

Antibacterial Efficacy of Chitosan Nanoparticles from *Musa paradisiaca* L. Peel Ethanol Extract against *Propionibacterium acnes*, Characterization, and Identification of the Compounds

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Abstract

Hijau lumut banana peel (*Musa × paradisiaca* L.) contains bioactive compounds with antibacterial properties and has potential as a natural anti-acne agent. The preparation and characterization of chitosan-based nanoparticles incorporating hijau lumut banana peel (HLBP) ethanol extract, evaluate their antibacterial activity against *Propionibacterium acnes*, and identify their chemical constituents were the goal of this study. Ethanol maceration was used to get HLBP extract, which was then combined with chitosan and sodium tripolyphosphate (NaTPP) in the ionic gelation process to create nanoparticles. The broth microdilution technique was used to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), while the agar diffusion assay was used to evaluate the antibacterial activity. Nanoparticles were characterized using particle size analysis (PSA), SEM, TEM, FTIR, and LC-MS/MS. The nanoparticles exhibited strong antibacterial activity against *P. acnes*, with inhibition zones of 31.47 ± 0.03 mm and 28.15 ± 0.10 mm for formulations containing 0.5% and 1% chitosan, respectively. MIC and MBC values were 1.25 and 2.50%, respectively. The optimal formulation (0.5% chitosan) had a particle size of 721.9 ± 25.57 nm, a polydispersity index of 0.509 ± 0.018 , and a zeta potential of -36.2 ± 0.60 mV. Morphological analysis revealed irregular flattened particles. These findings suggest that chitosan nanoparticles loaded with HLBP have encouraging antibacterial properties against *P. acnes* and could be used as a natural anti-acne formulation.

Keywords

Antibacterial Activity, Banana Peel, Chitosan, Nanoparticles, *Propionibacterium acnes*

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1. INTRODUCTION

Everyone, especially women, tends to want to have beautiful and healthy skin to increase their self-confidence. The skin is the outermost organ that makes up the human body which is located at the outermost level and covers the entire surface of the body, making the skin susceptible to disease (Abadi et al., 2021). Acne is blackheads that are infected by bacteria (Rudiyat et al., 2020). One of the causes of acne that is naturally found in normal skin is *Propionibacterium acnes*. Antibiotic-type chemical compounds such as clindamycin and tetracycline have long been used to treat acne, but sometimes they have side effects in the form of skin irritation and other health problems, so their use is limited. So natural ingredients are needed to treat acne (Rismana et al., 2014). One ingredient that can be used is hijau lumut banana peel (HLBP).

Based on a previous study investigating the antibacterial ac-

tivity of methanolic extracts from several local Balinese banana peels, hijau lumut banana peel extract at a concentration of 20% demonstrated inhibitory effects against *Staphylococcus aureus* and *Escherichia coli*, with inhibition zone diameters of 12.30 mm and 11.67 mm, respectively (Rita et al., 2020). However, the observed antibacterial activity was relatively moderate, indicating the need for strategies to enhance its efficacy. One promising approach is the application of nanotechnology.

Nanotechnology has attracted widespread interest because of its many beneficial properties, including reduced particle size, improved delivery of active compounds in food and pharmaceutical products, controlled release of bioactive substances, enhanced solubility, and increased absorption within the body (Ningsih et al., 2017). In recent years, green nanotechnology, which utilizes natural materials and environmentally friendly processes for nanoparticle synthesis, has been increasingly ex-

plored. The incorporation of nanotechnology can improve the penetration of active compounds through the dermal layer of the skin, thereby enhancing their therapeutic potential. Chitosan is widely employed as a biopolymer in nanoparticle formulations because of its excellent biocompatibility and encapsulation efficiency. Therefore, Encapsulation based on chitosan is a viable method for enhancing antibacterial properties and bioavailability of active compounds derived from banana peel extracts (Verma et al., 2019; Abd-Allah et al., 2020).

Chitosan encapsulation utilizes ionic gelation to entrap active ingredients within a protective biopolymeric matrix. Due to its insolubility at neutral pH, the encapsulation process typically involves dissolving chitosan in a weak organic acid, followed by the incorporation of the core material and this was followed by the incorporation of a cross-linking agent. This encapsulation strategy enhances antibacterial efficacy against acne-causing bacteria by combining the intrinsic antimicrobial properties of chitosan incorporated into advanced nanocarrier systems thereby enhancing the stability, increasing bioavailability, and enabling targeted delivery of bioactive compounds (Detsi et al., 2020; Luthfiyana et al., 2024).

Stronger antibacterial activity against *S. aureus* and *E. coli* was demonstrated by the chitosan-based nanoparticle formulation containing HLBP extract than the conventional (non-nanoparticle) extract formulation (Rita et al., 2023). Several studies have reported the anti-acne potential of nano-chitosan formulations containing plant extracts, including mangosteen peel (Rismana et al., 2014), Semarang guava (Sekar and Halim, 2017), and cork tree bark (Kuo et al., 2021). Characterization of nanoparticles generally involves the evaluation of particle size and stability, morphology, functional groups, and chemical composition. However, limited studies have focused on the preparation of nano-chitosan formulations derived from banana peel extracts for anti-acne applications. This study was designed to formulate HLBP ethanol extract into chitosan nanoparticles, characterize the resulting nanoparticle system, and determine its antibacterial potential against *Propionibacterium acnes*, one of the primary bacteria associated with acne development.

2. EXPERIMENTAL SECTION

2.1 Materials

The plant material used was hijau lumut banana peel obtained around Denpasar, Bali. The bioassay material was the *Propionibacterium acnes* culture utilized in the experiment was acquired from the Microbiology Laboratory, Faculty of Medicine, Udayana University, Denpasar.

The chemicals used in this research were ethanol (C₂H₅OH) (PT. Brataco, Indonesia), TLC plate GF 254 (PT Merck Tbk.), Macconkey powder (Merck Germany), goat blood (Sigma-Aldrich, USA), TSB (Tryptone Soy Broth) (Sigma-Aldrich, USA), erythromycin (Sigma-Aldrich, USA), Mac Farland standard (Sigma-Aldrich, USA), technical tween-80 (PT Merck Tbk.), sterile distilled water, acid glacial acetate (CH₃COOH) (PT

Merck Chemicals and Life Sciences Indonesia), NaTPP technical grade 85% and low molecular weight chitosan with a deacetylation degree of 75% (Sigma-Aldrich, USA).

The instruments used were DR-200Bc ELISA Microplate Reader, Fourier Transform Infrared Spectrometer (FTIR) (Shimadzu IR Prestige-21), Particle Size Analyzer (PSA) (Hori-ba Scientific SZ-100 Japan), Scanning Electron Microscopy (SEM) (SEM-EDX JEOL JSM-6510LA Japan), Transmission Electron Microscopy (TEM) (Hitachi HT7700 Japan), and Liquid Chromatography Tandem Mass Spectroscopy (LC-MS/MS) (Waters).

2.2 Methods

2.2.1 Sample Preparation and Extraction of Compounds in Banana Peels

HLBP were collected and air-dried, then ground into powder (water content less than 10%). Ethanol was used to macerate 2 kg of dried banana peel powder, with the powder kept fully immersed in the solvent for approximately 24 h. Furthermore, While the dregs were re-macerated with ethanol until all of the chemicals were removed, the filtrate was filtered. A rotatory vacuum evaporator was used to collect and evaporate the filtrate at low pressure and 40 °C until a concentrated ethanol extract (crude extract) was produced (Rita et al., 2023). The extract was stored to be continued in making of nanoparticles.

2.2.2 Preparation of Nanoparticles

Ionic gelation was used to produce nanoparticles, in which chitosan and NaTPP served as the encapsulating agents, following the best formulation reported by Rita et al. (2023). First, 1 g of an appropriate amount banana peel ethanol extract was dissolved in 35 mL of ethanol. The mixture was then mixed with 15 mL of distilled water in a 1000 mL beaker, followed by the addition of 50 mL of 1% chitosan solution formulated in 1% glacial acetic acid.

Subsequently, 50 mL of 1% NaTPP solution was introduced dropwise into the mixture while continuous stirring was maintained at 3800 rpm using a magnetic stirrer for 2 h, resulting in the formation of a precipitate. After a homogeneous colloidal suspension was obtained, Centrifugation at 3000 rpm for 15 minutes was used to separate the generated colloids.

The resulting sediment was then stored in a freezer at 3 °C for 2 days. After freezing, the material was dried using an air cooler until completely dry. After that, the sample was manually ground with a mortar and pestle until it was a fine powder. The same procedure was repeated using a chitosan concentration of 0.5% (Rita et al., 2023). This process was carried out repeatedly according to the quantity of nanoparticles required for the study.

2.2.3 Antibacterial Activity Assay

The Kirby-Bauer disc diffusion assay was used to evaluate the antibacterial qualities of the HLBP ethanol extract and its nanoparticle formulation. Their antibacterial performance was evaluated by measuring the diameter of the clear inhibition

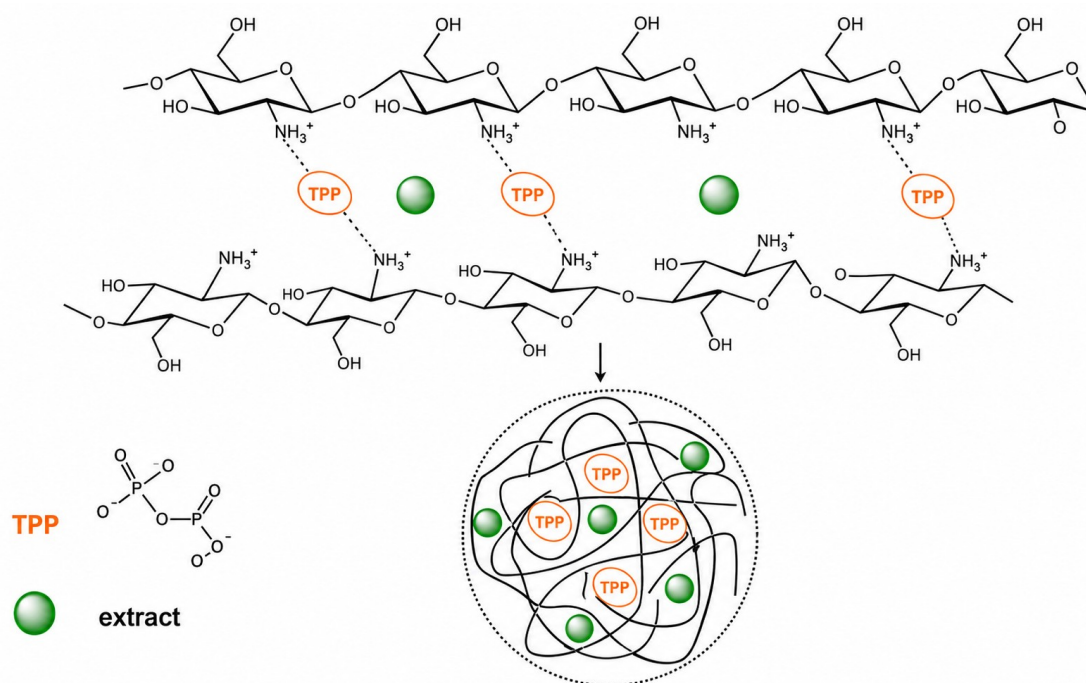


Figure 1. The Reaction of NaTPP, Chitosan, and Ethanol Extract of Hijau Lumut Banana Peel (Detsi et al., 2020)

zone surrounding each disc, indicating suppression of bacterial growth. The bacterial suspension was uniformly distributed across the agar medium's surface using a sterilized cotton swab and allowed to stand for approximately 5 minutes. Sterile disc papers were then placed using forceps sterilized over a Bunsen burner flame, and 20 μ L of each extract solution at a concentration of 10% was applied to the discs. Erythromycin (15 μ g/disc) was used as the positive control. Subsequently, using sterile forceps, the discs were positioned on Blood Agar plates and lightly pressed to establish firm contact with the agar surface. The plates were then incubated anaerobically using a gas-generating kit for approximately 5 days at 37 °C. After incubation, the diameter of the clear inhibition zone surrounding each disc was measured and recorded (Niederstebruch et al., 2017). To ensure that the experimental results were reliable and could be reproduced consistently, the antibacterial assay was performed in triplicate.

The broth microdilution method was employed to determine the MIC using serial concentrations between 20% and 0.3125% (w/v). The first well is filled with the highest concentration of antimicrobial solution. Serial dilutions (doubles) are made in subsequent wells to the sides until a range of concentrations is achieved. 100 μ L of microbial suspension with equal volume into each well. The mixture was incubated at 37 °C for 48 hours. The minimum concentration of antimicrobial present in the first clear well (no visible growth) is the MIC value. Optical density at 600 nm was evaluated with a microplate reader to calculate turbidity in the broth microdilution test. The MBC was determined by subculturing on

antibacterial-free agar, where no bacterial colonies are observed (David et al., 2021; Lallemand et al., 2016; Wiegand et al., 2008). A one-way analysis of variance (ANOVA) and Tukey's multiple comparison test were used to assess the data.

The percentage inhibition of *P. acnes* growth was determined using Equation (1):

$$\text{Inhibition (\%)} = \frac{\text{OD}_{\text{control}(+)} - \text{OD}_{\text{sample}}}{\text{OD}_{\text{control}(+)}} \times 100 \quad (1)$$

where:

- OD_{control(+)} = optical density of the positive growth control (bacteria without treatment)
- OD_{sample} = The treated bacterial culture's optical density

2.2.4 Characteristics of the Antibacterial Nanoparticle

This subsection focuses on interval estimation for the parameter of the XShanker distribution. The CIs considered here are developed under both asymptotic likelihood theory and bootstrap resampling frameworks. Characterization of the nanoparticles was carried out using PSA, SEM, TEM, and FTIR Spectroscopy. The size distribution of the generated particles was ascertained using PSA. The generated nanoparticles' surface morphology was examined using SEM, and their size and shape were more thoroughly assessed using TEM. Additionally, the functional groups of the nanoparticles were identified and characterized using FTIR spectroscopy (Fitri et al., 2020).

2.2.5 Analysis of Compound Content Using LC-MS/MS

The nanoparticles were dissolved in ethanol, filtered through Whatman filter paper, and loaded onto a reversed-phase C18

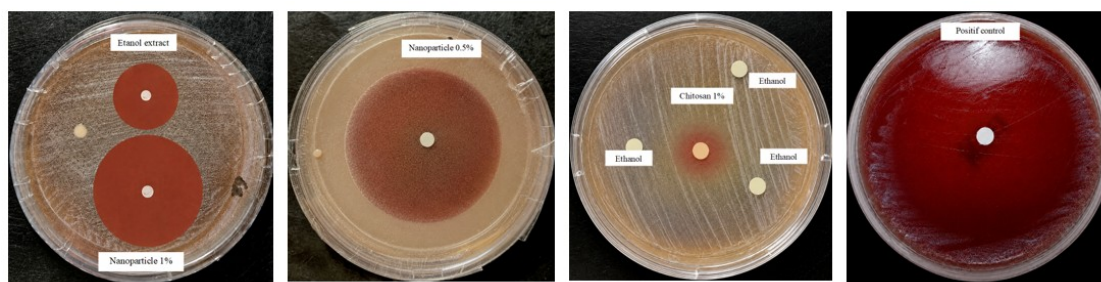


Figure 2. Antibacterial Assay Results of HLBP Ethanol Extract and Chitosan Nanoparticles against *Propionibacterium acnes* at Concentration 10%

pre-column (BEH, 1.7 μm , 2.1 mm internal diameter $\times$ 20 mm). Separation was performed using UPLC with mobile phases consisting of acetonitrile containing 0.05% formic acid (v/v) and water containing formic acid. The isolates were eluted at a flow rate of 0.2 mL/min for 30 minutes. An Acquity UPLC H-Class System connected to a quadrupole time-of-flight mass spectrometer (Xevo G2-S QToF) with a positive electrospray ionization (ESI) source was used to examine the resultant eluate. Data acquisition was performed using Xevo G2-S QToF software, and all mass spectrometry spectra were processed and analyzed using MassLynx version 4.1 (Riwanti et al., 2023).

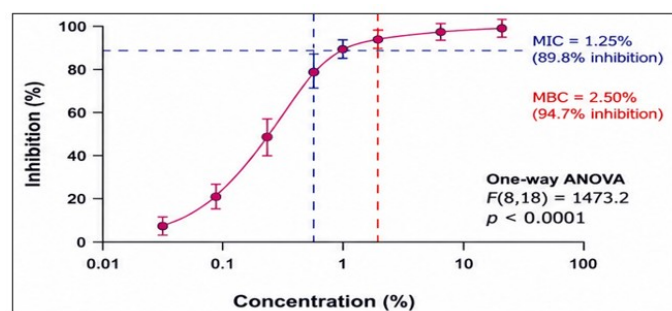


Figure 3. Graph of the Relationship between the Concentration of Chitosan Nanoparticles 0.5% Inhibition Value in Determining MIC and MBC

3. RESULTS AND DISCUSSIONS

3.1 Plant Preparation

The sample used in this study was HLBP (*Musa $\times$ paradisiaca* L.) obtained around Denpasar Bali. The determination of this plant was carried out at the Laboratory of the Bali-BRIN "Eka Karya" Botanical Garden. The results revealed that the plant investigated in this study can be ascertained to be *Musa $\times$ paradisiaca* L.

Fresh banana peels were collected, thoroughly rinsed to eliminate adhering impurities, subsequently cut into small sections, and air-dried to reduce their moisture content. This drying step helps prevent microbial growth, as humid condi-

tions can promote the proliferation of microorganisms that may interfere with both the extraction and storage processes (Qiu et al., 2024). After drying, the banana peels were blended into a fine powder for further processing.

3.2 Water Content Test

Water content testing was conducted as a physicochemical parameter because this plays a crucial role in determining the stability of bioactive constituents in herbal materials throughout the storage period. According to Zambrano et al. (2019), dried materials should have a moisture content of no more than 10%. Excessive moisture can promote fungal contamination, which may compromise the quality of the sample. As reported by Palacios et al. (2022), fungal growth can degrade bioactive compounds, leading to inaccurate activity test results because any observed inhibition may be attributed to fungal contamination rather than the extract itself. In this study, the water content was $7.78 \pm 0.04\%$, which complies with the Indonesian National Standard.

3.3 The HLBP Extraction

Extraction of HLBP was carried out using the maceration method. The maceration method was used to prevent damage to the active substance contained in the sample. From 1 kg of HLBP powder, 49.7406 g of ethanol extract was produced, which was blackish brown in color.

3.4 Preparation of Nanoparticles from HLBP Ethanol Extract

The nanoparticles were prepared using the ionic gelation process, and the resulting formulations are presented in Table 1. Using this technique, positively charged chitosan amino groups and negatively charged TPP ions electrostatically crosslink to produce colloidal particles in the nanoscale size range (Santo et al., 2021).

The addition of NaTPP serves as a cross-linking agent, promoting the formation of intermolecular bridges between chitosan chains. These cross-links create a stable polymeric matrix that acts as a wall surrounding and entrapping the drug. As a result, the nanoparticles exhibit enhanced mechanical strength and improved stability at neutral pH. During the nanoparticle formation process, the ethanol extract becomes encapsulated

Table 1. Nanoparticles Produced from the Ethanol Extract of HLBP

Chitosan Concentration (%)	Dry Nanoparticle Weight (grams)	Nanoparticle Color
0.5	1.62±0.10	Yellow
1	1.77±0.14	Yellow

Table 2. Antibacterial Assay Results of HLBP Ethanol Extract and Chitosan Nanoparticles against *Propionibacterium acnes* at Concentration 10%

Testing Materials	Inhibition Zone (mm)
Ethanol extract of HLBP	20.37±0.20 ^c
Chitosan Nanoparticles 0.5%	31.47±0.03 ^a
Chitosan Nanoparticles 1%	28.15±0.10 ^b
Chitosan 1%	13.30±0.23
Positive control (<i>Erythromycin</i>) 15 µg/disk	59.33±0.58
Negatif Control (Ethanol)	0.00±0.00

Note: numbers accompanied by different letters represent significantly different values at the 5% level

within the pores of the chitosan matrix (Stefanache et al., 2025) . In this study, nanoparticles containing banana peel ethanol extract were prepared using two different chitosan concentrations, namely 0.5% and 1.0%. The interaction among NaTPP, chitosan, and the banana peel ethanol extract during nanoparticle formation is illustrated in Figure 1.

3.5 Antibacterial Activity of Ethanol Extract and The Chitosan Nanoparticles

The inhibitory activity test of the crude extract and nanoparticles was conducted in triplicate and analysed using IBM SPSS Statistics 22.0. All of the data were found to be regularly distributed by the normality test. Subsequently, a homogeneity test was performed, revealing homogeneous variances among the groups ($p > 0.05$). Based on the results obtained, differences between the treatments were further examined using the Least Significant Difference (LSD) post hoc test. The antibacterial activities of the ethanol extract of HLBP and its nanoparticles against *Propionibacterium acnes* are presented in Table 2 and Figure 2.

The results presented in Table 2 show that the inhibition zone of the ethanol extract against *P. acnes* was 20.37 ± 0.20 mm, whereas the chitosan nanoparticles containing 0.5% and 1% chitosan exhibited inhibition zones of 31.47 ± 0.03 mm and 28.15 ± 0.10 mm, respectively. These findings demonstrate that nanoparticle formulations possess greater antibacterial activity than the crude ethanol extract. The enhanced activity can be attributed to the reduced particle size of the nanoparticles, which provides a larger surface area and increases the interaction between the active compounds and bacterial cells. The large surface area and high proportion of surface atoms facilitate penetration through the bacterial cell membrane, thereby improving antibacterial efficacy (Ly et al., 2024). The data presented in Table 2 indicate significant differences between the ethanol extract and the nanoparticles prepared with varying

chitosan concentrations. Interestingly, increasing the chitosan concentration from 0.5% to 1% caused the antibacterial activity to decline, as reflected by the smaller inhibition zone. This reduction may be associated with changes in nanoparticle characteristics, such as increased particle size or reduced release of bioactive compounds at higher chitosan concentrations. Nevertheless, chitosan itself contributes to the antibacterial effect by interacting with the negatively charged bacterial cell wall, forming a polymeric layer that disrupts nutrient transport into the cell. This interference ultimately inhibits bacterial growth and can lead to cell death (Mawazi et al., 2024).

Chitosan has been reported to exhibit significant antibacterial activity against *P. acnes*. However, chitosan nanoparticles possess a higher surface charge density due to their polycationic nature, enabling stronger interactions with bacterial cell surfaces compared to chitosan in its conventional form. Furthermore, chitosan nanoparticles can disrupt the integrity of bacterial membranes and cell walls, resulting in the leakage of intracellular constituents and ultimately leading to bacterial cell death (Chandrasekaran et al., 2020).

The antibacterial activity observed in both the ethanol extract and the activity displayed by the nanoparticle formulations is caused by the presence of secondary metabolites. These substances prevent bacterial development in a number of ways, such as by rupturing the cell wall and causing cell lysis, alteration of membrane permeability, denaturation of cellular proteins, and inhibition of intracellular enzymatic activities (Lobiuc et al., 2023).

Ethanol, used as the negative control, did not exhibit any inhibitory effect against bacterial growth, despite its well documented antiseptic properties. The absence of an inhibition zone may be attributed to the high volatility of pure ethanol, which evaporates rapidly and therefore does not remain in contact with the bacteria long enough to exert a sustained antimicrobial effect. Furthermore, the physicochemical prop-

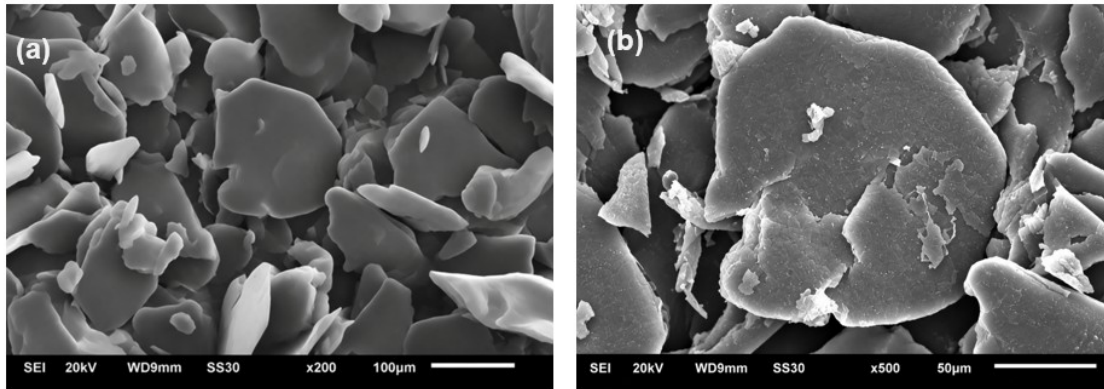


Figure 4. Results of the Nanoparticles' Scanning Electron Microscopy at 200× Magnification (a) and 500× Magnification (b)

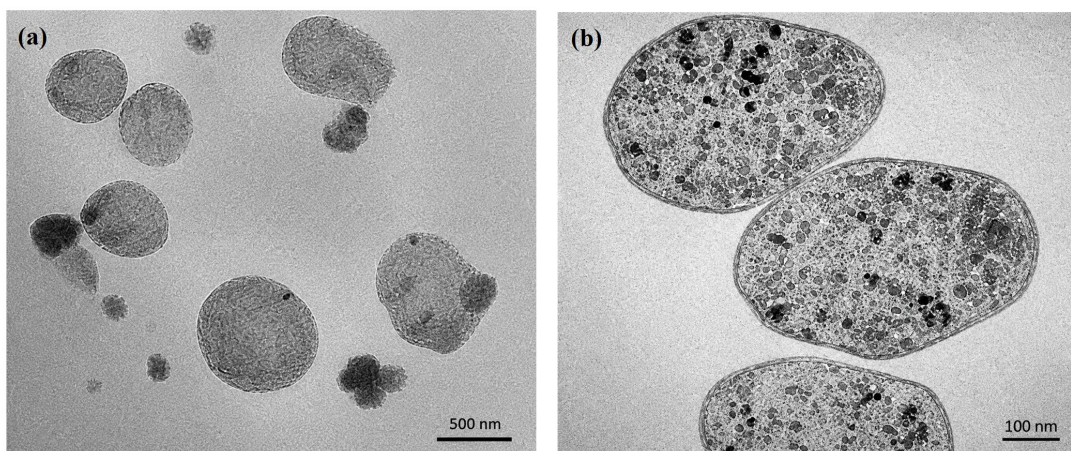


Figure 5. Transmission Electron Microscopy Results of The Nanoparticles with 10,000× Magnification (a), and 50,000× Magnification (b)

erties of ethanol limit its diffusion through the agar medium, resulting in uniform dispersion rather than the formation of a distinct inhibition zone. Pure ethanol is also considered less effective as an antimicrobial agent than diluted ethanol solutions. At high concentrations, ethanol causes rapid coagulation of bacterial surface proteins, forming a protective barrier that restricts further penetration of the alcohol into the cell. Consequently, intracellular targets are not effectively reached, leading to reduced antibacterial activity (Lim et al., 2023).

The nanoparticles with formulation 0.5% chitosan exhibited strong antibacterial activity against *P. acnes*. The MIC and MBC were determined to be 1.25% and 2.50%, respectively. Higher concentrations were associated with greater inhibition of bacterial growth, reaching 89.8% inhibition at the MIC level. Significant differences across the treatment groups were identified through one-way ANOVA ($F = 1473.2$, $p < 0.0001$), indicating potent antibacterial efficacy of the nanoparticles against *P. acnes*. Tukey HSD showed that concentrations $\geq 1.25\%$ were significantly different from the positive control ($p < 0.05$). The results are presented in the Figure 3. Inhibition was calculated

using Equation 1.

3.6 Characterization of Nanoparticles

3.6.1 Particle Size Analyzer (PSA)

Particle size characterization of the chitosan nanoparticles was performed with a PSA utilizing the Dynamic Light Scattering (DLS) technique. The 0.5% chitosan nanoparticle formulation exhibited the smallest particle size and fell within the nanometer range (Table 3). In contrast, the particle sizes of the 1% chitosan nanoparticle formulation and the ethanol extract exceeded 1000 nm. However, the particle sizes obtained were still relatively large, exceeding 500 nm. This may be attributed to the absence of stabilizing agents such as polyvinyl alcohol (PVA). According to Nafee et al. (2007), the addition of stabilizers such as PVA can help control particle size. At an optimal concentration, PVA can reduce particle diameter and improve nanoparticle characteristics. However, when the concentration of the stabilizer exceeds the optimal level ($>3\%$), nanoparticle stability may be compromised, leading to particle aggregation.

The particle size distribution and polydispersity index (PDI) are commonly used as parameters to assess particle size unifor-

Table 3. Particle Size (nm), Polydispersity Index, and Zeta Potential (mV) of the Ethanol Extract and the Nanoparticles

Formulation	Particle Size (nm)	Polydispersity Index	Zeta Potential (mV)
Ethanol extract	3,339 ± 67.18	0.9333 ± 0.1155	-0.62
Nano-chitosan 0.5%	721.9 ± 25.57	0.509 ± 0.0181	-36.2
Nano-Chitosan 1%	1,167.6 ± 18.61	0.623 ± 0.0281	-19.64

mity. The PDI indicates the extent of molecular mass distribution within a particular sample and provides an indication of the breadth of the particle size distribution. A lower PDI indicates a more homogeneous particle size distribution, which is associated with enhanced formulation stability. Conversely, an elevated PDI value reflects a wider variation in particle size, indicating a less homogeneous particle population, which may lead to faster flocculation and reduced stability of the formulation. The PDI ranges from 0 to 1. The best PDI result was obtained for the nano-chitosan formulation containing 0.5% chitosan, with a value of 0.509 ± 0.0181 . The PDI values of both nano-chitosan formulations fall within the intermediate range (0.08-0.7), which represents the range in which the particle size distribution algorithm operates most effectively. Particle populations with a PDI exceeding 0.7 are characterized by substantial size heterogeneity, and a higher likelihood of particle aggregation. Such a broad distribution can result in lower suspension stability (Al-Darwesh et al., 2024).

The difference in electrical potential between the particle surface and the surrounding diffuse layer of ions is known as the zeta potential that is strongly bound to the surface of a solid particle and the electroneutral region of the surrounding solution. A high surface charge on nanoparticles prevents aggregation due to the strong electrostatic repulsive forces between particles. The greater the magnitude of the zeta potential, the more effectively it prevents flocculation, which is the process by which small colloidal particles aggregate into larger particles. Particles with a high positive or negative zeta potential exhibit strong repulsive forces, whereas particles with a low zeta potential tend to experience attractive forces, leading to aggregation and reduced stability (Akbari et al., 2011).

Zeta potential values of approximately ± 5 mV indicate rapid aggregation. However, only short-term stability is provided by levels around ± 20 mV. Good stability is generally thought to be provided by a zeta potential of ± 30 mV, whereas very good stability is indicated by values of ± 60 mV. The zeta potential of the 0.5% nano-chitosan formulation was classified as good because it remained within the ± 30 mV stability range (Sathiyabama et al., 2024).

3.6.2 Scanning Electron Microscopy (SEM)

The morphological characteristics of the nanoparticles containing the ethanol extract of HLBP at a concentration of 0.5% were analyzed using SEM at magnifications of 200× and 500×, as shown in Figure 4, while the EDX results are presented in Table 4. The SEM micrograph revealed irregular plate-like particles with a tendency to agglomerate. Based on image cal-

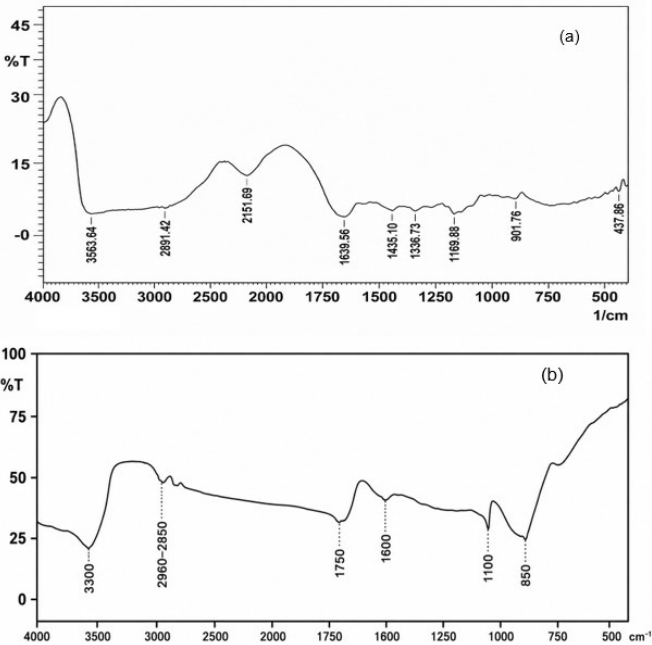


Figure 6. FTIR Spectrum of Chitosan (a) and The Chitosan Nanoparticles (b)

ibration using the 100 μm scale bar, the particle size ranged from approximately 15 to 97 μm , with an average particle size of 52.4 ± 21.3 μm . Most particles were distributed within the range of 30-70 μm .

The data obtained were in the form of two-dimensional images showing the surface morphology of the particle specimens. The particles exhibited a generally non-uniform and relatively flat morphology, which may facilitate nanoparticle agglomeration. This is due to the presence of numerous surface angles and irregularities that promote interactions between particles.

Similar results were also reported by Rita et al. (2023), who stated that nanoparticles of the ethyl acetate extract of HLBP tend to exhibit irregular shapes, including imperfect spherical and oval forms, as well as angular features on the particle surfaces. According to Ulusoy (2023), the greater the number of corners or edges on the particles, the higher the likelihood of agglomeration. This observation is consistent with the PSA results, which showed relatively large particle sizes, indicating that some particles had undergone agglomeration. The size difference between SEM and PSA results indicates that nanometer-sized primary particles (721,9 nm) tend to form micrometer-sized secondary agglomerates (15-97 μm)

Table 4. Surface Chemical Composition of Chitosan Nanoparticles at 500× Magnification Determined by EDX

Element Number	Element Symbol	Element Name	Weight Conc.
8	O	Oxygen	45.93
11	Na	Sodium	19.77
15	P	Phosphorus	16.33
6	C	Carbon	15.88
19	K	Potassium	1.54
17	Cl	Chlorine	0.39
12	Mg	Magnesium	0.09
14	Si	Silicon	0.07

during the drying and SEM sample preparation process.

The relatively large size and broad distribution suggests that further optimization of formulation parameters, such as polymer concentration, crosslinking conditions, stirring speed, and ultrasonication treatment, may be necessary to improve particle uniformity and reduce agglomeration (Danaei et al., 2018). The SEM system was additionally fitted with Energy-Dispersive X-ray Spectroscopy (EDX) to determine the elemental composition of the samples. EDX is a detector integrated with SEM and is used to analyze the chemical composition of a sample's surface. The EDX analysis presented in Table 4 shows that carbon (C) and oxygen (O) are the main components detected. The findings suggest that nanoparticles containing the chitosan biopolymer, represented by the chemical formula $(C_6H_9NO_3)_n$, were successfully produced, sodium tripolyphosphate (NaTPP), and bioactive compounds present in the ethanol extract. Mineral elements like sodium (Na), potassium (K), magnesium (Mg), phosphorus (P), and silicon (Si) were also found in banana peels.

Okorie et al. (2015) demonstrated that both ripe and unripe banana peels contain micronutrients, including zinc (Zn), iron (Fe), lead (Pb), and copper (Cu), as well as mineral elements such as magnesium (Mg), calcium (Ca), sodium (Na), potassium (K), and phosphorus (P). Hydrogen (H) was not identified by EDX because its atomic number ($Z = 1$) is too low for effective detection by this analytical technique, whereas commercially available energy-dispersive X-ray spectroscopy (EDX) systems are generally unable to detect elements with atomic numbers below 11 (Ye et al., 2022). Hydrogen can be identified using proton nuclear magnetic resonance (1H-NMR) spectroscopy rather than EDX.

3.6.3 Transmission Electron Microscopy (TEM)

The TEM analysis findings are illustrated in Figure 5. The synthesized chitosan nanoparticles exhibited diameters ranging from 500 to 700 nm. The nanoparticles displayed a predominantly spherical morphology with smooth surfaces, indicating the successful encapsulation of bioactive compounds derived from banana peels. The porosity of chitosan-tripolyphosphate nanoparticles may increase due to the acid solubility of chitosan. Furthermore, the incorporation of active compounds into the chitosan matrix can lead to morphological expansion

of the nanoparticles (Rita et al., 2023).

The mean particle size determined by DLS was greater than the size observed in TEM images, which is in agreement with the findings reported by Souza et al. (2016), which reported that broad particle size distributions can lead to inaccuracies in DLS measurements when compared with TEM results. DLS tends to indicate a greater proportion of larger particles because it measures the hydrodynamic diameter, which includes the hydration layer surrounding the particles, rather than the actual particle core size observed by TEM. In contrast, TEM images are acquired from dried samples, where particle agglomeration may occur during sample preparation. Furthermore, DLS measurements are performed in liquid media, providing a more representative assessment of particle size under actual dispersion conditions.

3.6.4 Thin Layer Chromatography (TLC)

The separation results obtained using a GF245 TLC plate with an n-hexane:ethyl acetate (5:5) mobile phase are presented in Table 5.

Overall, the findings indicate that the ethanol extract of HLBP contains a compound profile similar to that of the chitosan nanoparticles. This finding suggests that the bioactive compounds contained in the ethanol extract were successfully encapsulated within the nanoparticle matrix. A similar outcome was described in the study by Rita et al. (2023), who found that nine spots on the chromatographic plate of the ethyl acetate extract of HLBP, whereas only six spots were detected in the nanoparticle formulation when using A mobile phase consisting of chloroform and ethyl acetate in an 8:2 (v/v) ratio.

3.6.5 Fourier Transform Infrared (FTIR) Spectroscopy

FTIR spectroscopy was employed to characterize the synthesized nanoparticles and to identify alterations in functional groups that may reflect chemical interactions between the components. The cross-linking interactions between the HLBP ethanol extract, chitosan, and sodium tripolyphosphate (NaTPP) can be observed through shifts in wavenumbers and variations in the intensity of characteristic functional groups (Antasion-asti et al., 2020). In this study, FTIR analysis was performed over the spectral range of 4000-500 cm^{-1} . The FTIR spectra of chitosan and the HLBP ethanol extract nanoparticles are presented in Figure 6.

Table 5. Rf Value of The HLBP Ethanol Extract and Its Chitosan Nanoparticles

Spots	Rf Value	
	The HLBP Ethanol Extract	Chitosan Nanoparticles
1.	0.07	0.12
2.	0.12	0.18
3.	0.23	0.23
4.	0.47	0.57
5.	0.80	-

Table 6. The Results of The Analysis and Estimated Compounds

Retention Times (tR) (minute)	Ions (M+H) ⁺ (m/z)	Molecular formula	Alleged Compounds	Class of compounds
5.08	357.2316	C ₁₉ H ₃₂ O ₆	5,7-Megastigmadien-9-ol glucoside (a)	Glycoside
6.33	267.1599	C ₁₅ H ₂₂ O ₄	6-beta,8beta-Dihydroxyeremophilenolide (b)	isonorterpenoids
7.69	249.1493	C ₁₅ H ₂₀ O ₃	1a-1-Hydroxy-2,4(18),11(13)-eudesmatrien-12-Oic acid (c)	Sesquiterpene lactones
8.44	196.0902	C ₈ H ₁₂ N ₄ O ₂	1,3,9-Trimethylxanthine (Caffeine) (d)	Terpenoids
9.54	289.0876	C ₁₉ H ₁₃ O ₃	Hydroxyanigorufone (e)	Alkaloids
				phenylnaphthalenes

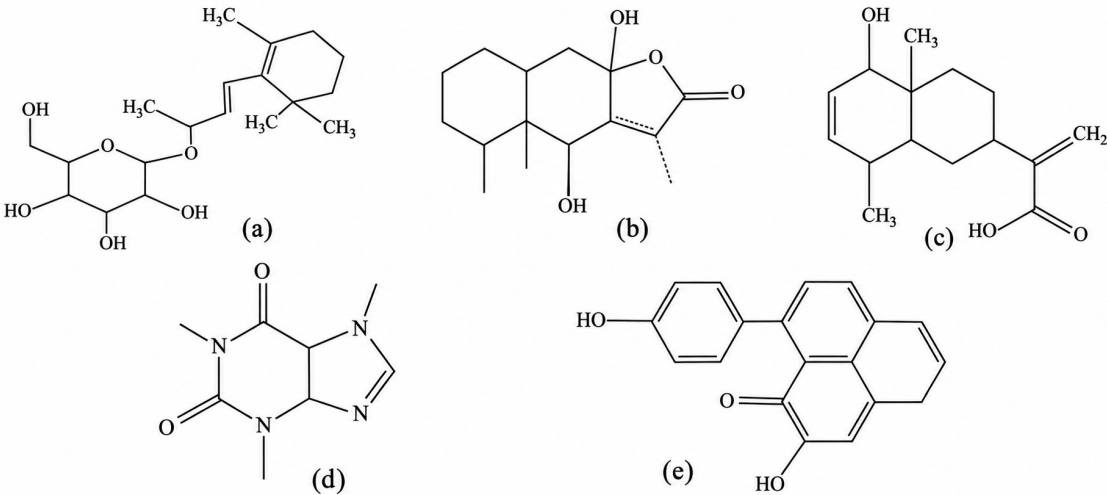


Figure 7. The Structure of The Compounds of The Nanoparticles. 5,7-megastigmadien-9-ol glucoside (a), 6-β,8-β-dihydroxyeremophilenolide (b), 1α-1-hydroxy-2,4(18),11(13)-eudesmatrien-12-oic acid (c), 1,3,9-trimethylxanthine (caffeine) (d), and hydroxyanigorufone (e)

Chitosan exhibits characteristic functional groups, primarily due to the overlapping absorption bands associated with stretching vibrations of amino (N–H) and hydroxyl (O–H) groups, which were observed at 3563.64 cm⁻¹ (Sathiyabama et al., 2024). The absorption peak at 2891.42 cm⁻¹ corresponds to aliphatic C–H stretching vibrations, while the band at 1639.56 cm⁻¹ is attributed to N–H bending vibrations of secondary amine groups, a characteristic feature of the chitosan structure. In addition, the absorption band at 1435.10 cm⁻¹

indicates C–N bending vibrations. A shift in the O–H/N–H stretching band from 3563.64 cm⁻¹ in chitosan to 3653.33 cm⁻¹ in chitosan nanoparticles was observed, accompanied by changes in band intensity and broadening. This shift suggests hydrogen bond formation between the bioactive compounds and the encapsulating chitosan matrix (Putri et al., 2019; Nahrowi et al., 2024). Furthermore, the shift in aliphatic C–H stretching vibrations from 2891.42 cm⁻¹ to 2885.63 cm⁻¹ indicates interactions between non-polar

core compounds and the hydrophobic regions of the carrier matrix (Zahed et al., 2023).

The NH bending vibration observed in chitosan at 1639.56 cm^{-1} shifted to a C=O stretching vibration at 1702.25 cm^{-1} in the nanoparticles. This change is attributed to the utilization of glacial acetic acid as a solvent for chitosan and may result from interactions involving hydroxyl (–OH) groups (Putri et al., 2019). Additionally, the C–N stretching vibration shifted from 1435.10 cm^{-1} in chitosan to 1509.35 cm^{-1} in the nanoparticles, indicating interactions between the encapsulated bioactive compounds and the chitosan polymer matrix. The appearance of a peak at 894.04 cm^{-1} in the nanoparticle spectrum further confirms the presence of phosphate (P=O) groups, which are characteristic of sodium tripolyphosphate (NaTPP) (Rita et al., 2023).

This result corresponds with the findings of Fatoni et al. (2023), who observed that the IR spectrum at 3448.72 cm^{-1} corresponds to the overlapping of –OH and N–H groups stretching vibrations. The weak absorption band observed at 2931.80 cm^{-1} is attributed to aliphatic C–H functional groups. Furthermore, the higher intensity of the peak at 3448.72 cm^{-1} indicates stronger hydrogen bonding interactions in the chitosan nanoparticles compared to pure chitosan.

3.7 Results of Analysis of Compounds from Chitosan Nanoparticle HLBP Ethanol Extract with LC-MS/MS

Identification of chemical compounds in the chitosan nanoparticles was performed using LC-MS/MS. The analysis generated LC chromatograms and mass spectra corresponding to different retention times. Compound identification was conducted using the MassLynx v4.1 software, along with the MassBank, ChemSpider, and PubChem databases. Each chromatographic peak at a specific retention time was characterized based on its protonated molecular ion ($[M+H]^+$), and the molecular formula was determined using the Elemental Composition submenu in MassLynx v4.1. The molecular formula selected was the one with the highest i-FIT percentage. The i-FIT value reflects the degree of agreement between the observed isotopic pattern and the theoretical isotopic distribution, thereby supporting the identification of compounds and their relative abundance in the mass spectrum. The results of the analysis and the estimated compounds can be seen in Table 6, while the proposed chemical structures are shown in Figure 7.

Megastigmanes are a class of terpenoid compounds characterized by a C13 carbon skeleton and are considered non-terpenoids derived from the degradation of carotenoids and related sesquiterpenoid structures. Numerous biological activities have been reported for megastigmane glycosides, including cytotoxic, antibacterial, antiviral, neuroprotective, antioxidant, hepatoprotective, anti-inflammatory, anti-melanogenic, and anti-allergic effects (Samra et al., 2024).

Almeida et al. (2006) reported that caffeine present in coffee extract exhibits antimicrobial activity against *Salmonella enterica*. In addition, caffeine at the $250\text{ }\mu\text{g/mL}$ concentration or higher significantly enhanced antibiotic efficacy, reducing

the MIC values of cefepime, ticarcillin, gentamicin, novobiocin, and azithromycin by approximately 50% against several Gram-negative bacteria, including *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*. Similarly, the findings of Woziwodzka et al. (2022) demonstrated that Caffeine has been shown to influence the efficacy of antibiotics with different chemical structures, exhibiting the greatest synergistic activity when combined with cefepime or azithromycin against Gram-negative bacteria.

Hydroxyanigorufone has showed promise as a natural antibacterial compound. Liu et al. (2014) stated that hydroxyanigorufone, isolated from the fruits of *Musa itinerans* Cheesm., demonstrated weak antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*. In addition, propylene glycol was found to be effective against *Streptococcus mutans*, *Enterococcus faecalis*, and *E. coli*, exhibiting bactericidal activities of 50%, 25%, and 50%, respectively (Nalawade et al., 2015). Furthermore, 3,8-dihydroxy-6-methoxy-7(11)-eremophyllene-12,8-olide, a sesquiterpene lactone, has shown significant antibacterial activity, particularly against Gram-positive bacteria such as *Bacillus subtilis* and *Staphylococcus aureus* (Irwandi et al., 2023).

4. CONCLUSIONS

The chitosan nanoparticle formulation successfully inhibited bacterial growth against *P. acnes*, at chitosan concentrations of 0.5% and 1%, respectively, resulting in inhibition zones of $31.47 \pm 0.03\text{ mm}$ and $28.15 \pm 0.10\text{ mm}$. The nanoparticles formulated with 0.5% chitosan particles possessed an average size of $721.9 \pm 25.57\text{ nm}$, a PDI of 0.509 ± 0.0181 , and a zeta potential of $-36.2 \pm 0.60\text{ mV}$, with a non-uniform flattened morphology. The compounds identified as potential contributors to the antibacterial activity included 5,7-megastigmadien-9-ol glucoside, 6 β ,8 β -dihydroxyeremophyllenolide, 1 α -hydroxy-2,4(18),11(13)-eudesmatrien-12-oic acid, caffeine, and hydroxyanigorufone. Based on these findings, chitosan nanoparticles loaded with HLBP ethanol extract show considerable potential for development as a natural antibacterial agent.

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