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Activity and Characterization of ZnO Nanoparticles from Ethanol Extract of *Plantago major L.* as Antibacterial against *Propionibacterium acnes*

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ABSTRACT: Acne infections are common in tropical regions such as Indonesia and are mainly caused by *Propionibacterium acnes*. Long-term use of antibiotics for acne treatment may lead to bacterial resistance, highlighting the need for safer natural antibacterial alternatives. Ethanol extract of broadleaf (*Plantago major* L.) has been reported to exhibit antibacterial activity, which can be enhanced through the application of nanotechnology in the form of zinc oxide nanoparticles (ZnO-NPs). Therefore, this study aims to synthesize and evaluate the antibacterial activity of ZnO-NPs derived from ethanol extract of broadleaf against P. acnes. The broadleaf extract was obtained through maceration with 96% ethanol, and ZnO-NPs were prepared by a precipitation method using zinc nitrate and zinc acetate precursors. The disc diffusion test results showed that 20 mg of ZnO-NPs acetate from broadleaf extract had strong antibacterial activity, with an inhibition zone of 18.11 ± 0.10 mm. The minimum inhibitory concentration of ZnO-NPs acetate from broadleaf extract is 0.078 mg/mL. The morphological characterization of ZnO-NPs acetate from broadleaf extract using Scanning Electron Microscopy-Energy Dispersive X-Ray (SEM-EDX) show a uniform granular particle shape with a size range of 200-228 nm. Based on the research results, the ZnO-NPs acetate from ethanolic extract of broadleaf has the potential to be an effective topical antibacterial alternative for treating acne infections. Based on these findings, ZnO-NPs synthesized from ethanol extract of broadleaf are expected to enhance antibacterial effectiveness and have potential as a safer and more effective topical antibacterial alternative for acne treatment.

Keywords: **acne vulgaris; nanoparticles; *Plantago major* L.; topical anti-infection; zinc acetate.**

Introduction

Acne vulgaris is a common skin problem in tropical regions, including Indonesia, and can impact self-confidence and quality of life. Acne is caused by infection with the Gram-positive anaerobic bacteria, primarily *Propionibacterium acnes*. Acne infection most commonly affects adolescents, with an incidence of 83–85% in women and 95–100% in men [1].

Acne is a chronic inflammatory skin condition characterized by lesions appearing on the face, neck, chest, back, or upper arms. Acne is caused by a pathogenesis involving excess sebum production, proliferation of *P. acnes*, and an inflammatory response. Treatment generally involves antibiotics such as erythromycin, clindamycin, and tetracycline, but long-term use can lead to side effects and resistances. Antibiotic resistance rates for *P. acnes* reach 12.9% for tetracycline, 45.2% for erythromycin, and 61.3% for clindamycin [2]. This has encouraged the development of safer and more effective natural antibacterial alternatives.

Broadleaf (*Plantago major* L.) have the potential to be natural antibacterials because they contain tannins, alkaloids, flavonoids, glycosides, triterpenoids, and saponins [3]. Ethanol extract was able to inhibit *P. acnes* at concentrations of 25% to 100% weigh per volume (w/v), but not optimally [4]. Broadleaf extract also has antibacterial inhibitory effect against *Staphylococcus epidermidis* at concentrations of 20% to 80% w/v [5]. The application of nanoparticle technology can be done to increase the antibacterial effectiveness of broadleaf extract, for example silver nanoparticles (Ag-NPs) from broadleaf extract are effective in inhibiting *Staphylococcus aureus, Pseudomonas aeruginosa,* and *Escherichia coli* [6].

Nanotechnology-based preparations can be synthesized using various precursors such as

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zinc, silver, gold, chitosan, titanium dioxide, and fullerenes. Their applications are broad, including antibacterial agents, medical device coatings, cancer drug delivery, gene therapy, ultraviolet (UV) protection, and even antivirals [7]. The development of nanotechnology is driven by the need for safer and more effective alternative therapies, as nanoparticles can penetrate bacterial cells and provide a stronger antibacterial effect than conventional antibiotics. Furthermore, nanoparticles can increase the bioavailability of active ingredients, achieving optimal concentrations to treat infections caused by pathogenic bacteria [8,9].

Nanoparticles from zinc precursors or called zinc oxide nanoparticles (ZnO-NPs) are known to be effective as antibacterials, antifungals, wound healers, UV filters, and have semiconductor, photochemical, and catalytic properties [7]. Zinc oxide has been recognized as safe by the Food and Drug Administration (FDA) and is widely used as an additive in various products despite its insoluble nature in water. Zinc oxide nanoparticles synthesis can be carried out using physical methods (steam decomposition, plasma, ultrasonic) or chemical methods (precipitation, microemulsion, chemical reduction, sol-gel, hydrothermal). Precipitation methods using zinc nitrate or zinc acetate precursors are a simple and economical option because they produce nanoparticles of controlled and uniform size through precursor precipitation reactions [10].

Several studies have demonstrated the antibacterial potential of ZnO nanoparticles against a broad spectrum of pathogenic bacteria. Rhamdiyah & Maharani [2022] reported that ZnO synthesized using *Moringa oleifera* leaf extract exhibited antibacterial activity against *S. aureus* and *E. coli* [11]. In addition, Ngangom et al. [2025] showed that ZnO nanoparticles synthesized with naringenin were highly crystalline, nanoscale in size, and displayed significant antibacterial activity against *S. aureus, P. aeruginosa*, *K. pneumoniae*, and E. faecalis [12]. Furthermore, Al-Momani et al. [2024] demonstrated that ZnO-NPs of *Peganum harmala* significantly inhibited both bacterial growth and biofilm formation in all tested *P. acnes* strains [13].

In preliminary research, ethanol extract of broadleaf was able to inhibit the growth of *P. acnes* [4]. Nanotechnology was applied to enhance the antibacterial activity of ethanol extract of broadleaf, using the precursor synthesis method of zinc nitrate and zinc acetate. The obtained zinc oxide nanoparticles were characterized using Scanning Electron Microscope-Energy Dispersive X-ray (SEM-EDX) to determine morphology, crystal size, and elemental composition, as well as Particle Size Analyzer (PSA) to assess its particle size distribution [7].

Based on this background, this study aims to synthesize and characterize zinc oxide nanoparticles (ZnO-NPs) using ethanol extract of *Plantago major* L. and to evaluate their antibacterial activity against *Propionibacterium acnes*. The results of this study are expected to provide scientific evidence that ZnO nanoparticles can enhance the antibacterial effectiveness of broadleaf extract as a safer and more effective alternative for acne treatment.

Methods

Research Objects

Broadleaf plants (*Plantago major* L.) were obtained from a plantation in Awan Village, Kintamani, Bangli Regency, Bali (8°14'51.9"S115°16'55.7"E). Plant determination was carried out at the National Research and Innovation Agency Eka Karya Bali Botanical Gardens. The *P. acnes* ATCC-6919 were obtained from the collection of the Microbiology Laboratory of Bali International University.

Broadleaf Extract Preparation

Fresh broadleaves were washed, chopped, drained, and then air-dried for 1 hour. The leaves were then oven-dried (Biobase) at 40°C for 24 hours, then blended (Waring) and sieved with a 60-mesh sieve to obtain a simplicia [14]. As much as 700 g of broadleaf simplicia was macerated with 3.5 L of 96% ethanol (JK Care, SKU S030906938) in a closed container for 24 hours with stirring every 4 hours, then filtered using Whatman paper no. 1. The residue was soaked again with 3.5 L of new solvent, and this process was repeated every 24 hours for 4 days until the filtrate was clear. All filtrates were combined and then evaporated using a rotary evaporator (Biobase) at 40°C until a thick extract was obtained [15].

Phytochemical Test of Broadleaf Ethanolic Extract

Phytochemical testing was conducted to detect flavonoids, saponins, tannins, alkaloids, triterpenoids, steroids, and phenolic compounds. Flavonoid testing was performed by adding concentrated chloride acid (HCl) and magnesium (Mg) powder to the ethanol extract of broadleaf. Saponin testing was performed by boiling the ground sample in distilled water, then shaking the solution vigorously after cooling [16].

The tannin test is carried out by dissolving the extract in ethanol until it is submerged, then 2–3 drops of 1% ferric chloride ($FeCl_3$) are added to 1 ml of the solution. The alkaloid test is carried out by dissolving 0.5 g of broadleaf extract in 0.5 ml of 1% HCl, then 1–2 drops of Dragendorff's reagent are added, this test follows the

procedure in previous research [17].

The triterpenoid and steroid tests were carried out by dripping anhydrous acetic acid (CH_3COOH) onto 50–100 mg of the extract until it was submerged and left for about 15 minutes, then 6 drops of the solution were transferred to a test tube and Liebermann–Burchard reagent was added [16]. The phenolic test was carried out by adding 2 drops of methanol and 5 drops of 10% $FeCl_3$ to 1 mL of broad leaf extract in a test tube [3].

Zinc Oxide Nanoparticles Synthesis of Broadleaf Ethanolic Extract

A total of 90 mL of 0.25 M zinc nitrate hexahydrate (Zn(NO3)2·6H2O) or 0.2 M zinc acetate dihydrate ($Zn(CH_3COO)_2 \cdot 2H_2O$) solution was stirred with a magnetic stirrer (Ika® C-MAG) at 2000 rpm at 60°C, then the pH was adjusted to 10.0 by adding 5 M NaOH. Next, 10 mL of 20% broadleaf ethanol extract was added to each solution, and stirred for 2 hours until the formation of ZnO-NPs was indicated by a color change to pale yellowish green. The solution was left for 24 hours at room temperature until the ZnO-NPs of the broadleaf ethanol extract were precipitated. The ZnO-NPs precipitate was then washed with distilled water for three times. The clean sediment was dried in an oven at 60°C for 24 hours until the powder of ZnO-NPs nitrate/acetate of broadleaf ethanol extract was obtained [18].

Antibacterial Activity Test against *P. acnes*

Disc diffusion assay test

The inhibitory effect of ZnO-NPs nitrate/acetate of broadleaf ethanol extract against *P. acnes* was carried out using a 6 mm diameter well diffusion method on Mueller Hinton Agar (MHA) media. The test samples included 20% w/v broadleaf ethanol extract, 20% w/v ZnO-NPs nitrate of broadleaf ethanol extract, 20% ZnO-NPs acetate of broadleaf ethanol extract, negative control of 10% v/v ethanol, and positive control of clindamycin concentration of 20 µg/mL. *Propionibacterium acnes* suspension equivalent to Mc Farland 0.5 was streaked on the surface of the MHA media. Then, the sample and negative/positive control were put into the wells as much as 100 µL. The petri dish was incubated at 37°C for 24 hours, then the diameter of the inhibition zone formed was measured.

Microdilution assay to determine the MIC

Determination of Minimum Inhibitory Concentration (MIC) of ZnO-NPs nitrate/acetate of broadleaf ethanol extract was carried out using the two-fold microdilution method on microplates containing Mueller Hinton Broth (MHB) media. The preparation of sample was two-fold diluted gradually with a concentration range of 20-1.25 mg/mL for broadleaf ethanol extract; 12.5-0.097 mg/mL for ZnO-NPs nitrate; 20-0.039 mg/mL for ZnO-NPs acetate; 12.5-0.097 mg/mL for ZnO-NPs nitrate of broadleaf ethanol extract; 20-0.039 mg/mL for ZnO-NPs acetate of broadleaf ethanol extract; negative control ethanol 10% v/v; and 5.0-0.019 µg/mL for positive control clindamycin. The *P. acnes* suspension used for the test was equivalent to a density of 7.5×10^5 CFU/mL. The microplates were incubated at 37°C for 18 hours, and the MIC values were determined using 0.01% resazurin staining [19].

Zinc Oxide Nanoparticles of Broadleaf Ethanol Extract Characterization

Morphology and elemental composition test using SEM-EDX

Samples of ZnO-NPs nitrate/acetate of broadleaf ethanol extract were dried, then placed on double carbon coated stubs. Scanning Electron Microscopy scanning (JEOL 6510LA) was used to view the surface morphology, while EDX analysed the X-rays to identify elements such as Zinc (Zn) and Oxide (O), as well as other elements such as Carbon (C) and Chloride (Cl) [20].

Particle Size using PSA

The ZnO-NPs nitrate/acetate of broadleaf ethanol extract was dispersed in ethanol and sonicated for 5–10 minutes to homogenize. At around 2 mL suspension was placed into a PSA cuvette (Malvern), then analysed with certain parameter settings (ZnO refractive index, temperature, viscosity). The tool utilizes laser light scattering due to Brownian motion to calculate the particle size distribution, displayed as a graph, Z-average (nm), and polydispersity index [20].

Data Analysis

The differences in the inhibition zone diameter from the disc diffusion antibacterial activity test results from various treatment groups were analysed using One Way-ANOVA using SPSS 19. If the conditions were met significant different ($p<0.05$), the ANOVA test results were continued with the LSD test to see the significance of the differences between groups [21].

Result and Discussion

Broadleaf Plant Determination and Extraction Result

Young broadleaves were obtained from a plantation in Awan Village, Kintamani, Bali, which is a fertile and

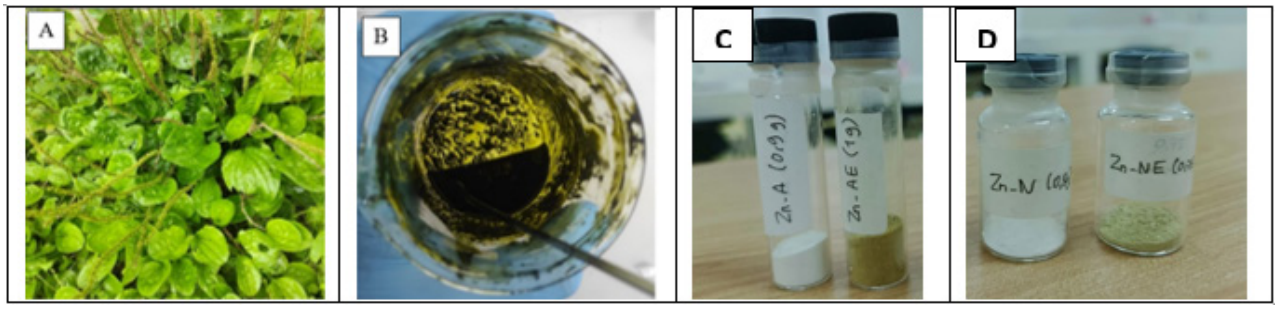


Figure 1. (A) Broadleaf plantain (Plantago major L.) from Awan Village, Kintamani, Bali, (B) broadleaf ethanol extract, (C) ZnO-NPs acetate and ZnO-NPs acetate of broadleaf ethanol extract, (D) ZnO-NPs nitrate and ZnO-NPs nitrate of broadleaf ethanol extract
Note: Zinc Oxide Nanoparticles (ZnO-NPs).

pollution-free area **(Figure 1 (A))**. The ethanolic extract of broadleaves (*Plantago major* L.) showed a thick consistency, greenish-black color, and aromatic odor **(Figure 1 (B))**. The extraction process produced 39.7 g of extract with a yield of 5.6%, lower than the standard 8–15%. [22]. This low yield is influenced by the use of 96% ethanol which is more non-polar than water, making it less effective in extracting water-soluble compounds such as flavonoids, glycosides, saponins, tannins, and water-bound phenolics [15].

Phytochemical Results of Broadleaf Ethanol Extract

Phytochemical screening showed that the ethanol extract of *Plantago major* L. contains flavonoids, saponins, tannins, alkaloids, and triterpenoids (Table 1), which have potential antibacterial, antifungal, antioxidant, and anti-inflammatory properties. These results are in line with the findings of Dewi (2021), which reported the presence of phenolic compounds, flavonoids, saponins, and tannins in broadleaf extract [3]. Rahamouz-Haghighi et al. (2023) was also identified terpenoid and siloxane compounds in broadleaf extract with antibacterial potential [20]. Research results from Wati (2017) found tannin, alkaloid, flavonoid, and saponin compounds in broadleaf extract [2]. Najafian et al. (2018) noted that the extract of broadleaves contains volatile compounds, triterpenoids, phenolics, and flavonoids [22].

Various compounds found in broadleaf extract are known to inhibit bacteria through various mechanisms. Flavonoids and phenolics disrupt membranes and enzymes, saponins damage membranes, tannins bind proteins, alkaloids inhibit protein synthesis, and triterpenoids increase the bacterial membrane permeability [26].

Antibacterial Test Result Towards *P. acnes*

This study aims to optimize the preparation of

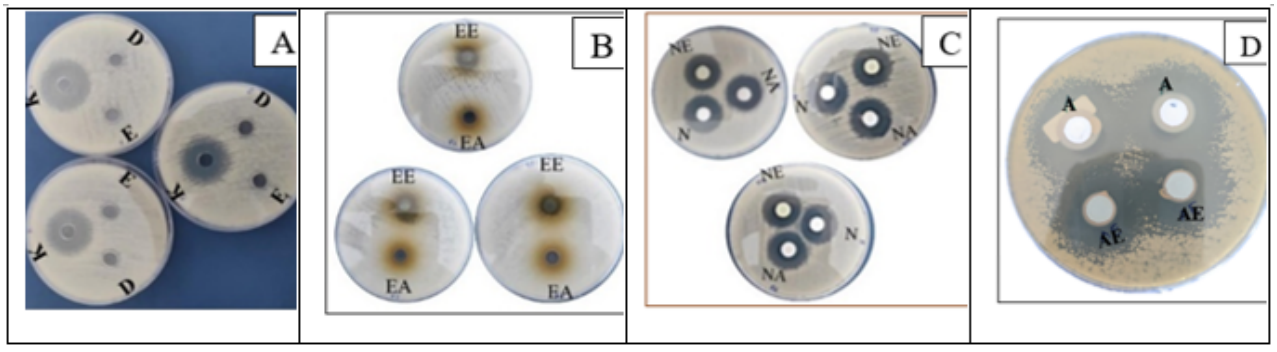
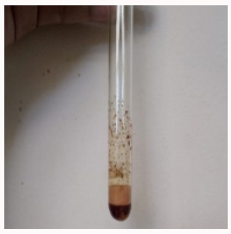
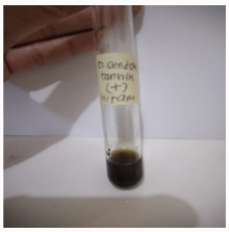
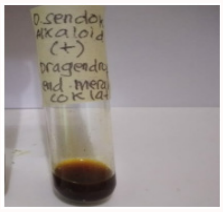
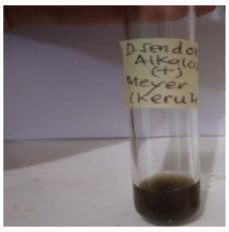
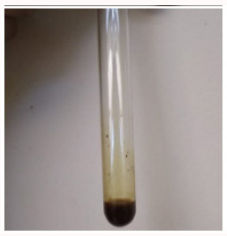
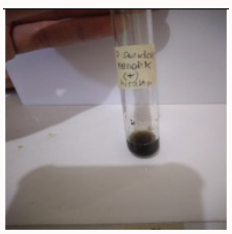


Figure 2. Antibacterial activity test results of zinc oxide nanoparticles of broadleaf ethanol extract (Plantago major L.), (A) DMSO [D], Ethanol [E], and Clindamycin [K], (B) Aqueous extract (Plantago major L.) [EA] and Ethanolic extract (Plantago major L.) [EE], (C) ZnO-NPs nitrate [N], ZnO-NPs nitrate of broadleaf ethanol extract (Plantago major L.) [NE] and ZnO-NPs nitrate of broadleaf aqueous extract (Plantago major L.) [NA], (D) ZnO-NPs acetate [A] and ZnO-NPs acetate of broadleaf ethanol extract (Plantago major L.) [AE]
Note: Zinc Oxide Nanoparticles (ZnO-NPs).

Table 1. Phytochemical screening results of broadleaf ethanol extract (*Plantago major* L.).

Parameter	Reagents	Results	Note	
Flavonoid	HCl + Mg powder	+	Brick red	
Saponin	Heated distilled water + HCl	+	Stable foam	
Tannin	$FeCl_3$ 1%	+	Black	
Alkaloid	Dragendorff	+	Brick red precipitate	
	Meyer	+	Cloudy	
Triterpenoid	Lieberman-Burchard	+	Brownish	
Phenolic	$FeCl_3$ 10%	+	Black	

Parameter	Reagents	Results	Note	
Steroid	Lieberman-Burchard	_	Brown	

ZnO-NPs from broadleaf extract (*Plantago major* L.) as an antibacterial against *P. acnes* and compare it with clindamycin through well diffusion and microdilution tests. The antibacterial test showed that the ZnO-NPs from ethanol extract of broadleaf was better than the extract alone to inhibit the growth of *P. acnes*. The ZnO-NPs acetate from ethanol extract of broadleaf was considered more effective than the ZnO-NPs nitrate from ethanol extract of broadleaf. When compared to the ZnO-NPs acetate control, the ZnO-NPs acetate from ethanol extract of broadleaf had a lower MIC value. When compared to the positive control of clindamycin, the ZnO-NPs acetate from ethanol extract of broadleaf was able to produce a stronger inhibition zone (14.13 ± 0.15 mm vs 18.11 ± 0.10, $p < 0.05$, respectively) (Table 2 and Figure 2).

Clindamycin has an antibacterial mechanism by inhibiting protein synthesis and has the potential to cause long-term resistance [2]. Meanwhile, the ZnO-NPs acetate of broadleaf ethanol extract has a dual antibacterial mechanism, first through the release of Zn^{2+} ions which help the formation of Reactive Oxygen Species (ROS) which damage bacterial cells, and secondly, it is strengthened by the bioactive compounds of broadleaf ethanol extract (flavonoids, tannins) which can increase membrane permeability and inhibit enzymes [13].

This research in line with the results from [Pangestin, 2017], that the phytochemical combination of pine flower extract and ZnO nanoparticles provides stronger antibacterial activity than the single components [27]. Combining plant extracts with nano-sized ZnO is also believed to reduce the MIC due to mechanisms that increase membrane penetration and retention of Zn^{2+} ions in the cytoplasm. This mechanism disrupts cellular functions, including active transport, metabolism, and enzymes activity, triggering the leakage of cell contents and ultimately causing bacterial damage and death due to Zn^{2+} ion toxicity [28].

This study differs from previous research by examining ZnO nanoparticles synthesized using *Plantago major* L. ethanol extract against *P. acnes*, a major bacterium

Table 2. Antibacterial activity test results of zinc oxide nanoparticles of broadleaf ethanol extract (*Plantago major* L.).

Treatment Group	Average of Inhibition Zone ± Standard Deviation (mm)	MIC(mg/mL)	Inhibition Category
Ethanol 10%	0.00 ± 0.00	-	-
Clindamycin	14.13 ± 0.15	0.078*	Strong
Broadleaf ethanol extract	8.14 ± 0.17	5.0	Moderate
ZnO-NPs nitrate	16.90 ± 0.10 [a, b]	0.195	Strong
ZnO-NPs nitrate of broadleaf ethanol extract	16.19 ± 0.10 [a, b]	0.195	Strong
ZnO-NPs acetate	20.20 ± 0.11 [a, b]	0.156	Strong
ZnO-NPs acetate of broadleaf ethanol extract	18.11 ± 0.10 [a, b]	0.078	Strong

Note: Minimum Inhibition Concentration (MIC), Zinc Oxide Nanoparticles (ZnO-NPs)
a Significantly different with Clindamycin
b Significantly different with ethanol 10%

* Concentration in µg/mL

Table 3. Results of physical and chemical characterization of zinc oxide nanoparticles of broadleaf ethanol extract (*Plantago major* L.).

Parameter	Test Method	Results			
		ZnO-NPs A	ZnO-NPs AE	ZnO-NPs N	ZnO-NPSs NE
Morphology	SEM	Granular-crystal; size 0.119- 0.215 µm	Granular; size 0.200- 0.228 µm	Crystal; size 0.115-0.259 µm	Irregular shape; size 0.119-0.236 µm
Elemental composition (%)	EDX	Zn (75.43); O (24.03); C (0.54)	Zn (49.53); O (46.74); C (3.73)	Zn (55.41); O (43.65); C (0.94)	Zn (75.50); O (23.43); C (1.07)
Particle Size	PSA	1253 nm; PI 0.4986; zeta potential (- 19.73 mV)	338.7 nm; PI 0.2695; zeta potential (- 28.04 mV)	NR	NR

Note: Scanning Electron Microscopy (SEM); Energy Dispersive X-Ray (EDX); Particle Size Analyzer (PSA); Zinc (Zn); Oxygen (O); Carbon (C); Zinc Oxide Nanoparticle (ZnO-NPs) Acetate (A), Acetate and broadleaf ethanol extract (AE), Nitrate (N), Nitrate and broadleaf ethanol extract (NE); Not Run (NR)

involved in acne development. Most earlier studies investigated the antibacterial activity of ZnO nanoparticles against common pathogenic bacteria using other plant extracts [13,29]. In this work, *Plantago major* L. was selected due to its high content of bioactive compounds. These compounds may contribute to a synergistic effect that enhances the antibacterial activity of the ZnO nanoparticles. Therefore, this study presents a plant-based ZnO nanoparticle approach that is more relevant for potential anti-acne applications.

Characterization of ZnO-NPs from Ethanol Extract of Broadleaf

Zinc oxide NPs synthesized from Zn acetate have a granular-crystalline morphology with a size of 0.119–0.215 µm. The addition of ethanol extract of broadleaf slightly increased the size to 0.200–0.228 µm, but the granular shape remained uniform (Table 3 and Figure 3). This indicates the role of ethanol extract as a surfactant that stabilizes the crystal shape without changing the morphology. Ethanol extract of plants plays a role in regulating particle size by binding metal ions and inhibiting crystal growth [18]. Uniform-sized granular morphology is important for photocatalytic applications because large surface area and even distribution can improve the catalytic performance of ZnO [30].

Meanwhile, ZnO-NPs from nitrate precursors exhibited a more varied particle size range (0.115-0.259 µm) with a heterogeneous crystal morphology. The addition of ethanol extract of broadleaf resulted in particle sizes in the range of 0.119-0.236 µm with increasingly irregular shapes, confirming that nitrate precursors tend to produce more heterogeneous crystals than acetate. These results are in line with previous studies showing that the type of precursor and synthesis conditions greatly influence the morphology of ZnO-NPs. Nitrate precursors tend to produce less uniform crystals due to differences in the rate of hydrolysis and precipitation of Zn^{2+} ions [31], while ethanol extract can modulate crystal growth thereby contributing to heterogeneous morphology [32].

The ZnO-NPs acetate contains 75.43% Zn and 24.03% O, close to the ideal composition, thus indicating high purity. In the combination of ZnO-NPs acetate with ethanol extract of broadleaf, the Zn content decreased to 49.53% with an increase in O and C, indicating the presence of organic residues that reduce the purity of ZnO. Zinc oxide nanoparticles nitrate has a Zn composition of 55.41% also indicating low purity with the possibility of the formation of a non-stoichiometric phase or precursor residue. In the ZnO-NPs nitrate with the ethanol extract of broadleaf, the Zn composition increased to 75.30%, equivalent to the ZnO-NPz acetate control (Table 3). In the ZnO-NPs forms, the presence of oxygen and carbon elements is useful for stability. However, adequate Zn levels make ZnO-NPs acetate of broadleaf extract superior to other types of ZnO-NPs, because it produces a more stable and optimal material according to the trend of ZnO-based nanomaterial research [31].

The results of particle size analysis using PSA show that ZnO-NPs acetate from broadleaf extract is smaller (338.7 nm) than ZnO-NPs acetate control (1253 nm), making it more effective for topical antibacterial applications because it can penetrate the skin, expand the surface area, and increase interaction with bacteria [33]. Zinc oxide nanoparticles acetate from ethanol extract of broadleaf also have a narrower particle distribution (PI 0.2695) and higher stability (-28.04 mV) compared to the ZnO-NPs acetate control, making it more homogeneous

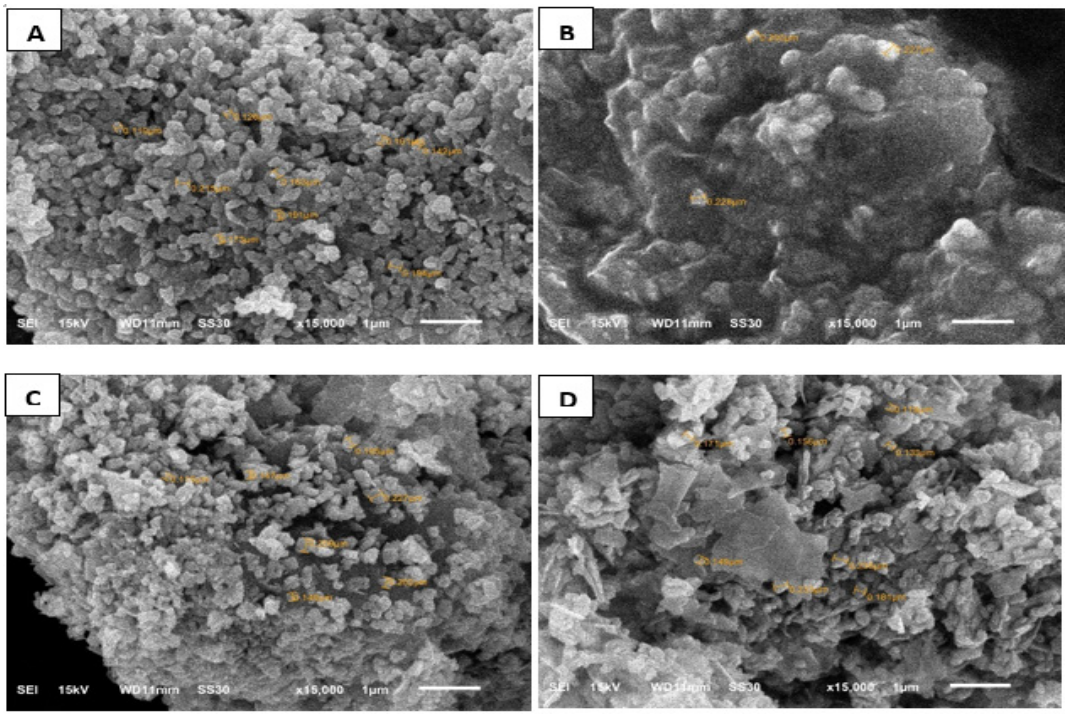


Figure 3. Morphology and crystal size analysis using Scanning Electron Microscopy (SEM), (A) ZnO-NPs acetate, (B) ZnO-NPs acetate of broadleaf ethanol extract (Plantago major L.), (C) ZnO-NPs nitrate, and (D) ZnO-NPs nitrate of broadleaf ethanol extract
Note: Zinc Oxide Nanoparticles (ZnO-NPs).

and resistant to agglomeration. Its small size, uniform distribution, and high stability make ZnO-NPs acetate of broadleaf extract more potential to be developed as an antibacterial topical active ingredient because it increases the antibacterial mechanism through ROS, Zn^{2+} ion release, and membrane damage, in line with recent studies that emphasize the importance of nanoscopic properties and stability for pharmaceutical effectiveness [34].

The results of this study are similar to research of Fakhari et al. (2019), showed that zinc precursors affect the morphology of ZnO-NPs, producing small and homogeneous particles for ZnO-NPs acetate, and forming flower structures for ZnO-NPs nitrate [35]. Small size and uniform morphology have been shown to support more effective antimicrobial activity [36]. Active substances from the flavonoid, tannin and saponin compounds, apart from increasing antibacterial activity, can also act as capping and chelating agents, helping to stabilize nanoparticles form [10].

Conclusion

Zinc oxide nanoparticles from ethanol extract of broadleaf (*Plantago major* L.) synthesized with zinc acetate precursor have been proven to be effective in inhibiting the growth of P. acnes with an MIC value of 0.078 mg/mL. The superiority of its antibacterial activity is influenced by its small particle size, narrow distribution, stable suspension, also oxygen and phytochemical content that enhances interaction with bacterial cells. Therefore, ZnO-NPs acetate of ethanol extract of broadleaf has the potential to be developed as a topical antibacterial for acne treatment.

Conflict of Interest

None.

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