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Effectiveness Test of Avocado Seed Extract (*Persea Americana* Mill.) in Inhibiting the Growth of *Staphylococcus aureus* Bacteria In Vitro

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A B S T R A C T

Staphylococcus aureus can cause several health problems in the oral cavity, including stomatitis, tooth abscess, soft tissue infections, gingivitis, and periodontitis. The exploration of new therapeutic modalities for *Staphylococcus aureus* infection is urgent. *Staphylococcus aureus* has developed resistance to many of the antibiotics commonly used to treat bacterial infections. This study aimed to determine the antibacterial effectiveness of avocado seed extract (*Persea americana* Mill.) in inhibiting bacterial growth *of Staphylococcus aureus* in vitro. This study is an in vitro study in which 28 petri dishes have been added with 1-2 oses of bacterial culture *Staphylococcus aureus* and MHA (Mueller Hinton Agar) were used in this study. There were 7 test groups, namely 0.2% chlorhexidine as a positive control (K1), negative control, DMSO (K2), avocado seed extract treatment group 2%, 4%, 6%, 8%, and 10% respectively as K3-K7. Treatment of avocado seed extract with a concentration of 6% has the ability to grow bacteria *Staphylococcus aureus* better than the bacterial growth ability of the positive control, chlorhexidine. The increase in extract concentration was in line with the increase in the diameter of the bacterial inhibition zone *Staphylococcus aureus*. Avocado seed extract (*Persea americana* mill.) showed effectiveness in inhibiting bacterial growth *of Staphylococcus aureus* in vitro.

1. Introduction

Staphylococcus aureus bacteria is one of the causes of oral cavity infections. The oral cavity is an environment rich in various types of bacteria, both beneficial and pathogenic. When this bacterial balance is disturbed, pathogenic bacteria such as *Staphylococcus aureus* can multiply and cause infection. *Staphylococcus aureus* can cause several health problems in the oral cavity, including stomatitis, tooth abscess, soft tissue infections, gingivitis, and periodontitis. The exploration of new therapeutic modalities for *Staphylococcus aureus* infection is urgent. *Staphylococcus aureus* has developed resistance to many of the antibiotics commonly used to treat bacterial infections. This resistance has made it difficult to treat *Staphylococcus aureus* infections, especially in cases of severe and persistent infection. Therefore, a new therapeutic approach is needed that can overcome the problem of antibiotic resistance. *Staphylococcus aureus* is one of the main causes of nosocomial infections (infections that occur in hospitals or other health facilities). Nosocomial infections tend to be more difficult to treat because these bacteria often already have resistance to many antibiotics, and such infections can have serious consequences in patients who are already weakened or have compromised immune systems. Some of the antibiotics used to treat *Staphylococcus aureus* infections can cause adverse side effects for patients. Therefore, it is important to look for new therapies that are more selective and have the potential to cause fewer side effects. Exploration and research of new therapeutic modalities for *Staphylococcus aureus* infection is very important. The

development of new drugs, especially those based on natural ingredients or compounds that have not been widely studied, can provide new hope in the treatment of this bacterial infection and help overcome the growing problem of antibiotic resistance.[1-5]

Avocado seed (*Persea americana* Mill.) has become an interesting research subject because it has potential as a potential antibacterial. This potential is mainly related to the content of bioactive compounds in avocado seeds which have been shown to have antimicrobial properties. Avocado seeds contain flavonoids, such as catechins and epicatechins, which have been known to have antimicrobial activity. These compounds can interfere with the growth and replication of bacteria, including *Staphylococcus aureus*. Tannins are polyphenolic compounds that are also found in avocado seeds. This compound has antimicrobial properties by binding to proteins on the surface of bacteria and inhibiting the growth of microorganisms. Saponins are compounds that have antimicrobial activity and are able to form complexes with cholesterol in bacterial cell membranes, resulting in bacterial damage and death. Avocado seeds also contain various polyphenolic compounds such as gallic acid, catechins, and epicatechins, which have potential as antimicrobials.[6-11] This study aimed to determine the antibacterial effectiveness of avocado seed extract (*Persea americana* mill.) in inhibiting bacterial growth *of Staphylococcus aureus* in vitro.

2. Methods

This study is an in vitro experimental study. This study uses the bacterial culture of *Staphylococcus aureus* on petri dishes obtained from the laboratory of microbiology, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, Indonesia. avocado seed (*Persea americana* mill.) 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 Sumatera 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 28 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 7 test groups, namely 0.2% chlorhexidine as a positive control (K1), negative control, DMSO (K2), avocado seed extract treatment group 2%, 4%, 6%, 8%, and 10% respectively as K3-K7. 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 software version 25. Univariate analysis was performed to present the data distribution for each test variable. Bivariate analysis was carried out to see statistical differences in each test variable, where $p < 0.05$.

3. Results and Discussion

Table 1 presents the average diameter of the bacterial inhibition zone for each group. Treatment of avocado seed extract with a concentration of 6% has the ability to grow bacteria *Staphylococcus aureus* better than the bacterial growth ability of the positive control, chlorhexidine. The increase in extract concentration was in line with the increase in the diameter of the bacterial inhibition zone *Staphylococcus aureus*.

Table 2 shows the comparison of effectiveness between treatment groups. The LSD post hoc test showed that there was a difference in the average diameter of the inhibition zone between the positive control and 6% avocado seed extract, statistically, $p > 0.05$. The diameter of the inhibition zone on the avocado seed extract showed an increase with increasing the concentration of the avocado seed extract.

Table 1. The average diameter of the bacterial inhibition zone for each group.

Treatment	Mean±SD
2%	11,22±0,25
4%	12,52±0,55
6%	14,02±0,34
8%	14,25±0,08
10%	15,22±0,45
K+ Chlorhexidine	13,85±0,31
K-DMSO	0±0

Table 2. Comparison of treatment effectiveness between groups.

Comparison	Between groups treatment	Mean difference	Sig.
2% extract	4% extract	-1.3000*	.002
	6% extract	-2.8000*	.000
	8% extract	-3.0250*	.000
	10% extract	-4.0000*	.000
	Positive control	-2.6250*	.000
	Negative control	11.2250*	.000
4% extract	6% extract	-1.5000*	.001
	8% extract	-1.7250*	.000
	10% extract	-2.7000*	.000
	Positive control	-1.3250*	.002
	Negative control	12.5250*	.000
6% extract	8% extract	-.2250	.552
	10% extract	-1.2000*	.004
	Positive control	.1750	.643
	Negative control	14.0250*	.000
8% extract	10% extract	-.9750*	.016
	Positive control	.4000	.295
	Negative control	14.2500*	.000

In avocado seeds, there are secondary metabolite compounds in the form of flavonoids, tannins, alkaloids, and saponins, where these compounds are active compounds that act as antibacterials.[12,13] Flavonoids act on bacterial cells by disrupting the cytoplasmic membrane and inhibiting enzymatic activity. The mechanism of flavonoids as antibacterial by denaturing and coagulating bacterial cell proteins. The process of protein denaturation results in protein coagulation in the bacterial cytoplasmic membrane, followed by the release of intracellular compounds.[14,15] If the integrity of the cytoplasmic membrane is damaged, macromolecules and intracellular ions will come out of the cell, which will lead to the death of the bacteria.[16] The antibacterial power of tannins is due to the toxicity of tannins which can damage the bacterial cell membrane. Besides that, tannin astringent compounds can also shrink the cell wall so that the permeability of the bacterial cell is disturbed.[17] If there is a disturbance in the permeability of the cell wall, the bacteria cannot withstand external influences and die immediately. The mechanism of action of alkaloids as antibacterials is by interfering with the constituent components of the poly peptidoglycan in bacterial cells so that the cell wall layer is not completely formed and causes cell death. Meanwhile, saponins work as an antibacterial by interfering with the stability of the bacterial cell membrane, causing the bacterial cell to lysis.[18-20]

4. Conclusion

Avocado seed extract (*Persea americana* mill.) showed effectiveness in inhibiting bacterial growth *Staphylococcus aureus* in vitro.

5. References

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