Effectiveness of Piper Betel Leaf Extract against The Growth of Staphylococcus Aureus (In Vitro)

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ABSTRACT

Infection of the oral cavity is an infection caused by the activity of pathological microorganisms such as Staphylococcus aureus bacteria. The use of herbal ingredients such as betel leaf extract, in the assumption, can be useful for inhibiting the growth of Staphylococcus aureus in the infection. This study aims to determine the effectiveness of antibacterial piper betel leaf extract 25% against the growth of Staphylococcus aureus. This research used posttest only control group design, composed of 27 samples of Staphylococcus aureus type A bacteria which were divided into 3 treatment groups, consists of : group I treated with 25% betel leaf extract, group II treated with lemongrass extract 25%, and control group treated with Amoxicillin 500 mg. Result obtained by means parametric statistic test, that is one way-ANOVA, obtained p value 0.001 ($p < 0.05$), so there is a significant difference in the inhibition zone of the three groups. Result of PostHoc Test, that is LSD (Least Significant Difference) obtained p value 0.001 ($p < 0.05$), there is significant difference in the measurement of inhibition zone between groups. Piper betel leaf extract 25% more effective than lemongrass extract 25% and amoxicillin 500mg, against the growth of Staphylococcus aureus.

Key Words: Piper Betel Leaf, Growth, Staphylococcus Aureus

INTRODUCTION

Staphylococcus aureus is known as pathogenic microorganisms that are associated with various clinical syndromes. Staphylococcus aureus is often found as a normal flora of germs in the skin and mucous membranes in humans, but it can also cause infection in both humans and animals. These bacteria caused disease with distinctive signs such as necrosis, inflammation and abscess formation in the oral cavity. [1,2] Periapical abscess is a condition that can be found on the teeth where the formation of local pus at the root end of the tooth and surrounding bone tissue. The pathologic cavity containing pus is caused by a mixed bacterial infection. Staphylococcus aureus is one of the bacteria that plays a role in the process of periapical abscess formation. [3,4]

In dentistry, the use of medicines from natural ingredients is considered safer and has relatively smaller side effects than the use of modern medicine. Another advantage is that the price is relatively cheap and the material more easily available. [5] Various herbal plant extracts have been studied to determine their antibacterial effects. Lemongrass (Cymbopogon citratus) and betel leaf (Piper betle Linn) are plants that grow or propagate on other tree trunks are usually used by the community to be used as herbal medicine. Scientific research on betel leaf in the field of medicine and dentistry has been done among others to determine the antioxidant properties and antibacterial properties. One of the antibacterial content of betel leaf is quite important is the polyphenol group compounds. In addition to polyphenols, other compounds such as alkaloids, tannins and steroids can also function as antibacterials. [6,7] Research conducted by

Dhika et al. (2007) to know the concentration of Minimum Inhibition Concentration (KHM) of betel extract to Streptococcus mutans bacteria cause dental caries where this research use various concentration that is 12.5%, 25%, 50%, and 100% and found the result that 25% power inhibition against Streptococcus mutans. [8]

The purpose of this research is to know the effectiveness of betel leaf extract to Staphylococcus aureus bacteria, compared with other herbs, namely lemongrass extract.

The role of Staphylococcus Aureus in Periapical Infection

Staphylococcus aureus is a species that is part of the genus Staphylococcus. Staphylococcus aureus is a circular gram-positive bacterium 0.8-1.0 μm in diameter, arranged in irregular groups such as grapes, facultative aerobs, non-sporeous, and immobile. These bacteria grow at an optimum temperature of 37ºC, but form the best pigment at room temperature (20-25ºC). Colonies on solid seed gray to golden yellow, round, smooth, protruding, and shiny. More than 90% of clinical isolates produce Staphylococcus aureus which has polysaccharide capsules or thin films that play a role in bacterial virulence. [1,9]

Staphylococcus aureus produces seven types of enterotoxins, namely A, B, C, C1, C2, D and E. Type A and B are the most common types. Type A causes various infections in humans, type B is often found in cases of food poisoning, osteomyelitis, and pneumonia. For type C, C1, C2 usually found in pharyngitis, type D is found in cases of impetigo, and type E is found in cases of vomiting. [10,11] Bacterial isolates obtained from periapical abnormalities due to dental tissue infection, indicate that Staphylococcus aureus is the most common bacteria in the case of the infection.

A periapical abscess is defined as a suppurative process around the root end of the tooth that occurs due to tissue destruction and is a continuing inflammatory response of periapical tissue to pulp irritation. [2,12] Periapical abscess caused by an infection of the pulp tissue is allowed, resulting in necrosis (death). The narrow pulp duct causes an imperfect drainage of the infected pulp, but may become the site of bacterial assembly and spread progressively into the periapical tissue. When the infection reaches the tooth root, the pathophysiology pathway of the infection process is affected by the amount, virulence of bacteria, host resilience, and tissue anatomy involved and the infection can enter the blood vessels to the periapical tissue through the apex. White blood cells which are the body's defenses against infection, move into the cavity and after phagocytic bacteria, the white blood cells will die. The dead white cells form the pus that fills the cavity. Due to the buildup of pus, the surrounding tissue will be pushed and become the abscess barrier wall. The periapical abscess is a pathological cavity in the apex area of the teeth that contains pus caused by a mixed bacterial infection. Bacteria that play a role in the process of formation of this abscess is Staphylococcus aureus and Streptococcus mutans. Staphylococcus aureus in this process has an active enzyme called koagulase whose function is to deposition fibrin. While Streptococcus mutans has 3 major enzymes that play a role in the spread of dental infections, namely streptokinase, streptodornase, and hyaluronidase. Hyaluronidase is an enzyme that destroys bridges between cells. [2,12]

Betel Leaf

Betel plants (*Piper betle linn*) is one of the elongated herbs with can reach 2-4meters plant height. The stem of the plant is round and soft, segmented, grooved and gray-green. Betel has a single leaf and its location is intermixed with varied shapes ranging from round to oval,

pointed leaf tip, heart-shaped base or slightly asymmetrical round. Betel leaf has a varied color that is yellow, green to dark green and aromatic smell. [13,14]
Betel leaf has active compounds namely essential oils, flavonoids, polyphenols, alkynids, and tannins. Essential oil is a volatile oil and contains a distinctive "aroma" or fragrance. The essential oil of betel leaf contains 30% phenol and some derivatives thereof. The main components of essential oils of phenol and their descendents, one of which chavicol can denature bacterial cell protein. [15,16] One of the content of betel leaf is quite important is a class of polyphenolic compounds. Polyphenol mechanism as an anti-bacterial agent acts as a toxin in protoplasm, destroys and penetrates walls and deposits bacterial cell proteins. Large molecular polyphenolic compounds capable of activating essential enzymes in bacterial cells though in very low concentrations. Polyphenols can cause damage to bacterial cells, protein denaturation, inactivate enzymes and cause cell leakage. Polyphenol content itself is obtained from the maceration process of betel leaves using ethanol 70%. [17, 18]


Fig. 1. Betel Leaf

Lemongrass

Lemongrass (*Cymbopogon citratus*) can also be called lemongrass, citronella grass or fever grass. Lemongrass is a perennial grass-shaped plant with a height of 50-100 cm. The stems are not woody, short-sided, and white, but some are purplish-white or reddish. The leaves are single, elongated like ribbons, lanceolate, glossy green, hugging rods, pointed tips, flat edges, 25-75 cm long, 5-15 cm wide, with parallel repeat. The lemongrass plants are clustered and bulbous, as well as soft and hollow. The stem has a midget root for shoots and yellowish white. The stems of lemongrass plants are rigid and easily broken. The stem of this plant grows perpendicular on the ground. Lemongrass plants of this type rarely have flowers, if there are generally flowers have no crown and contains grains. [19, 20]

Lemongrass contains essential oils consisting of among others: *citric*, *citronellol*, *α-Pinene*, *kamfen*, *sabinene*, *beta-Phellandrene*, *p-cymene*, *limonene*, *cis-ocimene*, *terpinol*, *borneol*, *terpinen-4-ol*, *α-Terpineol*, *geraniol*, *farnesol*, *methylheptenon*, *dipentene*, *methyl heptanone*, *bornylacetate*, *Geranyl formate*, *terpinyl acetate*, *citronyl acetate*, *geranyl acetate*, *beta-element*, *beta-Caryophyllene*, *beta-Bergamotene*, *trans-methylsoeugenol*, *beta-Cadinene*, *elemol* and *caryophyllene oxide*. Other compounds are *geranial*, *geranyl butyrate, eugenol and metileugenol*. [21, 22] Optimal segregation of citronellol content is generally obtained by distillation having a boiling point of 207˚C and a high vapor pressure of 60 mmHg. The use of temperature and vapor pressure is lower than the predetermined number, can cause the process faster and some of the existing sitronelol component into the trap and pump so that the results obtained less than optimal. [20, 23] Lemongrass is used as a body warmer, mouthwash, anti-fever, vomiting prevention, treat toothache, urinary disorders, gastritis, and colitis. [24, 25]

 Fig. 2. Lemongrass

MATERIAL AND METHODE

1.Making Betel Leaf Extract and Lemongrass Extract

Betel leaves and lemongrass 1 kg washed and dried in an oven with 50OC temperature, then blended into powder. Next is a maceration process using 70% ethanol and filtration using filter paper. The filtration result is evaporated in a rotary vacuum evaporator with a temperature of 40OC to concentrate (forming an extract). A total of 20 ml of a pure extract produced from 800 grams of dried powder is considered as an initial concentration of 100%. Dilution of the extract to obtain a concentration of 50% solution, by 1 ml of pure extract added 2 ml of physiological NaCl, then 1 ml of 50% solution was added 1 ml of physiological NaCl to obtain 25% solution.

2. Effectiveness Test of Leaf Betel Extract to *Staphylococcus Aureus*

Staphylococcus aureus was applied using cotton swab evenly and cultured on nine petri dishes containing medium Mueller Hinton Agar (MHA). In each petri dish, place 3 (three) pieces of paper disc on the surface of the media containing Staphylococcus aureus culture, then pressed with tweezers so that the paper discs are completely attached to the media. Furthermore, each paper disc was dripped with 10 µL 25% betel leaf extract, 25% lemongrass extract, and 500 mg amoxicillin using micropipette, then incubated at 37 ° C. for 24 hours. Inhibition zone measurements were carried out by observing and measuring clear areas on the surface of the *Mueller Hinton Agar* (MHA) medium around the paper disc using a sliding term: [(26)]

a. Clear zone diameter 20 mm or more: has a very strong inhibitory power
b. Clear zone diameter 10 - 20 mm: has a strong inhibitory power
c. Clear zone diameter 5 mm - 10 mm: has a moderate inhibitory power
d. Clear zone diameter <5 mm: has a weak inhibitory power

RESULT

The results showed that the average of inhibition zone in group I (betel leaf extract 25%) was 12,44 mm, group II (lemongrass extract 25%) was 0 mm, and control group (Amoxicillin 500 mg) was 31,88 mm. Group II results show no inhibitory power, possibly because the antibacterial content of lemongrass extract is not well soured at the time of the extraction process. The results of the inhibitory zone measurements were collected in all three groups, then statistical analysis was performed by using one way ANOVA test to find out the comparison of antibacterial effectiveness in group I, group II, and control group, which shows the amount of inhibitory power in each group. Based on the mean of post test in the three groups, the value of "p" in the three groups was 0,001 ($p < 0,05$). This shows that there is a significant difference in the inhibition zone of the three groups (table 1)

Table 1. One way ANOVA Test results

Group	N	F	P

Control group	9		
Group I	9	5021,520	0,001
Group II	9		

Furthermore, a Post Hoc test with LSD (Least Significant Difference) was conducted to see the significant differences between group I, group II, and control group (table 2).

Tabel 2. *Post Hoc* test result

Group		p
Group I	Group II	0,001
	Control group	
Group II	Group I	0,001
	Control group	
Control group	Group I	0,001
	Group II	

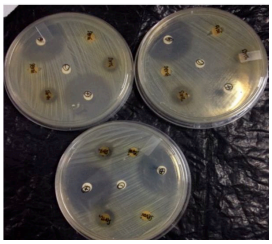
Fig. 3. Inhibition zone

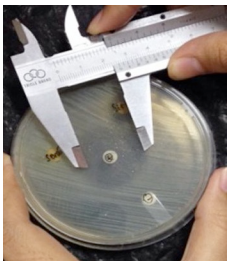
Fig. 4. Inhibition zone measurements using a sliding term

DISCUSSION

Betel leaf and lemongrass are examples of herbs that are widely available in Indonesia and can be used as an alternative medicine. Betel leaf itself is the most commonly used plant as a medicine. Kartasapoetra (1992) in his book Medicinal Plant Cultivation, said that betel leaf contains polyphenols that are proven as antibacterial agents. [(17)] These compounds act as toxins in protoplasm, destroying and penetrating walls and depositing bacterial cell proteins. In addition to polyphenols, other compounds such as alkaloids, tannins and steroids can also function as antibacterials. Polyphenol mechanism as an anti-bacterial agent acts as a toxin in protoplasm, destroys and penetrates walls and deposits bacterial cell proteins. Large molecular polyphenolic compounds capable of activating essential enzymes in bacterial cells in very low concentrations.

Polyphenols can cause damage to bacterial cells, protein denaturation, inactivating enzymes and the cause of cell leakage. The citronelol content of citronella has also been investigated for its effectiveness in the course of bacterial growth. In research conducted by Agustian et al. (2007) on the release of citronellol from citronella oil using a Bench scale fractional unit, citronelol deficiency in lemongrass can inhibit bacterial growth by adjusting to cell membranes, inactivation of essential enzymes, and the destruction or inactivation of function and genetic material. [23] Sitronelol is one of the ingredients contained in lemongrass which functions as an antibacterial, in which sitronelol is able to disturb the permeability of cell membrane and it is proved that lipid soluble essential oil of bacteria can damage and disrupt the permeability of microbes to supply nutrients, and water is disturbed so that it is unable to metabolize perfectly and lead to cell death.

Several studies have been conducted to determine the antibacterial effectiveness of betel leaf and lemongrass inhibiting bacterial growth. Betel leaf is an herb plant that is closely related to caries control, periodontal disease and halitosis control. [8.18,20,27,28,29,30] In research conducted by Health Diagnostic Laboratory of Indonesian Institute of Sciences (2012) using betel leaf extract with various concentration of 6.25%, 12.5%, and 25% against Staphylococcus aureus bacteria, resulting in the largest inhibition zone of 25 mm at 25% concentration. [31] Another study by Harman (2013) that is using 25% betel leaf extract of Enterococcus faecalis bacteria, the result of the inhibitory zone is 15,65 mm. [27] Research conducted by Poelongan (2009) using lemongrass extract of 25% to know the effectiveness of lemongrass extract to Escherichia coli and Staphylococcus aureus bacteria, obtained the mean of inhibitory zone results are 8 mm and 13 mm. From the results of these studies, we can see that with the same concentration these two extracts have different inhibitory zone, where the betel leaf extract is 25% larger than the lemongrass extract of 25%. [30] The results of these studies, support the results of this study is that 25% betel leaf extract has inhibitory power, but the inhibitory power by using lemongrass extract 25% very much different from the existing research. Post Hoc test showed that there were significant differences in the results of inhibitory measurements in each group, so that betel leaf extract in this study is inhibited bacterial growth (bacteriostatic). In this research can be said that the inhibitory power of betel leaf extract was more effective than lemongrass extract and amoxicillin.

CONCLUSION

Piper betel leaf extract 25% more effective than lemongrass extract 25% and amoxicillin 500mg, against the growth of Staphylococcus aureus.

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