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Antibacterial Activity Test of Gambas Fruit Extract (*Luffa Acutangula* (L.) Roxb.) against Bacterial Growth *Staphylococcus Aureus* In Vitro

Elwin Stanlee[1*], Indra Basar Siregar[2], Olivia Avriyanti Hanafiah[2], Isnandar[2]

[1]Faculty of Dentistry, Universitas Sumatera Utara, Medan, Indonesia

[2]Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Universitas Sumatera Utara, Medan, Indonesia

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***Corresponding author:**
Elwin Stanlee

E-mail address:
elwin.stanlee@gmail.com

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A B S T R A C T

One aspect that needs attention in dental and oral health is bacterial infection, including infections caused by *Staphylococcus aureus* bacteria. *Staphylococcus aureus* is a type of gram-positive bacteria that can be found in various parts of the human body, including the skin and nasal mucus. These bacteria usually do not cause problems if they remain within normal limits. However, when *Staphylococcus aureus* enters the oral cavity or surrounding tissue, it can cause various dental and oral health conditions. Gambas fruit (*Luffa acutangula* (L.) Roxb.), which is also known as Oyong fruit from the Cucurbitaceae family, has an antibacterial effect. This study aimed to determine the antibacterial activity of the ethanol extract of Gambas fruit (*Luffa acutangula* (l.) roxb.) on bacterial growth of *Staphylococcus aureus* in vitro. This type of research is a laboratory experimental design post-test only control group. Research data were analyzed using testone-way ANOVA with post-hoc LSD. The results showed that there was an inhibition diameter at each concentration of Gambas fruit extract. 25% Gambas fruit extract has an average diameter of 10.8 mm, 12.5% Gambas fruit extract has an average diameter of 9.4 mm, 6.25% Gambas fruit extract has an average diameter of 8 mm, 1 Gambas fruit extract 3.125% has an average diameter of 6.9 mm. Gambas fruit extract has antibacterial properties with a minimum inhibition level of 25% against the bacterial growth of *Staphylococcus aureus*.

1. Introduction

Dental and oral health is an important part of overall health. Good dental and oral care involves keeping the teeth, gums, and surrounding tissues clean and healthy. One aspect that needs attention in dental and oral health is bacterial infection, including infections caused by *Staphylococcus aureus* bacteria. *Staphylococcus aureus* is a type of gram-positive bacteria that can be found in various parts of the human body, including the skin and nasal mucus. These bacteria usually do not cause problems if they remain within normal limits. However, when *Staphylococcus aureus* enters the oral cavity or surrounding tissue, it can cause various dental and oral health conditions.[1-5]

One of the conditions that can be caused by *Staphylococcus aureus* is an infection of the gums, known as gingivitis. Gingivitis is an inflammation of the gums characterized by red, swollen, and bleeding gums when brushing your teeth. *Staphylococcus aureus* can be one of the causes of this gum infection if oral hygiene is not maintained properly. Apart from that, *Staphylococcus aureus* can also cause infections of the soft tissue around the teeth or oral cavity, such as a tooth abscess. A tooth abscess is a condition in which there is a buildup of pus inside or around a tooth, which can cause pain, swelling, and redness in the affected area. *Staphylococcus aureus* can be one of the bacteria that play a role in this infection. Infections due to *Staphylococcus aureus* are generally treated

with antibiotics. However, in the last decade, antibiotic resistance has increased, and many strains of *Staphylococcus aureus* have been found which are resistant to various antibiotics. Of course, efforts to explore new therapeutic modalities are needed in the management of infections due to *Staphylococcus aureus*.[6-8]

Gambas fruit (*Luffa acutangula* (L.) Roxb.), which is also known as Oyong fruit from the Cucurbitaceae family, has an antibacterial effect. This plant has been used as a traditional medicine to treat diabetes, gout, and sore throat. Gambas plants are widely cultivated in India, the People's Republic of China, Japan, Egypt, and several countries in Southeast Asia. More than 50 chemical compounds have been isolated from plants, such as flavonoids, anthraquinones, proteins, fatty acids, triterpene saponins, volatile components, and other compounds. Pharmacological studies show that luffa fruit has antimicrobial, antiparasitic, anticancer, antioxidant, hypoglycemic, anti-inflammatory, and analgesic effects. In addition, Gambas fruit can treat gout, sore throat, and anti-inflammation of the ear glands.[9-12] This study aimed to determine the antibacterial activity of the ethanol extract of Gambas fruit (*Luffa acutangula* (l.) roxb.) on bacterial growth of *Staphylococcus aureus* in vitro.

2. Methods

This study was experimental research in vitro and used bacterial culture *Staphylococcus aureus* on petri dishes obtained from the Microbiology Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Sumatra Utara, Medan, Indonesia. Gambas fruit (*Luffa acutangula* (l.) roxb.) as the test material, the extraction process was carried out using 96% ethanol solvent by maceration for 1x24 hours. The macerate resulting from maceration is thickened into an extract using a rotary evaporator. This study was approved by the medical and health research ethics committee of the Faculty of Medicine, Universitas Sumatra Utara, Medan, Indonesia.

Bacterial culture *Staphylococcus aureus* standardization of bacterial concentrations was carried out using McFarland 0.5 solution. The similarity of turbidity levels showed the same concentration of bacteria between test groups. A total of 24 petri dishes that had been added 1-2 ose of bacterial culture *Staphylococcus aureus* and MHA (Mueller Hinton Agar) were used in this study. There were 6 test groups, namely 0.2% chlorhexidine as a positive control (K1), negative control, DMSO (K2), 3.125%, 6.25%, 12.5% , and 25% Gambas fruit extract treatment group respectively as K3-K6. A total of 4 test petri dishes were used in each group. Furthermore, the inhibition of bacteria was measured by measuring the diameter of the inhibition zone of each treatment group. Data analysis was carried out using SPSS version 25 software. Univariate analysis was performed to present the distribution of each data variable test. Bivariate analysis was carried out to see statistical differences in each test variable, where $p < 0.05$.

3. Results and Discussion

Table 1 presents a comparison of the diameter of the inhibition zone between treatment groups. In groups, the control positive indicates a mean diameter of the inhibition zone of 11.53 ± 0.85. Group inhibition zone diameter control This positive result was still much higher than the treatment group with luffa fruit extract. The Gambas fruit extract consistently showed an increase in the diameter of the inhibition zone along with the increasing concentration of the Gambas fruit extract.

Table 2 shows a comparison of effectiveness between treatment groups. Post-hoc LSD test showed that there was no difference in the average levels of the diameter of the inhibition zone between control positives with 25% Gambas fruit extract, statistically, $p>0.05$. The diameter of the inhibition zone on the Gambas fruit extract showed an increase along with the increase in the concentration of the Gambas fruit extract.

Table 1. Comparison of the diameter of the inhibition zone between treatment groups.

Treatment	Mean±SD
25%	10,80±1,21
12,5%	9,45±0,82
6,25%	8,00±0,34
3,125%	6,97±0,47
K(+) Chlorhexidine 0,2%	11,53±0,85
K(-) DMSO	0

Table 2. Comparison of effectiveness between treatment groups.

Comparison	Between treatment groups	Mean difference	Sig.
25% extract	12.5% extract	1,3500*	0,032
	6.25% extract	2,8000*	0,000
	3,125% extract	3,8250*	0,000
	Positive control	-0,7250	0,222
12.5% extract	6.25% extract	1,4500*	0,022
	3,125% extract	2,4750*	0,001
	Positive control	-2,0750*	0,002
6.25% extract	3,125% extract	1,0250	0,092
	Positive control	-3,5250*	0,000
3,125% extract	Positive control	-4,5500*	0,000

* : p value < 0,05; post hoc LSD.

Gambas fruit extract can inhibit the growth of the bacteria *Staphylococcus aureus* because it contains flavonoids, saponins, and tannins as secondary metabolites that are antibacterial. Flavonoids have bioactivity as antibacterial compounds by forming complex compounds against extracellular proteins that disrupt the integrity of bacterial cell membranes, so they are widely used in the fields of nutrition, food safety, and health. The antibacterial activity of flavonoids is more towards several targets than only one target. One of its roles is to form complexes with proteins through hydrogen bonds and hydrophobic effects, so the antibacterial effect may be related to its ability to deactivate microbial adhesins, enzymes, and protein transport.[13,14]

Saponins are natural detergents and are identified by the formation of foam in the agitation test. The mechanism of action of saponins is included in the antibacterial group, which disrupts the permeability of the bacterial cell membrane, which results in damage to the cell membrane and causes the release of various important components from the bacterial cell, namely proteins, nucleic acids, and nucleotides. This eventually resulted in bacterial cells undergoing lysis. Meanwhile, tannins are organic substances found in water-soluble plant extracts. Tannin acts as an antibacterial targeting the cell wall polypeptides because tannins are polyphenolic compounds that can form complexes with polysaccharides and can precipitate proteins.[15-18]

In line with studies on the effects of Gambas Fruit extract (*Luffa acutangula* L.) on *Clostridium perfringens* bacteria showed, maceration extract of Gambas fruit at a concentration of 25% had strong antibacterial properties. The average inhibition zone formed was 15.6 mm (25% concentration), 12.5 mm (50% concentration), 20 mm (75% concentration), 21.5% (100% concentration), 43.5 mm (penicillin as a positive

control), and 0 mm (aquadest as a negative control). The results of this study are different from the statement that the higher the concentration of Gambas fruit extract, the larger the diameter of the inhibition zone on the bacteria *Staphylococcus aureus*. Other studies have shown that the methanol extract of Gambas fruit is able to inhibit the growth of gram-positive bacteria, namely *B. subtilis* and *S. aureus*. The antibacterial test of the methanol extract was made with a sample weight of 10 mg/hole or a concentration of 0.5 mg/µL, 15 mg/hole or a concentration of 0.75 mg/µL, and 20 mg/hole or a concentration of 1 mg/µL, with dimethyl sulfoxide (DMSO) solvent. And DMSO negative control 0 mg/µL. The inhibition zone formed against bacteria *S. aureus was* 12.51 mm with a sample weight of 15 mg/hole and 9.21 mm with a sample weight of 10 mg/hole, while the methanol extract of luffa fruit with a sample weight of 20 mg/hole obtained inhibition diameters that were not so clear and doubtful. Based on ANOVA analysis, sample weight had no effect on inhibiting bacterial growth *S. aureus*, *E. coli*, and *B. subtilis*. [19,20]

4. Conclusion

Gambas fruit ethanol extract (*Luffa acutangula* (L.) Roxb.) showed potential antibacterial activity against *Staphylococcus aureus* bacteria.

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