Testing Inhibitory Power of Avocado Seed Extract (*Persea americana Mill.*) for the Growth of *Staphylococcus aureus* and *Escherichia coli*

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Abstract: Infections are commonly treated using traditional medicine, especially made of natural ingredients such as plants. The use of medicinal plants for complementary or alternative traditional medicine, increases. Medicinal plants are considered to have fewer side effects when compared to chemical-based drugs. Apart from that, low price and accessibility are two aspects that medicinal plants contribute to be traditional medicine. One of the medicinal plants is avocado seeds (*P. americana*) which has the potential to be an alternative source of antibacterial ingredient. This study tested the inhibition power of avocado seed extract for the growth of *E. coli* and *S. aureus bacteria*; furthermore, it determined levels of phytochemical compounds (tannins and flavonoids) in avocado seed extracts. The experimental design used in this study was Completely Randomized Design (CRD). This study involved four types of avocado seed extract concentration: 10%, 15%, 20%, and control. Data analysis was carried out quantitatively using a computer statistics program (SPSS 22.0 for Windows). To test the normality of the data, the Kolmogorov-Smirnov test was carried out; if the data were not normal, the Kruskal Wallis test was carried out. To see the homogeneity of the variance, the Leven's test was employed. The effect of the treatment was tested using the One-way ANOVA test. If the test showed a significant result, the process was continued by the performing Duncan's multiple range test with a confidence level of 5% ($p < 0.05$).

1 INTRODUCTION

Avocado is a popular fruit in Indonesia. Generally, avocados have thick yellowish-green flesh and a brownish seed in the middle. The fruit has widely been used as traditional medicine to treat various diseases. The avocado's flesh can reduce pain and treat canker sores. Avocado leaves are usually used to treat nerve pain, stomach pain, reduce high blood pressure, and treat kidney stones (Anggrella, 2014; Widiyanti, Cacik, & Winata, 2017).

Currently, many infections are treated using traditional medicine, especially those made of natural ingredients such as plants. Natural ingredients are commonly used for either complementary or alternative traditional medicine. Medicinal plants are considered to have fewer side effects when compared to chemical-based drugs. Besides, they have affordable price and accessibility (Jiménez-Arellanes et al., 2013).

Persea americana Mill. is a generally edible fruit known as avocados which grow in tropical countries. However, not all parts of avocados are effectively used. For example, avocado seeds are less utilized than avocado flesh (Gunawan, Setiabudy, & Nafrialdi, 2007). Avocado seeds are known to give therapeutic effects, including antibacterial, anti-oxidant, anti-inflammatory, anti-fungal and analgesic effects (Lianti, 2014; Malangngi, Sangi, & Paendong, 2012).

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Pathogenic bacteria that often infect humans include *S. aureus* and *E. coli* causing intestinal inflammation and diarrhea (Hermawan, Hana, & Wiwiek, 2007). The bacteria are distinguished based on the composition of their cell walls and the nature of their staining. They include Gram-negative and Gram-positive bacteria that can cause diarrhea. Diarrhea can cause the body to lose a lot of fluids. It is commonly found and becomes a major contributor to mortality in Indonesia.

The number of infectious diseases in Indonesia increases every year due to several factors, e.g., poor public awareness of hygiene, shortages of trained health workers, increasingly dense population, insufficient knowledge, and lack of guidelines (Bontjura, 2015). Overuse dosage of antibiotics and the lack of government policies may lead to bacteria resistance (Omojate Godstime, Enwa Felix, Jewo Augustina, & Eze Christopher, 2014).

Research conducted by Idris et al. on the effect of 3.25 g ethyl acetate avocado seed extract (*Persea americana Mill*.) against *Staphylococcus aureus* and *Streptococcus pyogenes* shows antibacterial activity was found in 37-mm and 25-mm inhibition zones. Ethanol extract of avocado seeds becomes an alternative agent that can fight against *Enterococcus faecalis.* Avocado seed extract amounting to 10% plays a role in the antibacterial activity with an inhibition zone diameter of 2.3 ± 0.12 mm.

Back-to-nature lifestyle becomes quite popular at this time; hence, people start utilizing various natural materials, including medicinal plants for treatment (Dalimartha, 2008; Malangngi et al., 2012). Medicinal plants have more economic values and smaller side effects compared to synthetic drugs (Somchit et al., 2011). One of the medicinal plants is avocado seed (*P. americana*) which can provide an antibacterial effect. This study aimed to test the inhibition power of avocado seed extract for the growth of *E. coli* and *S. aureus bacteria.*

2 MATERIALS AND METHODS

This study was conducted at the Agricultural Laboratory and the Laboratory of Biosciences of Udayana University from January to April 2020. Completely Randomized Design (CRD) was used as the research design. This study involved four groups, namely:

1 Control group
2 Experiment group with 10% of avocado seed extract concentration
3 Experiment group with 15% of avocado seed extract concentration
4 Experiment group with 20% of avocado seed extract concentration

All groups were given different avocado seed extract concentrations to test the inhibition power of *S. aureus* and *E. coli*.

1. Making avocado seed extract

Preparation of avocado seed powder was done by washing the seeds first to remove dirt and then draining them. The seeds were then cut into small pieces using a knife. They were then dried using traditional drying methods under sunlight for six hours with occasional stirring. The dry weight was determined by subtracting the initial leaf weight from the final dry leaf weight multiplied by 100% until the total moisture content reached 13%. Seeds that were completely dry were then grinded using a blender. The dry powder was then sifted through a coffee filter and stored in a plastic container.

2. Avocado seed extraction

In extraction process, 100 grams of avocado seed powder was macerated in 1,000 ml of each solvent (ethanol, n-hexane and ethyl acetate) (1:10 ratio (w/v)) for 24 hours at 25^{o}C. The powder sample was then filtered with a filter paper. The residue was macerated again in the same way, up to two times (for two days). The macerated extract or filtrate was then collected together and evaporated to separate the solvent. Evaporation was carried out using a rotary evaporator at a temperature of 60^{o}C for n-hexane solvents and 70^{o}C for ethanol and ethyl acetate solvents, until the solvent evaporated completely. This process was done to gather a thick extract of avocado seeds.

3. Bacterial inhibitory test

The test was preceded by making Nutrient Agar (NA) media. To make the media, 100 µl of each suspension of *E. coli* and *S. aureus* were taken from the bacterial decay and then mixed in each medium and vortex. Once becoming homogeneous, the medium was poured into a petri dish to solidify. In the media with the bacteria, seven holes were made per petri dish using a sterile aluminum pipe with a diameter of 0.5 cm. Then, the extract solutions at various concentrations were inserted into the holes; the solvent used as the negative control group and chloramphenicol solution as the positive control group amounting to 100 µl each were poured into the other holes. The petri dishes were left for 10 minutes during the diffusion process and then incubated for 24 hours at 36 – 37^{0}C. After 24 hours, the area of bacterial growth and the hole diameter in millimeters were measured using a caliper at least three times.

4. Determination of minimum inhibitory concentration

The media containing the test solution, solidified positive control and negative control groups were observed visually. The growth of microbial colonies was slow down perhaps due to the turbidity of the media; the smallest concentration showed inhibition power for microbial growth after the diameter of the inhibition zone was measured by using a caliper. Observation was made after optimum incubation of microorganisms for one time. The inhibition power of the ethanol extract of avocado seed for the growth of *E. coli* and *S. aureus* was from the clear zone which was measured with calipers and then subtracted by the 0.5-cm diameter of the well.

3 STATISTICS

Data analysis was carried out quantitatively using a computer statistics program (SPSS 22.0 for Windows). To test the normality of the data, the Kolmogorov-Smirnov test was carried out; if the data were not normal, the Kruskal Wallis test was carried out. To see the homogeneity of the variance, the Leven's test was carried out. Furthermore, the One-Way Anova test was employed to see the effect of the treatment with avocado seed extract. If the test resulted in a significant result, the Duncan's multiple range test was utilized with a confidence level of 5% ($p < 0.05$).

4 RESULTS

This study was an experimental test to see whether there is inhibition of avocado leaf extract *(Persea americana Mill*) for the growth of *E. coli* and *S. aureus bacteria*. Four treatment groups consist of a control group and three different dose treatments, namely avocado leaf doses of 10%, 15% and 20%. Table 1 shows the results of antibacterial test after the administration of avocado seed extract.

Table 1: Antibacterial test results of avocado leaf extract (*Persea americana Mill*) for the growth of *S. aureus* bacteria.

No	Treatment	Diameter of Inhibition Zone against *S. aureus* (mm)			Length (mm)	Average (mm)
		I	II	III		
1	Extract 10%	3.83	5.65	4.43	13.91	4.63
2	Ekstrak 15%	4.62	4.46	5.84	14.92	4.97
3	Ekstrak 20%	6,89	6,77	6,54	20,2	6,73
4	Kontrol	21,44	21,44	21,44	64,32	21,44

Table 1. Shows the diameter of the inhibition zone at concentrations of 10%, 15% and 20% has an average value of: 4.63, 4.97, 6.73 and the control group, 21.44

Table 2: Antibacterial Activity Test Results of avocado leaf extract (Persea americana Mill) on the growth of *E. coli* bacteria.

No	Perlakuan	Hasil Diameter Zona HambatTerhadap Bakteri *S. aureus* (mm)			Jumlah (mm)	Rata-rata (mm)
		I	II	III		
1	Ekstrak 10%	3,63	5,23	4,33	13,19	4,39
2	Ekstrak 15%	5,77	4,23	5,67	15,67	5,22
3	Ekstrak 20%	7,34	6,34	6,67	20,35	6,78
4	Kontrol	21,44	21,44	21,44	64,32	21,44

Table 2. Shows the diameter of the inhibition zone at a concentration of 10%, 15% and 20% has an average value of: 4.39, 5.22, 6.78 and the control group, 21.44

Table 3: Categories of Inhibition Zone Diameter.

Inhibition Zone Diameter	Category
5 mm or less	weak
5 - 10 mm	Currently
10 - 20 mm	strong
20 or more	Very Strong

Based on the results of the normality test that was carried out using the Shapiro-Wilk Test statistic using SPSS version 2.2 software, it showed a significance number > 0.05 which indicated that the sample in this study was normal. The average diameter of the inhibition zone of avocado leaf extract in inhibiting the growth of Staphlococcus aureus and Escherichia coli is shown in Table 4 below.

Table 4: Mean Diameter of Inhibition Zone of Avocado Leaf Extract Against *S. aureus* and *E. coli.*

Extract concentration	*S. aureus*	*E. coli*
Ekstrak 10%	4,63± 0,45c	4,39± 0,33b
Extract 15%	4.97± 0.49d	5.22± 0.38c
Extract 20%	6.73 ± 0.76f	6.78± 0.48d
Control	21.44± 0.43g	21.44± 0.43g

Information ** = mean ± standard deviation is the average of six repetitions. Different letters in both columns and rows show significantly different results ($p < 0.05$) based on the test (ANOVA).

5 DISCUSSION

Based on Tables 1 and 2, concentration of 20% is the highest concentration with moderate inhibition for the growth of *S. aureus* and *E. coli*; as a reference from Table 3, the diameter of the inhibition zone 5-10 is in the moderate category.

Avocado leaves are plants that are widely used for treatment because they have an antibacterial effect. Avocado leaf extract is able to inhibit the growth of *S. aureus* and *E. coli*. The leaves consist of active compounds resulting in antibacterial properties such as flavonoids, saponins, alkaloids, tannins, and polyphenols.

Flavonoids have antibacterial effects by inhibiting bacterial DNA function. In this process, some errors might be found in bacterial replication and translation. Inhibition occurs when the bacterial cytoplasmic membrane which consists of lipids and amino acids removes the alcohol group in the flavonoid compound. This process will damage the cell walls, and thus the compound can enter the nucleus of the bacterial cells (Pisoschi & Pop, 2015).

Saponin compounds in avocado seeds can also have antibacterial properties which have the same mechanism of action as flavonoid compounds. Tannins are active compounds in avocado seeds. The way tannin produces an antibacterial effect is by precipitating bacteria and their proteins. The effect of the antibacterial content in tannin will disrupt the function of genetic materials and inactivate enzymes, causing bacterial cells not to form (Dwi, Tanjung, Chairani, Bikarindrasari, & Dewi, 2021).

The mechanism of alkaloids in inhibiting bacterial growth works by damaging the constituent components of peptidoglycan in bacterial cells; as a result, the cell wall layer is not formed completely and causes cell deaths. In addition, alkaloids have an inhibitory mechanism by binding to DNA presumably because the alkaloids have a base group that contains nitrogen. This base group will react to acidic compounds in bacteria such as DNA which is the main constituent of the cell nucleus. With the disruption of DNA, the synthesis of proteins and nucleic acids in cells will be disrupted. As a result, cell metabolism is distracted, and bacterial growth is inhibited or stopped (Isnarianti, Wahyudi, & Puspita, 2013).

6 CONCLUSIONS

Avocado leaf extract is effective in inhibiting the growth of *S. aureus* and *E. coli* with an average diameter of the inhibition zone that varies depending on the concentration of each extract. The content of metabolite compounds found in avocado leaves has the potential to inhibit the growth of these bacteria.

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