

KARAKTERISASI NANOPARTIKEL EKSTRAK ALPUKAT (*Persea americana* Mill) TERHADAP PERTUMBUHAN *PROPIONIBACTERIUM ACNE*

*Characterization of Avocado (*Persea americana* Mill) Extract Nanoparticles on the Growth of *Propionibacterium acne**

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Abstrak

Propionibacterium acne adalah salah satu bakteri penyebab jerawat. Salah satu tanaman yang dapat digunakan sebagai alternatif obat herbal adalah alpukat (*Persea americana* Mill.). Nanopartikel adalah partikel koloidal padat dengan diameter yang berkisar antara 1-1000 nm. Salah satu teknik yang umum digunakan dalam pembuatan ekstrak nanopartikel adalah teknik gelasi ion yang memiliki manfaat seperti proses yang sederhana, penggunaan pelarut non-organik, dan kemudahan pengendalian proses. Penelitian ini bertujuan untuk membandingkan efektivitas ekstrak buah alpukat dengan makerasi terhadap nanopartikel ekstrak pada pertumbuhan *Propionibacterium acne*. Buah alpukat diekstraksi dengan makerasi dan karakterisasi nanopartikel dilakukan dengan uji *Particle Size Analyzer* (PSA) dan *Fourier Transform Infra Red* (FTIR). Akhirnya, diukur zona penghambatan menggunakan metode difusi. Hasil menunjukkan bahwa daya hambat ekstrak alpukat pada konsentrasi 25% adalah 16,50 mm, pada konsentrasi 50% adalah 13,26 mm, dan pada konsentrasi 75% adalah 32,27 mm. Sementara itu, nanopartikel ekstrak alpukat pada konsentrasi 25%, 50%, dan 75% menghasilkan daya hambat yang sama, yaitu 6 mm. Hasil analisis statistik menggunakan uji nonparametrik Friedman menunjukkan nilai ($p < 0.05$) adalah 0.000 yang mengindikasikan perbedaan signifikan antara zona hambatan ekstrak buah alpukat dan nanopartikel ekstrak buah alpukat.

Kata Kunci:
Alpukat (*Persea americana* Mill)
Propionibacterium acne
Nanopartikel
Metode Difusi
Zona Hambat

Keywords:
Avocado (*Persea americana* Mill)
Propionibacterium acne
Nanoparticles
Diffusion method
Inhibition zone

Abstract

Propionibacterium acnes is one of the bacteria associated with acne. One plant that can potentially be used as an alternative herbal medicine is avocado (*Persea americana* Mill.). Nanoparticles are solid colloidal particles with diameters ranging from 1 to 1,000 nm. One technique commonly used for the preparation of extract nanoparticles is the ionic gelation technique, which offers several advantages, including a simple process, the use of non-organic solvents, and ease of process control. This study aimed to compare the antibacterial effectiveness of avocado fruit extract and avocado fruit extract nanoparticles against the growth of *Propionibacterium acnes*. Avocado fruit was extracted using the maceration method, and the resulting nanoparticles were characterized using *Particle Size Analyzer* (PSA) and *Fourier Transform Infrared* (FTIR) spectroscopy. The inhibition zone was then measured using the diffusion method. The results showed that avocado fruit extract at concentrations of 25%, 50%, and 75% produced inhibition zones with average diameters of 16.50, 13.26, and 32.27 mm, respectively. Meanwhile, avocado fruit extract nanoparticles at concentrations of 25%, 50%, and 75% produced the same inhibition zone diameter of 6 mm. Statistical analysis using the non-parametric Friedman test yielded a p -value of 0.000 ($p < 0.05$), indicating a significant difference between the inhibition zones of the avocado fruit extract and the avocado fruit extract nanoparticles.



INTRODUCTION

Infectious diseases are a common health problem experienced by the people of Indonesia and around the world. Infections caused by bacteria are the most common cause of infection, for example acne. Acne can be caused by several factors, including an unhealthy lifestyle, hormonal instability, lack of hygiene, and psychological factors. In addition, bacterial infections can also cause acne, and one type of bacteria known to cause acne is *Propionibacterium acne* (Yuliana et al., 2023). The mechanism of acne formation due to *Propionibacterium acne* bacterial infection is by damaging the stratum corneum, which is the outermost layer of the skin and the thinnest layer on the eyelids, cheeks, and stratum germinativum located at the base of the epidermis by secreting chemicals to damage the pore walls, causing inflammation and then blockage of fatty acids and oils in the skin (Fitriyanti et al., 2020). Indonesia is a country rich in various types of plants, some of which even have the potential as medicinal ingredients. Therefore, as time goes by, people have begun to return to using traditional medicine or alternative medicine by utilizing plants around them. Currently, various types of skincare are found that utilize fruits, even flowers are one of the main raw materials. One type of fruit that has the potential as a medicine is avocado (*Persea americana* Mill) (Noviyani, 2022). Avocado plants are plants that have various beneficial substances, including various secondary metabolites such as carbohydrates, proteins, vitamins A, B, C, D, and fat (Marini et al., 2020) and also in the form of saponins, polyphenols, alkaloids, and flavonoids that can act as antibacterials. Parts of the avocado plant that contain antibacterial compounds include the leaves, seeds, skin, and flesh of the avocado (Noviyani, 2022).

According to research conducted by Muchyar et al. (2018), the experiment resulted in the discovery that avocado fruit flesh extract showed the ability to inhibit the growth of *Staphylococcus aureus* bacteria at a concentration of 100%, reaching a size of around 15.55 mm, which can be classified as a strong inhibition zone. In a study conducted by Yuliana et al. (2023), it was shown that the inhibition zone obtained by avocado leaf extract gel against *Propionibacterium acne* bacteria at concentrations of 5%, 7%, and 7.5% was 0.2 ± 0.23 mm, 1.13 ± 0.20 mm, and 4.23 ± 0.20 mm. This inhibition zone is categorized as weak because the concentration used is considered too low. Nanotechnology has become one of the most interesting and significant domains in the fields of physics, chemistry, and biology. The aspects of nanotechnology that are experiencing rapid development involve nanomedicine, nanoemulsions, and nanoparticles. Nanoparticles refer to solid particles in colloids with a diameter of 1-1000 nm. Factors that influence drug effectiveness include particle shape and size, because particle dimensions can affect drug dissolution, absorption, and distribution. Until now, nanoparticle technology, especially in the context of herbal plants, is still being developed in Indonesia (Kumowal et al., 2019). Based on a study conducted by Fahira et al. (2023) the antibacterial Zone of Inhibition (ZOI) value of matoa leaf ethanol extract nanoparticles against *Streptococcus mutans* reached 8 mm at a concentration of 2.5%, 9.06 mm at a concentration of 5%, and 10.1 mm at a concentration of 7.5%. Furthermore, research from Saryanti and Nugraheni (2019) found that betel leaf extract nanoparticles could inhibit the growth of *Staphylococcus aureus*, with an inhibition zone diameter of around 8.9 mm. In the study of Kumowal et al. (2019) the results of measuring the inhibition zone of white galangal extract nanoparticles against the growth of *Klebsiella pneumonia* showed an average value of around 22.5 mm.

Based on this background, research on the potential of avocado fruit extract nanoparticles (*Persea americana* Mill) as an antimicrobial compound in inhibiting the growth of *Propionibacterium acne* needs to be conducted to determine the comparative effectiveness between avocado fruit extract without nanoparticles and avocado fruit extract nanoparticles (*Persea americana* Mill).

RESEARCH METHODS

This research has passed the ethics test at the Health Research Ethics Commission, Faculty of Dentistry, Airlangga University Surabaya with certification number: 0832/HRECC. FODM/VIII/2024. This study uses experiments with the aim of assessing antibacterial effectiveness of avocado extract nanoparticles (*Persea americana* Mill) against the growth of *Propionibacterium acne*. The design of this study uses an experimental method. The population and sample used in this study are avocados (*Persea americana* Mill) from Bangil Market with oval fruits, soft flesh, yellowish-green color, and still fresh. The *Propionibacterium acne* bacteria came from the Surabaya Health Laboratory Center. Samples were taken by the Nonprobability Sampling method, specifically by application of the Purposive Sampling method, which is the selection of samples based on certain criteria. The research was conducted in the Laboratory of Bacteriology and Pharmacology at the Faculty of Health Sciences, University of Muhammadiyah Sidoarjo. Phytochemical and Evaporation tests were carried out at the Organic Chemistry Laboratory, State University of Surabaya. The Fourier Transform Infra Red (FTIR) test was carried out at the Chemistry Laboratory of the State University of Surabaya. Meanwhile, the Particel Size Analyzer (PSA) test was carried out at the Chemistry Laboratory of the Sepuluh Nopember Institute of Technology Surabaya. This research was carried out for approximately three months, starting from August-October 2024.

Tools and materials

The necessary tools include a smoothing machine, autoclave, refrigerator, incubator, rotary vacuum evaporator, test tube, tube rack, bulb, measuring pipette, measuring flask, Erlenmeyer, beaker glass, measuring cup, stirring rod, substance spoon, droppipette, micropipette, blue tip, yellow tip, bunsen, petri cup, needle ose, round ose, vortex, colony counter, reagent bottle, KBr pellets, PSA, FTIR. Magnetic stirrer.

The required materials include avocado (*Persea americana* Mill) obtained from Bangil Market, *Propionibacterium acne* bacteria obtained from the Surabaya Laboratory Center, ethanol 96%, tissues, filter paper, Nutrient Agar (NA) media, Mueller-Hinton Agar (MHA) media, NaCl 0.9%, aquiaades, dimethyl sulfoxide 10%, McFarland 0.5 (BaCl₂ 1.75%, H₂SO₄ 1%), Blood Agar Plate (BAP) media, HCl 2 N, 10% chloride, anhydrous acetic acid, concentrated sulfuric acid, Liebermann-Burchard, chloroform, concentrated HCl, magnesium metal, Dragendorff, Mayer, NaOH 1N, chitosan 0.1, Na-TPP 0.1%, H₂SO₄ 1%, BaCl₂, Mg, FeCl₃.

Method and result analysis

1. Preparation of maceration extract

The avocado pulp that has been collected as much as 3 kg will be cleaned first, then split into two parts, and the avocado pulp is taken by scraping. After that, the avocado pulp will be dried to reduce its moisture content before mashing or blending. A total of 300 grams of crushed avocado pulp will be put into a dark container and 1000 mL of 96% ethanol will be added. Next, the maceration process is carried out for 72 hours (3 x 24 hours) in a cool place protected from light, with stirring every 4 hours. After that, the solution obtained from the process is filtered with a funnel coated with filter paper. The filtrate is separated and the ethanol is removed through an evaporation process in a rotary evaporator with a temperature of no more than 50°C, so that an extract containing water remains. The viscous extract obtained is calculated as the yield result. Yield calculation formula:

$$\frac{\text{Total weight of the extract}}{\text{Total weight of powder}} \times 100\%$$

2. Phytochemical tests

- a) The flavonoid test was performed by adding 1 mL of the extract to 3 mL of 70% ethanol. The mixture was shaken, heated, and shaken again, followed by filtration. The resulting filtrate was then added with 0.1 g of Mg and 2 drops of concentrated HCl. A positive result for flavonoids was indicated by the development of a red color.
- b) The saponin test was performed by placing 1 mL of the extract into a test tube and adding 10 mL of water. The mixture was heated over a Bunsen burner, vigorously shaken, and allowed to stand for 15 minutes. The formation of stable, persistent foam indicated a positive result for saponins.
- c) The tannin test was performed by adding 1 mL of the extract to a test tube and boiling it with 20 mL of water, followed by filtration. Then, 2-3 drops of 1% FeCl₃ solution were added to the resulting filtrate. A positive result for tannins was indicated by the development of a greenish-brown or bluish-black color.
- d) The alkaloid test was performed by adding 1 mL of the extract, 1 mL of chloroform, and 1 mL of ammonia (NH₃) to a test tube. The mixture was heated, shaken, and filtered. The resulting filtrate was divided into three portions and transferred into three test tubes. Three drops of 2 N sulfuric acid were added to each test tube, followed by the addition of Mayer's, Wagner's, and Dragendorff's reagents, respectively. A positive result was indicated by the formation of a white precipitate with Mayer's reagent, an orange precipitate with Wagner's reagent, and a brown precipitate with Dragendorff's reagent.
- e) The steroid test was performed by mixing 1 mL of the extract with 3 mL of 70% ethanol, followed by the addition of 2 mL of concentrated sulfuric acid and 2 mL of Liebermann-Burchard reagent. A positive result for steroids was indicated by a color change from purple to blue or green.
- f) The triterpenoid test was performed by adding 2 mL of chloroform and 3 mL of concentrated H₂SO₄ to 1 mL of the extract. A positive result for triterpenoids was indicated by the formation of a brownish-red color.
- g) The phenolic test was performed by adding 1 mL of the extract to a test tube, followed by the addition of 1 mL of 1% NaCl and 10% gelatin solution. A positive result for phenolic compounds was indicated by the formation of a white precipitate.

3. Avocado fruit extract nanoparticle manufacturing

The manufacture of extract nanoparticles and the characteristics of extract nanoparticles, namely first making a solution of chitosan and Na-TPP. A total of 0.1 grams of chitosan was weighed, then 100 mL of 1% acetic acid solution was added to a 250-milliliter beaker glass. Then, it was continued by weighing 0.035 grams of Na-TPP and adding 35 mL of aquades to a 250-milliliter beaker glass. After that, 1 gram of avocado extract will be weighed. Then, avocado ethanol extract was mixed with 35 mL of 96% ethanol, 15 mL of distilled water, 100 mL of 0.1% chitosan solution and 35 mL of Na-TPP into a 1000 mL beker glass, then stirred using a 1500 rpm magnetic stirrer for 20 minutes and stirred again using a magnetic stirrer at 1000 rpm for 2 hours. Furthermore, colloidal chitosan and Na-TPP nanoparticles derived from avocado fruit extract were separated by centrifugation at 4000 rpm for 20 minutes.

4. Extraction characterization of avocado fruit nanoparticles (*Persea americana* Mill)

a) Particle Size and Distribution

Before use, the Particle Size Analyzer (PSA) is preheated for ± 20 minutes. After that, the computer device connected to the appliance is turned on. Then the settings on the tool began to be made. Using a vortex mixer for ± 1 minute, the avocado nanoparticle extract solution is shaken. Then put it in a clean cuvette until it is 2/3 filled with the cuvette. Then, a standard solution is fed into the tool and covered with a sensor. Before the measurement is taken, the temperature is pre-set at 25^o C by pressing the "Temp.Panel" menu. Then, by pressing the "Auto1" menu, the tool will measure the particle size automatically.

b) Fourier Transform Infra Red (FTIR)

A total of 0.0020 g of avocado nanoparticle extract samples and 0.1980 g KBr were weighed and then crushed and printed to form thin plates (transparent). The samples were read using an FTIR device with infrared absorption at a wave number of 4000–450 cm⁻¹. Furthermore, the resulting chromatogram is further analyzed.

5. Antibacterial effectiveness testing

The test was carried out with avocado fruit ethanol extract with concentrations of 25%, 50% and 75% and avocado fruit ethanol nanoparticle extract with concentrations of 25%, 50% and 75% against *Propionibacterium acne* using the disc diffusion method. The negative control used was sterile aquadest and the positive control used was chloramphenicol antibiotic disc paper. Each concentration is repeated 3 times. The antibacterial activity test was carried out using 15 mL of MHA medium with a temperature of 45-50°C in each petri dish and left to solidify. Sterile cotton swabs are dipped into the suspension of *Propionibacterium acne* bacteria using a sterile technique. Excess inoculum is removed by pressing saturated cotton against the inner wall of the tube, then scraped evenly over the entire surface of the MHA medium to the edge of the dish to ensure dense and even growth then allowed to dry for 5 minutes. One by one, the discs that have been dipped into each concentration of the avocado fruit ethanol extract test solution and the avocado fruit ethanol nanoparticle extract are placed at equal distances using sterile tweezers. To ensure that the discs adhere to the surface of the MHA media, gently press down on each disc with tweezers. The cup is incubated upside down at 37°C for 24 hours. After incubation, the inhibition zone marked by a clear area around the disc is measured in

diameter using a digital caliper in millimeters (mm) until the Zone of Inhibition (ZOI) value or inhibition zone value is obtained.

6. Data analysis

The data obtained was statistically analyzed with the SPSS program and then the normality of the data was seen using the Shaphiro-Wilk test. If the data distribution is normal, then it will be continued with the Two Way Anova and Post Hoc Duncan parametric statistical tests. If the data is not normal, a non-parametric test is carried out using the Friedman test.

RESULT AND DISCUSSION

1. Maceration and Phytochemical Tests

Avocado fruit (*Persea americana* Mill) was macerated using 96% ethanol. After maceration, the yield was calculated and the yield was 30%. The yield is a comparison between the results of the amount of metabolites obtained after the extraction process with the weight of the sample used. The yield is said to be good if the value is more than 20% (Putriani et al., 2022). Good yield in the extraction process illustrates high efficiency in obtaining the desired results from raw materials. Optimal yield indicates that the extraction process successfully isolates the desired components maximally, maximizing the amount of extract obtained from the raw materials used (Aristyanti et al., 2017). The characteristics of good yield involve several important aspects, such as extraction efficiency, final product quality, process consistency, and optimization of extraction conditions. High extraction efficiency indicates that the target components can be isolated effectively, but high yield does not always guarantee good product quality. The quality of the final product is very important, because the extract obtained must meet the established purity standards, free from unwanted components (Wijaya et al., 2022). In addition, consistency in the extraction process is essential so that the results obtained do not fluctuate from one batch to another, which often occurs due to variations in process settings (Aristyanti et al., 2017). Another important factor is the optimization of extraction conditions, such as the selection of solvent type, temperature, extraction time, and material to solvent ratio. All of these factors must be set properly to achieve optimal yield, ensuring that extraction runs efficiently, produces high-quality products, and can be repeated with consistent results (Wijaya et al., 2022).

Phytochemical tests are a series of tests or analyses to detect and identify active chemical compounds (phytochemicals) found in plant materials. These compounds, such as alkaloids, flavonoids, saponins, tannins, and terpenoids, have the potential as medicinal ingredients or other bioactive substances. Phytochemical tests are carried out using specific reagents and methods for each type of compound, so that the type and amount of active compounds contained in a particular plant can be known (Rumagit, 2015). The results of the phytochemical test on avocado extract (*Persea americana* Mill) can be seen in Table I.

Table I. Results of Phytochemical Test of Avocado Fruit Extract (*Persea americana* Mill)

No.	Phytochemical Tests	Reactants	Results (Formation)	Conclusion (+)/(-)
1	Alkaloids	Mayer	White precipitate	+
		Wagner	Chocolate precipitate	+
		Dragendorff	Orange prescipitate	-

2	Flavonoids	Mg + HCl pekat + etanol	Red	+
3	Saponins	-	The presence of stable foam	+
4	Steroids	Libermann-Burchard	Puprple to blue/green	+
5	Triterpenoids	Kloroform + H ₂ SO ₄ pekat	Brownish red	+
6	Phenolic	NaCl 10% + Gelatin 1 %	White precipitate	+
7	Tannins	FeCl ₃ 1%	Greenish brown	+

Description:

(+) : Contains compounds

(-) : Does not contain compounds

The compound content in avocado fruit is influenced by the extraction process carried out. Factors that affect the extraction results include the duration of extraction, type, and amount of solvent used (Suharto et al., 2012). The results of phytochemical tests show that avocado fruit contains alkaloids, flavonoids, saponins, steroids, triterpenoids, phenolics, and tannins. This content can be influenced by environmental factors that affect growth, as well as physiological and biochemical changes in avocado fruit (Venkatachalam, 2019). Avocado fruit extract contains flavonoids that show a color change to red, which functions effectively as an antibacterial. Flavonoids act as antibacterials by forming complexes with extracellular proteins that can disrupt the integrity of bacterial cell membranes (Rachmawaty et al., 2009). Saponins in avocado fruit extract show positive results with the formation of stable foam due to the physical properties of saponins which are easily soluble in water (Mailuhu et al., 2017). Saponins are active substances that can increase the permeability of cell membranes, which can cause hemolysis. When saponins interact with bacterial or fungal cells, these cells will experience damage or lysis (Utami, 2008). Meanwhile, steroid compounds show positive results with a green or bluish color change. The color change that usually occurs is caused by the reaction between steroids and acetic anhydride, which produces an acetyl steroid complex (Rahmah, 2018). The mechanism of action of steroids as antibacterials is by damaging bacterial cell membranes (Monalisa et al., 2011). The mechanism of action of steroids as antibacterials in inhibiting the growth of *Propionibacterium acne* is related to lipid membranes and sensitivity to steroid components that cause leakage in liposome bacteria. Steroids can interact with cell phospholipid membranes that are permeable to lipophilic compounds, causing decreased membrane integrity and changes in cell membrane morphology, causing fragile cells and lysis (Siregar et al., 2023). Tannins in avocado fruit extract showed positive results with a color change to greenish brown, due to the reaction of tannin compounds with Fe³⁺ (Sangi et al., 2008). Tannins have antibacterial properties by shrinking cell walls or membranes, which disrupt bacterial permeability and inhibit their life activities, so that their growth stops (Ajizah, 2004). Phenolic compounds showed positive results with the presence of white precipitation formed from the use of 10% NaCl and 1% Gelatin. The mechanism of action of phenolic compounds as antibacterials is by denaturing proteins in cells. The formation of hydrogen bonds between phenol and protein causes damage to the protein structure. This damage can disrupt the permeability of cell walls and cytoplasmic membranes, which in turn cause the integration of macromolecules and ions in cells, which can ultimately cause lysis (Bouarab-Chibane et al., 2019). Triterpenoids produce a reddish brown color change, which occurs due to the reaction of avocado fruit extract with chloroform and concentrated H₂SO₄. Triterpenoid compounds can cause lysis in bacterial cells by binding to proteins, lipids, and carbohydrates in the cell membrane. Triterpenoids inhibit growth and kill microbes by disrupting the process of membrane or cell wall formation, so that the membrane or cell wall is formed imperfectly or even not formed at all (Sari et al., 2015). Alkaloid

testing is carried out using three reagents: Mayer which produces white precipitation, Wagner which produces brown precipitation, and Dragendorff which produces orange precipitation. Alkaloids work by inhibiting the formation of peptidoglycan in bacterial cells, so that the cell wall layer is not formed perfectly and causes bacterial death (Rachmawaty et al., 2009).

From the phytochemical tests conducted, it was shown that the results of flavonoids, saponins, steroids, triterpenoids, phenolics, tannins, and alkaloids were positive. Although the Alkaloid test showed that the three Mayer and Wagner reagents showed positive results and Dragendorff showed negative results, the alkaloid test results were still said to be positive. The difference in positive results in the Mayer and Wagner tests but negative in the Dragendorff test in detecting alkaloids can be explained by the differences in sensitivity and specificity of each reagent. Mayer's reagents (potassium mercury iodide) and Wagner's (iodine in potassium iodide) are more sensitive to various types of alkaloids and tend to give positive results even though the alkaloid concentration is low or the chemical structure of the alkaloid is not very specific. Both of these reagents work by forming a precipitate that indicates the presence of alkaloids, so they often show positive results for many types of alkaloids (Shaikh and Patil, 2020). On the other hand, the Dragendorff reagent (bismuth potassium iodide) is more specific and requires certain types of alkaloids and higher concentrations to produce a positive reaction. The chemical structure of alkaloids that have steric or electronic properties that prevent interaction with bismuth ions in Dragendorff's reagent can cause the test results to be negative, even though the alkaloids were detected by Mayer and Wagner. This is especially true for alkaloids that have chemical structures that do not match the Dragendorff reaction mechanism (Kumar and Mathew, 2014).

2. Avocado Extract Nanoparticles (*Persea americana* Mill)

Nanoparticles are solid particles of colloidal size with a diameter ranging from 1-1000 nm. The manufacture of nanoparticles in the form of colloids produces white nanoparticle sediment from centrifuge. One of the effective and commonly used methods in the manufacture of nanoparticles is the ionic gelation method. This ionic gelation method is an effective method in the formation of nanoparticles because of its advantages, such as a simple process, the use of non-organic solvents, and ease of the process. However, the disadvantages of this ionic gelation method are that the particle size distribution can vary and the stability of the nanoparticles depends on the pH and environment (Fitri et al., 2020). The ionic gelation method produces nanoparticles through a cross-linking process between polyelectrolytes and multivalent ion pairs. The electrostatic interaction between the amine group (NH_3^+) in chitosan and the negatively charged group (PO_4^{3-}) of Na-TPP causes the formation of cross-links, which increases the mechanical strength of the particles made in this study (Dwitarani et al., 2021). This study used chitosan polymer because it has bioactive, biocompatible, chelating, antibacterial, and biodegradable properties. However, chitosan rapidly absorbs water and has a high degree of swelling in aqueous environments, making the drug delivery and release system for biological and medical applications less effective. To produce chitosan derivatives with better biocompatibility and lower degree of swelling, NaTPP needs to be added. The use of NaTPP crosslinker agent in low doses aims to avoid excessive

binding between TPP polyanions and amine groups on chitosan. The ionic gelation method is used with a pair of chitosan and NaTPP polymers. The electrostatic interaction between the amine groups on chitosan and the negative groups on polyanions, such as tripolyphosphate, causes the formation of chitosan nanoparticles. Compared with other methods, the ionic gelation method is simpler to use. Chitosan dissolved in acetic acid is then mixed with polyanions or anionic polymers. By using a magnetic stirrer at room temperature, nanoparticles are formed spontaneously. Changing the ratio between chitosan and NaTPP allows the adjustment of the size and surface structure of the particles (Kurniasari and Atun, 2017).

3. Particle Size Analyzer (PSA)

Particle size is an important characteristic of nanoparticles because it determines drug distribution, toxicity, affects drug loading, drug release, and the stability of the nanoparticle system (Julianawati et al., 2020). The size and size distribution of nanoparticles can be measured using a Particle Size Analyzer (PSA).

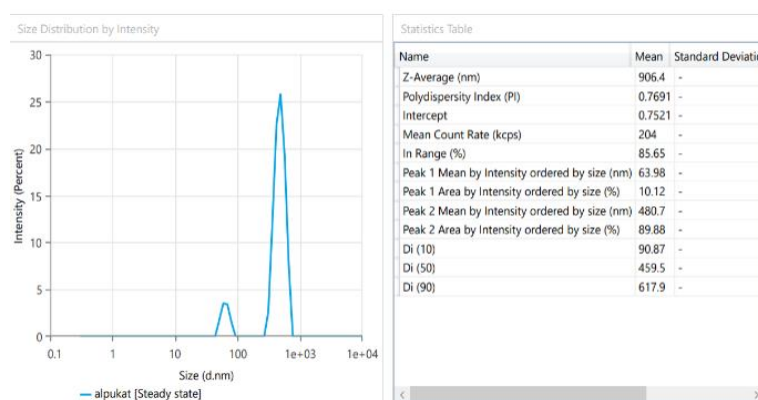


Figure 1. Particle Size Analyzer (PSA) Test Results

Figure 1. Shows the results of nanoparticle testing of avocado fruit extract (*Persea americana* Mill) carried out using a PSA tool showing a particle size of around ± 906.4 nm and having a Polydispersity Index (PI) value below 1, this indicates that it has a homogeneous particle size and tends to be uniform. A polydispersity index value higher than one results in larger particles being formed and tends to be non-uniform (Ramdani dan Sibero, 2015). The PSA tool is widely used to test samples in nanometer and submicron sizes. Usually materials or samples have a high tendency to clump. In checking using this PSA, the powder sample is dispersed into the media, so that the particles do not agglomerate (clump) together. This can result in the particle size that is read is the size of a single particle. The form of the measurement results is in the form of a distribution, so that the condition of the measurement results of the samples taken can be assumed to describe the overall condition of the sample (Hoten, 2020).

4. Functional Group Analysis with Fourier Transformed Infrared (FT-IR) on Avocado (*Persea americana* Mill) Extract Nanoparticle Material

The FT-IR stage aims to see the chemical characterization, namely to determine the functional groups in the avocado (*Persea americana* Mill) fruit extract nanoparticle solution. FT-IR is used for qualitative analysis by examining the absorbance of infrared light. In addition, characterization with FT-IR aims to identify the type of vibration of bonds between atoms in certain functional groups detected at wave numbers of 4,000-500 cm^{-1} (Akbar et al., 2016). The results of FT-IR characterization on the avocado (*Persea americana* Mill) fruit extract nanoparticle solution can be seen in Figure 2

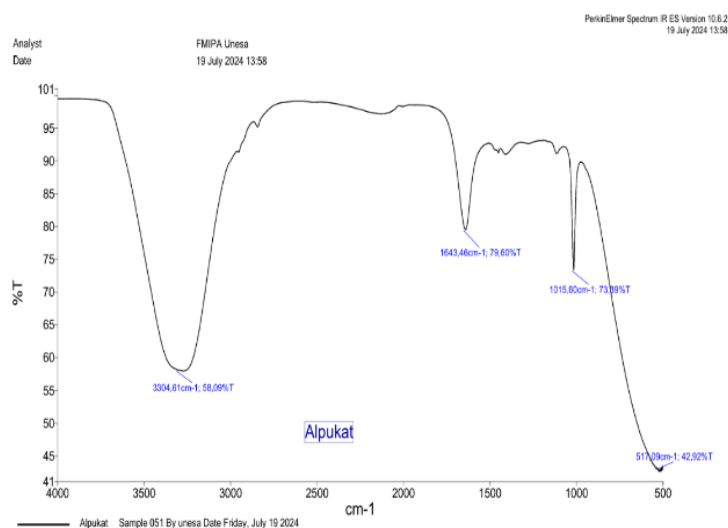


Figure 2. FT-IR Characterization Results of Avocado Fruit Extract Nanoparticles (*Persea americana* Mill)

The results of the FT-IR spectrum analysis indicate the presence of several significant absorption bands, each of which represents a specific functional group in the compound being analyzed. In the spectrum shown in Figure 3, the first absorption band was detected at a wave number of 3304.61 cm^{-1} with a transmission rate of 58.09%. This band indicates stretching of the hydroxyl group (OH), which originates from the vibration of intramolecular hydrogen bonds. The presence of this OH group often indicates the presence of alcohol or carboxylic acid in the compound. The second absorption band is located at a wave number of 1643.46 cm^{-1} with a transmission rate of 79.60%, indicating the presence of a carbonyl group (C=O) which is usually found in aldehydes, ketones, or carboxylic acids. The third absorption band was detected at a wave number of 1015.60 cm^{-1} with a transmission rate of 73.39%, indicating stretching of the C-O-C (ether) or C-O-H (alcohol) group. This peak indicates the presence of oxygen-carbon bonds in the compound structure. Finally, there is an absorption band at wave number 517.09 cm^{-1} with a transmission rate of 42.92%, indicating stretching of the aromatic C-H group, usually found in aromatic compounds containing benzene rings. Overall, the FT-IR spectrum of avocado (*Persea americana* Mill) fruit extract nanoparticles identified the presence of hydroxyl (OH), carbonyl (C=O), ether/alcohol (C-O-C/C-O-H), and aromatic C-H groups, which provide insight into the chemical composition and molecular structure of the compound. Fourier Transform Infrared Spectroscopy (FTIR) plays an important role in identifying active compounds that contribute to bacterial inhibition. FTIR is used to characterize functional groups in a compound, both in the form of pure extracts and after the

nanoparticle synthesis process. The presence of functional groups such as -OH (hydroxyl), -C=O (carbonyl), and -NH (amine) detected in the FTIR

spectrum can indicate the presence of antibacterial compounds such as flavonoids, tannins, alkaloids, and saponins. These compounds are known to have the ability to damage bacterial cell walls, inhibit protein synthesis, or interfere with microbial metabolism, thus contributing to the formation of inhibition zones in inhibition tests. FTIR is also used to ensure the successful encapsulation of active compounds in nanoparticles without degradation, as well as to analyze structural changes after interaction with bacteria (Siregar et al, 2023).

5. Inhibitory Power Test of Avocado Fruit Extract (*Persea americana* Mill) and Avocado Fruit Extract Nanoparticles (*Persea americana* Mill)

This study used the diffusion method to test the effectiveness of avocado fruit extract (*Persea americana* Mill) against *Propionibacterium acne*. The clear zone formed around the paper disc indicates inhibition of the growth of *Propionibacterium acne* bacterial colonies. The diameter of the inhibition zone formed was measured with a caliper and expressed in millimeters (mm). The larger the inhibition zone formed, the higher the antibacterial activity of the avocado fruit. The diameter of the inhibition zone produced by variations in avocado extract concentrations of 25%, 50%, and 75% was compared with the inhibition zone around the paper disc containing 250 mg Chloramphenicol used as a positive control and distilled water as a negative control. If the size of the inhibition zone is ≤ 15 mm, it is categorized as resistant, in the range of 16-20 it is categorized as intermediate, and if ≥ 21 mm it is categorized as sensitive (CLSI, 2011).

Table II. Results of Antibacterial Effectiveness Test of Avocado Fruit Extract (*Persea americana* Mill) and Results of Antibacterial Effectiveness Test of Nanoparticles of Avocado Fruit Extract (*Persea americana* Mill)

Results of Antibacterial Effectiveness Test of Avocado Fruit Extract (<i>Persea americana</i> Mill)			
No.	Concentration (%)	Average Diameter (mm)	Information
1	Chloramphenicol Positive Control 100%	6	Resistant
2	Sterile Aquades Negative Control	0	Resistant
3	Avocado Extract 25%	16,50	Intermediates
4	Avocado Extract 50%	13,26	Intermediates
5	Avocado Extract 75%	32,27	Sensitive
Results of Antibacterial Effectiveness Test of Nanoparticles of Avocado Fruit Extract (<i>Persea americana</i> Mill)			
No.	Concentration (%)	Average Diameter (mm)	Information
1	Chloramphenicol Positive Control 100%	6	Resistant
2	Sterile Aquades Negative Control	0	Resistant
3	Nanoparticles Avocado Extract 25%	6	Resistant
4	Nanoparticles Avocado Extract 50%	6	Resistant
5	Nanoparticles Avocado Extract 75%	6	Resistant

Based on the results presented in Table II, the avocado extract at a concentration of 25% produced an inhibition zone with an average diameter of 16.50 mm, which was classified as intermediate. At a concentration of 50%, the inhibition zone had an average diameter of 13.26 mm and was also classified as intermediate. Meanwhile, the 75% concentration produced an inhibition zone with an average diameter of 32.27 mm, which was

classified as sensitive. The formation of the inhibition zone may have been influenced by the bioactive compounds present in avocado, including alkaloids, flavonoids, saponins, steroids, triterpenoids, phenolic compounds, and tannins. These compounds have potential antioxidant and antibacterial activities that may inhibit bacterial growth.

The results of the inhibition zone measurement of avocado extract nanoparticles in Table II at concentrations of 25%, 50%, and 75% show a size of 6 mm. This shows that avocado extract nanoparticles have a resistant inhibition. In the negative control, no inhibition zone was found at all, while the positive control with the antibiotic chloramphenicol showed a resistant inhibition. The formation of the inhibition zone on the nanoparticles is influenced by the chitosan solution, which has the ability to inhibit bacteria. There are two possible mechanisms of chitosan as an antibacterial. First, chitosan adheres to the surface of bacterial cells, forming a polymer layer that can prevent the entry of nutrients into the cells, causing the bacterial cells to die. Second, chitosan with a low molecular weight can enter the cells and cover the cells, because chitosan can adsorb electronegative substances in the cells, which interfere with bacterial activity (Kumowal et al., 2019).

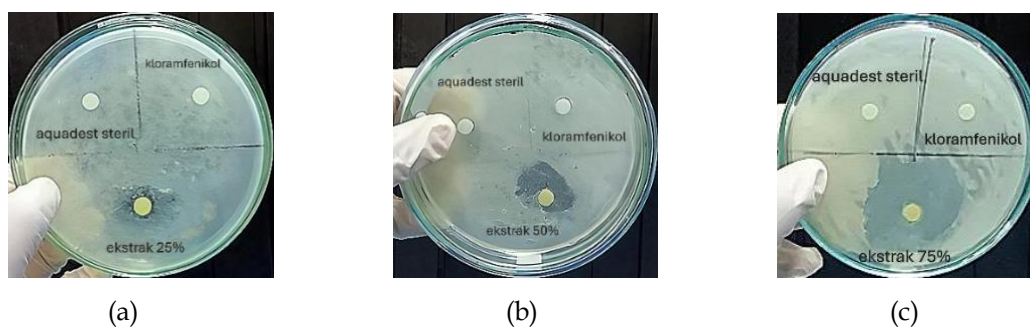


Figure 3. Results of Antibacterial Effectiveness Test of Avocado Extract (*Persea americana* Mill) and Control Group

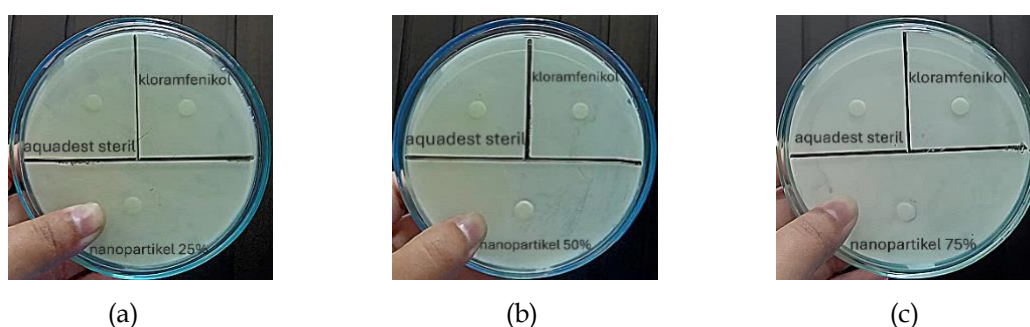


Figure 4. Results of Antibacterial Effectiveness Test of Avocado Extract Nanoparticles (*Persea americana* Mill) and Control Group

Based on the results of the inhibition zone measurements that have been carried out, it can be seen in table 2 that avocado extract without nanoparticles shows a larger diameter of the inhibition zone against *Propionibacterium acne* bacteria. This is in line with previous research by Muchyar et al. (2018) which used avocado extract with 96% ethanol found antibacterial activity against gram-positive bacteria *Staphylococcus*

aureus at a concentration of 100%. Lenny (2016) also reported that avocado extract using the maceration method with 96% ethanol had antibacterial activity against gram-positive bacteria *Staphylococcus aureus* and *Staphylococcus epidermidis* at concentrations of 25%, 50%, 75%, and 100%. In addition, there is also a study conducted by Yuliana et al. (2023) that uses avocado extract with 96% ethanol and found antibacterial activity against Gram-positive bacteria *Propionibacterium acne* at concentrations of 5%, 7%, and 7.5%. Meanwhile, in tests with nanoparticles that have been carried out at concentrations of 25%, 50%, and 75%, the results show resistance to *Propionibacterium acne* bacteria.

Avocado extract can be more effective at inhibiting the growth of bacterial inhibition zones than extract nanoparticles due to its more stable physical and chemical properties and more easily accessible to bacteria. The viscous extract contains bioactive compounds such as flavonoids, tannins, alkaloids, and saponins in a more concentrated form. These compounds have natural antibacterial properties that are effective in inhibiting bacterial growth by damaging bacterial cell membranes or inhibiting the function of bacterial essential enzymes (Lenny, 2016). Meanwhile, unstable or clumped nanoparticles will reduce the active surface area that can interact with microorganisms, thus decreasing their effectiveness. The nanoparticle synthesis process also determines the size, shape, and distribution of particles, which ultimately affects the antibacterial activity in the inhibition zone test. In addition, particle size also affects the effectiveness of nanoparticles against bacterial growth, the smaller the nanoparticle size, the more unique its chemical physical properties, such as larger surface area and increased reactivity which can strengthen the interaction between charges on the surface of bacteria and produce a stronger antimicrobial effect. With a large surface area, nanoparticles can more easily attach to the surface of bacterial cells, causing instability of the cell membrane and leakage of intracellular components, which ultimately leads to cell death (Siregar et al., 2023).

The normality test of the inhibition zone was carried out by the Shapiro-Wilk method, because the number of data was less than 50, and a p-value of 0.071 was obtained for avocado extract, while the p-value for avocado extract nanoparticles was 0.000. The data were considered normally distributed if the p-value was >0.05 , so it can be concluded that the inhibition zone data of avocado extract nanoparticles were not normally distributed. So to test the hypothesis, Friedman's non-parametric test will be used which is used to assess significant differences between several related groups or repeated measurements on non-parametric data. The results of Friedman's non-parametric test showed a significance value of $p < 0.05$, which was 0.000. Based on these values, it can be concluded that there are differences in inhibition zones between avocado fruit extract and avocado extract nanoparticles.

CONCLUSION

Based on the research that has been done, it can be concluded that the antibacterial test of avocado extract without nanoparticles against *Propionibacterium acne* at concentrations of 25% and 50% is able to form an inhibition zone that is classified as intermediate and at a concentration of 75% is able to form an inhibition zone with a sensitive category. Meanwhile, the antibacterial test of nanoparticle extract against the growth of *Propionibacterium acne* with concentrations of 25%, 50%, and 75% produces an inhibition zone that is classified

as resistant. Based on the analysis of Friedman's nonparametric test data, the value ($p < 0.05$) is 0.000, which indicates a significant difference in the inhibition zone between avocado fruit extract and avocado fruit extract nanoparticles.

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