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Synergistic Effect Antibacterial Activity of The Combination of Andaliman (*Zanthoxylum acanthopodium* DC) Fruit Essential Oil and Erythromycin against *Streptococcus mutans* and *Streptococcus pyogenes*

(*Efek Sinergisme Aktivitas Antibakteri Kombinasi Minyak Atsiri Buah Andaliman (Zanthoxylum acanthopodium DC) dan Eritromisin terhadap Streptococcus mutans dan Streptococcus pyogenes*)

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ABSTRACT

Background: The concept of synergism is one of the approaches taken to combat the problems related to antibiotic resistance. **Objectives**: The goal of this study was to determine the synergistic effect of the antibacterial activity of a combination of essential oil of Zanthoxylum acanthopodium DC fruit and erythromycin against *Streptococcus mutans* and *Streptococcus pyogenes*. **Methods**: The broth microdilution assay was used to determine the Minimum Inhibitory Concentration (MIC) of *Z. acanthpodium* DC fruit essential oil and erythromycin. The synergistic effects were assessed using the checkerboard method. **Results**: MIC value of Z. acanthopodium DC fruit essential oil against both bacteria was 2500 mg/mL. The combination of *Z. acanthopodium* DC fruit essential oil and erythromycin had a synergistic effect against *S. mutans* and *S. pyogenes* with fractional inhibitory concentration index (FICI) values of 0.375 and 0.0872, respectively. **Conclusions**: These results suggest that a combination of herbal plants and conventional antibiotics could be used as an alternative therapy for bacterial infections.

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INTRODUCTION

Antibiotics are substances that can either kill or suppress the growth of bacteria (Ledingham, Hinchliffe, Jackson, Thomas, & Tomson, 2019). Antibiotic resistance is a major worldwide health issue associated with antibiotics and a significant cause of infection therapy failure. In 2019, Murray et al. reported that it was estimated that antimicrobial resistance caused approximately 4.95 million deaths (Murray et al., 2022). Gram-negative bacteria are more resistant than gram-positive bacteria, however, occurrences of antibiotic resistance in gram-positive bacteria are also increasing (Centers for Disease Control and Prevention (CDC), 2019). *Streptococcus mutans* and *Streptococcus pyogenes* constitute the oral microbiota. According to previous studies, oral pathogens are also linked to systemic diseases, such as cardiovascular disease. World Health Organization (WHO) has reported that many bacteria linked to oral-maxillofacial infections are also linked to antibiotic resistance, posing a severe health threat worldwide (Meinen et al., 2021; Zhou & Li, 2015).

Gram-positive bacterium that commonly found in the human oral cavity is *Streptococcus mutans* and contributes significantly to dental caries (Adiguna & Santoso, 2017; Andries, Gunawan, & Supit, 2014). *Streptococcus pyogenes* is a gram-positive bacterium and its primary infection is commonly identified as *Streptococcus* pharyngitis. It is estimated that hundreds of millions of people experience serious *S. pyogenes* infections each year, of which 600,000 cases of invasive infections and 616 million cases of pharyngitis contributed to 163,000 deaths from 2009 and 2014 (Kebede, Admas, & Mekonnen, 2021). Streptococcus infections in patients with allergies to the β-lactam group can treated with macrolide antibiotics as an alternative. The use of macrolides is known to experience resistance, which is supported by previous research, where erythromycin antibiotics showed resistance to gram-positive bacteria isolated from dental plaque in 83.3% of cases. The use of herbal plants as an alternative therapy for infectious diseases has increased, due to the high content of antimicrobial compounds (Khasanah, Muslim, & Welkriana, 2019) .

Andaliman (*Zanthoxylum acanthopodium* DC) is one of the herbal plants that can be effectively utilized for this purpose. *Z. acanthopodium* DC is a wild plant native to North Sumatra (Nurlaeni, Iskandar, & Junaedi, 2021). Among the secondary metabolites found in *Z. acanthopodium* DC seeds are flavonoids, saponins, tannins, triterpenoids, and alkaloids. *Z. acanthopodium* DC fruit also has a significant essential oil content, with prior studies showing that there are 29 chemical components, with geranyl acetate being the primary component of *Z. acanthopodium* DC fruit essential oil. *Z. acanthopodium* DC fruit possesses a variety of pharmacological properties, including antibacterial activity (Moektiwardoyo, Muchtaridi, & Halimah, 2014). Previous research has found that *Z. acanthopodium* DC fruit extract in ethyl acetate inhibits the growth of *Escherichia coli*. Terpene chemicals found in *Z. acanthopodium* DC fruit suppress

E. coli growth by causing cell damage (Sitanggang, Duniaji, & Pratiwi, 2019). The ethyl acetate fraction of Andaliman fruit extract is also active against *Staphylococcus aureus* (Muzafri, 2019).

The concept of drug synergism, combining known antibiotics with plant extracts, is a novel idea. They can have beneficial and harmful effects, whereas harmful effects can be antagonistic or toxic (Mabeku, Emmanuel, Kouam, Zra, & Louis, 2013). Combination or synergistic therapy may improve therapeutic efficacy compared to single-drug use, increase the spectrum of antibacterial activity, prevent therapeutic failure when antimicrobial resistance is suspected, prevent the development of resistance, and lower the dose associated with toxicity (Rai & Kon, 2013). No studies have been conducted to investigate the synergistic effect of *Z. acanthopodium* DC fruit essential oil with erythromycin or any other antibiotics. The present study aimed to evaluate the synergistic antibacterial activity of andaliman (*Zanthoxylum acanthopodium* DC) fruit essential oil and erythromycin against *Streptococcus mutans* and *Streptococcus pyogenes*.

MATERIAL AND METHODS

Materials

Fresh fruit of *Z. acanthopodium* DC, *Streptococcus mutans* and *Streptococcus pyogenes* ATCC® 19615™ that was provided by by the Research Centre for Pharmaceutical Ingredients and Traditional Medicine, *Nutrient Agar* (Himedia®), *Mueller Hinton Broth* (MHB) (Himedia®), dimethyl sulphoxide (DMSO), McFarland 0.5 Standard (Himedia®), NaCl (Merck®), and H_2O.

Methods

Essential Oil Preparation

Z. acanthopodium fruits were collected. The fresh crushed *Z. acanthopodium* DC fruit weight 100 g is then taken into a 500 mL distillation flask, 300 mL water was added and by using Clevenger-type apparatus, hydrodistilled was done for 2-3 hours. Recovered oils were stored in amber bottles at 4 ℃ (Soulaimani et al., 2021). Using the following equation, EO yield was calculated:

$$\text{Yield of the Essential oil (\% w/v)} = \frac{\text{volume of the extracted EO (mL)}}{\text{Weight of the fresh material (g)}} \times 100$$

Gas Chromatography Mass Spectrometry (GC-MS) Analysis

Gas Chromatography-Mass Spectrometry (GC-MS) was used to analyze the Chemical components contained in the essential oil of *Z. acanthopodium* DC fruit (Altun & Yapici, 2022) with the instrument Agilent 7890B GC, 5977B MSD (Agilent Technologies) equipped with an Agilent DB-5MS UI 5% Phenyl Methyl Silox (30 m × 250 μm × 0.25 μm) at 325 °C. Helium (He) was used as the carrier gas. The front inlet was kept at 250 °C in the splitless mode 1 μL of sample was injected into the GC-MS instrument. The GC oven was programmed at 40 ℃ for 1 minute, then increased by 10 ℃ per minute until it reached a temperature of 300 ℃, and maintained for 4 minutes. The temperature of the transfer line was maintained at 270 ℃, the MS source at a temperature of 230 ℃, and the quadrupole MS at a temperature of 150 ℃. Chemical components were identified by comparing the mass spectra of the samples with a database from the NIST 17 Library.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

Determination of the MIC value of *Z. acanthopodium* DC fruit essential oil and erythromycin in this study was performed using the microdilution (broth) method. Each sample was dissolved in DMSO 0.5% before being diluted with MHB to the desired concentration, in which erythromycin and essential oil at 1,000 and 10,000 mg/mL, respectively. Briefly, 96-well microplates were filled with 100 µL of MHB. Essential oil at a concentration of 10,000 mg/mL was added to the first row (A1) of 96-well microplates and serially diluted. Erythromycin at 1,000 mg/mL concentration was added to the second row (A2) and serially diluted. After that, 100 µL of bacterial suspension at a concentration of 1×10^6 CFU/mL was inoculated. The microplate was incubated for 24 hours at 37 ℃. MIC was determined by looking at the turbidity formed, where the well with the lowest concentration that showed no turbidity was considered the MIC value. All assays were performed in triplicate (Crevelin et al., 2015; Haq et al., 2022; Maftuhah, Harnina, & Mustikaningtyas, 2015). To determine MBC values, one loop of the broth from wells with no turbidity during MIC assay was transferred to a MHA plate and incubated at 37 ℃ overnight. The bactericidal action was indicated by the lowest concentration at which the test organisms did not grow in the MHA plate (Balali, Yar, & Sylverken, 2023; Hossain et al., 2017).

The Synergistic Effect of *Z. acanthopodium* DC Fruit Essential oil with Erythromycin

The synergistic effect of *Z. acanthopodium* DC fruit Essential Oil with Erythromycin was determined using the checkerboard technique (n=3). Working solutions of erythromycin and *Z. acanthopodium* DC fruit essential oil were prepared based on the MIC value. 96-well microplates were used and filled with 100 µL of MHB. Essential oil was introduced horizontally and two-fold serial dilution was performed along the x-axis. Erythromycin was introduced vertically into the same plate, and two-fold serial dilution was performed along y-axis. Subsequently, 100 µL of bacterial suspension at a concentration of 1×10^6 CFU/mL was inoculated in all wells. Two wells were reserved for negative and medium controls (Altun & Yapici, 2022; Soudeiha, Dahdouh, Azar, Sarkis, & Daoud, 2017). The interaction between essential oil and erythromycin was determined by FICI values using the following formula (Altun & Yapici, 2022):

$$\sum \text{FICI} = \text{FIC essential oil} + \text{FIC erythromycin}$$

$$\text{FIC}_{\text{essential oil}} = \frac{\text{MIC EO in combination}}{\text{MIC of EO alone}}$$

$$\text{FIC}_{\text{erythromycin}} = \frac{\text{MIC Erythromycin in combination}}{\text{MIC of erythromycin alone}}$$

The FICI results were interpreted as follows: synergism, FICI ≤0.5; additive, 0.5< FICI ≤1; indifferent, ≥1.0 FICI ≤4.0, and antagonism, FICI >4.

RESULTS AND DISCUSSION

Essential Oil Preparation and GC-MS Analysis

Developing new antibiotics takes time, combining antibiotics with other antibacterial agents in new regimens is key to managing AMR (Coates, Hu, Holt, & Yeh, 2020). Essential oils are known to have potential as antibacterial agents, due to their chemical composition and broad spectrum of antimicrobial activity. However, some essential oils' therapeutic application has limitations, due to their moderate or low antimicrobial activity. Combining essential oil and conventional antibiotics has been indicated as an effective strategy for enhancing their efficacy and producing additional antimicrobial activity (Soulaimani et al., 2021). This study evaluated the synergistic antibacterial activity of combinations of *Z. acanthopodium* DC fruit essential oil and erythromycin against *S. mutans* and *S. pyogenes*. The essential oil of *Z. acanthopodium* DC in this study was obtained through hydrodistillation. Hydrodistillation of fruit part of the studied plants resulted in oil with a light-yellow color and a distinctive fragrance. The result showed that essential oil yield of fresh fruit was 1.107% (v/w). The GC-MS analysis successfully identified chemical constituents in essential oil. In total 52 constituent were identified, with the main constituents of Z. acanthopodium DC essential oil were D-limonene (31,6499%), geranyl acetate (20,4238%), α-pinene (11.208%), dan other constituent such as geraniol, citronellol, citronellal, and linalool as seen in **Table 1**.

Table 1. Chemical Compositions of *Zanthoxylum acanthopodium* DC Fruit Essential Oil

Compound	Class	RT (min)	Composition (%)
D-limonene	Monoterpene	8.2209	31.6499
Geranyl acetate	Monoterpene	13.3629	20.4238
α-pinene	Monoterpene	6.5573	11.208
Geraniol	Monoterpene	11.5607	9.4701
Citronellol	Monoterpene	11.1574	2.8371
Cinnamic acid	Ester	13.5394	2.6713
Sabinene	Monoterpene	7.2	2.4955
α-Selinene	Sesquiterpene	14.9635	2.4227
Citronellal	Monoterpene	10.0483	2.145
β-Myrcene	Monoterpene	7.4395	1.8808

Linalool	Monoterpene	9.2291	1.7094
Other			11.0862
Total (%)			99.9998

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

Results of individual antibacterial activities of *Z. acanthopodium* DC fruit essential oil and antibiotic are given in **Table 2**. The data showed that both *Z. acanthopodium* DC essential oil and erythromycin were able to inhibit *S. mutans* and *S. pyogenes*, where the MIC value for *Z. acanthopodium* DC EO was 2500 mg/mL, while the MIC value of erythromycin ranging from 1.95 mg/mL to 250 mg/mL. The test was then carried out from the MIC value obtained to determine the MBC value. *Z. acanthopodium* DC fruit essential oil exhibited bactericidal activity against *S. pyogenes* with an MBC value of 2500 mg/mL. In contrast, it did not exhibit bactericidal activity against *S. mutans*, with an MBC value > 2500 mg/mL. This result could be due to the capability of *S. mutans* to form biofilms, which can increase the resistance of *S. mutans* (Ács et al., 2018). Research on *Z. acanthopodium* DC fruit conducted by Susanti et al. (2020) also showed that *Z. acanthopodium* DC has antibacterial activity, where the n-hexane extract was able to inhibit the growth of *Salmonella* typhi. Another study from the genus Zanthoxylum with the species *Z. armatum* DC showed positive results against *S. mutans* with an inhibition zone of 12.66 ± 0.33 mm (Mirza et al., 2019). *Z. nitidum* DC fruit essential oil from Vietnam also has antibacterial activity against gram-positive bacteria *Bacillius subtilis* and gram-negative bacteria *E. coli* with MIC value of 100 mg/mL (Tuyen et al., 2021). These results indicate that the genus *Zanthoxylum* has antibacterial activity against both gram-positive and gram-negative bacteria.

Table 2. Antibacterial Activity of *Zanthoxylum acanthopodium* DC Essential oil and Erythromycin against tested bacteria

Sample	*S. mutans*	*S. pyogenes*	*S. mutans*	*S. pyogenes*
	MIC (mg/mL)		MBC (mg/mL)	
Essential oil of *Z. acanthopodium* DC	2500 ± 0	2500 ± 0	> 2500	2500
Erythromycin	250 ± 0	1.953125 ± 0	> 250	1.953125

MIC values are presented as mean ± SD (n=3)

Erythromycin is a macrolide antibiotic that inhibits protein synthesis and it's commonly used to treat infections caused by gram-positive bacteria, including streptococci and staphylococci. Several studies have shown that overuse of macrolides is associated with increased cases of antibiotic resistance, one of which is mediated by the Erm gene which causes changes in ribosomal targets (Percival, Williams, Randle, & Cooper, 2014; Wijesundara, Lee, Cheng, Davidson, & Rupasinghe, 2021). *S. mutans* and *S. pyogenes* are gram-positive bacteria, which is one factor affecting the action of essential oils. Previous studies have reported that essential oils have better antibacterial activity against gram-positive. This is related to the difference in membrane structure between the two types of bacteria, where gram-negative bacteria have an outer membrane containing lipopolysaccharide (LPS) molecules. LPS prevents the targeted cell from being penetrated by macromolecules and hydrophobic compounds (Li, Cai, Liu, Sun, & Luo, 2019).

The antibacterial action of *Z. acanthopodium* DC essential oil could be attributed to the fact that its primary constituents are monoterpenes, which are known to have high antibacterial activity. The main constituents of *Z. acanthopodium* DC essential oil were D-limonene (31,6499%) and other constituents such as geraniol, citronellol, citronellal, and linalool (**Table 1**). According to the study conducted by (Kintamani et al., 2023), the GC-MS analysis of *Z. acanthopodium* DC fruit essential oil was primarily composed of D-limonene, geranyl acetate, geraniol, and citronellal. (Yang et al., 2022) reported that the essential oil of *Z. acanthopodium* DC fruit from China has the main component of limonene. These results differ from previous research, where the essential oil of andaliman fruit from Indonesia was extracted with n-hexane and the main compounds were carveol (47.7%) and mirtenyl acetate (12.55%) (Yanti & Limas, 2019). Extraction techniques and environmental factors, such as light and temperature may be responsible for these varying components of Z. *acanthopodium* DC fruit essential oil.

Limonene has been reported to exhibit antibacterial activity. According to the previous study conducted by Li et al. (2019) finger citron essential oil possessed antibacterial activity against *Staphylococcus aureus* and *E. coli*, in which limonene was the main component of finger citron essential oil, causing damage to cell membranes, resulting in leakage of nucleic acids and proteins and causing death in both bacteria. Research conducted by (Han, Chen, & Sun, 2021) also supports this result, where limonene showed antibacterial activity against *S. aureus* with a MIC value of 20,000 mg/mL. Sun et al. reported that lemon essential oil was able to inhibit the growth of *S. mutans* and able to reduce biofilm formation, with the main component of oil being limonene (48%) (Sun et al., 2018). Geraniol is an aliphatic monoterpene. Several studies have reported that geraniol has several pharmacological activities, such as anti-diabetes, anti-inflammatory, and antibacterial activities. The mechanism of geraniol is its ability to adhere to the lipids of the cell membrane of microorganisms, interacting with components contained in the lipids of the cell membrane to cause cell membrane to be more permeable which ultimately damages the structure of the microorganism cell membrane (Lira et al., 2020). The citronellal content of andaliman fruit essential oil is associated with its antibacterial properties. Citronellal, the primary component of *Cymbopogon nardus* essential oil, possessed antibacterial activity against *S. mutans* at a concentration of 25%, according to research by (Kamal, Ismail, Arief, & Ponnuraj, 2020).

The Synergistic Effect of *Z. acanthopodium* DC Fruit Essential Oil with Erythromycin

The synergistic effect of *Z. acanthopodium* fruit essential oil and erythromycin against *S. mutans* and *S. pyogenes* was evaluated using the checkerboard assay. FICI values were used to interpret the interactions. The results showed that *Z. acanthopodium* DC fruit essential oil generally reduced the MIC value of erythromycin against *S. mutans* and *S. pyogenes*, with a decrease ranging from 4 to 32fold. As shown in **Table 3**, Combining *Z. acanthopodium* DC fruit essential oil and erythromycin produced a synergistic effect. The EO in combination with erythromycin demonstrated a synergistic effect (FICI ≤ 0.5) for all tested bacteria at a sub-MIC. Sreepian et al. (2022) reported that the combination of Citrus aurantifolia fruit peel essential oil from the Rutaceae family showed a synergistic effect when combined with gentamicin with a FICI value of 0.012-0.016 against methicillin-resistant *S. aureus* (MRSA). In another study reported by Magi et al., carvacrol, in combination with erythromycin, was able to reduce the MIC value of erythromycin against *S. pyogenes* by 2-2048fold (Magi, Marini, & Facinelli, 2015). Another study using a combination of cinnamon essential oil and chloramphenicol found that essential oil was able to reduce the MIC of chloramphenicol (2fold) against *E. coli* and produce a synergistic effect with FICI value ≤ 0.5 (El Atki et al., 2019).

Table 3. Synergistic Effect of *Zanthoxylum acanthopodium* DC fruit Essential oil on antibacterial activity of Erytromycin against tested bacteria

	MIC_1 (mg/mL)	MIC_2 (mg/mL)	FIC	FICI	Interpretation
S. mutans					
Z. acanthopodium DC Essential oil	2500	312.5	0.125	0.375	Synergistic
Erythromycin	250	62.5	0.25		
S. pyogenes					
Z. acanthopodium DC Essential oil	2500	9.765625	0.0039	0.0873	Synergistic
Erythromycin	1.953125	0.16267	0.0834		

Data are presented as mean (n = 3); MIC_1, MIC of one sample alone; MIC_2, MIC of samples in combination; FIC, fractional inhibitory concentration; FICI, fractional inhibitory concentration index.

Excessive use of antibiotics contributes to health problems in the form of antibiotic resistance (Panichayupakaranant, Septama, & Sinviratpong, 2019). One approach that can be adopted is a combination of antibacterial agents. Different combinations can enhance or help antibiotics work on their targets in bacterial cells, and some compounds work through different mechanisms. Synergism can increase the spectrum of antibacterial activity, minimize cases of antibiotic resistance, and reduce dose-related toxicity owing to the lower concentrations or doses of both antibacterial compounds (Sanhueza et al., 2017).

CONCLUSION

This study showed that the main components of *Z. acanthopodium* DC fruit essential oil are D-limonene, geranyl acetate, and α-pinene. *Z. acanthopodium* fruit essential oil showed activity against gram-positive bacteria and synergistic antibacterial activity when combined with erythromycin. These results suggest that a combination of herbal plants and conventional antibiotics could be an alternative therapy for treating bacterial infections.

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CONFLICT OF INTEREST

Authors declare no conflict of interest

REFERENCES

Ács, K., Balázs, V. L., Kocsis, B., Bencsik, T., Böszörményi, A., & Horváth, G. (2018). Antibacterial activity evaluation of selected essential oils in liquid and vapor phase on respiratory tract pathogens. *BMC Complementary and Alternative Medicine*, *18*(1), 1–9. https://doi.org/10.1186/S12906-018-2291-9/TABLES/6

Adiguna, P., & Santoso, O. (2017). Pengaruh Ekstrak Daun Serai (*Cymbopogon citratus*) Pada Berbagai Konsentrasi Terhadap Viabilitas Bakteri *Streptococcus mutans*. *Jurnal Kedokteran Diponegoro*, *6*(4), 1543–1550.

Altun, M., & Yapici, B. M. (2022). Determination of chemical compositions and antibacterial effects of selected essential oils against human pathogenic strains. *Anais Da Academia Brasileira de Ciencias*, *94*(1). https://doi.org/10.1590/0001-3765202220210074

Andries, J. R., Gunawan, P. N., & Supit, A. (2014). Uji Efek Anti Bakteri Ekstrak Bunga Cengkeh Terhadap Bakteri *Streptococcus mutans* Secara *In Vitro*. *Jurnal E-GiGi (EG)*, *2*(2).

Balali, G. I., Yar, D. D., & Sylverken, A. A. (2023). Antimicrobial activities of *Hibiscus sabdariffa* and *Aspilia africana* against clinical isolates of *Salmonella typhi*. *Scientific African*, *20*. https://doi.org/10.1016/j.sciaf.2023.e01667

Centers for Disease Control and Prevention. (2019). *Antibiotic resistance threats in the United States, 2019*. Atlanta, Georgia. https://doi.org/10.15620/cdc:82532

Coates, A. R. M., Hu, Y., Holt, J., & Yeh, P. (2020). Antibiotic combination therapy against resistant bacterial infections: synergy, rejuvenation and resistance reduction. *Expert Review of Anti-Infective Therapy*, *18*(1), 5–15. https://doi.org/10.1080/14787210.2020.1705155

Crevelin, E. J., Caixeta, S. C., Dias, H. J., Groppo, M., Cunha, W. R., Martins, C. H. G., & Crotti, A. E. M. (2015). Antimicrobial Activity of the Essential Oil of *Plectranthus neochilus* against

Cariogenic Bacteria. *Evidence-Based Complementary and Alternative Medicine*, *2015*. https://doi.org/10.1155/2015/102317

El Atki, Y., Aouam, I., El Kamari, F., Taroq, A., Nayme, K., Timinouni, M., … Abdellaoui, A. (2019). Antibacterial activity of cinnamon essential oils and their synergistic potential with antibiotics. *Journal of Advanced Pharmaceutical Technology and Research*, *10*(2), 63–67. https://doi.org/10.4103/japtr.JAPTR_366_18

Han, Y., Chen, W., & Sun, Z. (2021). Antimicrobial activity and mechanism of limonene against *Staphylococcus aureus*. *Journal of Food Safety*, *41*(5). https://doi.org/10.1111/jfs.12918

Haq, S. U., Wang, L., Guo, W., Aqib, A. I., Muneer, A., Saqib, M., … Liang, J. (2022). Enhancing activity of β-lactam and fluoroquinolones antibiotics by artemisinin and its derivatives against MDR *Escherichia coli*. *Frontiers in Veterinary Science*, *9*. https://doi.org/10.3389/FVETS.2022.1048531/FULL

Hossain, S., Heo, H., De Silva, B. C. J., Wimalasena, S. H. M. P., Pathirana, H. N. K. S., & Heo, G.-J. (2017). Antibacterial activity of essential oil from lavender (*Lavandula angustifolia*) against pet turtle-borne pathogenic bacteria . *Laboratory Animal Research*, *33*(3), 195. https://doi.org/10.5625/lar.2017.33.3.195

Kamal, H. Z. A., Ismail, T. N. N. T., Arief, E. M., & Ponnuraj, K. T. (2020). Antimicrobial activities of citronella (*Cymbopogon nardus*) essential oil against several oral pathogens and its volatile compounds. *Padjadjaran Journal of Dentistry*, *32*(1), 1. https://doi.org/10.24198/pjd.vol32no1.24966

Kebede, D., Admas, A., & Mekonnen, D. (2021). Prevalence and antibiotics susceptibility profiles of *Streptococcus pyogenes* among pediatric patients with acute pharyngitis at Felege Hiwot Comprehensive Specialized Hospital, Northwest Ethiopia. *BMC Microbiology*, *21*(1). https://doi.org/10.1186/s12866-021-02196-0

Khasanah, H. R., Muslim, Z., & Welkriana, P. W. (2019). Uji Sensitifitas Bakteri Gram Positif pada Plak Gigi terhadap Antibiotika. *AVICENNA*, *14*(2), 36–41.

Kintamani, E., Batubara, I., Kusmana, C., Tiryana, T., Mirmanto, E., & Asoka, S. F. (2023). Essential Oil Compounds of Andaliman (*Zanthoxylum acanthopodium* DC.) Fruit Varieties and Their Utilization as Skin Anti-Aging Using Molecular Docking. *Life*, *13*(3), 754. https://doi.org/10.3390/life13030754

Ledingham, K., Hinchliffe, S., Jackson, M., Thomas, F., & Tomson, G. (2019). *Antibiotic resistance: using a cultural contexts of health approach to address a global health challenge*. Retrieved from www.euro.who.int

Li, Z. H., Cai, M., Liu, Y. S., Sun, P. L., & Luo, S. L. (2019). Antibacterial Activity and Mechanisms of Essential Oil from *Citrus medica L. Var. Sarcodactylis*. *Molecules*, *24*(8). https://doi.org/10.3390/molecules24081577

Lira, M. H. P. de, Andrade Júnior, F. P. de, Moraes, G. F. Q., Macena, G. da S., Pereira, F. de O., & Lima, I. O. (2020, May 3). Antimicrobial activity of geraniol: an integrative review. *Journal of*

Essential Oil Research, Vol. 32, pp. 187–197. Taylor and Francis Inc. https://doi.org/10.1080/10412905.2020.1745697

Mabeku, L. B. K., Emmanuel, T., Kouam, J., Zra, T., & Louis, O. E. J. (2013). Synergetic Effects of Plant Extracts and Antibiotics on *Vibrio cholerae* O1 Strains Isolated From Clinical Specimens. *International Journal of Biology*, *5*(3), 64–72. https://doi.org/10.5539/ijb.v5n3p64

Maftuhah, A., Harnina, S. B., & Mustikaningtyas, D. (2015). Pengaruh Infusa Daun Beluntas (*Pluchea indica*) Terhadap Pertumbuhan Bakteri *Staphylococcus epidermidis*. *Unnes Journal of Life Science*, *4*(1), 60–65. Retrieved from http://journal.unnes.ac.id/sju/index.php/UnnesJLifeSci

Magi, G., Marini, E., & Facinelli, B. (2015). Antimicrobial activity of essential oils and carvacrol, and synergy of carvacrol and erythromycin, against clinical, erythromycin-resistant Group A Streptococci. *Frontiers in Microbiology*, *6*(MAR). https://doi.org/10.3389/FMICB.2015.00165/ABSTRACT

Meinen, A., Reuss, A., Willrich, N., Feig, M., Noll, I., Eckmanns, T., … Markwart, R. (2021). Antimicrobial Resistance and the Spectrum of Pathogens in Dental and Oral-Maxillofacial Infections in Hospitals and Dental Practices in Germany. *Frontiers in Microbiology*, *12*. https://doi.org/10.3389/fmicb.2021.676108

Mirza, A. U., Khan, M. S., Nami, S. A. A., Kareem, A., Rehman, S., Bhat, S. A., & Nishat, N. (2019). Copper Oxide Nanomaterials Derived from Zanthoxylum armatum DC. and Berberis lycium Royle Plant Species: Characterization, Assessment of Free Radical Scavenging and Antibacterial Activity. *Chemistry and Biodiversity*, *16*(8). https://doi.org/10.1002/cbdv.201900145

Moektiwardoyo, M., Muchtaridi, M., & Halimah, E. (2014). Chemical composition and locomotor activity of Andaliman fruits (Zanthoxylum acanthopodium DC.) essential oil on mice. *International Journal of Pharmacy and Pharmaceutical Sciences*, *6*(2), 547–550. Retrieved from https://www.researchgate.net/publication/286601487_Chemical_composition_and_locomotor_activity_of_Andaliman_fruits_Zanthoxylum_acanthopodium_DC_essential_oil_on_mice

Murray, C. J., Ikuta, K. S., Sharara, F., Swetschinski, L., Robles Aguilar, G., Gray, A., … Criollo-Mora, E. (2022). Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *Lancet*, *399*(10325), 629–655. https://doi.org/10.1016/S0140-6736(21)02724-0

Muzafri, A. (2019). Uji Aktivitas Antimikroba ekstrak Andaliman (*Zanthoxylum acanthopodium* DC.) pada *Staphylococcus aureus*. *Jurnal Sungkai*, *7*(1), 122–126.

Nurlaeni, Y., Iskandar, J., & Junaedi, D. I. (2021). Ethnoecology of Zanthoxylum acanthopodium by local communities around lake Toba, North Sumatra, Indonesia. *Biodiversitas*, *22*(4), 1806–1818. https://doi.org/10.13057/biodiv/d220426

Panichayupakaranant, P., Septama, A. W., & Sinviratpong, A. (2019). Synergistic activity of lawsone methyl ether in combination with some antibiotics and artocarpin against *methicillin-resistant Staphylococcus aureus, Candida albicans, and Trychophyton rubrum*. *Chinese Herbal Medicines*, *11*(3), 321–325. https://doi.org/10.1016/j.chmed.2019.06.001

Percival, S. L., Williams, D. W., Randle, J., & Cooper, T. (Eds.). (2014). *Biofilms in Infection Prevention and Control* (1st ed.). Academic Press.

Rai, M. K., & Kon, V. K. (2013). *Fighting Multidrug Resistance with Herbal Extracts, Essential Oils and Their Components* (M. K. Rai & K. V. Kon, Eds.). United States of America: Academic Press. Retrieved from www.elsevierdirect.com/rights

Sanhueza, L., Melo, R., Montero, R., Maisey, K., Mendoza, L., & Wilkens, M. (2017). Synergistic interactions between phenolic compounds identified in grape pomace extract with antibiotics of different classes against Staphylococcus aureus and Escherichia coli. *PLoS ONE*, *12*(2). https://doi.org/10.1371/journal.pone.0172273

Sitanggang, F. M. C., Duniaji, A. S., & Pratiwi, I. D. P. K. (2019). Daya Hambat Ekstrak Buah Andaliman (*Zanthoxylum acanthopodium* DC) Dalam Etil Asetat Terhadap Pertumbuhan *Escherichia coli*. *Jurnal Ilmu Dan Teknologi Pangan*, *8*(3), 257–266.

Soudeiha, M. A. H., Dahdouh, E. A., Azar, E., Sarkis, D. K., & Daoud, Z. (2017). In vitro evaluation of the colistin-carbapenem combination in clinical isolates of a. baumannii using the checkerboard, Etest, and time-kill curve Techniques. *Frontiers in Cellular and Infection Microbiology*, *7*(MAY). https://doi.org/10.3389/fcimb.2017.00209

Soulaimani, B., Hidar, N. El, Ben El Fakir, S., Mezrioui, N., Hassani, L., & Abbad, A. (2021). Combined antibacterial activity of essential oils extracted from *Lavandula maroccana* (Murb.), *Thymus pallidus* Batt. and *Rosmarinus officinalis* L. against antibiotic-resistant Gram-negative bacteria. *European Journal of Integrative Medicine*, *43*. https://doi.org/10.1016/j.eujim.2021.101312

Sreepian, A., Popruk, S., Nutalai, D., Phutthanu, C., & Sreepian, P. M. (2022). Antibacterial Activities and Synergistic Interaction of Citrus Essential Oils and Limonene with Gentamicin against Clinically Isolated Methicillin-Resistant Staphylococcus aureus. *Scientific World Journal*, *2022*. https://doi.org/10.1155/2022/8418287

Sun, Y., Chen, S., Zhang, C., Liu, Y., Ma, L., & Zhang, X. (2018). Effects of sub-minimum inhibitory concentrations of lemon essential oil on the acid tolerance and biofilm formation of *Streptococcus mutans*. *Archives of Oral Biology*, *87*, 235–241. https://doi.org/10.1016/j.archoralbio.2017.12.028

Susanti, N., Situmorang, E., & Fitri, W. (2020). Effectiveness of the Antibacterial Activity of n-Hexane Andaliman (*Zanthoxylum Acanthopodium* DC) Extract Against *Bacillus subtilis*, *Salmonella typhi*, and *Staphylococcus aureus*. *Journal of Physics: Conference Series*, *1462*(1). Institute of Physics Publishing. https://doi.org/10.1088/1742-6596/1462/1/012072

Tuyen, T. T., Quan, P. M., Thu Le, V. T., Toan, T. Q., Nghi, D. H., Bach, C. P., … Van, N. T. H. (2021). Chemical Composition, Antimicrobial, and Cytotoxic Activities of Leaf, Fruit, and Branch Essential Oils Obtained From *Zanthoxylum nitidum* Grown in Vietnam. *Natural Product Communications*, *16*(1), 1–7.

Wijesundara, N. M., Lee, S. F., Cheng, Z., Davidson, R., & Rupasinghe, H. P. V. (2021). Carvacrol exhibits rapid bactericidal activity against *Streptococcus pyogenes* through cell membrane damage. *Scientific Reports*, *11*(1). https://doi.org/10.1038/s41598-020-79713-0

Yang, J., Song, X., Hu, H., Zhong, W., Cao, R., Xu, Y., & Li, R. (2022). Chemical Composition and Antifungal, Anti-Inflammatory, Antiviral, and Larvicidal Activities of the Essential Oils of *Zanthoxylum acanthopodium* DC. from China and Myanmar. *Molecules*, *27*(16). https://doi.org/10.3390/molecules27165243

Yanti, & Limas, R. W. (2019). Chemical profiling of *zanthoxylum acanthopodium* essential oil and its antidiabetic activity. *Food Research*, *3*(5), 422–427. https://doi.org/10.26656/fr.2017.3(5).065

Zhou, X., & Li, Y. (2015). *Atlas of oral microbiology : from healthy microflora to disease*. China: Elsevier Inc.