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Research Paper

Formulation and Evaluation of Antibacterial and Anti-Inflammatory Capsules Containing *Phyllanthus emblica* L. Fruit Nanoparticles

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Abstract
Phyllanthus emblica fruit has diuretic, antibacterial, hepatoprotective, antitumor, hypocholesterolemic, antioxidant, and antiulcerogenic activities making it possible as a traditional medicine in capsule form. Capsule preparations have the advantage of covering the unpleasant taste and smell of medicinal ingredients, easy to swallow, so they are practical to use. This study aimed to formulate *Phyllanthus emblica* fruit nanoparticles in capsules and to test the antibacterial activity against *Streptococcus mutans* and *Pseudomonas aeruginosa* and anti-inflammatory activity by observing denaturation inhibition in vitro. The method used to manufacture *Phyllanthus emblica* fruit simplicia nanoparticles was used using a High Energy Ball mill grinding machine. Capsule formulation was carried out with various doses of nanoparticles (100, 150, and 200 mg). Evaluation includes weight uniformity and disintegration time. Test of its antibacterial activity against *Streptococcus mutans* and *Pseudomonas aeruginosa* and its anti-inflammatory activity in vitro. The study found that all formulas could be made into capsules that met the evaluation test requirements. The results of the evaluation of disintegration time ranged from 6.17-11.33 minutes. For the evaluation results of weight uniformity, it was found that weight deviations in columns A1 and A2 were in the range between 0.9% to 2.8% and 0.9 to 1.3%. The study reports on the antibacterial activity of *Phyllanthus emblica* fruit nanoparticle capsules (PFNP) against *Streptococcus mutans* and *Pseudomonas aeruginosa*. The results indicate that PFNP exhibits a dose-dependent antibacterial effect, with inhibition zone diameters of 10.83 mm, 11.6 mm, and 12.63 mm observed at 100 mg, 150 mg, and 200 mg, respectively, against *Streptococcus mutans*. Similarly, PFNP demonstrated a dose-dependent antibacterial effect against *Pseudomonas aeruginosa*, with inhibition zone diameters of 10.7 mm, 11.4 mm, and 12.1 mm observed at doses of 100 mg, 150 mg, and 200 mg, respectively. PFNP capsules showed inhibition results with a value of 5.63%, 6.13%, and 6.80%. It was concluded that *Phyllanthus emblica* fruit nanoparticles (*Phyllanthus emblica* L.) could be formulated in capsule dosage forms with doses of 100 mg, 150 mg, and 200 mg; has antibacterial activity against *Streptococcus mutans* and *Pseudomonas aeruginosa*, and has no anti-inflammatory activity.

Keywords
Formulation, Antibacterial, Anti-Inflammatory, Capsule, Nanoparticle, *Phyllanthus emblica* L.

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1. INTRODUCTION

Phyllanthus emblica L. is a botanical species with significant medicinal properties extensively utilized in traditional medicine. The herbs above have been utilized as therapeutic and nourishing tonics, comprising tannins, essential amino acids, and vitamins. The previous botanical specimen provides essential nutrients such as vitamin C and minerals. Throughout history, the fruit has been used for medicinal purposes in treating a range of conditions, including diabetes, hyperlipidemia, CNS disorders, and eye diseases (Ahmad et al., 2021).

Phyllanthus emblica fruit has diuretic, hepatoprotective, antitumor, hypocholesterolemia, antioxidant, and antiulcerogenic activities. *P. emblica* is also reported to have antiviral, antibacterial, antifungal, anthelmintic, and anti-inflammatory properties. Several bioactive compounds of *P. emblica*, such as flavonoids (quercetin), ascorbic acid, gallic acid, alkaloids (filantin, filantidin), hydrolyzed tannins (emblicanins A and B), punigluconins and pedunculagins have been identified. The antioxidant activity of *P. emblica* has been linked to the presence of tannins such as emblikanin A and emblikanin B (ur Rehman et al., 2007).

Nanoparticles are particles with a size of about 1-1000 nm.

Nano-size have advantages compared to the same material in larger sizes. The smaller the particle size, the wider the surface area. This large surface area will cause the material to become more reactive. The superiority of materials on the nanometer scale causes nanoparticle materials to be widely applied in various fields, one of which is the pharmaceutical field. Its small size allows this material to penetrate the intercellular spaces and increases the system's affinity due to the increased contact surface area (Yokoyama et al., 2008).

One of the development of nanoparticles to increase the release of secondary metabolites into tissues is simplicia nanoparticles. according to Masfria et al. (2021), the antimutagenic activity of extracts and nanoparticles of simplicia rhaphidohora pinnata leaves shows that the activity of simplicia nanoparticles is much stronger than that of ethanol extract due to the absorption of simplicia nanoparticles in the intestine will be better due to increased solubility, increased enterocyte membrane permeability, and opening of tight paracellular junctions between enterocytes (Masfria et al., 2021; Masfria et al., 2017).

In addition, there is research related to the synergistic mechanism of nanoparticles in the absorption process in the body showing that the pharmacological effects of isolated herbal compounds are sometimes weaker than simplicia/herbal extracts. hence the notion that purer herbal extracts are sometimes less effective. in the pharmacokinetic process, there is a synergistic relationship between primary and secondary metabolites in plants, thereby increasing the promotion of the intestinal absorption of active constituents by improving solubility, inhibiting first-pass elimination mediated by drug-metabolizing enzymes or drug transporters, increasing the membrane permeability of enterocytes, and reversibly opening the paracellular tight junction between enterocytes. therefore there is a synergistic relationship between the metabolites as well as being enhanced by forming them into nanoparticles which help the release of metabolites better due to the larger surface area (Abdifetah and Na-Bangchang, 2019; Zhao et al., 2020).

According to Ministry of Health of the Republic of Indonesia (2020), Capsules are a type of pharmaceutical formulation that comprises a medication enclosed in a shell that can be either soluble or hard. The shell typically comprises gelatin, although it may be fabricated from starch or other appropriate substances.

Capsules are one of the practical dosage forms that patients can accept. Other advantages of capsule preparations are: capsules can cover the less unpleasant taste and smell of the medicinal substance, are easier to swallow, are pretty stable in storage, can be filled with single or mixed medicinal ingredients and medicinal ingredients in the form of granules, and the manufacturing process is faster and more practical because it does not require a lot of additional ingredients/auxiliaries such as tablets (Hoag, 2017).

Bacteria are unicellular prokaryotic organisms or simple associations of similar cells. Cell multiplication is usually by binary fission. Bacteria are microscopic; most are around 0.5 to 1.0 μm in diameter (Goldman and Green, 2015). *Streptococcus mutans* belong to the type of Streptococcus bacteria, which are gram-positive, round-shaped, non-motile facultative anaerobic bacteria that can grow optimally at temperatures ranging from 18-40°C. *Streptococcus mutans* is an essential etiological agent in dental caries. The natural habitat of *Streptococcus mutans* is in the human oral cavity, specifically in dental plaque, in the form of multispecies biofilms that form on the hard surfaces of teeth. *Streptococcus mutans*, a human pathogen, has been linked to subacute bacterial endocarditis, a severe inflammation of the heart valves. Additionally, certain strains of this bacterium have been associated with various extraoral pathologies, including cerebral microbleed hemorrhage, IgA nephropathy, and atherosclerosis (Lemos et al., 2019).

Pseudomonas aeruginosa is a bacterium that is gram-negative and facultatively aerobic. It can grow through aerobic and anaerobic respiration, with nitrate as the terminal electron acceptor. *Pseudomonas aeruginosa* exhibits the capability of anaerobic growth in the presence of arginine, and its fermentative capacity is restricted, leading to sluggish or negligible growth. *Pseudomonas aeruginosa* is commonly characterized as an "opportunistic" pathogen due to its infrequent occurrence in healthy individuals. From a clinical perspective, patients with compromised immune systems, such as those with cystic fibrosis (CF), cancer, AIDS, medical devices, burns and eye injuries, and non-healing diabetic wounds, are at greater risk for potential complications (Silva et al., 2020).

The bacterial sensitivity test is a diagnostic technique employed to ascertain the degree of vulnerability or receptivity of a bacterium to antimicrobial or antibacterial agents. The test serves the additional function of identifying pure substances that exhibit antibacterial properties. The Kirby and Bauer method is widely used for assessing antimicrobial agents' efficacy. The antimicrobial agent plate is positioned onto the agar medium inoculated with microorganisms, which will subsequently diffuse throughout the agar medium. The absence of microbial growth in distinct regions of agar media denotes the manifestation of antimicrobial agent-induced inhibition of microorganisms (Yousufi, 2012).

Inflammation is a local protective response caused by damage to tissues caused by physical trauma, damaging chemical substances, or microbiological substances. The anti-inflammatory test in this study was carried out in-vitro by measuring denaturation inhibition of the Bovine Serum Albumin (BSA) protein (Janani et al., 2020).

Based on the previous description, the researchers developed capsule preparations from *Phyllanthus emblica* fruit nanoparticles, evaluated the preparations, and then tested the antimicrobial and anti-inflammatory activities.

2. EXPERIMENTAL SECTION

2.1 Materials

The tools used in this study were glassware such as beaker glass (Iwaki), Erlenmeyer (Iwaki), measuring cups (Pyrex), and petri dishes. The instruments used were an incubator (Memmert IN55), analytical balance (Fujitsu FS-AR 210), autoclave

(Daihan WAC-47), oven (Memmert UN55), UV-Vis spectrophotometry (Shimadzu-1800), Fourier Transform Infra Red Spectrophotometer (Shimadzu-IRPrestige21) and micropipette (Eppendorf).

The materials and reagents used in this study were pro-analytical quality materials and reagents obtained from Merck and Sigma-Aldrich, such as Avicel PH-102, magnesium stearate, talc, lactose, Mueller-Hinton Agar, Nutrient Agar media, Nutrient Broth media, Bovine Serum Albumin (BSA) and methanol. While other ingredients such as diclofenac sodium (pharmaceutical chemistry), distilled water, *P. aeruginosa* bacteria, *S. mutans* bacteria, and size 00 gelatin capsule shells were obtained from the USU faculty of pharmacy laboratory, and *Phyllanthus emblica* fruit samples were obtained from Badung Regency, Bali Province.

2.2 Method

2.2.1 Preparation of *Phyllanthus emblica* Fruit Simplicia

Phyllanthus emblica fruit was washed thoroughly from impurities with running water until clean, drained, cut into small pieces, and dried in a drying cupboard with a temperature of 40°C-50°C. Then the dried simplicia was powdered using a grinding machine, stored in a clean container that was given, and then tightly closed, and stored in a place protected from heat and sunlight.

2.2.2 Preparation of *Phyllanthus emblica* Fruit Nanoparticles

Phyllanthus emblica fruit simplicia powder that has been made was then crushed into a nanoscale using a High-Energy Ballmill. *Phyllanthus emblica* nano simplicia was made at PT Nanotech Herbal Indonesia (Gd. Nanoplex, Batan Lama no A12, Setu-Tangerang Selatan). Then the characterization of *Phyllanthus emblica* nano simplicia using the Particle Size Analyzer (PSA) was carried out at PT Nanotech Herbal Indonesia, Fourier Transform Infra Red (FTIR) Spectrophotometer and Scanning Electron Microscope (SEM).

2.2.3 Preparation of *Phyllanthus emblica* Fruit Nanoparticle Capsules

Capsule mass preparation was carried out by mixing until homogeneous *Phyllanthus emblica* fruit nanoparticle powder, fillers, expanders, and lubricants, which were weighed according to the required amount in a mortar using a spatula, then pre-formulation tests were carried out before being put into 00 size hard capsule shells (Jyothi et al., 2017).

The administered formula consisted of capsules containing nanoparticles derived from *Phyllanthus emblica* fruit (PFNP) and was administered in doses of 100 mg, 150 mg, and 200 mg. Table 1 displays the proposed formulation of nanoparticle capsules containing *Phyllanthus emblica* (PFNP) fruit.

2.2.4 Capsule Preformulation Test

The capsule pre-formulation test method was tapping density, flow time, and angle of repose. The determination of the tap density was carried out in the following way: The granules were inserted into the measuring cup up to the marked line and expressed as the initial volume (V_1), then the measuring cup was shaken 20 times with a modified device to obtain the final volume (V_2) (Nasreen and Narayanan, 2011). The tap density requirement is less than 20% which is calculated by the following formula:

Table 1. PFNP Capsule Formula

Ingredients	Weight (mg)		
	F1	F2	F3
Phyllanthus emblica Fruit Nanoparticles	100	150	200
Avicel PH 102	27.5	26.5	25.5
Talcum	5.5	5.3	5.1
Mg Stearate	5.5	5.3	5.1
Lactose	311.5	342.9	374.9
Weight	550	530	510

$$\text{Tap density} = \frac{V_1 - V_2}{V_2} \times 100\% \quad (1)$$

Determination of granule time was carried out by Weighing 100 g of granules, then putting them in a funnel assembled, and then leveling the surface. The bottom cover was opened when the stopwatch was turned on. The stopwatch was stopped when the granules ran out through the funnel, and the flow time was recorded. The granule flow time requirement is less than 10 seconds (Harahap et al., 2019).

Determination of the angle of repose of the granules was carried out by 100 g of granules, weighed, and then put into a flow funnel that has been assembled. The surface of the granules in the funnel was leveled, then the funnel cover was opened so that the granules flowed until they ran out. The height of the granule pile was measured. Granules with free flow will have an angle of repose between 20° to 40° (Nasreen and Narayanan, 2011). The angle of repose can be calculated using the formula:

$$\tan\theta = \frac{2H}{D} \quad (2)$$

Description: θ = angle of rest
H = height of granule pile (cm)
D = diameter of the pile of granules (cm)

2.2.5 Capsule Evaluation Test

Tablet evaluation testing was carried out using two methods: the weight uniformity and time were destroyed. Weight uniformity testing was carried out by taking twenty capsules and weighing them. Each capsule was weighed again. The contents of all capsules are then removed, then all parts of the capsule shell

are weighed, and then the contents of the capsule and the average weight of each content of the capsule are calculated. The difference in the percentage of the weight of the contents of each capsule to the average weight of each capsule's contents should not be more than that specified in column A and, for every two capsules, not more than that specified in column B (Sutiswa and Rahman, 2020).

Table 2. Terms of Weight Deviation

Average Weight	Deviation of capsule contents weight in %	
	A	B
120 mg or more	± 10%	± 20%
more than 120 mg	± 7.5%	± 15%

The disintegration time test was carried out by inserting one capsule into each basket tube, then one disc was inserted into each tube, and the tool was run. As the medium used water with a temperature of 37±1°C. At the end of the time limit stated as the capsule disintegration time, the capsule is declared destroyed if no more capsules are left on the wire mesh. The test was carried out with six capsules, and within 15 minutes, the capsules disintegrated and passed through the gauze in the tube (Osei-Asare et al., 2021).

2.2.6 Antibacterial Activity Test

Antibacterial activity was tested on samples of *Phyllanthus emblica* fruit simplicia nanoparticles and *Phyllanthus emblica* fruit simplicia capsules. The test was carried out by dispersing the entire sample solution into a centrifuge tube using distilled water. The solution was then centrifuged at 10,000 rpm for 10 minutes. Then the filtrate was taken from the centrifuge tube (Vieitez et al., 2018).

Put 0.1 mL of bacterial inoculum into a sterile petri dish, then pour 15 mL of sterile Mueller-Hinto Agar media that has been thawed and wait until the temperature is 45°C, homogenize by rotating and leave until the media solidifies. Sterile tray paper saturated with a *Phyllanthus emblica* fruit nanoparticle capsule powder solution was then placed on the surface of the MHA media, which had been compacted in a petri dish. Petri dishes containing filter paper were incubated at 36–37°C for approximately 24 hours. Furthermore, the diameter of the inhibition area around the buffer paper was measured using a vernier caliper (Widjajanti et al., 2021).

2.2.7 In Vitro Anti-inflammatory Activity Test

A test was conducted to evaluate the in vitro anti-inflammatory activity, as per the methodology described in the literature by Janani et al. (2020). The negative control solutions were prepared to utilize 0.2% bovine serum albumin, while the positive controls were prepared to utilize Diclofenac sodium at concentrations of 4000 g/mL, 2000 g/mL, 1000 g/mL, and 500 g/mL. The test solution was prepared by utilizing a capsule in a methanol solvent and transferring it to a 25-mL volumetric flask. The main solution had a concentration range of 4000-8000 g/mL, which was subsequently diluted to a concentration of 1000 g/mL (Janani et al., 2020).

The anti-inflammatory activity can be measured by utilizing 50 L of the test and positive control solutions. Subsequently, a 0.2% BSA solution can be added to the mixture until the volume reaches 5 mL. This process will result in a 20 g/mL concentration for each hydrogel preparation concentration. Additionally, concentrations of 5 g/mL, 10 g/mL, 20 g/mL, and 40 g/mL diclofenac sodium concentration solutions can also be obtained. Subsequently, the sample was subjected to incubation at 25°C for 30 minutes, followed by heating at 72°C for 5 minutes and later allowed to rest for 25 minutes at a temperature of 23°C. Following the cooling process, the solution underwent vortexing, and subsequent absorbance measurements were taken via UV-visible spectrophotometry, specifically at a wavelength of 660 nanometers (Janani et al., 2020).

3. RESULTS AND DISCUSSION

3.1 Results of *Phyllanthus emblica* Fruit Nanoparticles

The results of *Phyllanthus emblica* fruit nanoparticles obtained from *Phyllanthus emblica* fruit simplicia, which were sent to PT Nanotech Herbal Indonesia to be processed into nanoparticles, showed the size of the nano simplicia obtained was 731±168 nm using particle size analysis (PSA). This is also supported by the SEM results which can be seen in Figure 1, having an irregular surface shape, uneven surface texture and forming sharp and obtuse corners with very small particle sizes (<200 μm). However, it can be seen that there are some quite large particles up to <1000 μm. According to Martien et al. (2012), nanoparticles are particles with sizes within 1-1000 nm, so the results of these nanoparticle sizes are still in the nanoparticle size range.

The findings derived from the Fourier Transform Infrared (FTIR) analysis are visually presented in Figure 2. The absorption wave numbers observed at 3282, 2926, 1716, 1614, and 1446 cm^{-1} can be attributed to the stretching frequencies of various functional groups. Precisely, the wave number at 3304 cm^{-1} corresponds to the stretching frequency of the -OH functional group, while the wave number at 2902 cm^{-1} corresponds to the stretching frequency of the -C-H functional group. Similarly, the wave number at 1726 cm^{-1} corresponds to the stretching frequency of the -C=O functional group, the wave number at 1612 cm^{-1} corresponds to the stretching frequency of the C-O (carbonyl) functional group, and the wave number at 1341 cm^{-1} corresponds to the stretching frequency of the -C-O-C functional group. Identifying these functional groups suggests the existence of polyphenolic compounds, pectin, and vitamin C within the nanoparticles derived from *P. emblica* fruit simplicia.

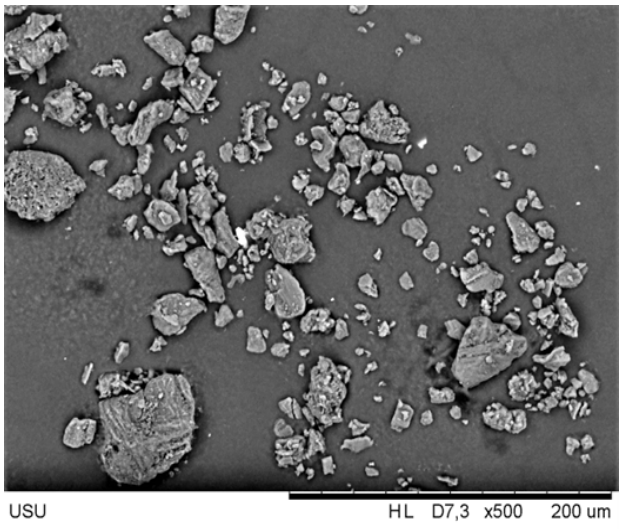

Figure 1. Morphological Results of *Phyllanthus emblica* Fruit Nanoparticles using SEM

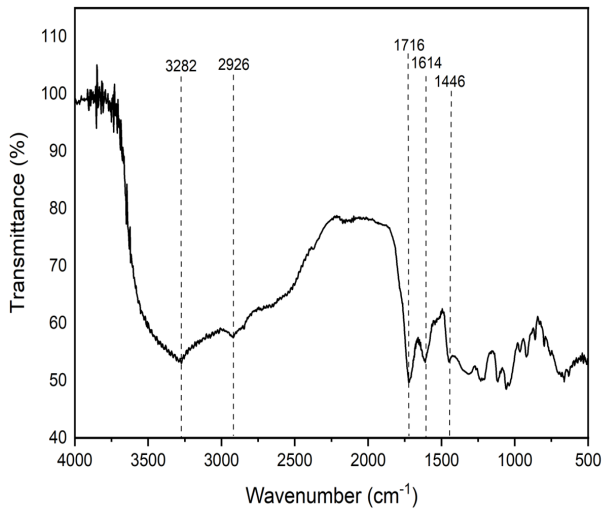

Figure 2. FTIR Spectra of *Phyllanthus emblica* Fruit Nanoparticles

3.2 Formulation and Preformulation

The capsule formulation is carried out by first granulating the filler material and mixing the other additives and simplicia nanoparticles. Making granule mass using 10% lactose which gets a good mass so that it is easy to put in a capsule container. The results of making *Phyllanthus emblica* fruit nanoparticle capsule preparations can be seen in Figure 3.

Table 3 displays the outcomes of the pre-formulation test (PFNP) conducted on the nanoparticle capsule containing *Phyllanthus emblica* fruit.

Table 3. The Results of the PFNP Capsule Granule Pre-Formulation Test

Formulas	Preformulation Test		
	Flow Time (seconds)	Tap density	Remaining Angle
100	1.56	27.13°	14.67%
150	2.02	25.91°	16%
200	3.32	26.09°	19.3
Terms	<10 seconds	20° ≤ x < 40°	≤ 20%

Based on Table 4. above shows that the results of the pre-formulation test flow time, angle of repose, and Tap density have met the requirements. An angle of repose that we smaller than 30° usually indicates that the material can flow freely, and if the angle is above 40°, the flow properties are usually poor (Harahap et al., 2019). This flow property was determined by the particle size, particle size distribution, and particle shape of the active ingredient and is assisted by adding additional materials such as lubricants and fillers (Harahap et al., 2019).

In the flow time and Tap density test, all formulas had a flow time and Tap density that met the requirements. Although the values fluctuated between formulas with low and high doses, the higher the dose, the increased flow time. This is due to the more significant percentage of fines at large doses, so flow time and Tap density increase (Harahap et al., 2019).

3.3 Capsule Evaluation Test Results

The results of the evaluation test for *Phyllanthus emblica* (PFNP) fruit nanoparticle capsules can be seen in Table 4.

Based on Table 4 above shows that the results of the capsule weight uniformity test have met the requirements contained in the Indonesian Pharmacopoeia Edition III (Indonesian Ministry of Health, 1979). namely not more than two capsules, each weight deviating from the average weight of the given price. Determined in column A, namely 7.5%, and not a single capsule whose weight deviated from the average weight of the price specified in column B, namely 15%. The weight deviation obtained from all formulas ranged from 0.9% to 2.80%. The capsule mass of each formula was different because of the variation in the amount of filler material and the size of the granule particles, which are different so that as the percentage of granules in the capsule increases with a smaller dose, the mass that the same capsule volume can accommodate smaller (Augsburger and Hoag, 2017).

Based on Table 4 above shows that the results of the capsule evaluation test at doses of 100, 150, and 200 mg showed that the three capsule formulas met the disintegration time requirements contained in the Indonesian Pharmacopoeia Edition III, which is no more than 15 minutes (Indonesian Ministry of Health, 1979). The difference in the amount of filler with the nanoparticle powder affects the disintegration time of the capsule. Based on the results obtained, capsules with lactose fillers that are more hydrophilic with a higher percentage are more easily destroyed than those with a low percentage (Augsburger and Hoag, 2017).

Figure 3. *Phyllanthus emblica* Fruit Nanoparticle Capsule Preparation

Table 4. The Results of the PFNP Capsule Evaluation Test

Formulas	Evaluation Test	Parameter	Results
F1	Weight uniformity	A1 (%)	2.12
		A2 (%)	1.73
		B1 (%)	2.12
	Time is destroyed	Time (minutes)	6.78
F2	Weight uniformity	A1 (%)	2.80
		A2 (%)	0.93
		B1 (%)	2.80
	Time is destroyed	Time (minutes)	8.91
F3	Weight uniformity	A1 (%)	0.9
		A2 (%)	0.9
		B1 (%)	0.9
	Time is destroyed	Time (minutes)	9.77

Description
F1: PFNP capsule formula with a dose of 100 mg
F2: PFNP capsule formula with a dose of 150 mg
F3: PFNP capsule formula with a dose of 200 mg

3.4 Antibacterial Activity Test Results

3.4.1 Antibacterial in *P. emblica* Fruit Nanoparticles

Table 5 and Figure 4 show the results of examining the antibacterial activity on nanoparticles derived from *Phyllanthus emblica* fruit.

Table 5. Results of Antibacterial Activity Test of *Phyllanthus emblica* Fruit Nanoparticles

Concentration (mg/mL)	Inhibition Zone Diameter	
	S. mutans (mm)	*P. aeruginosa* (mm)
50	7.77 ± 1.46	8.57 ± 0.60
100	10.83 ± 0.15	11.27 ± 0.12
150	12.37 ± 0.64	11.87 ± 0.45
200	13.57 ± 1.04	12.87 ± 0.60
300	14.90 ± 0.61	13.87 ± 1.12
400	15.97 ± 0.64	15.13 ± 0.99

The findings presented in Table 5 indicate that the utilization of *Phyllanthus emblica* fruit nanoparticles results in a notable suppression of *S. mutans* bacterial growth. As per Davis and Stout (1971), four distinct categories of bacterial inhibition exist. According to established criteria, a bacterial inhibition zone is considered weak if its diameter measures 5 mm or less. Secondly, it can be classified as moderate if the diameter of the inhibition zone falls within the range of 5–10 mm. Thirdly, an inhibition zone with a diameter ranging from 10 to 20 mm is classified as trong. Fourthly, an inhibition zone with a diameter exceeding 20 mm for bacteria is classified as highly potent. It is widely recognized that the dose range of 100-200 units represents the minimum effective dose for bacterial activity, making it suitable for capsule dosing (Indonesian Ministry of Health, 1979).

Nanoparticles have a higher affinity for interacting with bacterial cell wall components due to the increased contact surface area. In addition, its small size causes substances that have an antibacterial activity to enter cells more easily, which in turn leads to the inhibition of bacterial intracellular enzymes (Bouarab-Chibane et al., 2019).

Table 6. Results of Antibacterial Activity Test of *Phyllanthus emblica* Fruit Nanoparticles

Concentration (mg/mL)	Inhibition Zone Diameter	
	S. mutans (mm)	*P. aeruginosa* (mm)
F1	10.83 ± 0.76	10.7 ± 0.10
F2	11.60 ± 0.36	11.4 ± 0.26
F3	12.63 ± 0.91	12.1 ± 0.40

Description
F1: PFNP capsule formula with a dose of 100 mg
F2: PFNP capsule formula with a dose of 150 mg
F3: PFNP capsule formula with a dose of 200 mg

3.4.2 Antibacterial in *P. emblica* Fruit Nanoparticle Capsules

Table 6 and Figure 5 display the outcomes of the antibacterial activity assessment conducted on the *Phyllanthus emblica* fruit

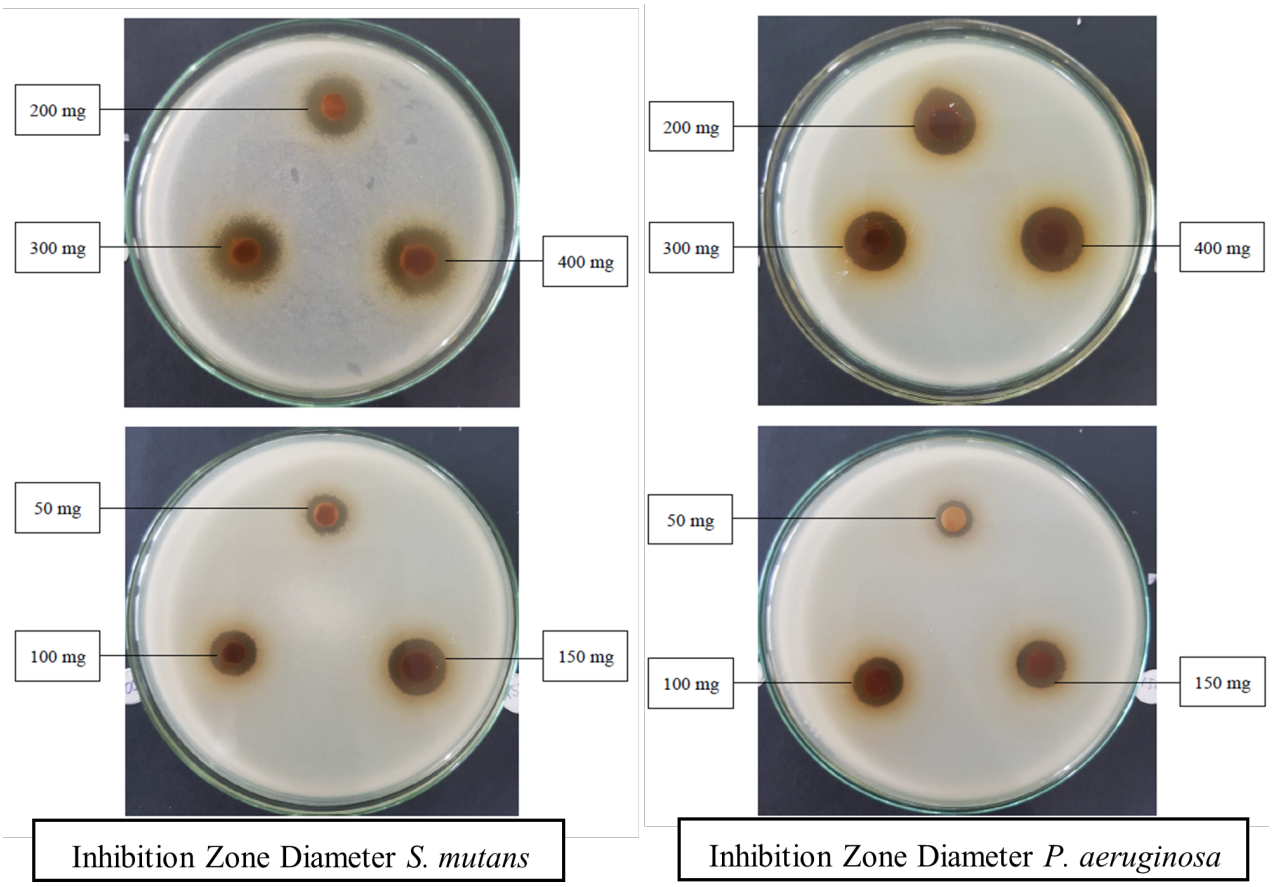

Figure 4. Results of Antibacterial Activity Test of *Phyllanthus emblica* Fruit Nanoparticles

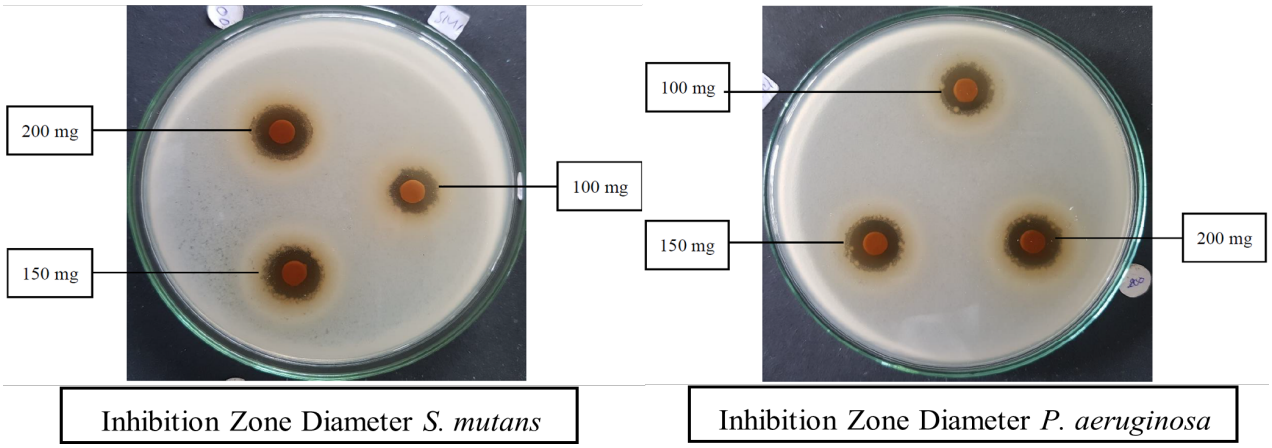

Figure 5. Results of the PFNP Capsule Antibacterial Activity Test

nanoparticle capsule (PFNP).

As per the findings of Davis and Stout (1971), there are four discrete classifications of bacterial inhibition. Per the predetermined standards, a bacterial inhibition zone is deemed feeble if its measurement in diameter is equal to or less than 5 mm. It is essential to highlight that a moderate inhibition zone is characterized by a diameter within the 5 to 10 mm range. The third criterion about the strength of bacterial inhibition zones is satisfied when the diameter of the zone above is 10 to 20 mm. The fourth criterion is deemed to be met when the diameter of the bacterial inhibition zone is greater than 20 mm, which indicates a highly potent inhibition zone. The results suggest that the nanoparticle capsule obtained from *Phyllanthus emblica* fruit demonstrated significant antibacterial properties

against *S. mutans* and *P. aeruginosa*. This was shown by the high antibacterial activity observed at all tested doses.

Antibacterial activity can be caused by components of secondary metabolites, namely phenolic compounds in the form of flavonoids and tannins. Flavonoids and tannins are bactericidal but not sporicidal (Setyoningrum et al., 2021). Phenolic compounds interact with bacterial cell wall components, which can cause changes in the cell wall's permeability, then cause cell coagulation, which can also lead to the inhibition of bacterial intracellular enzymes (Aljadi and Yusoff, 2003).

3.4.3 Anti-inflammatory Activity Test Results of *P. emblica* Fruit Nanoparticle Capsules

The results of anti-inflammatory activity against positive controls with concentrations of 5 μg/mL, 10 μg/mL, 20 μg/mL, and 40 μg/mL and a test solution of 10 μg/mL can be seen in Table 7.

Table 7. Test Results for the Anti-Inflammatory Activity of the PFNP Capsule

Concentration (μg/mL)	Absorbance	% inhibition
Negative control	2.397	-
Positive control (5 μg/mL)	2.157	10.01
Positive control (10 μg/mL)	2.143	10.6
Positive control (20 μg/mL)	2.117	11.68
Positive control (40 μg/mL)	2.059	14.10
F1 capsule	2.262	5.63
F2 capsule	2.250	6.13
F3 capsule	2.234	6.80

Description
F1: PFNP capsule formula with a dose of 100 mg
F2: PFNP capsule formula with a dose of 150 mg
F3: PFNP capsule formula with a dose of 200 mg

Anti-inflammatory activity was observed in the negative control based on the percentage of inhibition results. The rate of inhibition outcomes in the positive control exhibited an upward trend with the rise in concentration, which was also observed in the capsule test solution. The F3 capsule showed the highest level of inhibition, as evidenced by the test solution results, which yielded a %inhibition value of 6.80.

4. CONCLUSION

Phyllanthus emblica fruit nanoparticle powder can be formulated in capsule dosage forms with 100 mg, 150 mg, and 200 mg. The study found that capsules containing nanoparticles of *Phyllanthus emblica* fruit did not exhibit anti-inflammatory properties. However, they were found to possess antibacterial activity against *Streptococcus mutans* bacteria, with effective concentrations beginning at a dose of 100 mg and resulting in an inhibition zone diameter of 10.83 mm. Similarly, the capsules were effective against *Pseudomonas aeruginosa* at a dose of 100 mg, resulting in an inhibition zone diameter of 10.7 mm.

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