

Green Synthesis and Characterization of ZnO Nanoparticles Using *Rhizophora apiculata*: Evaluation of Sun Protection Activity

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Abstract: ZnO nanoparticles (ZnO NPs) have sunscreen activity and are popularly used in cosmetics. The synthesis of environmentally friendly ZnO NPs is needed because environmental protection has now become rooted in society's expectations. The research aimed to synthesize ZnO NPs with leaves, bark, and roots of *Rhizophora apiculata* with zinc nitrate hexahydrate precursor. The method used is green synthesis with characterization of particle size, zeta potential, polydispersity index (PDI), functional groups, and in vitro sun protection factor (SPF). The results show that the synthesis of ZnO NPs mediated by the leaves, bark, and roots of *Rhizophora apiculata* with zinc nitrate hexahydrate precursor has been successful. This is confirmed by the presence of functional groups in FTIR analysis. Overall, the synthesis results have nanometer-scale characteristics, moderate zeta potential, moderate-high polydispersity index, and sunscreen activity in vitro. This research concludes that the synthesis with *Rhizophora apiculata* bark showed the best results. It produced the smallest particle size, the highest zeta potential value, PDI in the moderate category, and the highest SPF value.

Keywords: nanoparticles; ZnO; *Rhizophora apiculata*; green synthesis; SPF.

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1. Introduction

Metal oxides have been used in sunscreen products since the early 1980s [1]. Commercial sunscreens containing nano-sized zinc oxide (ZnO) provide superior UV protection and reduce skin whitening compared to larger particle sizes, preventing skin cancer, sunburn, and photoaging [2,3]. The popular use of ZnO nanoparticles (ZnO NPs) also needs to consider other aspects, such as environmentally friendly synthesis processes. ZnO NPs can be synthesized through physical, chemical, and green synthesis pathways [4].

The synthesis of ZnO using physical methods has the disadvantage of requiring a large space for the machine, expensive equipment, high temperature, and high pressure [5]. Besides that, the chemical method uses hazardous chemicals, requires high-pressure equipment, high energy, careful control of reaction conditions, and batch-to-batch reproducibility may be an issue [6]. As the applications using ZnO NPs increase, their synthesis using environmentally

friendly methods has become a matter of widespread concern, especially since the concept of environmentally friendly has now become deeply rooted in society's expectations [7].

Green synthesis provides a solution to overcome these problems. Synthesis of ZnO NPs using the green synthesis method has the advantages of being much safer, low energy consumption, easily obtained plant sources, and environmentally friendly compared to physical or chemical methods [7,8]. Green synthesis of ZnO NPs has been successfully achieved using extracts of plant parts such as leaves, bark, and roots [9,10].

Rhizophora apiculata is a plant that has the widest distribution and is economically important in Asia, with a tropical climate [11]. The leaves, roots, and bark of the *Rhizophora apiculata* plant contain flavonoid compounds [12,13]. This research explores a wider range of alternative plant parts as mediators in the green synthesis of ZnO NPs. The presence of OH groups in flavonoids can reduce zinc compounds to ZnO NPs and also act as capping agents or stabilizers and reducing agents [14].

The background that has been explained indicates the importance of this research to find an alternative synthesis of ZnO NPs using *Rhizophora apiculata*. The sunscreen activity of ZnO NPs needs to be known to obtain better development information.

2. Materials and Methods

2.1. Materials.

Rhizophora apiculata leaves, bark, and roots were collected from the Mangrove Learning and Restoration Center in Pekalongan, Indonesia. This synthesis uses zinc nitrate hexahydrate (Sigma Aldrich, USA), NaOH (Merck, Germany), and demineralized water (Brataco, Indonesia).

2.2. Preparation of extracts *Rhizophora apiculata*.

The preparation of the extract was carried out according to previous research with some modifications. The leaves, stem bark, and roots of *Rhizophora apiculata* were washed with demineralized water, cut into small pieces, and dried at 40°C in a solar dryer dome. Then each sample was reduced to powder size and extracted with demineralized water at a concentration of 2%. The extraction process uses a hot plate at 4500 rpm and a temperature of 60°C for 20 minutes. The extract was filtered [15]. Identification of flavonoid compounds using the Shibata test, NaOH test, and Pew's test [16,17].

2.3. Green synthesis ZnO NPs.

Zinc nitrate hexahydrate dissolved in demineralized water at a concentration of 3%. The precursor solution is stirred at 7500 rpm on a hotplate and heated at 80°C. Then, 5mL of extract is added dropwise to the precursor solution. 16 grams of NaOH is dissolved in 100 mL of demineralized water and added dropwise to the extract-precursor solution until the pH is 13. The mixture remains on the hotplate during the synthesis process for 2 hours. Then centrifugation at 300 rpm for 15 minutes. The pellet was washed and then dried at 120°C for 1 hour. The final stage is calcination at 200°C for 30 minutes [18,19].

2.4. Determination of synthesis yield.

The yield (%) of green synthesis ZnO NPs was calculated as follows [20]:

$$\text{Yield (\%)} = \frac{\text{Weight of product}}{\text{Weight of precursor}} \times 100 \quad (1)$$

2.5. Determination of particle size, zeta potential, and polydispersity index (PDI).

The sample was analyzed using a particle size analyzer. ZnO NPs were dispersed into demineralized water until concentrations of 10 mg/100mL. The process of dispersing using an ultrasonic for 30 minutes. Then, it was put into a cuvette and analyzed [19,21,22].

2.6. Fourier transform infrared (FTIR) spectroscopy analysis.

The samples were analyzed using the FTIR instrument using the UATR (Universal Attenuated Total Reflectance) method. ZnO NPs were placed on the detector of the instrument. Then, it was analyzed with a wave range of 400–4000 cm⁻¹ [23].

2.7. Measurement of UV protector.

The samples were analyzed using spectrophotometry to investigate the in vitro sun protection factor (SPF). ZnO NPs were dispersed in absolute ethanol until a concentration of 25 mg/50ml. The process of dispersing using ultrasonics for 30 minutes. The solution was put into a cuvette and read at 5 nm intervals at a wavelength of 290–320 nm [24]. The measurement of SPF value used the Mansur equation. Where the correction factor (CF) is 10, A is the absorbance of ZnO NPs, I is the solar intensity spectrum, and EE is the erythemal effect. EE and I are constants. The Equation is as follows:

$$\text{SPF} = \text{CF} \sum_{290}^{320} A(\lambda) I(\lambda) \text{EE}(\lambda) \quad (2)$$

3. Results and Discussion

3.1. Preparation of *Rhizopora apiculata* extract.

The leaves, bark, and root of *Rhizopora apiculata* water extract are liquid, brown in colour, and smell like herbs. The extraction process uses water as a solvent. It can extract flavonoid compounds from samples; this solvent is also environmentally friendly [25]. The results of the leaves, bark, and root extract are shown in Figure 1.

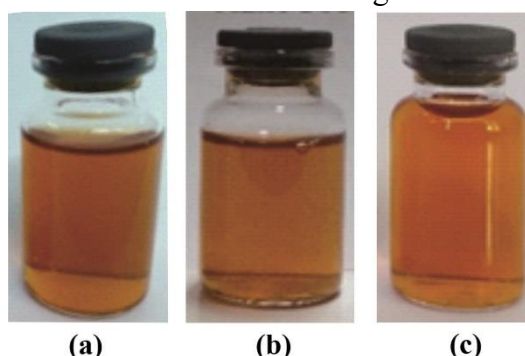


Figure 1. Water extract of *Rhizopora apiculata* (a) leaves; (b) bark; (c) roots.

A flavonoid phytochemical test is used to ensure the presence of flavonoid content. As is known, flavonoids have an important role in the synthesis process. The results of the phytochemical test are shown in Table 1. The results showed that all extracts contained flavonoids. The Shibata test, the red solution indicates flavonol, and the orange colour indicates

flavone. The NaOH test, the appearance of yellow-red, coffee-orange, purple-red, or blue indicates the presence of xanthenes and or flavones, flavonols, limon, and anthocyanins. The Pew's test, the red colour indicates the presence of flavonol.

Table 1. Flavonoid phytochemical test.

Reagent	Rhizopora apiculata extract		
	Leaves	Bark	Root
Shibata test	+ (orange)	+ (orange)	+ (orange)
NaOH test	+ (yellow-red)	+ (yellow-red)	+ (yellow-red)
Pew's test	+ (red)	+ (red)	+ (red)

3.2. Green synthesis of ZnO NPs.

All ZnO NPs synthesis results produced light brown and fine characteristics. These results are from previous research, which stated that the results of green synthesis nanoparticles have a fine form and a light cream colour [26]. This fine form indicates that real particles of ZnO have been formed. At the same time, the light cream colour is possible due to the effects of using plant extracts. Figure 2 shows the results of ZnO NPs.

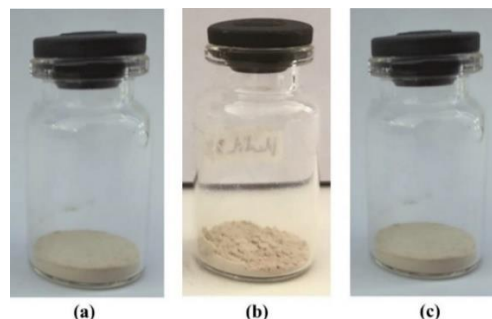


Figure 2. ZnO NPs are synthesized by (a) leaves; (b) bark; (c) roots of *Rhizopora apiculata* extract.

The phytochemical test of flavonoids shows the important role of the synthesis process as a stabilizer, capping agent, and reductor [27]. The aromatic hydroxyl group in flavonoid compounds is bound to the Zn^{2+} ion of zinc nitrate hexahydrate, which forms a stable complex in the process of forming ZnO NPs. In the centrifugation and calcination stages, this complex system produces ZnO NPs. The possible mechanism of synthesis of ZnO NPs is shown in Figure 3 [28].

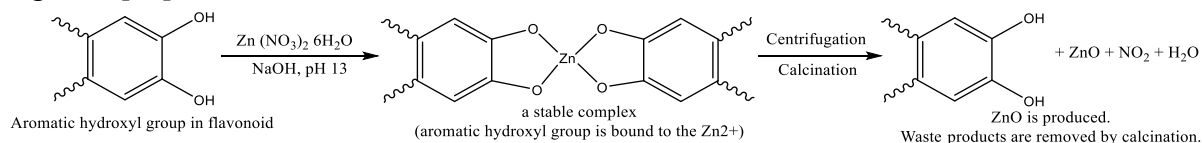


Figure 3. Mechanism synthesis of ZnO NPs.

3.3. Yield of synthesis ZnO NPs.

The synthesis yield was measured to calculate the percentage of ZnO NPs obtained compared to the precursor. Yield measurements are shown in Table 2. In previous research, green synthesis produced yields ranging from 4.58% to 43.59% [20]. In this research, the highest yield was 24.8%, and the lowest was 12.5%. This research shows that synthesis with plant parts will give different results in yield.

Table 2. Yield of green synthesis ZnO NPs.

<i>Rhizopora apiculata</i> extract	Synthesis results	Yield
Leaves	650 mg	21,6%
Bark	408 mg	13,6%
Root	390 mg	12,5%

3.4. Determination of particle size, zeta potential, and polydispersity index (PDI).

The results of the characterization of ZnO NPs are shown in Table 3. Particle size characterization was used to ensure the formation of ZnO on a nanometer scale. Nanoparticles are defined as solid particles with sizes in the range of 10-1000 nm [29]. The research results obtained from leaf, bark, and root extracts, each with a size scale of 532.6 nm, 142.4 nm, and 206.7 nm, respectively. These results indicate that ZnO was successfully synthesized on a nanometer scale. This research has a smaller size compared to previous research; the particle size of ZnO NPs mediated by *Coriandrum sativum* L. leaves is 552.3 nm, *Kalopanax septemlobus* bark is 500 nm, and *Codonopsis lanceolata* roots is 500 nm [30–32]. However, the results of this research have a larger size compared to using *Lycopersicon esculentum* fruit peel extract, which is 10.75 nm [15].

Table 3. Characterization of particle size, zeta potential, and PDI.

<i>Rhizopora apiculata</i> extract	Particle size	Zeta potential	PDI
Leaves	532.6	-31.1	0.716
Bark	142.4	-36.2	0.368
Root	206.7	-35.1	0.253

Zeta potential characterization is a measure of the charge on particles that describes the stability of a material, and it can be positive or negative [33,34]. There are several categories of zeta potential values. The categories are coagulation (0 to ± 5 mV), incipient instability (± 10 to ± 30 mV), moderate stability (± 30 to ± 40 mV), good stability (± 40 to ± 60 mV), and excellent stability ($> \pm 60$ mV) [35]. The research results obtained from leaf, bark, and root extracts each had a zeta potential of -31.1 mV, -36.2 mV, and -35.1 mV. All results are included in the moderate stability category. The results of this research have a more stable zeta potential value compared to the synthesis using fruit extracts of *Myristica fragrans*, which is -22.1 mV [36]. However, the research of other researchers shows more stable results when using pectin, which is -52.245 mV [19].

Polydispersity index (PDI) is important to measure; it describes the degree of heterogeneity based on the particle size of the sample [37]. PDI value can range from 0 to 1, and the categories are monodisperse (≤ 0.1), moderately (0.1–0.7), and highly polydisperse (> 0.7) [38]. The research results obtained from leaf, bark, and root extracts each had a PDI of 0.716 (highly polydispers), 0.368 (moderately), and 0.253 (moderately). The results of this research are similar to previous research using *Silybum marianum* extract, which is 0.443 [39].

3.5. Fourier transform infrared (FTIR) spectroscopy analysis.

FTIR analysis is used to determine the presence of functional groups of ZnO [40]. The results of the FTIR analysis are shown in Figure 4. The presence of ZnO is shown in the sharp peak on the wavenumber range of 400-500 nm^{-1} [41]. The research results showed sharp peaks in the synthesis mediated by leaves, bark, and roots at wavenumbers 418.70 cm^{-1} , 400.74 cm^{-1} , and 418.70 cm^{-1} . The presence of this peak indicates the success of ZnO synthesis. There is another peak that appears, caused by residues that have not been completely removed during the synthesis process. This is also the same as in previous research using pineapple peel extract; in FTIR analysis, there are ZnO functional groups and extract residues [42].

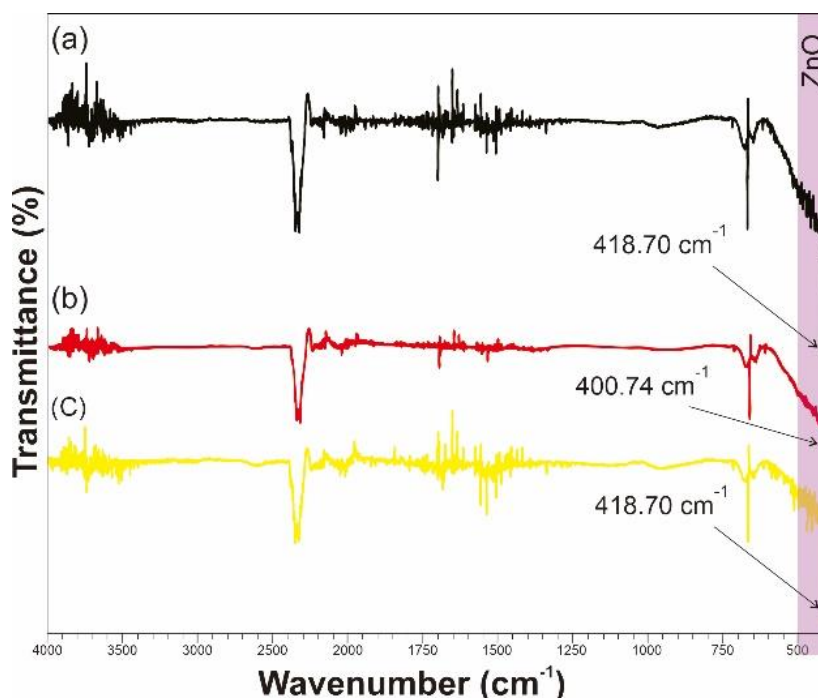


Figure 4. FTIR analysis of ZnO NPs is synthesized by **a)** leaves; **b)** bark; **c)** roots of *Rhizopora apiculata*.

3.6. UV protector.

Sun Protection Factor (SPF) is defined as an indicator that describes the effectiveness of a product or active substance's protection against ultraviolet rays [43]. The higher the SPF value, the higher the ability to protect against ultraviolet. The SPF values of ZnO NPs are shown in Table 4. The research results showed the SPF value of the synthesis mediated by leaves, bark, and roots at 9.71, 33.57, and 23.15. The highest SPF is produced by *Rhizopora apiculata* bark extract. It is higher than previous research. Synthesis using *Solanum lycopersicum* produced a ZnO NPs SPF value of 16.8 [24].

Table 4. SPF value measurements of ZnO NPs.

<i>Rhizopora apiculata</i>	λ (nm)	Abs	EE x I	Abs x EE x I	$\sum_{290}^{320} A(\lambda) I(\lambda) EE(\lambda)$	CF	SPF
Leaves	290	0.937	0.015	0.01406	0.97058	10	9.71
	295	0.945	0.0817	0.07721			
	300	0.951	0.2874	0.27332			
	305	0.979	0.3278	0.32092			
	310	0.987	0.1864	0.18398			
	315	0.991	0.0839	0.08314			
	320	0.997	0.018	0.01795			
Bark	290	3.847	0.015	0.05771	3.35653	10	33.57
	295	3.414	0.0817	0.27892			
	300	3.044	0.2874	0.87485			
	305	3.505	0.3278	114.894			
	310	3.444	0.1864	0.64196			
	315	3.479	0.0839	0.29189			
	320	3.459	0.018	0.06226			
Root	290	2.335	0.015	0.03503	2.31516	10	23.15
	295	2.323	0.0817	0.18979			
	300	2.304	0.2874	0.66217			
	305	2.31	0.3278	0.75722			
	310	2.322	0.1864	0.43282			
	315	2.329	0.0839	0.1954			
	320	2.374	0.018	0.04273			

Previous studies have shown that green-synthesized ZnO NPs exhibit lower cytotoxicity and better biocompatibility compared to chemically synthesized ZnO NPs [44]. Additionally, the repeated use of chemically synthesized ZnO NPs in sunscreen products has been found to be safe, with no evidence of ZnO NP penetration into the viable epidermis or toxicity to the underlying skin layers [45]. Based on these findings and supported by the SPF evaluation results, green-synthesized ZnO NPs demonstrate promising potential for further development as cosmetic ingredients.

4. Conclusions

The synthesis of ZnO NPs mediated by the leaves, bark, and roots of *Rhizophora apiculata* with zinc nitrate hexahydrate precursor has been successful. This is confirmed by the presence of functional groups in FTIR analysis. The best synthesis results were obtained using *Rhizophora apiculata* bark, which produced the smallest particle size, the highest zeta potential value, PDI in the moderate category, and the highest SPF value.

Author Contributions

Conceptualization, N.C.E.; supervision, N.C.E.; methodology, N.C.E. and M.S.; software, M.S.; project administration, R.; Resource, B.A.W.; investigation, M.R.A.N., R., and B.A.W.; writing-original draft, N.C.E., M.R.A.N., R., and B.A.W.; writing-review & editing, M. S.. All authors have read and agreed to the published version of the manuscript.

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Not applicable.

Data Availability Statement

Data supporting the findings of this study are available upon reasonable request from the corresponding author.

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Conflicts of Interest

The authors declare no conflict of interest.

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