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# Antibacterial Activity of Essential Oils Against Streptococcus Mutans

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## Abstract

**Background/Objectives:** The purpose of this study was to investigate the antimicrobial activity against bacteria in oral cavity by selecting tea tree and pepper, which are natural essential oils with aroma suitable for oral use

**Method/Statistical Analysis:** *Streptococcus mutans* (*S. mutans*) was used for the antibacterial activity measurement after subculture in brain heart infusion (BHI; Sigma-Aldrich, St. Louis, MO, USA). Tea tree and peppermint essential oil concentrations of 0.5%, 1%, 5%, 10%, 20% and 30% were divided into $5\times10^5$ colony forming units; CFUs/ml diluted *S. mutans* 10ul, BHI 900ul, 10% Tween 20 90ul was applied to prepare the final 1mL to check the CFUs according to 24h at 37°C to evaluate the antimicrobial effect.

**Findings:** The CFUs of the essential oils of tea tree and peppermint showed a reduction of $10^{-6}$ in 30% tea tree compared to the control without natural essential oil, 30% Peppermint showed 10-8 deaths. Although the difference in natural essential oils is different, it is shown that the higher the concentration of the essential oil, the higher the bacterial death.

**Improvements/Applications:** Peppermint and tea tree essential oils were found to be antimicrobial. Especially, tea tree essential oil has excellent antimicrobial activity compared to peppermint essential oil.

***Keywords:*** *Essential oils, Antibacterial activity, Streptococcus mutans, Oral health, Dental caries.*

## Introduction

The oral cavity is a space that is always invasive due to saliva and has an environment suitable for bacteria to reside. More than 700 kinds of bacteria exist in the oral cavity of these people by being involved in oral health and morbidity[1].

Dental caries is the most common oral disease[2]. It is a multi factorial disease, caused by harmful changes in bacterial ecology due to the formation of bio films that adhere to the tooth surface[3]. In the past decades, many reports around the world have shown an overall decline in tooth decay. However, recent studies have shown a surprising increase in caries prevalence, especially in the underprivileged[4].

*Streptococcus mutans* (*S. mutans*) is related to dental caries[5]. S. mutans can colonize the oral cavity, and bacterial bio film formation. There are other microorganisms with the ability to interact and this ecosystem to survive in the acidic environment in the colony[3].

Dental caries are caused by an imbalance between demineralization and demineralization of tooth structure. Acid bacteria ferment dietary carbohydrates to produce organic acids to dissolve tooth enamel and disintegrate tooth tissue[6].

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The extent of the pH fall is affected by a number of factors, including the composition of the micro flora, as well as the type and frequency of sugar intake[7]. Streptococcus mutans produce glucosyltransferase (GTF) enzymes, which are recognized as virulence factors in the pathogenesis of caries. GTF enzymes synthesize extra cellular glucans and contribute significantly to the formation of polysaccharides in dental plaque matrices. Sucrose-dependent mechanism of plaque formation is based on GTF produced by Streptococcus mutans[8].

It is known that this microorganism is involved not only in smooth surface caries, but also in fissure and in muscle caries[9-11].

The use of topical antimicrobial agents, widely used to prevent dental caries[12], the effect is exhibited by reducing the number of bacteria or inhibiting the formation of bacterial membrane at the tooth surface, but it causes side effects such as tooth coloring[13-14], substances with low toxicity to humans and the environment are required and studies on natural substances capable of satisfying this is actively conducted[15].

Oral treatment agents used to kill or inhibit microorganisms in the oral cavity by chemical method, prevent oral cleanliness, bad breath removal, and prevent dental caries and periodontal disease[16]. Oral treatment of chemicals contributes to the suppression of oral microorganisms, but due to side effects, continuous oral hygiene management requires the development of natural oral products that can be safely used for a long time without side effects[17]. Recently, interest in natural products for the prevention of dental caries or periodontal disease associated with dental plaques is increasing, and as the resistance to antibiotics has increased, interest in natural antibiotics as a new next-generation antibiotic material has increased[18].

Essential oil refers to volatile substances that are physically separated from fragrant plants. In general, essential oils are named after the extracted plant and have been called essential oils as the essence of taste and aroma[19].

Essential oil has been used for a long time since ancient times and is mainly used as fragrance, cosmetics, perfume, soap, detergent, spice[20]. Antimicrobial, anti-inflammatory, antifungal, antiviral and anti-cancer effects of Essential oil have recently been reported, and they have been applied to various industrial fields such as animal feed, insecticide, dental products and alternative medicine[21-23].

These materials have various scents and show useful bio active effects by their unique ingredients, but they contain odors that are inappropriate for use, and despite their excellent effects, they have been considered somewhat limited in clinical treatment including oral diseases[18].

Peppermint, called 'mint' has been used by the Egyptians and Romans in its digestive system for history and is known as the "mate of the intestines." Peppermint is the antipathetic medication equivalent to aspirin in medicine. The main ingredient, menthol, helps to cool the skin and muscles, and has a strong local analgesic effect, which is used as a pain relief and massage oil or cream for the neck. The oral effect has a refreshing feeling, which prevents bad breath and has excellent sterilizing effect[24-27].

Tea tree was introduced to Europe around 1927 and quickly gained attention due to its disinfectant properties. It is the most powerful immunostimulant with antibacterial, antiviral and anti fungal effects. It activates leukocytes to establish a line of defense against invading organisms. It is also effective against viral diseases such as chickenpox, shingles, herpes and warts, and fungal diseases and candida rhinitis. It is also widely used for sore throats and mouth gargles, and boosts immunity, so doctors in France use it as an aid to immune systems such as AIDS and cancer[24-27].

Therefore, in this study, we tried to investigate the antibacterial activity against oral bacteria by selecting tea tree and peppermint, which are natural essential oils with fragrance suitable for oral use.

In addition, as part of the interest in the new generation of antimicrobial materials, by confirming the value of tea tree and pepper essential oil extracted from plants as a natural antimicrobial agent against oral bacteria, it provides the possibility of being used as an oral disease treatment agent. The purpose of this study is to present practical basic data on the prevention and treatment of oral infectious diseases.

## Method

Two natural essential oils, tea tree and peppermint, refined in Kaput, England, were purchased through Dong-seong science. *S. mutans* (KCTC 3065/ATCC 25175)

was used for the antibacterial activity measurement after subculture in brain heart infusion (BHI; Sigma-Aldrich, St. Louis, MO, USA). S. mutans was diluted at a 5×105 ratio, which was anaerobically incubated at 37°C for 24 h.

Tea tree and peppermint essential oil concentrations of 0.5%, 1%, 5%, 10%, 20% and 30% were divided into $5 \times 10^5$ colony forming units; CFUs/ml diluted S. mutans 10ul, BHI 900ul, 10% Tween 20 90ul was applied to prepare the final 1mL to check the CFUs according to 24h at 37° C to evaluate the antimicrobial effect.

## Result and Discussion

CFUs according to the essential oil concentrations of refined tea tree and peppermint are *S. mutans* (control; $8.8 \times 10^{11}$), Peppermint CFUs are 0.5% ($1.6 \times 10^{10}$), 1% ($8.0 \times 10^9$), 5% ($1.5 \times 10^9$), 10% ($2.5 \times 10^8$), 20% ($2.4 \times 10^7$), and 30% ($9.5 \times 10^5$). In contrast, the tea tree's CFUs were 0.5% ($5.4 \times 10^9$), 1% ($3.4 \times 10^9$), 5% ($3.8 \times 10^8$), 10% ($1.4 \times 10^7$), 20% ($8.2 \times 10^4$), and 30% ($2.3 \times 10^3$) [Figure 1].

Compared with the control without natural essential oil, the 30% tea tree showed a 10-8 reduction, and the 30% peppermint showed $10^{-6}$ death [Figure 2].

The difference in the antibacterial effect of natural essential oils was shown, but as the concentration of% was increased, it showed higher killing of bacteria. Also, tea tree showed higher antibacterial effect than peppermint.

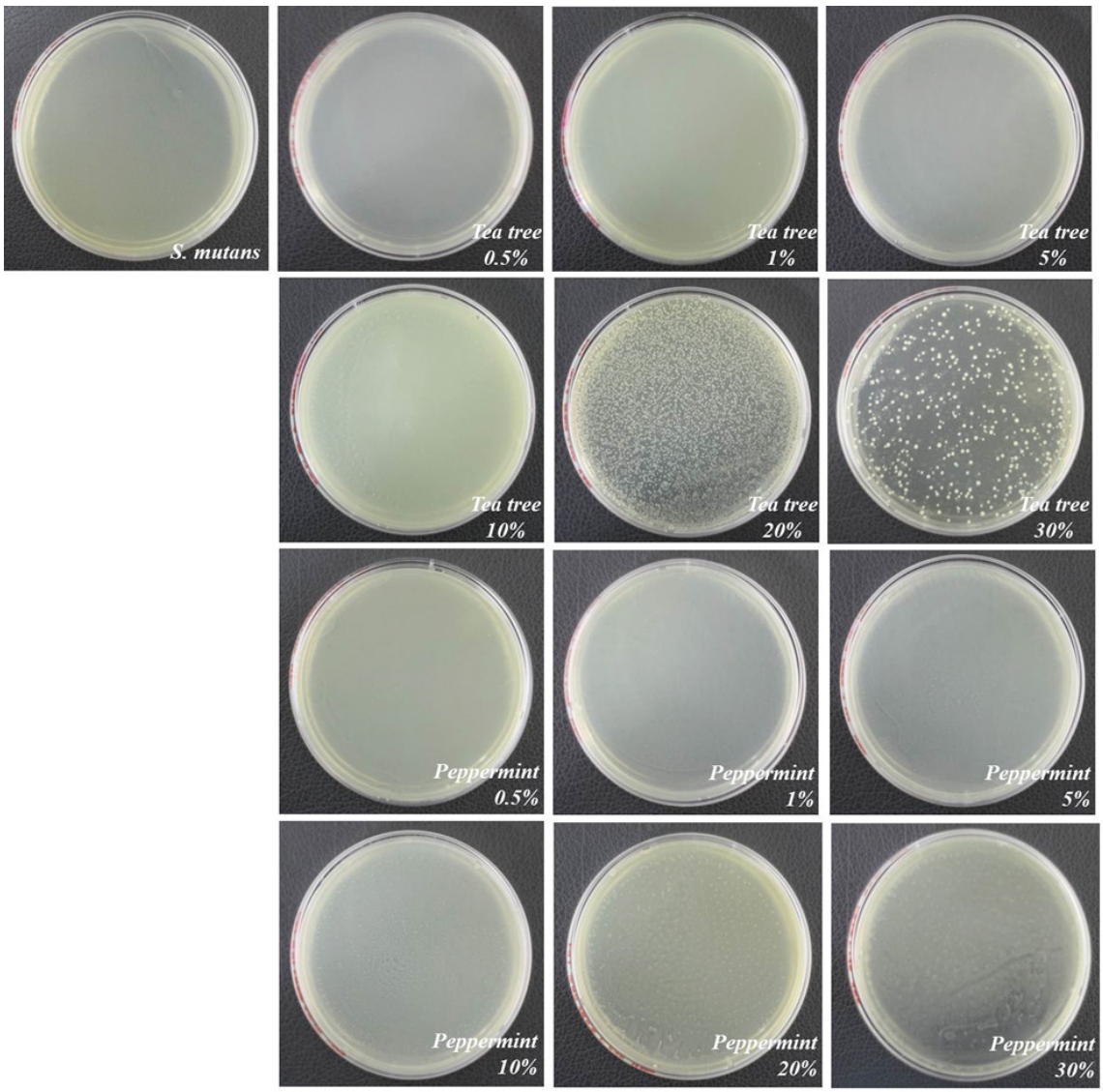


**Figure 1. Antibacterial effect of various concentrations of essential oils on *S. mutans***

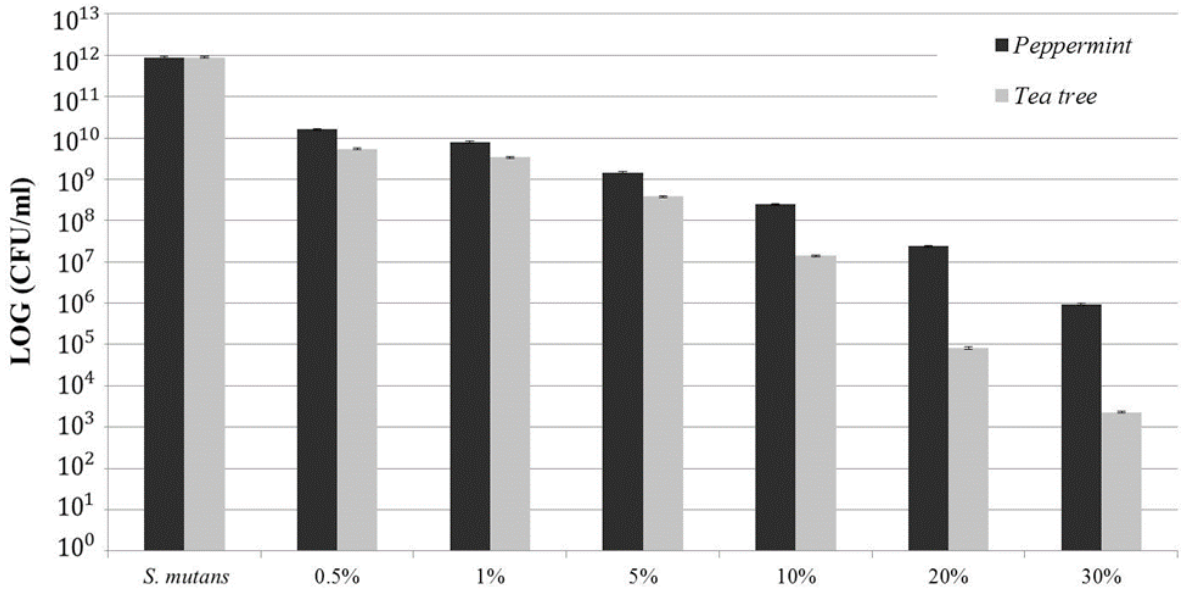


**Figure 2. The survivalrateof CFU by application of essential oils on *S. mutans***

## Discussion

Essential oils are known to destroy bacteria by destroying their cell walls and disrupting their enzymatic activity[28]. Essential oil also reduces oral bacteria by preventing the colonization of Gram-positive bacteria that cause plaque, slowing the replication of bacteria and removing endotoxins from Gram-negative bacteria[28]. Essential oil used in this experiment showed antimicrobial effect against oral bacteria.

In addition, because of comparing the effects of aroma oil mixed with tea tree, lemon, and peppermint with the experiment group, tantum and saline, the degree of bad breath was significantly lower in the experimental group[29]. After providing oral care using aroma solution of tea tree, peppermint and lemon, the concentration of bad breath and volatile sulfur compounds decreased significantly[30].

*S.mutans* is a Gram-positive bacterium, discovered by Clark in human caries lesions in 1924, that breaks down monosaccharides and fructose to produce organic acids, causing demineralization of teeth and causing dental caries, it is known as a major causative agent of dental caries by actively producing lactic acid in acidic environment due to its acid resistance[31].

Previous studies have shown that the use of natural extracts in oral treatments has the effect of inhibiting *S.mutans*[16], this results showed similar results to this study showing antibacterial effect.

In addition, there was a significant difference in the antibacterial effect of natural essential oils, but as the concentration was increased, the bactericidal effect of higher bacteria appeared. Comparing the effects of different types of aromatic oils, tea tree was found to have a higher antibacterial effect even at lower concentrations than peppermint.

Therefore, based on the results of this study, if various types of research are conducted in the future, it is expected that next-generation antibacterial materials using essential oils can be developed for the prevention and treatment of infectious diseases in the oral cavity.

## Conclusion

The purpose of this study was to investigate the antimicrobial effects on oral bacteria by selecting tea tree and peppermint, which are natural essential oils with fragrance suitable for oral treatment.

As a result of this study, the following suggestions are made.

First, it is necessary to repeat research to confirm the antibacterial effect using various essential oils.

Second, it is suggested to carry out an iterative study to confirm the antibacterial effect according to the concentration difference of various essential oils.

Third, continuous attention is needed to develop next-generation antimicrobial materials that use essential oils as a natural oral treatment that can be safely used for a long time without side effects.

**Ethical Clearance:** Not required

**Source of Funding:** Self

**Conflict of Interest:** Nil

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