HASIL CEK_Persea americana (Mill.), avocado, antibacterial activity, mechanism of action

by Nanik Sulistyani Role Of Flavonoid-rich Fraction From Persea

Submission date: 25-Mar-2023 08:46AM (UTC+0700)
Submission ID: 2045912787
File name: 19-11077-SP.pdf (351.4K)
Word count: 4872
Character count: 26086

doi.org/10.36721/PJPS.2022.35.6.SP.1805-1811.1

Role of flavonoid-rich fraction from *Persea americana* (Mill.) in bacterial leakage of *Staphylococcus aureus*

Nanik Sulistyani[1], Nurkhasanah[2], Lola Angelita[3],
Ichwan Ridwan Rais[1]* and Zainul Amiruddin Zakaria[4]*
[1]Departement of Pharmaceutical Biology, Ahmad Dahlan University, Faculty of Pharmacy, Yogyakarta, Indonesia
[2]Departement of Pharmaceutical Chemistry, Ahmad Dahlan University, Faculty of Pharmacy, Yogyakarta, Indonesia
[3]Departement of Pharmaceutical Biology, College of Pharmacy "Yayasan Pharmasi", Semarang, Indonesia
[4]Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Universiti Malaysia Sabah, Sabah, Malaysia

Abstract: This study aimed to determine the ability of *Persea americana* (Mill.) or avocado peels ethanolic extract and its fractions to cause bacterial cell leakage in *Staphylococcus aureus*. The interaction of antibacterial compound with bacterial cells induces several changes leading to the damage of membrane permeability, followed by intracellular bacterial cell leakage. The experiment started with the determination of minimum inhibitory concentration and minimum bactericidal concentration by micro dilution method. Following the determination of MIC and MBC values, the samples, at the concentrations of 1xMIC and 2xMIC, were tested and analyzed by UV-Vis spectrophotometer at 260 and 280 nm to determine the leakage of bacteria cells. The value of K^+ ion leakage was determined using atomic absorption spectrophotometry while the value of electrical conductivity was measured by conducto meter to determine the leakage of the cell membrane. The recorded MIC and MBC values of samples were 10% w/v. At the concentrations of 10% and 20% w/v, the samples caused an increase in nucleic acid, protein and DNA levels as well as an increase in extra cellular electrical conductivity. Prolonged exposure of the extract increased the leakage of bacterial cell contents and the electrical conductivity, indicating the damage of bacterial cell membrane.

Keywords: *Persea americana* (Mill.), avocado, antibacterial activity, mechanism of action.

INTRODUCTION

Persea americana (Mill.) (avocado) is a member of *Lauraceae* family which is traditionally cultivated in most of tropical countries (Kavaz and Ogbonna, 2019). It has been known as food with high nutrients and medical purposes with large therapeutics effects (Bhuyan *et al*., 2019). Avocado production worldwide reached 5.0 million tons in 2014 (Araújo *et al*., 2018). A huge industrialization of avocado pulp processing was generating a vast quantity of waste. Avocado seeds and peels showed 16% and 11%, respectively, of total waste weight from the fruit (Jimenez *et al*., 2020). The secondary metabolites as main bioactive contents in avocado peels are polyphenols (Lara-Flores *et al*., 2018). A higher total flavonoid content (TFC) was exhibited with group of polar solvents such like ethanol, ethyl acetate and water, presenting the effect of solvent on extraction and fractionation process (Wakeel *et al*., 2019). Polyphenols with flavonoid are contained in avocado peels, which are traditionally used as antimicrobial (Akan, 2021) and present high antioxidant activity (Rahman *et al*., 2022). Avocado waste peels have performed antimicrobial activity. An aqueous extract of Hass avocado peels exhibited *H. pylori* urease inhibitory activity (Liu *et al*., 2018).

Staphylococcus aureus is a normal flora on the skin, respiratory tract and digestive tract. However, *Staphylococcus aureus* can cause widespread infection. Skin infections can occur when the skin surface is exposed due to diseases such as eczema, surgical wounds or because of the use of infusion devices (Schmidt *et al*., 2015). *Staphylococcus aureus* infection can also trigger endocarditis, osteomyelitis and meningitis (Tong *et al*., 2015). *Staphylococcus aureus* is a bacterium that has high resistance to various antibiotics such as penicillin, erythromycin, tetracycline and clindamycin. (Lowy, 2000).

Bacterial cytoplasmic membrane is semipermeable membrane which will select molecules that can enter the cell. Cell membrane permeability to small ions such as H^+, K^+, Na^+ and Ca^{2+} is regulated by the structure and chemical components of the cell membrane. The interaction that occurs between an active compound and the bacterial cell membrane can cause interference with this permeability and even cause cell leakage. One indication of the occurrence of cell leakage is an increase in high levels of K^+ ions outside the cell due to the flow of K^+ ions from inside to outside the cell. In addition, the interaction of antibacterial compounds with proteins or enzymes in cell membranes will cause damage to membrane permeability and cause leakage of intracellular components such as sodium glutamate, sodium hydrogen

*Corresponding author: e-mail: ichwan.rais@pharm.uad.ac.id; zaz@ums.edu.my

phosphate, nucleotides, glutamate and amino acids (Tassou *et al*., 2000).

One of the ways to identify antibacterial mechanisms is by identifying damage to cell walls and cell membranes by analyzing cell leakage that occurs using ultraviolet-visible spectrophotometry at wavelengths of 260 nm and 280 nm (Naufalin and Herastuti, 2017). The reason for this is that RNA and DNA are compounds detected at the wavelength of 260 nm while proteins are identified at the wavelength of 280 nm. In addition, the leakage of K^+ ions that can be absorbed at the wavelength of 766.5 nm and the increase in extracellular electrical conductivity were also measured to support the findings of several previous studies on the potential of the extract to induce cell leakage (Diao *et al*., 2014; Veranitisagul *et al*., 2019). Hence, this study aims to determine the antibacterial mechanism of avocado peels extract against *Staphylococcus aureus* bacteria.

MATERIALS AND METHODS

Chemicals

All chemicals used were purchased from Merck (USA) with pro analyte grade and Oxoid (UK) for microbial test.

Bacterial strain and growth

The bacterial used in this present study was *Staphylococcus aureus* (ATCC 259253). The strain was subcultured in Mueller-Hinton Agar (MHA) at 37°C, 24 h before use.

Preparation of avocado peels ethanolic extract

Avocado peels were collected from Central Java, Indonesia in August 2020. The avocado was identified by taxonomist Mr. Hery Setiyawan and kept as a voucher specimen (258/Lab,Bio/B/XI/2020) at the Laboratory of Biology, Faculty of Applied Science and Technology, University of Ahmad Dahlan, Yogyakarta, Indonesia. Avocado peels were collected and prepared based on the method reported by Mardiati *et al*. (2017) with slight modification. The dried avocado peels were crushed and sieved by using 18-mesh and 40-mesh sieves to equalize the particle size of the powder. Avocado peels powder was macerated by using 96% ethanol solvent for 24 hours and then filtered to collect the supernatant. The undissolved peels were re-macerated with 96% ethanol for another 24 hours and the supernatant was also collected and combined together with the first batch of supernatant. The collected supernatant was evaporated at 50°C using a rotary evaporator to obtain a thick and dark brown extract named as EPa.

Preparation of flavonoid-rich fractions from avocado peels ethanolic extract

Flavonoid-rich fractions were prepared from avocado peels ethanolic extract (EPa) by liquid-liquid fractionation technique using polarity degradation of solvents from *n*-hexane, ethyl acetate and methanol, respectively (Efendi, 2019). Firstly, extract was diluted in warm water to reach 500mg/L of sample concentration. *n*-Hexane was then added to the solution with intermittent shaking to form two different layers in separating funnel. Organic layer was then kept and aqueous layer was evaporated to remove n-hexane residual. The process was repeated with ethyl acetate and methanol to get residual of ethyl acetate and methanolic fraction. The fractions were named as HPa (*n*-Hexane), EtPa (ethyl acetate) and MPa (methanol).

Total flavonoid content (TFC)

Total flavonoid content was measured by method that was described in Herbal Pharmacopeia of Indonesia (FHI). Shortly, a series concentration of quercetin in the range of 20 to 100 ppm were prepared as standards. TFC values of EPa, HPa, EtPa and MPa were exhibited as microgram of quercetin equivalency each gram of extract (mg QE/g extract). Absorbances were determined in triplicates at 439 nm of wavelength.

Antibacterial activity assays

Assay of minimum inhibitory concentration (mic) and minimum bactericidal concentration (MBC)

Determination of the minimum inhibitory concentration (MIC) was carried out using the microdilution method described by Amado *et al*. (2019) with slight modifications. Bacterial stock was cultured in BHI media to prepare fresh colonies and diluted with 0.9% NaCl to obtain a suspension of 0.5 McFarland. Further, the bacterial suspension was inoculated in double-strength BHI medium. A series of samples from 2.5% to 20% (w/v) were added to BHI medium in microtube. These mixtures were then incubated at 37°C for 24 hours. Following the incubation, turbidity in the tested microtubes was observed virtually. The MIC value was determined by the presence of a clear solution at the lowest concentration. Positive and negative controls used were vancomycin and BHI media, respectively. The minimum bactericidal concentration (MBC) was tested by Kirby-Bauer method. Antibacterial activity assay was started with bacteria suspension that was inoculated on MHA surface, then 6 mm of blank disk paper, vancomycin disk, and a series of samples in 10% of DMSO were dripped onto the surface (Andayani *et al*, 2016). Further, petri dish was then incubated for 24h at 37°C.

Physicochemical characterization of bacterial surfaces

Bacteria cell leakage test

Cell leakage (nucleic acid, DNA, and protein leakage) test was carried out with reference to the study of Lou *et al*. (2011). A total of 10mL of bacterial suspension, which had been incubated for 18 hours at 37°C, was then centrifuged for 20 minutes at 3500 rpm. The supernatant

solution was discarded, while the precipitate was collected and washed with phosphate buffer solution (pH 7.4). The precipitate was then resuspended with phosphate buffer solution and added with extracts concentration of 1 MIC and 2 MIC. For the control purposes, the extracts, at 1 MIC and 2 MIC, were mixed with phosphate buffer (pH 7.4) without the presence of bacteria and labeled as extract control. For the bacterial control (0 MIC), the bacterial cell precipitate was mixed with phosphate buffer solution without the presence of extract. All of the suspension was incubated for 4 hours and 24 hours before being centrifuged for 15 minutes at 2500 rpm. After centrifugation, the supernatant was collected, and the cell leakage was identified at 260 nm and 280 nm using a UV-Vis Spectrophotometer.

Pottasium efflux

Measurement of K^+ ion leakage out of *Staphylococcus aureus* bacterial cells after administration of ethanol extract of avocado skin. The bacterial suspension that had been incubated for 18 hours at 37°C was taken as much as 10 mL and centrifuged at 3500 rpm for 20 minutes. The supernatant solution was discarded and the precipitate was washed with phosphate buffer solution (pH 7.4) until the pH reached the pH of the phosphate buffer solution and resuspended with phosphate buffer solution. After that 1 MIC and 2 MIC of concentrations of extracts were added. As a control for extract, phosphate buffer solution (pH 7.4) was added into 1 MIC and 2 MIC of extracts. As a control of 0 MIC of bacteria, a phosphate buffer solution was added into the bacterial precipitate. Then, it was incubated for 4 hours and 24 hours. The suspension was centrifuged for 15 minutes at a speed of 2500 rpm and the supernatant solution was identified for K^+ ion leakage using an Atomic Absorption Spectrophotometer at a wavelength of 766.5 nm (Lou *et al*., 2011).

Electrical conductivity test

The permeability of the bacterial cell membrane is determined by the relative electrical conductivity. The test bacteria were incubated at 37°C for 10 hours and then centrifuged for 10 minutes at 5000g. The bacteria were washed with 5% glucose until the conductivity was close to the 5% glucose conductivity, indicating that the bacteria were in isotonic conditions. Samples with three concentrations, namely control, 1 MIC and 2 MIC, were added to 5% glucose and then their conductivity was measured (L1). Then the three concentrations of samples (control, 1 MIC, and 2 MIC) were added to isotonic bacteria and homogenized, then the mixture was incubated at 37°C for 10 hours and every 2 hours the conductivity was measured (L2). As a control (L0), the bacterial conductivity was measured in 5% glucose treated with boiling water for 5 minutes (Veranitisagul *et al*., 2019).

STATISTICAL ANALYSIS

Nucleic acid, DNA and protein leakage data were analyzed by using the SPSS program. The data were tested using one-way Anova and LSD with a 95% confidence level.

RESULTS

Preparation of avocado peels ethanolic extract and *flavonoid-rich fractions*

Considering the extraction process, type of solvents and biological factors, the yield value of the extracts obtained was 15.81%, slightly lower than that in the previous report, which was 17.04% (Sulistyani *et al*., 2022; Wulandari *et al*., 2019). As reported in our previous study, the yield of n-hexane fractionation collected was the highest, which was 27.41%, followed by the yields of methanol and ethyl acetate fractionations, which were 15.83% and 8.34%, respectively.

Total flavonoid content (TFC)

TFC assay conducted on EPa, HPa, EtPa and MPa revealed that the highest total flavonoid content was in EtPA (173.18 mg QE/g of extract), followed by EPa (8.84 mg QE/g of extract), MPa (1.76mg QE/g of extract) and *n*-hexane (1.28 mg QE/g of extract) as seen on table 1).

Table 1: Total flavonoid content of samples

Sample	Flavonoid (mg QE/g) extract*
EPa	8.84 ± 0.01
HPa	1.28 ± 0.02
EtPa	173.18 ± 2.06
MPa	1.76 ± 0.04

Table 2: Inhibition zones of samples (±SD) correspond to MIC/ MBC

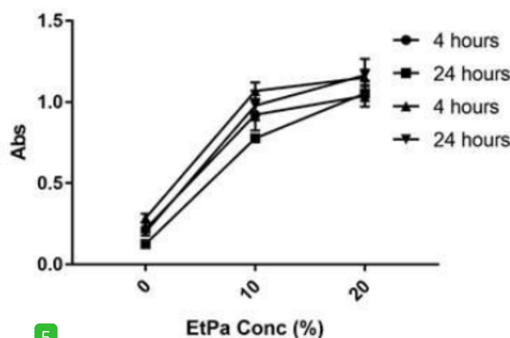
Sample	Inhibition Zone (mm)	MIC/MBC (mg)
Vancomycin (positive control)	12.17 ± 0.29	-
DMSO (negative control)	0.00 ± 0.00	-
EPa	5.67 ± 0.29	2.00
HPa	0.00 ± 0.00	2.00
EtPa	8.33 ± 0.58	2.00
MPa	2.83 ± 0.29	2.00

Data mean ± SD (n = 3) *significant ($p<0.05$) compared to negative and positive control

Antibacterial activity assays

Assay of minimum inhibitory concentration (mic) and minimum bactericidal concentration (MBC)

According to our previous report (Sulistyani *et al*., 2022), the MIC and MBC of extract showed 10% of concentration. The clear zone surrounding the disk paper was defined as the inhibition ability in millimeter (mm) which corresponded to MIC values in milligrams (mg) (table 2).



Data mean ± SD (n = 3) *significant ($p<0.05$) compared to the 0% concentration

Fig. 1: Absorbance values of cellular leakage from *Staphylococcus aureus* bacteria induced by EtPA (•,▪ on 260 nm; ▲,▼ on 280 nm)

Physicochemical characterization of bacterial surfaces

Bacterial cell leakage test

The mechanism of damaged cell membranes can be described as leakage of intracellular ions and substances such as ATP ions, nucleic acids, amino acids, sodium glutamate, sodium hydrogen phosphate, nucleotides, glutamate, K^+ ions and phosphate ions. As shown on fig. 1, EtPa exhibited an increasing UV absorbance at wavelengths of 260 nm and 280 nm.

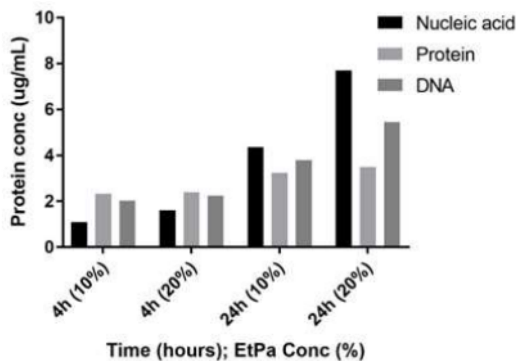


Fig. 2: Nucleic acid, protein and DNA levels compared to 0% of extract concentration

Profoundly, the bacterial cell leakage test showed the higher level of nucleic acid, protein and DNA increase in groups with extract treatment than in the group with 0% extract (fig. 2).

Pottasium efflux

The results of the test for leakage of K^+ ion from *Staphylococcus aureus* after the addition of EtPa can be seen in fig. 3. The results showed that the concentration of EtPa affected the levels of K+ ions in the extra cellular fluid. The greater the concentration of the EtPa was the higher the concentration of K+ ions became compared to the group with the concentration of 0% (without the addition of EtPa).

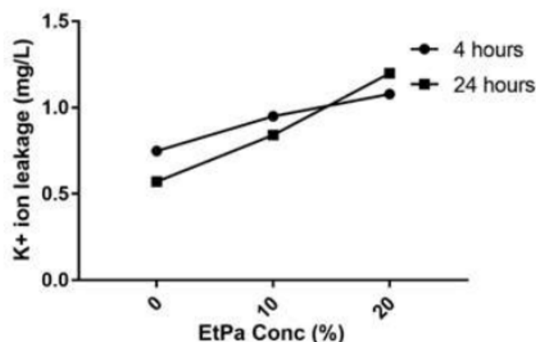


Fig. 3: Leakage of K^+ ions for *Staphylococcus aureus*

Electrical conductivity test

The results of the electrical conductivity test (table 3) showed that the treatment of 0% EtPa (containing only bacterial suspension with 5% glucose) could result in the discharge of cell contents from *Staphylococcus aureus* bacteria which was characterized by an increase in electrical conductivity by 39.411±0.357% at 4 hours and by 40.877±0,067% at 24 hours. The treatment of EtPa at a concentration of 10% increased electrical conductivity by 45.594±1.373% at 4 hours and by 48.306±1.192% at 24 hours. The treatment of EtPa at a concentration of 20% increased electrical conductivity by 50.422±0.388% at 4 hours and by 50.546±1.237% at 24 hours.

DISCUSSION

The effectiveness of extraction to obtain the yields was dependent on the method, particle size of the sample, solvent type, and the length of the process (Salamah *et al*., 2017). The yields were also influenced by the plant part, species, time and location of harvesting (Sulistyani *et al*., 2022) In this present study, the EPa obtained was 15.81%, then fractionated to reach the yield of ethyl acetate fractions (8.34%) with the highest TFC (173.18±2.06 mg QE/g) as the flavonoid-rich fraction (FRF).

As mentioned above on table 2, the TFC increasing profiles of the sample's concentrations were performed the same increasing profiles of inhibition zones that

Table 3: Electrical conductivity values

Time (hour)	Electrical Conductivity Values (%)		
	EtPa 0%	EtPa 10%	EtPa 15%
4	39.41±0,36	45.60±1,373*	50.42±0,39*
24	40.88±0,07	48.31±1,192*	50.55±1,24*

Data mean ± SD (n = 3) *significant ($p<0.05$) compared to the 0% concentration

leaded by EtPA (8.33 mm) as the highest, followed by EPa (5.67 mm), MPa (2.83 m0), and *n*-hexane (0.00 mm). These profiles indicated the link between flavonoid content and inhibitory ability of the samples, even though, EtPa as the highest TFC (173.18 mg QE/g of extract) with the biggest inhibition zone (8.33±0.58 mm) exhibited medium antibacterial strength (Mawardi *et al*., 2020). Further, EtPa was then observed for the physicochemical characterization of bacterial surfaces assays.

As the first assay, bacterial cell leakage was tested according to cell membrane damaged mechanism. Compounds that can be absorbed at a wavelength of 260 nm are DNA polymers and RNA derivatives, namely nucleotides. At a wavelength of 280 nm, it can detect proteins (Xu *et al*., 2017). Purines, pyrimidines and ribonucleotides are compounds that can be detected at a wavelength of 260 nm. Tyrosine and tryprophan in small amounts are detected at a wavelength of 280 nm (Park *et al*., 2003; Xu *et al*., 2017). Based on fig. 1, EtPa showed an increasing UV absorbance at wavelengths of 260 nm and 280 nm. The longer the contact time between EtPa and *Staphylococcus aureus* bacteria was, the more the absorbance value escalated. So, this indicates an addition in the number of compounds released from bacterial cells. At a wavelength of 280 nm, the absorbance value was greater than at 260 nm. This means that bacterial cells leak protein compounds from the cytoplasm or periplasm (intracellularly) (Branen and Davidson, 1993). Further, the bacteria cell leakage test showed that the higher the level of nucleic acid was, the more protein and DNA increased compared to the 0% extract (fig. 2), linear to the increasing concentration variable of EtPa. Similarly, the levels of nucleic acids, proteins and DNA based on contact time variable increased at 24 hours compared to those at contact time of 4 hours. The contact time between EtPA and *Staphylococcus aureus* bacteria at 24 hours was longer than 4 hours. This condition gave more time for nucleic acids, proteins, and DNA to exit bacterial cells to the supernatant.

According to the mechanism of potassium ion as the main ion in the cell cytoplasm which functions for cell growth (Ultee *et al*., 1998), K^+ ions are useful for activating cytoplasmic enzymes so that turgor pressure is maintained and regulates pH stability in the cytoplasm (Nikaido and Vaara, 1985). The K^+ ion has an important function in maintaining the unity of the ribosome (Jawetz *et al*., 1996). Metal ions that percolate through the cell will affect the permeability of the cell membrane or bacterial cell wall. An indication of damage to the cytoplasmic membrane is leakage of low molecular weight compounds such as nucleotide components (purines, pyrimidines, pentoses and inorganic phosphates), amino acids and inorganic ions such as K^+ ions (Johnston *et al*., 2003). The presence of K^+ ion levels read on the Atomic Absorption Spectrophotometer indicates the presence of channels that cause leakage in the cytoplasmic membrane of bacterial cells (Johnston *et al*., 2003). The permeability of the cell membrane decreases to smaller uncharged hydrophilic molecules such as water, urea and glycerol. Cell membranes are not easily passed to charged ions or electrolytes such as H^+, Na^+, HCO^-, K^+, Ca^{2+}, Cl^- and Mg^{2+} ions (Supu, 2015). Damage to bacterial cell membranes is characterized by leakage of intracellular molecules such as nucleic acids, proteins, and ions. Increased levels of ions in extracellular fluid cause electrical conductivity to increase so that electrical conductivity can be used as a marker of damage to the permeability of bacterial cell membranes (Diao *et al*., 2014).

Study of electrical conductivity test (Mahae *et al*., 2011) revealed that high concentrations of glucose can cause osmotic pressure, water content decrease, then dehydration and cell death (Chirife *et al*., 1983). It is similar to the finding in this study, which was that the higher concentration of FRF from EtPa added to the test bacteria was, the greater of the value from the electrical conductivity became. The increasing extra cellular electrical conductivity indicates an increase in the number of electrolytes in the extra cellular fluid, so that this exhibits electrolyte leakage from the bacterial cell due to the damaged bacterial cell membrane. Bacterial cells require electrolytes to facilitate cell membrane function, maintain normal metabolic activity and maintain enzyme activity (Diao *et al*., 2014).

CONCLUSION

EtPa has an antibacterial action mechanism against *Staphylococcus aureus* bacteria, by disrupting the function of cell membranes causing electrolyte leakage which is characterized by an increase in the value of electrical conductivity. In addition, EtPa increased the levels of nucleic acids, proteins, DNA and K^+ ions which were detected in the extra cellular fluid of *Staphylococcus aureus* bacteria and this was associated with a simultaneous decrease in the number of viable bacteria.

ACKNOWLEDGEMENT

This present research was funded by the Ministry of Research and Technology/ National Innovation and Research Board through the Master's Thesis Research Grant scheme with grant number PTM-015/SKPP.TT/LPPM UAD/VI/2020.

REFERENCES

Akan S (2021). Phytochemicals in avocado peel and their potential uses. *Food Health*, **7**(2): 138-149.

Amado DAV, Helmann GAB, Detoni AM, de Carvalho SLC, de Aguiar CM, Martin CA, Tiuman TS and Cottica SM (2019). Antioxidant and antibacterial activity and preliminary toxicity analysis of four varieties of avocado (*Persea americana* Mill.). *Braz. J. Food Technol*., **22**(2): 1-11.

Andayani R, Mubarak Z, Rinanda DR, Kuala US, Pendidikan P, Gigi D, Gigi FK and Kuala US (2016). Aktivitas antibakteri tepung cacing tanah (*Lumbricus rubellus*) terhadap *Enterococcus faecalis* secara *in vitro*. **Syiah Kuala** Dent. Soc., **1**(2): 201–210.

Araújo RG, Rodriguez-jasso RM, Ruiz HA, Pintado MME and Noe C (2018). Avocado by-products: Nutritional and functional properties. *Trends Food Sci Technol*., **80**: 51-60.

Bhuyan DJ, Alsherbiny MA, Perera S, Low M, Basu A, Devi OA, Barooah MS, Li CG and Papoutsis K (2019). The Oddysey of bioactive compounds in Avocado (*Persea americana* Mill.) and their health benefits. *Antioxidants,* **8**(10): 426.

Branen AL and Davidson PM (1993). Antimicrobial in foods. *Second edition*. Marcel Dekker, Inc, New York.

Chirife J, Herszage L, Joseph A and Kohn ES (1983). In vitro study of bacterial growth inhibition in concentrated sugar solutions: microbiological basis for the use of sugar in treating infected wounds. Antimicrob. Agents Chemother., **23**(5): 766-773.

Diao WR, Hu QP, Zhang H and Xu JG (2014). Chemical composition, antibacterial activity and mechanism of action of essential oil from seeds of fennel (*Foeniculum vulgare* Mill.). *Food Control*, **35**(1): 109–116.

Efendi MR (2019). Skrining aktivitas antibakteri fraksi kelopak bunga *Mussaenda frondosa* L. *J. Pharm. Sci*., **2**(1): 38-44.

FHI (2017). Farmakope Herbal Indonesia. 2nd Ed. Ministry of Health of Republik Indonesia, pp.530-531.

Jawetz E, Melnick LJ and Adelberg A (1996). Mikrobiologi kedokteran. Diterjemahkan oleh Bagian Mikrobiologi Fakultas Kedokteran Universitas Airlangga. EGC, Jakarta, pp.317-326.

Jimenez P, Garcia P, Quitral V, Vasquez K, Parra-Ruiz C, Reyes-Farias M, Garcia-Diaz DF, Robert P, Encina C and Soto-Covasich J (2020). Pulp, leaf, peel and seed of avocado fruit: A review of bioactive compounds and healthy benefits. *Food Rev. Int*., **37**(6): 619-655.

Johnston MD, Hanlon GW, Denyer SP and Lambert RJW (2003). Membrane damage to bacteria caused by single and combined biocides. *J. App. Microbiol*., **94**(6): 1015-1023.

Kavaz D and Ogbonna C (2019). Comparative study of biological activity and chemical composition of methanolic and ethanolic plant extracts of *Persea americana* leaves *in-vitro*. *Eur. J. Sci. Technol*., **17**: 261-270.

Lara-Flores AA, Araújo RG, Rodríguez-Jasso RM, Aguedo M, Aguilar CN, Trajano HL and Ruiz HA (2018). Bioeconomy and biorefinery: Valorization of hemicellulose from lignocellulosic biomass and potential use of avocado residues as a promising resource of bioproducts. **Waste to Wealth**, Springer, pp 141–170.

Liu Q, Meng X, Li Y, Zhao CN, Tang GY, Li S, Gan RY and Li HB (2018). Natural products for the prevention and management of *Helicobacter pylori* infection. Compr Rev Food Sci, Food Saf., **17**(4): 937-952.

Lou Z, Wang H, Zhu S, Ma C and Wang Z (2011). Antibacterial activity and mechanism of action of chlorogenic acid. *J. Food Sci*., **76**(6): 398-403.

Lowy FD (2000). Is *Staphylococcus aureus* an intracellular pathogen, *Trend Microbiol.,* **8**(8): 341-343.

Mahae N, Chalat C and Muhamud P (2011). Antioxidant and antimicrobial properties of chitosan-sugar complex. *Int. Food Res. J*., **18**(4): 1543–1551.

Mardiati Y, Chaidir Z and Dharma A (2017). Antimicrobial activity of *Persea americana* peel extract from North Sumatra, Indonesia, against gram-positive and gram-negative bacteria *in vitro*. *Am. Sci Res. J. Eng, Technol. Sci*., **38**(2): 247-251.

Naufalin R and Herastuti SR (2017). Antibacterial activity of *Nicolaia speciosa* fruit extract. *Intl. Food Res. J*., **24**(1): 379-385.

Nikaido H and Vaara M (1985). Molecular basis of bacterial outer membrane permeability. *Microbiol. Rev*., **49**(1): 1-32.

Park SJ, Park HW and Park J (2003). Inactivation kinetics of food poisoning microorganisms by carbon dioxide and high hydrostatic pressure. *J. Food Sci*., **68**(3): 976-981.

Rahman N, Sabang SM, Abdullah R and Bohari B (2022). Antioxidant properties of the methanolic extract of avocado fruit peel (*Persea americana* Mill.) from Indonesia. *J. Adv. Pharm. Technol. Res*., **13**(3): 166-170.

Mawardi RH, Sulistyani N, Nurkhasanah and Putri RDR (2020). Antibacterial activity and TLC-Bioautography analysis of the active fractions of Muntingia calabura L. leaves against *Staphylococcus aureus*. *J. Farm. Sains. dan Kom*, **17**(2): 69-75.

Salamah N, Rozak M and Al Abror M (2017). Pengaruh metode penyarian terhadap kadar alkaloid total daun

jembirit (*Tabernaemontana sphaerocarpa*. BL) dengan metode spektrofotometri visibel. *Pharmaciana*, **7**(1): 113–122.

Schmidt A, Bénard S and Cyr S (2015). Hospital cost of staphylococcal infection after cardiothoracic or orthopedic operations in France: A retrospective database analysis. *Surg*. *Infect*, **16**(4): 428-435.

Sulistyani N, Angelita L and Nurkhasanah (2022). Inhibitory activity of *Persea americana* Mill. peels extract and fraction containing phenolic compound against *Staphylococcus aureus* ATCC 25923. *J. Pharm*. *Sci*. *Community*, **19**(1): 1-7.

Supu I (2015). Mekanisme transport ion melalui karakterisasi sifat listrik pada membran jeruk lemon (*Citrus medica* Linn). *J. Dinamika*, **6**(1): 11-24.

Tassou C, Koutsoumanis K and Nychas GJE (2000). Inhibition of *Salmonella enteritidis* and *Staphylococcus aureus* in nutrient broth by mint essential oil. *Food Res*. *Int*., **33**(2000): 273-280.

Tong SYC, Davis JS, Eichenberger E, Holland TL and Fowler VGJ (2015). *Staphylococcus aureus* infections: Epidemiology, pathophysiology, clinical manifestations and management. *Clin*. *Microbiol*. *Rev*. **28**(3): 603-661.

Ultee A, Gorris LGM and Smid EJ (1998). Bactericidal activity of carvacrol towards the food-borne pathogen Bacillus cereus. *J*. *App*. *Microbiol*., **85**(2): 211-218.

Veranitisagul C, Wattanathana W, Wannapaiboon S, Hanlumyuang Y, Sukthavorn K, Nootsuwan N, Chotiwan S, Phuthong W, Jongrungruangchok S and Laobuthee A (2019). Antimicrobial, conductive and mechanical properties of AgCB/PBS composite system. *J*. *Chem*., Article ID 3487529.

Wakeel A, Jan SA, Ullah I, Shinwari ZK and Xu M (2019). Solvent polarity mediates phytochemical yield and antioxidant capacity of *Isatis tinctoria*. *Peer J*., **7**(2019): e7857.

Wulandari G, Rahman AA and Rubiyanti R,(2019). Uji aktivitas antibakteri ekstrak etanol Kulit buah alpukat (*Persea americana* Mill) terhadap *Staphylococcus aureus* ATCC 25923. *Media Informasi*, **15**(1): 74-80.

Xu W, Chan KM and Kool ET (2017). Fluoroscent nucleobases as tools for studying DNA and RNA. *Nat*. *Chem*., **9**(11): 1043-1055.

HASIL CEK_Persea americana (Mill.), avocado, antibacterial activity, mechanism of action

ORIGINALITY REPORT

7%	5%	6%	1%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

#	Source	%
1	www.pjps.pk Internet Source	2%
2	www.frontiersin.org Internet Source	1%
3	Marni Kaimudin, Komers R.W. Manduapessy, Sumarsana. " Potential of Seaweed . As inhibitors of and ", IOP Conference Series: Earth and Environmental Science, 2020 Publication	1%
4	Patra, Jayanta Kumar, Hyewon Hwang, Joon Weon Choi, and Kwang-Hyun Baek. "Bactericidal Mechanism of Bio-oil Obtained from Fast Pyrolysis of Pinus densiflora Against Two Foodborne Pathogens, Bacillus cereus and Listeria monocytogenes", Foodborne Pathogens and Disease, 2015. Publication	1%
5	mdpi-res.com Internet Source	1%

6	www.scielo.br Internet Source	1 %
7	Claira Arul Aruldass, Santhana Raj Louis Masalamany, Chidambaram Kulandaisamy Venil, Wan Azlina Ahmad. "Antibacterial mode of action of violacein from Chromobacterium violaceum UTM5 against Staphylococcus aureus and methicillin-resistant Staphylococcus aureus (MRSA)", Environmental Science and Pollution Research, 2017 Publication	1 %
8	Muhammad Sadiq, Joel Tarning, Tay Aye Cho, Anil Anal. "Antibacterial Activities and Possible Modes of Action of Acacia nilotica (L.) Del. against Multidrug-Resistant Escherichia coli and Salmonella", Molecules, 2017 Publication	1 %

Exclude quotes	On	Exclude matches	< 1%
Exclude bibliography	On		