, while efforts are made to discover new antibacterial compounds and strategies to combat this significant health issue. The management of coagulase-negative Staphylococci, particularly *S. epidermidis*, has grown more difficult in light of the growing importance of these bacteria as the cause of nosocomial infections and the rise in antimicrobial resistance cases in recent years [9]. Our study results indicate that tea tree oil exhibits antibacterial properties, aligning with a prior study by Mumu and Hossain's study confirms that tea tree oil (TTO) has shown antibacterial activity against various bacterial species [27]. Consistent with the research carried out by Dobler et al., this oil has exhibited the capacity to hinder the proliferation of oral bacteria [14]. It has been demonstrated that a pronounced synergy exists among augmentin, amoxicillin, and azithromycin when combined with tea tree oil. This combination resulted in a noticeable expansion in the inhibition zone diameter, effectively impeding the growth of *S. epidermidis* bacteria. This enhanced effectiveness of the antibiotics was observed. These findings align with a study conducted by Fadli et al., reinforcing the efficacy of essential oils in conjunction with various antibiotics [17].

![Figure 1: The three stages of combining antibiotics and tea tree oil against *S. epidermidis*.](image)
Table 1: The Average effect of Tea tree oils with Antibiotics against *S. epidermidis* bacteria.

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Antibiotic alone</th>
<th>Tea tree oil alone</th>
<th>Antibiotic and Tea tree oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMC</td>
<td>18.8±5.38</td>
<td>13.8±5.81</td>
<td>23.13±5.78</td>
</tr>
<tr>
<td>AMX</td>
<td>19.6 ±2.85</td>
<td>12.8±4.85</td>
<td>24.3±4.96</td>
</tr>
<tr>
<td>AZM</td>
<td>24.3±4.96</td>
<td>14.8±7.09</td>
<td>27.57±7.5</td>
</tr>
<tr>
<td>MET</td>
<td>00±00</td>
<td>13.6±5.28</td>
<td>13.4±5.44</td>
</tr>
</tbody>
</table>

AMC= Augmentin, AMX=Amoxicillin, AZM = Azithromycin, and MET= Metronidazole, Average of Triplicate Inhibition Zone in mm (Mean value ± standard deviations).

Figure 2: The three stages of combining antibiotics and tea tree oil on Petri dishes with Mueller-Hinton agar against *S. epidermidis*. 
Figure 3: The three stages of combining Toothpastes and tea tree oil against *S. epidermidis*.

Figure 4: The three stages of combining toothpastes and tea tree oil on Petri dishes with Mueller-Hinton agar against *S. epidermidis*. 
4. Conclusions

The current study showed that toothpaste brands (Sensodyne, Ipana, Denta, and Cariatx Gingival Kin) and antibiotics (Augmentin, Amoxicillin, and Azithromycin) combined with tea tree oil had a synergistic effect against S. epidermidis bacteria. Notably, Metronidazole proved ineffective and lacked synergy with tea tree oil. This combination significantly improved the effectiveness of antibiotics and toothpastes, suggesting a vital strategy for advancing oral and Dental health. The findings also pave the way for further exploration of combining natural products with antimicrobials and testing them on diverse microbial species.

Acknowledgments

The authors extend their sincere appreciation to Mr. Taher Alshegmani, Azddin Ben Ehmeda, the dedicated personnel at the laboratory in Misurata Oncology Centre, and all individuals in the Biomedical Engineering Department at Karabuk University for their invaluable support throughout this study.

Conflicts of Interest

The authors declare no conflict of interest.

References


Table 2: The average effect of Tea tree oils with Toothpastes against S. epidermidis bacteria.

<table>
<thead>
<tr>
<th>Toothpastes</th>
<th>Toothpaste alone</th>
<th>Tea tree oil alone</th>
<th>Toothpaste and Tea tree oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensodyne (TP1)</td>
<td>00±00</td>
<td>16.7±3.29</td>
<td>21.27±8.11</td>
</tr>
<tr>
<td>Ipana (TP2)</td>
<td>14.5±0.44</td>
<td>15.27±3.29</td>
<td>23.47±2.73</td>
</tr>
<tr>
<td>Denta (TP3)</td>
<td>17.6±2.14</td>
<td>13.97±0.06</td>
<td>28.43±2.7</td>
</tr>
<tr>
<td>Cariatx Gingival Kin (TP4)</td>
<td>16.1±1.33</td>
<td>18.33±4.16</td>
<td>29.6±2.97</td>
</tr>
</tbody>
</table>

TP= Toothpaste, Average of Triplicate Inhibition Zone in mm (Mean value ± standard deviations).


