



Evaluation Of Antibacterial Properties of Essential Oils Against Oral Pathogens

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Abstract

Background/Objectives: The purpose of this study was to investigate the antibacterial activity against bacteria in the oral cavity by selecting natural essential oils for use as an oral material.

Methods/Statistical analysis: In order to measure the antibacterial effect, the concentration of essential oil of each of myrrh, geranium, and lemon is divided into 0.5%, 1%, 5%, 10%, 20%, 30%, and 40% classified into BHI (Brain Heart Infusion, Difco Co., USA). *S. mutans* 1×10^5 colony forming units (CFU) were applied and the mixed solution was incubated at 37°C for 24 hours, and then CFUs were checked to evaluate the antibacterial effect.

Findings: The growth inhibitory effect of *Streptococcus mutans* (*S. mutans*) bacteria according to the concentration of natural essential oils of three kinds of refined myrrh, geranium, and lemon was confirmed. At 00 concentration of myrrh essential oils and geranium essential oils, *S. mutans* showed complete antibacterial effect. At 40% concentration of myrrh essential oils and geranium essential oils, *S. mutans* showed complete antibacterial effect. Lemon essential oils showed a more pronounced antibacterial effect at a lower concentration than the other two oils, and complete antibacterial effect was found at 20%.

Improvements/Applications: The antibacterial activity of essential oils is thought that if these are applied to oral hygiene products, they will be very useful in the prevention and treatment of oral diseases.

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

1. Introduction

There are many kinds of microorganisms in human saliva, and among them, there are about 500 kinds of pathogens that cause diseases[1]. They can cause bad breath and diseases such as dental caries and periodontal diseases, causing eating function problems, pain, and tooth loss. In particular, oral diseases such as dental caries or periodontal disease have a high tendency to remain permanently damaged once damage is applied, so it is considered important not only to treat them, but also to prevent the

disease and manage the prognosis of treatment[2]. Dental caries is a bacterial infectious disease that is widely affected by humans around the world and is the most prevalent disease among oral diseases. Various microorganisms exist in the oral cavity. Among them, mutans streptococci, especially *Streptococcus mutans* (*S. mutans*), are the bacteria most closely related to dental caries and are mainly found in human saliva and plaque[3].

S. mutans has H⁺(proton)-translocating ATPase in the cell wall, so

it has high acid resistance compared to other bacteria, so it continuously maintains metabolism even at low pH[4]. Currently, a number of sanctions are being used to prevent dental caries, including fluoride, caseinphosphotide amorphous calcium phosphate (CPP), anti-caries sweeteners such as xylitol, biopharmaceutical extracts, bamboo salts, and essential oils[5].

Among them, essential oils are volatile oils extracted from scented medicinal plants' flowers, roots, leaves, stems, and fruits of trees. They retain the power of the soil and the vitality of plants grown by receiving solar energy. It plays a very important and diverse role, such as playing a hormone-like role in plant metabolism or regulating physiological functions[6]. Essential oil is a volatile fragrance component that is a complex of various biochemical components made during the secondary metabolism of plants. In addition to its antibacterial effect, it is widely used in skin beauty and cosmetics, as well as for skin astringent and soothing effects, and helps prevent skin aging[7]. In addition, it is known that most of the chemical properties are determined during the extraction process and the biosynthesis process[8]. Essential oils restore health by boosting the body's immune system, and use 100% pure essential oils extracted from nature, which helps to improve health with few side effects and maintain the body's homeostasis[9]. Essential oils are extracted differently according to the flowers, leaves, fruits, stems, and roots of various plants, and their therapeutic efficacy varies according to the extraction site[10]. In addition, as a natural organic substance produced from various materials, it is known to have

antibacterial and antifungal effects[11]. Essential oils are excellent in disinfecting and preservative effects and have vitality with 100% pure natural ingredients[12].

Myrrh is a substance used as an astringent, disinfectant, and anti-inflammatory, and uses oleoresin from the shell. It is also used for topical oral treatment, sore throat, gingivitis, and other gum diseases, and is used for tonsillitis and oral ulcers[13]. Geranium extracts oil from leaves, stems, and flowers using a steam distillation method, and has the functions of antidepressant, antilipid, anti-inflammatory, antifungal, and anti-astringent, and is effective for acne, bruises, burns, skin congestion, eczema, and oily skin[14]. In addition, since it has cell regeneration ability, it has been reported to improve skin inflammation or inhibit skin aging, so it can be usefully used for skin care, burns and wound treatment[15]. Lemon is a plant belonging to the family of Unhyang, and the oil extracted from the light yellow fruit peel has been traditionally used in perfumes because of its rich, citrus scent [16], because of its antibacterial effect, it is used as a treatment for infectious diseases in Europe and is known to bring psychological relief[9].

Antibiotics for antibacterial activity have been mainly used as chemical agents for inhibiting various pathogens. However, antibiotics can make resistant bacteria when used for a long period, and there is a possibility of removing the resident bacteria of the oral cavity as well as the pathogens. Therefore, the use of natural extracts rather than synthetic chemicals is recommended as part of an effort to suppress only pathogens while keeping

oral resident bacteria healthy[16]. As the understanding of the utility of natural extracts expands and research is active, various ways to use the results are being sought. Research is actively being conducted to observe the antibacterial effect of plant extracts or essential oils against the causative agents of dental caries, periodontitis, fungal infections, and other oral infections[17].

In this study, we selected natural essential oils such as myrrh, geranium, and lemon essential oils that have a scent suitable for use as an oral material to investigate the antibacterial activity against bacteria in the oral cavity. In addition, by confirming the possibility of being used as a therapeutic agent for oral diseases, we tried to present practical basic data for the prevention and treatment of oral-related infectious diseases.

2. Materials and Methods

2.1. Use strain

S. mutans (KCTC 3065/ATCC 25175) was used for the antibacterial activity after subculture in brain heart infusion (BHI; Sigma-Aldrich, St. Louis, MO, USA). *S. mutans* was anaerobically incubated at 37°C for 24 h. *S. mutans* was diluted at a 1×10^5 ratio.

2.2. Antibacterial activity test

Three kinds of natural essential oils, myrrh, geranium, and lemon, refined in Kaput, England, were purchased through Dongsung Science. In order to measure the antibacterial effect, the concentration of essential oil of each of myrrh, geranium, and lemon is divided into 0.5%, 1%, 5%, 10%, 20%, 30%, and 40% classified into BHI (Brain Heart Infusion, Difco Co., USA). *S. mutans* 1×10^5 colony

forming units (CFU) were applied and the mixed solution (*S. mutans* 10ul, BHI 900ul, and 10% Tween 20 90ul) was incubated at 37°C for 24 hours, and then CFU were checked to evaluate the antibacterial effect.

2.3. Statistical analysis

The data were analyzed by the statistical software program (SPSS v.24.0, SPSS Inc., Chicago, IL, USA) to evaluate antibacterial activity through one-way ANOVA and Duncan test for post-hoc. The statistical significance was set at 0.05.

3. Results

The growth inhibitory effect of *S. mutans* bacteria according to the concentration of natural essential oils of three kinds of refined myrrh, geranium, and lemon was confirmed. It was confirmed that CFU inhibited the growth of bacteria from 0.5% myrrh essential oils and geranium essential oils applied. Myrrh essential oils and geranium essential oils had no bacterial growth was observed in 40 mg/mL (Figure 1 and Figure 2). On the other hand, when 20% lemon essential oils were applied, the bacteria were completely killed (Figure 3). The results of statistical analysis based on the results of quantifying CFU are shown in Figure 4. Compared to the control without applying essential oils, all three essential oils showed a statistically significant killing effect from 0.5% application ($p < 0.05$), and the higher the concentration of % contained, the higher the death of bacteria. In particular, numerically, it was confirmed that lemon essential oils most effectively suppressed the growth of bacteria, and when 20% lemon essential oils were applied, the bacteria did not grow

completely. Statistical differences according to the application of three essential oils according to each concentration showed differences between myrrh, geranium, and lemon

essential oils at all concentrations among which lemon essential oils showed excellent anti-antibacterial effects ($p < 0.05$).

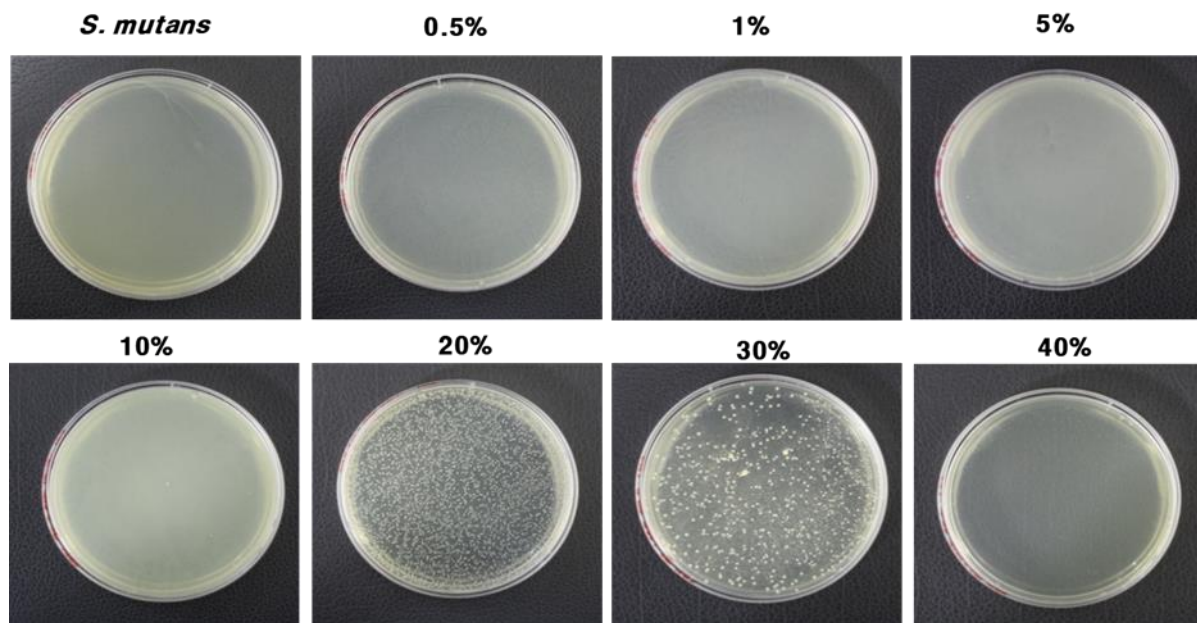


Figure 1. Inhibitory effects of growth of *S. mutans* by myrrh essential oils.

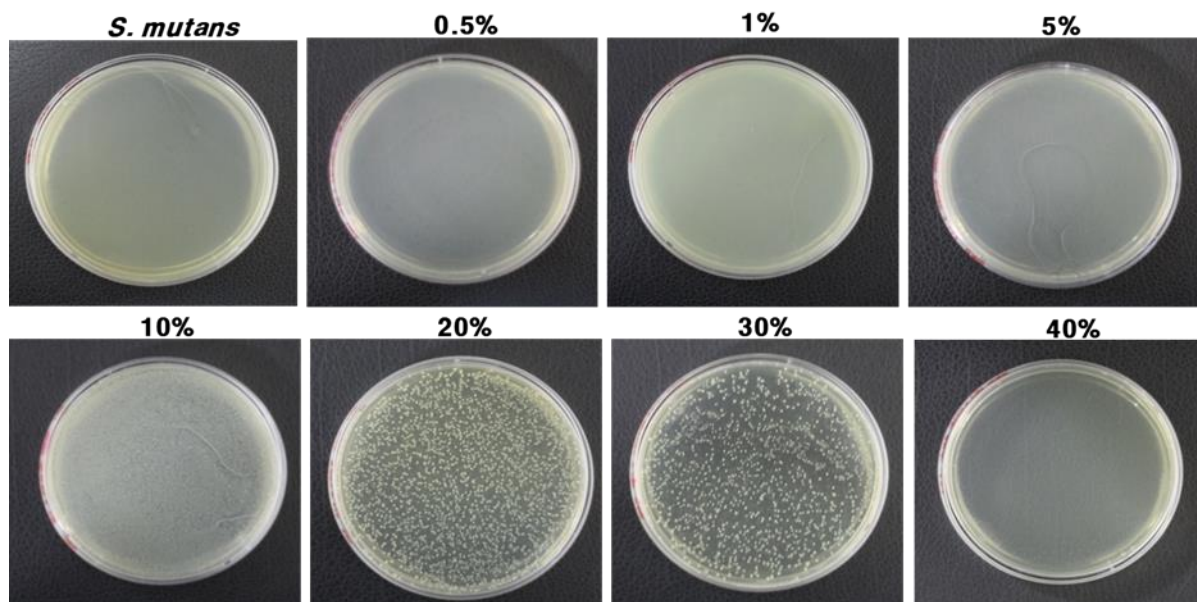


Figure 2. Inhibitory effects of growth of *S. mutans* by geranium essential oils.

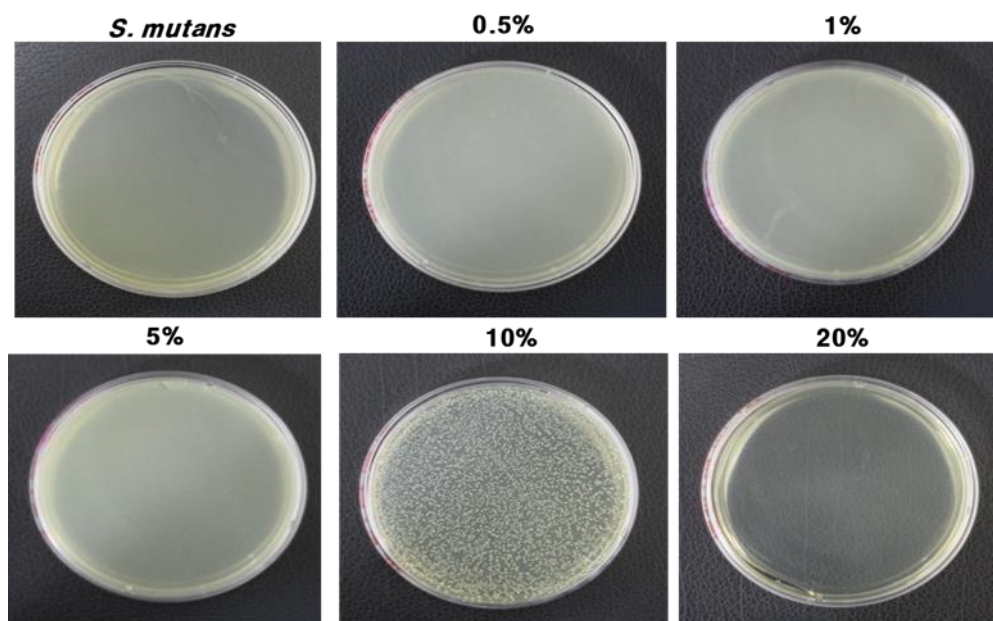


Figure 3. Inhibitory effects of growth of *S. mutans* by lemon essential oils.

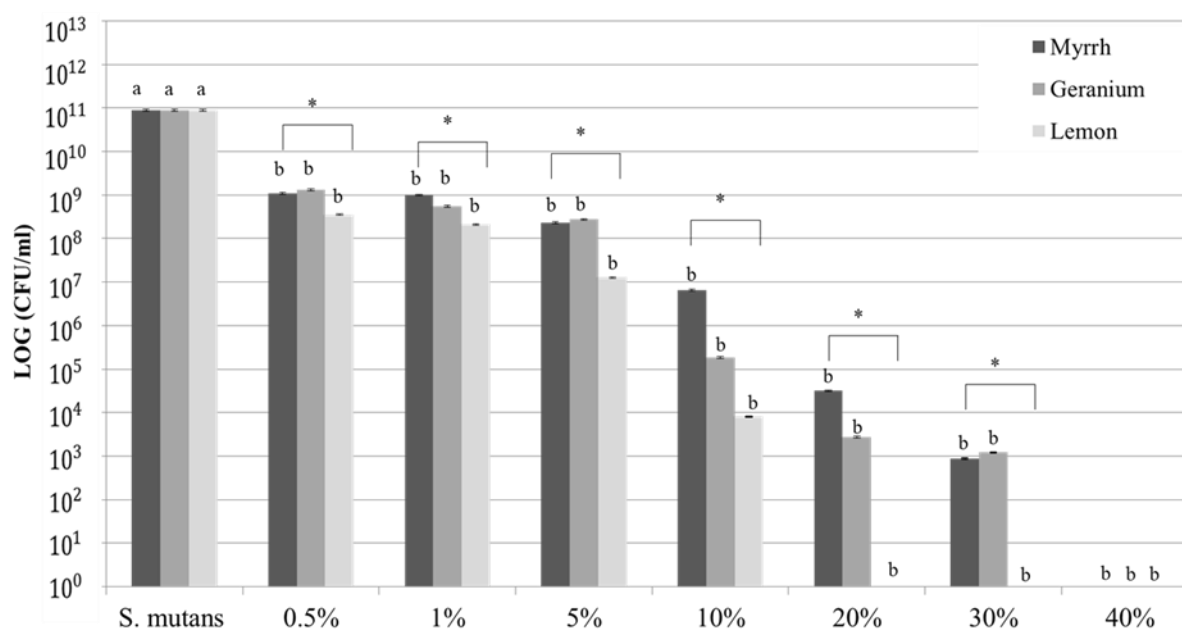


Figure 4. Antibacterial effect according to the three kinds of essential oils by number of CFU of *S. mutans*

4. Discussion

Recently, due to the continuous use of chemical antibiotics, the number of pathogens resistant to antibiotics is increasing. In this reality, attempts are being made to clinically use natural substances with antibacterial effects. Accordingly, it is considered meaningful

to study what kind of antimicrobial effect natural substances such as myrrh, geranium, and lemon exert against pathogenic microorganisms in the oral cavity and can be used clinically for therapeutic purposes in the oral cavity.

Dental caries is an irreversible disease caused by demineralization of the

hard tissue of teeth. When a glycoprotein film is formed from saliva on a clean tooth, bacteria in the oral cavity adhere to it and form a colony[18]. *S. mutans*, the bacteria most closely related to the activity of dental caries, creates an acid from sucrose, lowers the tooth surface bacterial membrane and oral pH, creates an acidic environment and demineralizes the teeth to cause dental caries[19]. In addition, *S. mutans* plays an important role in the formation of tooth surface bacterial film as it continues to colonize after inducing adhesion of other bacteria to the tooth surface by producing insoluble glucan[20]. It is thought to be an important factor in causing dental caries because it has the ability to tolerate acid well[21].

Essential oils are natural chemical substances that have a pharmacological action formed by plants themselves, and are volatile aromatic oils extracted from flowers, fruits, stems, leaves, and roots of various plants. There are more than 300 types of oils that can be used for the human body, of which about 60 types of oils are used[22]. Essential oils extracted from various plants are known to have antimicrobial activity[23]. These chemical products are currently receiving special attention as a good alternative to synthetic chemical substances for the prevention of dental caries[24]. It has been reported that essential oils have excellent balancing effects, cell growth promotion, phytohormones, anesthesia, disinfection and preservation effects, and that research has been attempted[22].

The use of myrrh as a disinfectant can be found in the Bible, and it is used alone or in combination with other herbal products for topical oral treatment, sore throat, gingivitis, other gum diseases,

tonsillitis, and oral ulcers. It is harvested in the wild in Somalia, Ethiopia and Kenya, and is applied to the necessary body parts for topical use. In the industrial field, it is mainly used as a component of toothpaste and fragrance, an irritant tonic, and as a protective agent in the pharmaceutical industry[13]. Myrrh plays a role in activating the immune system before pathogens infiltrate the body and produce antibodies to respond. The guggulsterone component of myrrh regulates toll-like receptors and protects the host from pathogens[13]. In some studies, oral functional fluids and toothpaste containing myrrh have been effective in treating gum inflammation[25], two sesquiterpenic ingredients extracted from myrrh resin have been shown to have an anti-bacterial effect[26]. In addition, myrrh essential oil is a substance that has astringent, bactericidal and anti-inflammatory effects. Antibacterial activity or antibacterial effects against oral pathogens such as *S. mutans*, *Porphyromonas gingivalis* (*P. gingivalis*), and *Candida albicans* (*C. albicans*) were confirmed, and similar to this study [2]. Geranium has long been used as a treatment for wounds and tumors, and is widely used as a fragrance in various types of cosmetics such as soaps, detergents, creams, lotions, and perfumes[27]. Geranium oil acts as a diuretic, promotes lymph circulation, is useful for cellulite, fluid retention, ankle treatment, astringent action, and is useful for healing wounds and bruises[28]. Lemon helps remove dead skin cells, improves complexion, and has an astringent effect that prevents excessive sebum production, so it has a cleansing effect on oily skin or seborrheic hair, and

has an antibacterial effect useful for acne, boils, and warts treatment[9]. Lemon oil is known to be effective in treating infectious insect bites, treating malaria, and atherosclerosis. In addition, the ancient Egyptians used it as an antidote to food poisoning from meat or fish and fevers such as typhoid fever, and as a tonic for endocrine glands[29]. It is used for hand care and broken nail care, and has a mental stability effect as it softens the wounded tissues and refreshes and clears the mood[10]. In a study that used an oral solution containing lemon, tea tree, and peppermint essential oil and tantium in patients in the intensive care unit, the oral solution containing essential oils significantly reduced bad breath than tantium[30]. In a study that confirmed the effect on oral health status of home-aged elderly by using an oral water solution using myrrh, tea tree, and mandarin essential oil, a significant difference was found between saliva pH and oral symptoms[31].

Myrrh, geranium, and lemon essential oils all showed antibacterial activity from the application of 0.5% concentration, and as the concentration of essential oil increased, higher killing effects on bacteria appeared. In addition, lemon essential oil was found to inhibit the growth of bacteria more effectively than myrrh and geranium, and when 20% lemon essential oil was applied, the bacteria did not grow completely.

From the results of this study, the antibacterial activity of essential oils of myrrh, geranium and lemon was effective against *S. mutans*, a pathogen in the oral cavity. It is thought that if these are applied to oral hygiene products, they

will be very useful in the prevention and treatment of oral diseases.

5. Conclusion

In this study, an experiment was conducted to investigate the antibacterial activity against *S. mutans*, an oral bacterium, by selecting the essential oils such as myrrh, geranium, and lemon, which have a scent suitable for use as a natural oral material, as follows.

The three essential oils, myrrh, geranium, and lemon, all showed statistically significant bacterial apoptosis effects from 0.5% application compared to controls without essential oils, and the higher the concentration of % contained. lemon essential oil most effectively inhibits the growth of bacteria, and when 20% lemon essential oil is applied, it was confirmed that the bacteria did not grow completely, showing the best anti-antibacterial effect.

Based on the results of this study, the following proposals are made: First, repeated studies are needed to confirm the antibacterial and bactericidal effects of various essential oils. Second, it is proposed to conduct repeated studies to confirm the antimicrobial effects of myrrh, geranium, and lemon essential oil according to the difference in concentration. Third, it is believed that support is needed for continued interest in the development of next-generation antibacterial materials using essential oil, a natural extract, as an oral treatment.

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