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# Antibacterial Activity of Thymus vulgaris against Dental Caries

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**A B S T R A C T**

**Introduction:** Essential oils possess antibacterial properties that can successfully combat disorders caused by microorganisms, such as bacteria, viruses, and protozoa. The aim of this study was to assess the effectiveness of *Thymus vulgaris* essential oils against bacterial strains associated with dental caries. **Methods:** Saliva specimens were obtained from individuals with dental caries at the Karanganyar Community Health Center during January–February 2023. The essential oil extracted from *Thymus vulgaris* was diluted to various concentrations ranging from 0.20 mg/mL to 100 mg/mL. Each concentration was made in 25–30 ml containers, with ten containers for each selective medium. **Results:** The different amounts of essential oil stopped all the isolates from growing. In summary, the oil exhibits the least inhibitory effect at a concentration range of 16 µL/mL. At a concentration of 128 µl/mL, *Thymus vulgaris* EO effectively inhibited all isolates, except for *Streptococcus mutans-1* and *Escherichia coli*. **Conclusion:** Essential oil from *Thymus vulgaris* showed strong activity against clinical strains belonging to *Staphylococcus* sp., *Pseudomonas* sp., *Escherichia* sp., *Bacillus* sp., *Klebsiella* sp., *Streptococcus* sp., and *Lactobacillus* sp isolated from the oral cavity at a dose of 64 and 128 µl/mL.

## 1. Introduction

Throughout millennia, plants have been extensively utilized for many purposes, such as the management of infectious diseases, the preservation of food, and the manufacturing of perfumes. Currently, the growing resistance of bacteria to existing antimicrobials, together with the emergence and resurgence of diseases, has limited the range of treatment choices for infections caused by germs. To address the evolution of antibiotic resistance, it is necessary to create new antimicrobial agents. Thymus vulgaris, also known as thyme, is a member of the Lamiaceae family. Thyme essential oil is highly valued worldwide due to its antioxidant, antibacterial, and antifungal characteristics and its ability to preserve food. The antibacterial activities of the substance are exceptional due to the inclusion of phenolic, carvacrol, and thymol components.[1-3]

Essential oils have demonstrated antibacterial characteristics that can effectively address issues caused by microorganisms, including bacteria, viruses, and protozoa. Notably, many organisms are capable of producing antimicrobial compounds that can detect and combat infectious illnesses. Certain individuals possess the ability to produce chemical compounds that can trigger chemotherapeutic processes, while others include molecular components that can effectively kill bacteria. In addition to the rise of antimicrobial resistance in microorganisms, particularly bacteria, the lack of effective selective

toxicity is also a significant disadvantage of antibiotics produced in laboratories. Nevertheless, in both scenarios, it has been demonstrated that natural antimicrobial substances contain the capacity to overcome antimicrobial resistance and selectively eradicate microorganisms.[4,5] The objective of this study was to examine the efficacy of Thymus vulgaris essential oils against bacterial isolates in dental caries.

## 2. Methods

### Plant preparation

The Thymus vulgaris leaves utilized in this investigation were acquired from the Tawangmangu botanical conservatory. A botanist at the Department of Biology, Faculty of Science, Universitas Setia Budi, Indonesia, identified these plant species and stored voucher specimens in the herbarium. Thymus vulgaris plants were air-dried in a shaded area and then ground into fine powders. The plant's fine powders (300 g) were combined with 3000 ml of distilled water in a 4-liter round-bottom flask. The ratio of plant material to extraction solvent was 1/100 (w/v). The mixture was then exposed to water distillation for 6 hours using Clavenger-type equipment, maintaining a temperature range of 45 to 55°C. The measured quantity of oil was determined in milliliters, dehydrated using anhydrous sodium sulfate, and preserved in amber vials at a temperature of 4°C until it was utilized.

### Isolation and identification of cariogenic bacteria

Saliva specimens were obtained from individuals with dental caries at the Karanganyar Community Health Center during January–February 2023. The samples were vigorously mixed using a vortex, then diluted by a factor of ten using sterile water before being cultured on agar plates. The media used to target different pathogenic bacteria associated with dental issues were as follows: Mitis Salivarius (MS) agar is supplemented with potassium tellurite for Streptococci, de Man, Rogosa, and Sharpe (MRS) agar for Lactobacilli, mannitol salt agar (MSA) for Staphylococcus aureus, and MacConkey agar for other pathogens. In summary, suitable quantities of sterilized semi-solid medium were put onto Petri plates and left to settle at room temperature. 100 µL of the diluted sample was equally distributed onto each plate and incubated at 37°C for a period of 18 to 24 hours.

The isolates were inoculated onto a blood agar medium to conduct the hemolysis test. The susceptibility of the bacterial isolates was determined using the agar-well diffusion method on Muller-Hinton agar. In summary, a group of the experimental organisms was selected using a sterile wire loop and introduced into 5 ml of normal saline in a test tube. The standardization process involved adjusting the solution to a 0.5 McFarland standard using a colorimeter. An aseptic technique was employed to apply the suspension to the surface of a solidified Mulluer-Hinton agar using a sterile swab stick. Additionally, six depressions with a diameter of 6 mm were created using a sterile cork borer. The essential oils were diluted in a 3% solution of DMSO to achieve dosages of 16 µL/mL, 32 µL/mL, 64 µL/mL, and 128 µL/mL. These diluted solutions were then distributed into individual wells. The fifth and sixth wells were filled with 3% DMSO and 3% H2O2 as negative quality control (NQC) and positive quality control (PQC), respectively.

### Determination of minimum inhibitory concentration (MIC) and minimum bacterial concentration (MBC)

The European Committee for Antimicrobial Susceptibility Testing (EUCAST) of the European Society of Clinical Microbiology and Infectious Diseases provided instructions on how to calculate the minimum inhibitory concentration (MIC) using the agar dilution method. In order to ascertain the minimum inhibitory concentration (MIC), 20 mL of Muller-Hinton agar (MHA) were utilized on 9-cm Petri dishes for the purpose of agar dilution. To achieve a total volume of 20 mL, 19 mL of molten MHA was combined with 1 mL of each EO dose. In summary, MHA was made according to the makers' instructions

and then cooled to a temperature of 50°C using a water bath.

The essential oil extracted from Thymus vulgaris was diluted to various concentrations ranging from 0.20 mg/mL to 100 mg/mL. Each concentration was made in 25–30 ml containers, with ten containers for each selective medium. Each container was filled with 19 mL of molten MHA, which was then mixed completely before being put into pre-labeled sterile Petri plates on a level platform. The plates were left to air-dry at ambient temperature to prevent any moisture droplets from forming on the agar surface. Bacterial suspensions cultivated in tryptic soy broths enriched with 0.2% glucose and cultured for 18–24 hours were introduced onto the desiccated plates. The wells were filled with varying concentrations of the essential oils (EOs) and left undisturbed at 37°C for 18–24 hours. The minimum inhibitory concentration (MIC), which refers to the lowest concentration of the extracts that totally halted observable development, was determined through visual observation. The determination of the minimum bactericidal concentration (MBC) involved obtaining scratches from minimum inhibitory concentration (MIC) experiments and attempting to cultivate bacteria on fresh agar plates. When colonies were grown from scratches obtained from the MIC test plates, it was important to note that the MIC was not treated as the MBC, and vice versa.

**Statistical analysis**

The data were expressed as the mean ± SEM for each group. A computer program (SPSS version 20) was used for statistical analysis. The differences among the groups were performed using one-way analysis of variance (ANOVA); p-values < 0.05 were considered statistically significant.

**3. Results and discussion**

**Identification of cariogenic bacteria**

The identified bacterial isolates consist of *Klebsiella pneumoniae, Bacillus licheniformis, Streptococcus mutans, Staphylococcus aureus, Lactobacillus acidophilus, Pseudomonas aeruginosa, and Escherichia coli. Among the P. aeruginosa isolates*, two are specifically designated as *P. aeruginosa-1 and P. aeruginosa-2.* Similarly, two of the Streptococcus mutans isolates are designated as Streptococcus mutans-1 and Streptococcus mutans-2. They all generated yellow hues through the process of fermenting the sugars. All of them were cultivated in a broth that was enhanced with arginine and resulted in a brick-red hue. The Gram stain of the isolates showed the presence of both Gram-positive and Gram-negative isolates.

**Antibacterial activity of Thymus vulgaris against bacterial isolates**

The different amounts of essential oil stopped all the isolates from growing. In summary, the oil exhibits the least inhibitory effect at a concentration range of 16 µL/mL. At 16 µl/mL, the test EO did not show any mean inhibitory zones against *Streptococcus mutans-1* and E. coli. However, the mean maximum inhibitory zone of 14.00 ± 0.8 mm was seen against *S. aureus*. At a dose of 32 µL/mL, there was no inhibition zone observed against S. mutans-1 and E. coli. However, the greatest inhibition zone (18.5 ± 0.4 mm) was observed against S. aureus. Furthermore, at a concentration of 64 µL/mL, the essential oil (EO) exhibited superior inhibition of isolates compared to the results obtained at a concentration of 32 µL/mL. At this dosage, S. mutans-1 and E. coli remained resistant to the oil, while S. aureus remained susceptible, with the highest average susceptibility zone of 25.0 ± 0.7 mm. Bacillus licheniformis came in second with an average susceptibility zone of 21.3 ± 0.1 mm. *Klebsiella pneumoniae* came in third with 15.5 ± 0.3 mm, *Pseudomonas aeruginosa-2* came in at 13.8 ± 0.8 mm, *Lactobacillus acidophilus* came in at 11.3 ± 0.9 mm, *Pseudomonas aeruginosa-1* came in at 11.7 ± 0.2 mm, and *Streptococcus mutans-1* came in at 10.1 ± 0.0 mm.

At a concentration of 128 µl/mL, Thymus vulgaris EO effectively inhibited all isolates, except for *Streptococcus mutans-1* and *Escherichia coli.* The mean

inhibition zones for these two isolates were significantly higher than the other dosages tested and the positive control (3% H2O2). The greatest mean inhibition zone for *Staphylococcus aureus* was recorded at 46.0 ± 1.0 mm. The positive control, on the other hand, had a bigger inhibition zone (31.0 ± 0.8 mm), bigger than the largest mean inhibitory zones for 16, 32, and 64 µl/mL. Nevertheless, in all of the experiments, the negative control (3% DMSO) did not exhibit any inhibitory effects against any of the isolates. Therefore, the dosages of 128 µl/mL and 16 µl/mL exhibited the highest and lowest levels of inhibitor concentrations against the isolates, respectively. Typically, the positive control exhibited inhibition zones that were greater than those observed with various doses. Overall, a concentration of 128 µl/mL of EO was found to be the most potent among the other concentrations.

**MIC and MBC of Thymus vulgaris EO against bacterial isolates**

For all isolates except Streptococcus mutans-1 and Escherichia coli, the test essential oil's minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were found. These isolates did not show any zones of inhibition when compared to Thymus vulgaris EO. Klebsiella pneumoniae had a concentration of 0.80 µl/mL, while Streptococcus mutans-2 had a concentration of 0.20 µl/mL. Lactobacillus acidophilus and Escherichia coli exhibited concentrations of 0.20 µl/mL and 0.40 µl/mL, respectively. Both Pseudomonas aeruginosa strains (1 and 2) exhibited identical minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of 0.20 µl/mL and 0.40 µl/mL, respectively. The MIC (minimum inhibitory concentration) values of 1.58 µl/mL and MBC (minimum bactericidal concentration) values of 3.05 µl/mL were observed to be high for both Streptococcus mutans-1 and Staphylococcus aureus.

Thymus vulgaris essential oil (EO) has shown significant activity against various isolates, including *Klebsiella pneumoniae, Streptococcus mutans-2, Lactobacillus acidophilus,* and the *Pseudomonas aeruginosa-2* strain. These isolates demonstrated identical minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of 0.20 µl/mL. The MIC and MBC values for Bacillus licheniformis were both determined to be 0.40 µl/mL. In contrast, the MIC and MBC values for the Pseudomonas aeruginosa-1 strain were found to be 0.20 µl/mL and 0.40 µl/mL, respectively. Unfortunately, the MIC and MBC values for Streptococcus mutans-1 and Escherichia coli could not be obtained due to their resistance to Thymus vulgaris EO.

The current investigation demonstrates that *Bacillus licheniformis, Streptococcus mutans-2,* and *Staphylococcus aureus* exhibited susceptibility to doses of 64 µl/mL and 128 µl/mL. *Streptococcus mutans-1, Lactobacillus acidophilus,* and *Escherichia coli* were all found to be vulnerable when exposed to a dosage of 128 µl/mL. Our analysis found that the Gram-positive strains had a higher MIC/MBC ratio compared to the Gram-negative bacteria. This provides a plausible explanation for the observed drop in susceptibility levels against gram-positive bacteria.

Prior study has demonstrated that thyme exhibits antimicrobial efficacy against *Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella pneumonia, Escherichia coli*, and Bacillus.[6] Thyme essential oils have long been recognized for their antibacterial qualities, with Gram-positive bacteria being more vulnerable to these oils than Gram-negative pathogens. In this investigation, the gram-positive bacteria Bacillus licheniformis, Staphylococcus aureus, and Lactobacillus acidophilus were significantly suppressed by the essential oil of Thymus vulgaris, with the exception of the two strains of Streptococcus mutans. Similarly, the gram-negative pathogens investigated in this study exhibited a high sensitivity rate to Thymus vulgaris EO, with the exception of Escherichia coli. Also, our research showed that the main parts of Thymus vulgaris essential oil—phenol (thymol and/or carvacrol)—were

probably what made it so effective at killing bacteria that cause cariogenic infections.

The antibacterial properties of this essential oil may be attributed to the bioactivities of its primary constituents or the synergistic effects of all its components.[7-9] On the other hand, the bactericidal and fungicidal properties of numerous essential oils make them highly valuable in the pharmaceutical, food, and cosmetics industries as substitutes for synthetic chemicals. Additionally, they are employed as additives and preservatives in food, in addition to their beneficial properties as natural medicines. Studies have been conducted on animals to examine the mechanisms by which essential oils and their components work.[10-13] These investigations have been done using both in vitro (outside of a living organism) and in vivo (inside a living organism) examinations.

Essential oils possess cytotoxic abilities due to their pro-oxidant nature, making them highly effective as antiseptic and antibacterial agents. In addition, essential oils often do not pose any long-term genotoxic hazards. Moreover, certain individuals demonstrate potent and evident antimutagenic characteristics, which may be closely linked to their anticarcinogenic effects. Despite their numerous benefits, essential oils can be harmful not only to bacteria, fungus, and viruses but also to the human body, where they can have severe effects if used in excessive amounts.[14,15] Essential oils in large amounts can depolarize mitochondrial membranes in eukaryotic cells by lowering the membrane potential, stopping the cycling of calcium ions and other ionic channels, and lowering the pH gradient.[16] The role and application of essential oils in the treatment of various human diseases, remarkably infectious diseases due to multidrug resistant bacterial strains, may be an alternative therapy to synthetic drugs that show adverse side effects.

## 4. Conclusion

Essential oil from Thymus vulgaris showed strong activity against clinical strains belonging to Staphylococcus sp., Pseudomonas sp., Escherichia sp., Bacillus sp., Klebsiella sp., Streptococcus sp., and Lactobacillus sp isolated from the oral cavity at a dose of 64 and 128 µl/mL.

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