Antibacterial activity of Sentul fruit peel extract (Sandoricum koetjape) against Streptococcus mutans and Staphylococcus aureus

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Antibacterial activity of Sentul fruit peel extract (*Sandoricum koetjape*) against *Streptococcus mutans* and *Staphylococcus aureus*

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ABSTRACT

Introduction: Infectious mouth diseases are caused by microorganisms such as *Staphylococcus aureus* and *Streptococcus mutans*. Sentul fruit peel extract contains several phytochemical compounds, flavonoid compounds, saponins and tannins are aromatic hydroxyl groups that act as antibacterial so that they can be used as a treatment for infections of the mouth. This study aimed to determine the inhibitory ability of Sentul fruit peel extract (*Sandoricum koetjape*) against *Streptococcus mutans* and *Staphylococcus Aureus* bacteria which can cause oral infections.

Method: Sentul fruit peel simplicia was extracted by maceration method with ethanol for 24 hours. The maceration method was chosen in this study because it is a method that is easy to do and uses simple tools, which is enough to soak the sample in a solvent. A filtering process followed this and the filtrate was then evaporated with a vacuum rotary evaporator at a temperature of 45oC, so that a thick extract was produced. The maceration process was repeated 2 times. After the extraction process, then proceed with liquid-liquid fractionation using distilled water. The thick extract was put into a separating funnel and distilled water was added. Then it was shaken and the aquadest fraction was taken, followed by evaporation with a vacuum rotary evaporator at 45°C, so that the aqua fraction of the ethanol extract was produced.

Result: The results showed that the Sentul fruit peel extract was able to inhibit the growth of *S. mutans* and S. aureus bacteria as seen from the formation of a clear zone. Based on the ANOVA test, the extract concentration treatment had a significant effect ($p<0.005$) on the diameter of the clear zone on *S. mutans* and *S. aureus* bacteria. Positive control (streptomycin) showed significant differences in Duncan's test, because it produced the greatest antibacterial activity against test bacteria compared to negative control and various extract concentrations. The diameter of the clear zone in the positive control against *S. mutans* and *S. aureus* bacteria were 17.63±0.28 mm and 17.62±1.04 mm, respectively. Sentul peel ethanol extract of aqua fraction at a concentration of 100% gave the highest inhibition zone compared to concentrations of 75%, 50%, and 25%, although it was still smaller than the diameter of the clear zone in the positive control. In bacteria *S. mutans*, the ethanol extract of Sentul peel aqua fraction at a concentration of 100% gave a clear zone diameter of 14.31±1.06 mm and in S. aureus bacteria it gave a clear zone diameter of 15.34±1.81 mm.

Conclusion: Sentul fruit peel extract (Sandoricum koetjape) has the ability to inhibit *Streptococcus mutans* bacteria and *Staphylococcus Aureus* bacteria.

Keywords: Sentul fruit, antibacterial, *Streptococcus mutans*, *Staphylococcus aureus*, oral infection.
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INTRODUCTION

Infectious diseases are diseases caused by microorganisms. A disease will arise when bacteria cause both functional and structural damage.[1] Staphylococcus aureus is a microorganism that resides in the mouth. These bacteria are normal flora in the mouth that can cause disease if there are predisposing factors such as changes in the number of microorganisms, increasing or unbalanced and a decrease in the host's immune system.[2] Infection by Staphylococcus aureus can cause disease with characteristic signs, namely inflammation, necrosis and abscess formation.[3]

Other bacteria such as *Streptococcus mutans*, these bacteria are able to adhere to the tooth surface; produce the enzyme glucuronyl transferase. These enzymes produce glucans that are insoluble in water and play a role in causing plaque and colonies on the tooth surface. The World Health Organization (WHO) reports that 10-15% of the world's population suffers from periodontal disease, 80% of young children suffer from gingivitis, while almost all of the adult population suffers from gingivitis.[4] Gingivitis is an inflammation of the gingiva that causes

bleeding accompanied by swelling, redness, exudate and changes in the normal contour of the gingiva. Gingivitis is caused by the accumulation of bacteria in plaque, and plaque that accumulates in the mouth will experience mineralization to form tartar.[5] Tartar is a medium for the growth and proliferation of bacteria that can cause inflammation of the gums.

The most common treatments for gingivitis are scaling and root planing. Scaling is an attempt to remove plaque, calculus and stains on the surface of the crown and root of the tooth. Root planing is an act of cleaning and smoothing the surface of the root of the tooth from necrotic tissue and residual bacteria and their products attached to the surface of the tooth root.[5]

Several studies have shown that mouthwash can inhibit plaque formation and has been shown to reduce the severity of gingivitis. In general, mouthwash has the same way of working: destroying bacterial cells, breaking down enzymes in the plaque matrix, inhibiting bacterial aggression, or inhibiting bacterial attachment to tooth surfaces.[6]

Sentul fruit can be eaten and is also used in traditional medicinal herbs such as the roots can treat diarrhea, the leaves can relieve fever, and the powdered part of the stem can be used as an anthelmintic.[7] Several researchers have proven the efficacy of the Sentul plant as a vaginal discharge medicine, namely reporting that the methanol extract of Sentul bark can inhibit the growth of Candida albican fungus by 39.65%.[8] In addition, the ethyl acetate extract of the leaves of the Sentul harp plant also has anti-bacterial activity.[9] The results of the phytochemical screening examination of Sentul fruit peel simplicia powder showed the presence of groups of alkaloid compounds, flavonoids, tannins, saponins, glycosides, anthraquinone glycosides and steroids.

The research results on Sentul fruit peel extract from several phytochemical compounds, flavonoid compounds, saponins and tannins are aromatic hydroxyl groups that act as antibacterial.[10] This study aimed to determine the inhibitory ability of Sentul fruit peel extract (Sandoricum koetjape) against *Streptococcus mutans* and Staphylococcus Aureus bacteria.

RESEARCH METHODS

Materials and tools

The materials used in this study were samples of Sentul fruit peel, aluminum foil, ethanol, distilled water, filter paper, disc, alcohol, mice, 20% DMSO, Muller Hinton Agar (MHA) media, isolates of *S. mutans* and *S. aureus* bacteria.

The tools used in the research were beakers, measuring cups, measuring flasks, petri dishes, separating funnels, vacuum rotary evaporator (Buchi, Switzerland), erlenmeyer, Laminar air flow, micropipette, analytical balance (Ohaus, USA), stir bar, spatula, funnel, loop needle, bunsen burner, and calipers.

Work procedures

a. Sample Preparation and Processing
Sentul fruit (Sandoricum koetjape) was obtained from Sayan Village, Ubud District, Gianyar Regency, Bali Province. Sentul fruit obtained was washed clean, then separated the fruit's skin and sliced thinly, dried at room temperature. The dried fruit peel was mashed with a blender, then the sample powder was stored in a jar.

b. Sentul Fruit Peel Extract
Sentul fruit peel simplicia was extracted with ethanol for 24 hours by maceration method. The maceration method was chosen in this study because it is a method that is easy to do and uses simple tools, which is enough to soak the sample in a solvent. A filtering process followed this and the filtrate was then evaporated with a vacuum rotary evaporator at a temperature of 45°C, so that a thick extract was produced. The maceration process was repeated 2 times. After the extraction process, then proceed with liquid-liquid fractionation using distilled water. The thick extract was put into a separating funnel and distilled water was added. Then it was shaken and the aquadest fraction was taken, followed by evaporation with a vacuum rotary evaporator at 45°C, so that the aqua fraction of the ethanol extract was produced.

c. Antibacterial Test
The stages of preparation include growth of bacteria, bacterial suspension, paper discs preparation, negative control and positive control, and manufacture of concentration series, such as the concentration of 300; 400; and 500 mg/mL. The antibacterial activity test using the Disc Diffusion method (Kirby-Bauer Test). The test bacteria suspension as much as 20 L was inserted into the media in a petri then streaked with an o'ses needle on the test media. A paper disc with a diameter of 6 mm. Positive control was streptomycin or amoxicillin, negative control was DMSO 20%, ethanol sentul fruit peel extract with concentrations of 300 mg/ml, 400 mg/ml and 500 mg/ml, respectively. Then the disc is placed on the surface of the media in accordance with the desired position. The media was then incubated at 37°C for 24 hours, then the diameter of the inhibition zone was measured using a caliper expressed in millimeters.

RESULTS

Flavonoid compounds, saponins and tannins are phytochemical compounds that have an antibacterial role. Sentul ethanol extract of aqua fraction was then tested for antibacterial against *S. mutans* and *S. aureus* bacteria (Table 1 and Figure 1).

The results showed that sentul extract was able to inhibit the growth of *S. mutans* and S. aureus bacteria as seen from the formation of a clear zone. This indicates that the sentul extract has antibacterial ability to the concentration of the extract. Based on the ANOVA test, the extract concentration treatment had a significant effect ($p<0.005$) on the diameter of the clear zone on *S. mutans* and S. aureus bacteria. The positive control showed a significant difference in Duncan's test, because it produced the greatest antibacterial activity against the test bacteria compared to the negative control and various extract concentrations. The diameter of the clear zone in the positive control against *S. mutans* and *S. aureus* bacteria with values of 17.63 ± 0.28 mm and 17.62 ± 1.04 mm, respectively. A positive control using the antibiotic streptomycin.

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Table 1. Antibacterial test results sentul ethanol extract aqua fraction.

Study group	Zone diameter (mm)	
	S. mutans	*S. aureus*
Negative control	0.00±0.00	0.00±0.00
Positive control	17.63±0.28	17.62±1.04
Sentul fruit peel extract 25%	10.79±0.28	9.50±0.34
Sentul fruit peel extract 50%	11.78±0.41	11.02±0.60
Sentul fruit peel extract 75%	11.28±0.35	11.34±0.57
Sentul fruit peel extract 100%	14.31±1.06	15.34±1.81

Description: The average value followed by the same letter in the same column shows a non-significant difference (Duncan 5%)

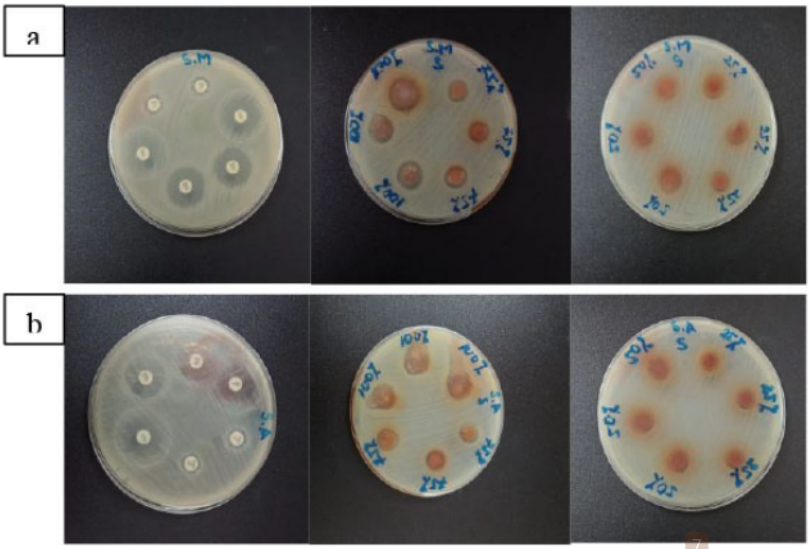

Figure 1. a) the results of the antibacterial test against *S. mutans* and b) the results of the antibacterial test against the bacteria S. aureus.

The results showed that sentul extract was able to inhibit the growth of *S. mutans* and S. aureus bacteria as seen from the formation of a clear zone. This indicates that the sentul extract has antibacterial ability to the concentration of the extract. Based on the ANOVA test, the extract concentration treatment had a significant effect ($p<0.005$) on the diameter of the clear zone on *S. mutans* and S. aureus bacteria. The positive control showed a significant difference in Duncan's test, because it produced the greatest antibacterial activity against the test bacteria compared to the negative control and various extract concentrations. The diameter of the clear zone in the positive control against *S. mutans* and S. aureus bacteria with values of 17.63 ± 0.28 mm and 17.62 ± 1.04 mm, respectively. A positive control using the antibiotic streptomycin.

Duncan's test on the diameter of the inhibition zone of *S. mutans* and *S. aureus* bacteria, for the negative control, showed significant differences with the positive control and various extract concentrations. The negative control used was distilled water which showed no inhibition zone. This indicates that the control used has no effect on the antibacterial test.

Sentul peel ethanol extract of aqua fraction at a concentration of 100% gave the highest inhibition zone compared to concentrations of 75%, 50%, and 25%, although it was still smaller than the diameter of the clear zone in the positive control. In bacteria *S. mutans*, the ethanol extract of sentul peel aqua fraction at a concentration of 100% gave a clear zone diameter of 14.31±1.06 mm and in S. aureus bacteria it gave a clear zone diameter of 15.34±1.81 mm.

Sentul peel ethanol extract of aqua fraction at concentrations of 75% and 50% showed the formation of inhibition zones on *S. mutans* and *S. aureus* bacteria which were not statistically significantly different between the two concentrations. 75% and 50% Concentrations produced a smaller inhibition zone than the concentration of 100%. Sentul peel ethanol extract of aqua fraction at a concentration of 25% produced the smallest inhibition zone for *S. mutans* and *S. aureus* bacteria compared to other extract concentrations (Figure 2).

DISCUSSION

Sentul fruit peel was collected, washed, and dried in an oven to obtain simplicia. After drying, the sample was powdered. Then extraction is done by maceration method with ethanol. The maceration method was chosen in this study because it is a method that is easy to do and uses simple tools, which is enough to soak the sample in a solvent. After the extraction process, it was continued with liquid-liquid fractionation using hexane, ethyl acetate and aquades as solvents. The choice of solvent for fractionation is based on the solvent's polarity level. The fractionated extract used in antibacterial testing is distilled water fractionation. The choice of ethanol solvent and aquades fraction was based on previous research where the sentul ethanol extract of the aqua fraction gave the highest levels of flavonoid compounds, saponins and tannins with flavonoid levels of 11476.16 mg/100g QE, tannins 88.605 mg/g and 6.862 mg/g.[10]

Lower concentration certainly contains fewer antibacterial compounds. This is in accordance with Rahmawati et al.[11] that the greater the interaction concentration of a given extract, the greater the diameter of the inhibition formed, because the more bioactive components contained in the extract.

Davis and Stout[12] stated the criteria for antibacterial power as follows: an inhibition zone diameter of 5 mm or less were categorized as weak, an inhibition zone of 5-10 mm was categorized as moderate, an inhibition zone of 10-20 mm was categorized as strong and an inhibition zone of 20 mm or more was

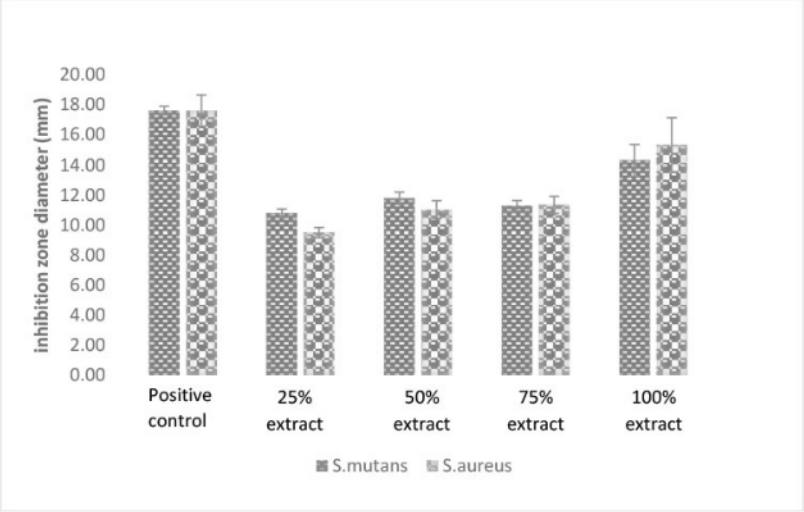

Figure 2. Graph of clear zone diameter of ethanol extract sentul aqua fraction.

categorized as very strong. This means that the aqua fraction of Sentul peel ethanol extract at a concentration of 25%-100% against *S. mutans* bacteria is categorized as strong antibacterial, while S. aureus bacteria from aqua fraction Sentul ethanol extract at a concentration of 50%-100% is categorized as strong antibacterial and 25% categorized as moderate antibacterial (Figure 2).

Sentul peel ethanol extract of aqua fraction contains flavonoid compounds, tannins and saponins which have antibacterial roles. Each compound has a mechanism of action in inhibiting bacterial growth. The mechanism of action of flavonoids as antimicrobials can be divided into 3: inhibiting nucleic acid synthesis, inhibiting cell membrane function and inhibiting energy metabolism.[13] The antibacterial action mechanism of tannins has antibacterial power by means of protein precipitation. The antibacterial effect of tannins through reactions with cell membranes, inactivation of enzymes and inactivation of the function of genetic material. The mechanism of action of tannins as antibacterial is to inhibit the enzyme reverse transcriptase and DNA topoisomerase so that bacterial cells cannot be formed.[14] The mechanism of action of saponins as antibacterial is that it can cause leakage of proteins and enzymes from the cell. Saponins can be anti-bacterial because their surface active substances are similar to detergents, as a result, saponins will reduce the surface tension of bacterial cell walls and damage membrane permeability. This damage to the cell membrane greatly interferes with the survival of bacteria.[15]

CONCLUSION

Based on the research above, it can be concluded that the extract of sentul fruit peel (Sandoricum koetjape) has the ability to inhibit the bacteria *Streptococcus mutans* and Staphylococcus aureus bacteria. The highest ability to inhibit bacterial growth was obtained at a concentration of 100%.

CONFLICT OF INTEREST

All author declares there is no conflict of interest regarding publication of current study.

ETHICAL CONSIDERETION

This study has been approved by ethical committee Politeknik Kesehatan Denpasar, Bali-Indonesia, with ethical clearance reference number: LB.02.03/EA/KEPK/ 0272 /2022.

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AUTHOR CONTRIBUTION

All authors had contributed in manuscript writing and agreed for the final version of the manuscript for publication.

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