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Potential of Red Betel Leaf Extract (*Piper crocatum*) and Siwak (*Salvadora persica*) Against *Staphylococcus Aureus* Bacteria

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ABSTRACT

Background. One of the bacteria that cause oral cavity infection is *Staphylococcus aureus*. This study aims to explore the antibacterial potential of red betel extract and siwak extract against *Staphylococcus aureus* bacteria, in vitro. **Methods.** This research is an in vitro experimental study to assess the diameter of the inhibition zone of red betel leaf extract and siwak against *Staphylococcus aureus*. The extract was made from red betel leaf simplicia and siwak wood with a dose of 50% each. The bacterial suspension was made by taking one pure colony from a test tube. Data analysis was performed using SPSS version 25 software. **Results:** Red betel leaf extract 50% showed the largest diameter of the inhibition zone, namely 9.1 ± 0.68 mm. Meanwhile, the siwak extract showed a smaller inhibition zone diameter of 7.9 ± 0.68 mm. One-way ANOVA analysis showed that there was a significant difference between the diameters of the inhibition zones of each test group. **Conclusion:** Red betel leaf extracts are 50% greater in inhibiting the growth of *Staphylococcus aureus* than 50% siwak extract.

1. Introduction

An oral cavity is an ideal place for the growth and development of micro-organisms because the mouth has moisture and has regular food intake. Diseases of the mouth are closely related to oral hygiene. Oral diseases caused by microbes that multiply in the mouth, including plaque and tartar (calculus), inflammation of the gums (gingivitis), cavities (dental caries), inflammation of the tonsils and throat, inflammation of the mouth (stomatitis), and bad breath. (halitosis). To prevent acid imbalance in the oral cavity, it can be done by preventing the formation of plaque. One of the bacteria that cause infectious diseases in the oral cavity is *Staphylococcus aureus*. Infectious diseases caused by these bacteria have

characteristics such as necrosis, inflammation, and abscess formation.^{1,2} *Staphylococcus aureus* is a gram-positive bacterium that is spherical like grapes, contains polysaccharides and proteins that function as antigens which are important substances in the cell wall structure, do not form spores, and do not have flagella.^{3,4}

Medicinal plants are types of plants that are believed by the community to have efficacy and have been used as raw materials for traditional medicines. One of the plants that have the potential as a medicinal ingredient and is often used by the community is red betel (*Piper crocatum*). The red betel plant is a plant that contains flavonoids, alkaloids, polyphenolic

compounds, tannins, and essential oils. Red betel leaf contains chemical compounds that are thought to have potential as antimicrobial properties such as alkaloids, flavonoids, tannins, and essential oils. Other chemical constituents contained in red betel leaf are hydroxychavicol, kavikol, cavibetol, allylprokatekol, kalrvalkrol, eugenol, p-cymene, cineole, calryofelen, caldimen estralgol, terpenenal, and phenyl propaldal.⁵ Studies have shown that red betel leaf extract (*Piper crocatum*) can inhibit the growth and kill gram-positive bacteria using the *Staphylococcus aureus* at a concentration of 25%.⁶ Siwalk (*Salvadora persica*) is an alternative plant that contain antimicrobials. Siwak extract contains compound active antimicrobials, such as saponins, terpenoids, and phenols. The content is effective in killing and inhibiting some growth bacteria oral. Siwak contains ingredient antiseptic, tannic which character astringency, oil essentials that enhancing saliva, fluoride to prevent caries (additives to commercial toothpaste), silicates which could whiten teeth, and sulfur to eliminate plaque dental studies show that siwak wood extract can hinder some bacteria cavity oral aerobic and anaerobic. extract wood Siwak which is made as to the liquid for gargling is effective to prevent plaque and can hinder bacteria grams negative.^{7,8} This study aims to explore the antibacterial potential of red betel extract and siwak extract against *Staphylococcus aureus* bacteria, in vitro.

2. Methods

The research design was an in vitro experimental study to assess the diameter of the inhibition zone of red betel leaf extract and siwak against *Staphylococcus aureus*. The red betel leaf and siwak wood were first cleaned with running water and dried in an oven at 60°C. Next, the betel leaf and siwak wood were cut and refined to obtain red betel leaf simplicia and siwak wood simplicia. The simplicia was then extracted by the maceration method using 96% ethanol solvent for 3x24 hours. Next, the macerate was separated from the pulp and thickened using a rotary evaporator to obtain a thick extract of red betel leaf and siwak.

The preparation of the bacterial suspension was started by taking one pure colony from a test tube using a sterile loop and putting it into an inoculum tube containing physiological NaCl, rotating it with a vortex and equalizing it with a turbidity of 0.5 Mc. Farlralnd uses Densi-CHECK®. Next, the bacteria growth media was made, namely, agar media, about 28 grams of Nutrient Agar powder was weighed and put into an Erlenmeyer, added some water, then heated until dissolved. Add distilled water to 1 liter and sterilized it by autoclave at 121°C for 15 minutes. Media that has been heated as much as 20 ml, put into a test tube using parchment paper to cover and wrap it. Then sterilized in an autoclave for 15 minutes at a temperature of 121°C with a pressure of 15 psi. The tube containing the agar medium is placed at an inclination of 30-45°, observed that it does not touch the tube cap and that it is allowed to cool until it hardens. Medial agar as much as 20 ml was poured into a petri dish while being shaken on the table surface to mix well and allowed to solidify. Sterile cotton buds were immersed in bacterial suspension, then scraped evenly onto the surface of the solidified agar medium. Nine blank discs were soaked in 50% red betel leaf extract, 50% siwak extract, and DMSO. Then wait for the disc paper to diffuse completely for 10 minutes.

The research data were first tested for normal data using Shapiro-Wilk. After that, the research data analysis was continued with the one-way AlNOVA statistical test. All research data was processed using SPSS version 25 software.

3. Results

Table 1 shows the diameter of the inhibition zone of each test group. Red betel leaf extract 50% showed the largest diameter of the inhibition zone, namely 9.1 ± 0.68 mm. Meanwhile, the siwak extract showed a smaller inhibition zone diameter of 7.9 ± 0.68 mm. One-way ANOVA analysis showed that there was a significant difference between the diameters of the inhibition zones of each test group, $p=0.000$.

Table 1. Inhibitory zone diameter per test group

Group	Mean±SD (mm)	p-value
Siwak extract 50%	7.9±0.68	0.000*
Red betel leaf extract 50%	9.1±0.68	
DMSO	0.0±0.00	

*one-way ANOVA, p <0.05

4. Discussion

Based on the measurement results, it is known that the red betel leaf extract has an average inhibition zone diameter of 9.1 mm. Siwak extract showed the average diameter of the inhibition zone was 7.9 mm. The strength of the antibacterial compounds of siwak extract and red betel leaf extract was moderate, due to the diameter of the inhibition zone formed < 10 mm, both siwak extract and red betel leaf extract. However, from both extracts, it was found that red betel leaf extract was more potent in inhibiting bacteria than siwak extract. According to the Dalvis Stout method, an extract is said to be very strong if the inhibition area is 20 mm or more, it is said to be strong if the inhibition area is 10-20 mm, it is said to be moderate if the inhibition area is 5-10 mm, and said to be weak if the inhibition area is < 5 mm.⁹

The difference in the effectiveness of siwak extract and red betel leaf extract is due to differences in the concentration of the content contained in the siwak extract and red betel leaf extract. Red betel leaf contains chemical compounds such as alkaloids, polyphenolic compounds, flavonoids, tannins, saponins, Catechin, and essential oils. The antifungal power of this leaf may be due to the presence of alkaloid compounds, polyphenolic compounds, flavonoids, tannins, saponins, and essential oils. The essential oil content of red betel leaf using the Stahl distillation method was 0.727% (v/b). Siwak (*Salvadora persica*) contains Sallvaldourea and salvadorine, saponins, tannins, vitamin C, silicates, resins, cyanogenic glycosides and benylsothio-cyanate. The catechins and tannins work competitively with the glycosyltransferase enzyme in reducing salkalridal as the base material for glycosylation. Glycosyltransferase enzymes play a role in the process of adding sugar groups to proteins or lipids. If this enzyme is inhibited, the formation of bacterial polysaccharides is inhibited. The effect of

traffic as an antibacterial from tannins is through reactions with cell membranes, inactivation of enzymes, and destruction or inactivation of the function of genetic material. Flavonoids have a function as an antibacterial by forming complex compounds against extracellular proteins that disrupt the integrity of the bacterial cell membrane.¹⁰⁻¹⁵

5. Conclusion

Red betel leaf extract is 50% greater in inhibiting the growth of *Staphylococcus aureus* than 50% siwak extract.

6. References

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