ISSN: 1410-8917
Jurnal Kimia Sains & Aplikasi
e-ISSN: 2597-9914

Jurnal Kimia Sains dan Aplikasi 29 (5) (2026): 354-360

Jurnal Kimia Sains dan Aplikasi
Journal of Scientific and Applied Chemistry

Journal homepage: http://ejournal.undip.ac.id/index.php/ksa

The Characteristic and Free Radical Scavenging Activity of Zinc Oxide Nanoparticles (ZnO-NPs) Synthesized using Noni (*Morinda citrifolia* L.) Fruits and Leaves Extract

Selvina Koesyamin [1], Tagor Marsillam Siregar [1,*], Antonius Herry Cahyana [2]

[1] Department of Food Technology, Faculty of Science and Technology, Universitas Pelita Harapan, Tangerang, Indonesia
[2] Department of Chemistry, Faculty of Mathematics and Natural Science, Universitas Indonesia, Depok, Indonesia

* Corresponding author: tagor.siregar@uph.edu

https://doi.org/10.14710/jksa.29.5.354-360

Article Info

Article history:

Received: 17th February 2026
Revised: 26th May 2026
Accepted: 28th May 2026
Online: 15th June 2026

Keywords:
Zinc oxide nanoparticles; free radical scavenging activity; characteristic; calcination; synthesis

Abstract

Zinc oxide nanoparticles (ZnO-NPs) have gained considerable attention due to their unique properties, including high chemical stability, excellent sensing ability, and antibacterial activity. Biological synthesis of ZnO-NPs is a promising approach because of its sustainability and the availability of diverse natural reducing agents. Noni (*Morinda citrifolia* L.), traditionally used as a medicinal plant for over 2,000 years in Polynesia, contains phenolics and flavonoids that act as natural reducing agents. This study aimed to evaluate the effects of different plant parts (fruits and leaves) and solvent polarities (deionized water, ethyl acetate, and hexane) on the reducing ability of the extracts, as measured by ZnO-NP yield and free radical scavenging activity. The characteristics of ZnO-NPs after calcination were also evaluated. Solvent polarity significantly influenced reducing power and antioxidant activity, while no notable differences were observed between fruits and leaves. Fruit extracts prepared with ethyl acetate (semi-polar solvent) produced the highest ZnO-NPs yield (2.88 ± 0.11 mg), the strongest free radical scavenging activity (10.63 ± 0.65% inhibition/mg ZnO-NPs), and nanoparticles averaging 115.9 nm in size. Calcination at 100°C, conducted to eliminate residual moisture and stabilize the particles, resulted in an increase in the mean particle size from 115.9 nm to 313.6 nm. *Morinda citrifolia*-mediated ZnO-NPs could be utilized as effective bioactive additives in the development of active food packaging systems to prevent oxidative degradation.

1. Introduction

Nanotechnology is a new, promising field with a broad range of novel uses, and permits the controlled synthesis of materials in which at least one dimension of the structures is less than 100 nm [1, 2, 3, 4]. While nanotechnology is defined by the 100 nm threshold, biological synthesis often produces larger submicron aggregates that still exhibit unique nanoscale characteristics. The extraordinary properties come from their nanoscale sizes and large surface area enabled by their high surface-to-volume ratio. Zinc oxide (ZnO), a nanoparticle, is a multifunctional material because of its unique physical and chemical properties. One of its unique features is its high chemical stability profile [5]. Furthermore, zinc oxide nanoparticles (ZnO-NPs) are relatively inexpensive, exhibit excellent gas-sensing properties, and possess antioxidant and antibacterial activities. In addition, they have a favorable safety profile and are generally recognized as safe (GRAS) by regulatory authorities when used within specified concentration limits [6, 7, 8].

Noni (*Morinda citrifolia* L.) is one of the common medicinal plants that has been used for over 2,000 years by the Polynesians. It has been investigated for its significant health benefits. Some diseases that have been reported are cancer, arthritis, diabetes, asthma, hypertension, infections, and pain [9]. Other research has shown that Noni (*Morinda citrifolia* L.) has been traditionally used in Polynesian cultures to treat menstrual cramps, bowel irregularities, diabetes, liver disease, and urinary tract infections. Almost all parts of the tree are used, such as leaves, fruit, and root (rhizomes) [10]. Approximately 160 phytochemical compounds have already been identified in the Noni (*Morinda citrifolia* L.) plant. The major phytochemicals are phenolic compounds and flavonoids [9].

There are some ways to synthesize ZnO-NPs: physical, chemical, and biological. However, biological synthesis is an interesting method to explore, as biological methods for nanoparticle synthesis offer significant diversity, with sources ranging from plants, bacteria, and fungi to even viruses [11]. In this research, Noni (*Morinda citrifolia* L.) leaves and fruit extracts were used for the synthesis of ZnO-NPs.

In the biological method, plant extracts are used as reducing and capping agents for the synthesis of nanoparticles, owing to their phytochemicals with reducing properties. Cations from metal ions combine with anions from the plant extracts, reducing their oxidation states and, in turn, altering the physical appearance and color of the culture media [10]. Plant metabolites serve as reducing agents from their alkaloids, polyphenols, and flavonoids. Plant-mediated nanoparticle synthesis is gaining favor and showing greater reliability because plant materials are easier to obtain and more economical than microorganisms [11].

The study by Pai *et al.* [10] demonstrated that leaf extracts are excellent reducing agents for the synthesis of silver nanoparticles. Moreover, in another study by Kumar *et al.* [1]. ZnO-NPs were synthesized from grapefruit (*Citrus paradisi*) peel extracts. The study showed that ZnO-NPs exhibit significant photocatalytic and free radical-scavenging activity. It was found that ZnO-NPs exhibit 80% free radical scavenging activity at 1.2 mM. From the two studies, it can be hypothesized that Noni (*Morinda citrifolia* L.) plant extracts may be a potential reducing agent for the synthesis of ZnO-NPs. In both studies, the plant extracts were extracted with deionized water, a polar solvent.

Therefore, in this research, plant extracts will be obtained using polar, less polar, and nonpolar solvents to investigate whether other phytochemicals not extracted with polar solvents may serve as potential reducing agents for the synthesis of ZnO nanoparticles. Furthermore, in the synthesis of ZnO-NPs, some methods required calcination, while others did not. Calcination is usually used to make some changes in the physical and chemical structure. Commonly used temperatures included 100, 200, 400, and 500°C [12]. Thus, in this study, the effect of calcination on the characteristics of ZnO-NPs (size and morphology) was observed.

The aim of this study is to evaluate the effect of different samples and solvent polarities, as well as the best combination of sample and solvent, on the amount of reducing power of plant extracts and free radical scavenging activity of ZnO-NPs. Furthermore, the characteristic (size and morphology) of ZnO-NPs after calcination was studied.

2. Experimental

2.1. Materials

The materials used in this research were Noni (*Morinda citrifolia L.*) leaves and fruit. Chemicals used for extraction and synthesis were deionized water, pro-analysis ethyl acetate, pro-analysis hexane, zinc acetate powder, and sodium hydroxide (NaOH) solution. Chemicals used for analysis were methanol, DPPH (Diphenyl-picryl-hydrazyl) solution, folin-ciocalteu 10% reagent, gallic acid, quercetin, sodium carbonate (Na_2CO_3) 7.5%, and aluminium chloride ($AlCl_3$) 2%.

2.2. Sample Preparation

Fresh fruits and leaves of Noni (*Morinda citrifolia* L.) were washed with clean water three times to remove dirt. The fruits were cut into smaller sizes and dried in the sunlight. While the leaves dried directly under sunlight, without undergoing a size-reduction process. The fruits and leaves were dried in the sunlight for a week (moisture content < 8%). After drying, the samples were ground into a powder and passed through a 40-mesh sieve to obtain a uniform particle size. The moisture content of the resulting powders was determined using the oven-drying method.

2.3. Noni (*Morinda citrifolia* L.) Plant Extraction

The plant extracts were used to synthesize ZnO-NPs as reducing agents. The preparation of the plant extract used the maceration process, which involves soaking and agitating the sample with the solvent. The solvents used were deionized water (polar), ethyl acetate (semi-polar), and hexane (non-polar). The purpose of using solvents with different polarities was to extract different phytochemicals based on their polarity. Since, according to Widyawati *et al.* [13], different polarities of solvents might determine the types, compositions, and antioxidant activities of the phytochemicals extracted. Thus, in this research, several solvents with different polarities were used.

2.4. Synthesis of Zinc Oxide Nanoparticles (ZnO-NPs)

A total of 0.5 g of dried material obtained from the extraction process of Noni (*Morinda citrifolia*) was mixed with 50 mL of 0.6 M zinc acetate solution, and the pH was adjusted to 8 using 1 M NaOH. 0.5 g of dry matter from the extracts was determined by the oven method. The mixture was stirred for 3 hours at 75–80°C using magnetic stirrers, then observed until the precipitate showed no further color change. The whiter color indicated the formation of ZnO-NPs. The resulting ZnO-NPs were separated by centrifugation at 5000 rpm for 10 minutes, washed with deionized water three times, and dried in a hot-air oven at 60°C overnight. The ZnO-NPs were then analyzed for their free radical scavenging activity (DPPH). The control used for the analysis was the zinc acetate solution [1].

2.5. Calcination of ZnO-NPs

The synthesized ZnO-NPs were subjected to thermal treatment to evaluate the effect of calcination on their structural properties. The dried nanoparticles were placed in a muffle furnace and heated to 100°C at a constant heating rate of 5°C/min. This temperature was maintained for 3 hours to ensure structural stabilization and removal of residual moisture.

2.6. Total Phenolic Content

Total phenolic content (TPC) analysis was performed to determine the phenolic compounds present in the extract, as these compounds act as reducing agents during the synthesis of ZnO-NPs. For the analysis, 0.5 mL of *Morinda citrifolia* L. extract was transferred into a test tube, followed by the addition of 2.5 mL of 10% Folin–Ciocalteu reagent and 2.0 mL of 7.5% Na_2CO_3 solution. The mixture was thoroughly mixed and incubated at room temperature for 1 hour. Subsequently, the absorbance was measured at 725 nm using a UV–Vis spectrophotometer. A reagent blank consisting of distilled water and all reagents was prepared under the same conditions. The total phenolic content was determined from a gallic acid calibration curve using linear regression analysis and expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE/g extract) [14].

2.7. Total Flavonoid Content

The total flavonoid content (TFC) of *Morinda citrifolia* L. fruit and leaf extracts was determined using the aluminum chloride colorimetric method. Briefly, 1 mL of the extract was mixed with 1 mL of 2% $AlCl_3$ solution in methanol. The mixture was homogenized with a vortex mixer and allowed to stand at room temperature for 10 minutes. Subsequently, the absorbance was measured at 415 nm using a UV–Vis spectrophotometer. A blank solution consisting of 1 mL of methanol and 1 mL of 2% $AlCl_3$ in methanol was prepared under the same conditions. The total flavonoid content was calculated using a quercetin calibration curve and expressed as milligrams of quercetin equivalents per gram of extract (mg QE/g extract) [14].

2.8. Free Radical Scavenging Activity

The free radical scavenging activity of the ZnO-NPs was evaluated using the DPPH assay. Briefly, 50 mg of ZnO-NPs powder was dispersed in 1 mL of deionized water and vortexed for 15 min to facilitate the extraction of soluble antioxidant compounds, as ZnO-NPs are sparingly soluble in water. The suspension was then centrifuged at 5000 rpm for 5 minutes. Subsequently, 1 mL of the supernatant was mixed with 1.5 mL of DPPH solution and incubated in the dark at room temperature for 30 minutes. The absorbance of the reaction mixture was measured at 517 nm using a UV–Vis spectrophotometer.

The remaining precipitate containing undissolved ZnO-NPs was collected, dried, and weighed. The free radical scavenging activity was expressed as percentage inhibition (% inhibition) relative to the mass of the undissolved ZnO-NPs (mg) [15].

2.9. Particle Size Analyzer

Particle size analysis (PSA) was performed using a PSA300 Static Image Analysis System. This instrument consists of a microscope equipped with a digital camera that captures images of particles as the sample-mounted slide is scanned.

2.10. Scanning Electron Microscopy (SEM)

The morphology of the ZnO-NPs was characterized using SEM. SEM analysis was performed using a JEOL JSM-6510 scanning electron microscope. Prior to analysis, the ZnO-NPs powder was dispersed in deionized water and sonicated to obtain a homogeneous suspension. A small drop of the suspension was deposited onto a glass slide and allowed to dry at room temperature. The prepared sample was then placed in the SEM chamber for morphological observation.

3. Results and Discussion

3.1. Noni (*Morinda citrifolia* L.) Plant Extraction

The plant extracts were calculated to contain the dry matter required for the reduction of ZnO-NPs. The dry matter was calculated by using the oven method. Each sample was taken in 2 mL, and the samples were dried in the oven until the weight was constant. The amount of dry matter was expressed in grams/mL. Dry matter refers to the amount of phytochemicals extracted. It can be seen that polar solvent (deionized water) extracted the highest amount of phytochemicals compared to the semi-polar solvent (ethyl acetate) and the non-polar solvent (hexane).

Table 1 shows the dry matter of Noni (*Morinda citrifolia* L.) fruit and leaves extracts. The result was supported by Krishnaiah *et al.* [9] and Ramamoorthy and Bono [16], who stated that deionized water (polar solvent) could extract polysaccharides, saponins, tannins, lectins, terpenoids, anthocyanins, starches, and polypeptides from Noni (*Morinda citrifolia* L.). While for ethyl acetate, only alkaloids, aglycones, and glycosides could be extracted. Followed by hexane, which could extract waxes and fats only. Based on the types of compounds that could be extracted by each solvent, it was clear that the dry matter of the sample extracted using deionized water (polar solvent) was the highest. Between samples, fruits have higher dry matter than leaves in all solvent types. Fruits have larger kinds of compounds compared to leaves. Thus, the dry matter of the fruit sample is higher than that of the leaves since they have higher phytochemicals [16, 17].

Table 1. Dry matter of Noni (*Morinda citrifolia*) fruit and leaves extracts

Type of solvent	Dry matter (g/mL)	
	Fruit extract	Leaves extract
Water	0.08335± 0.0066	0.05975 ± 0.0039
Ethyl acetate	0.0483 ± 0.0038	0.0438 ± 0.0045
Hexane	0.0157 ± 0.0004	0.0156 ± 0.0013

3.1.1. Reducing Agents of Extract

Phytochemicals such as phenolics, flavonoids, and alkaloids were the main compounds responsible for reducing ionic bulk metallics to nanoparticle formation [18, 19, 20, 21]. However, according to Krishnaiah *et al.* [9], in Noni (*Morinda citrifolia* L.), the major phytochemicals acting as reducing agents were phenolics and flavonoids. Thus, the quantification of phenolic and flavonoid compounds contained in the extract was determined.

3.1.2. Total Phenolics of Noni (*Morinda citrifolia* L.) Plant Extracts

In the extraction of Noni (*Morinda citrifolia* L.)'s fruits, ethyl acetate was the most effective solvent for extracting phenolic compounds. However, for the extraction of Noni (*Morinda citrifolia* L.) leaves, water was the most effective solvent. Deeper observation of the phenolic content of fruits and leaves extracted with ethyl acetate and water, respectively, indicated that Noni (*Morinda citrifolia* L.) fruit contains higher semi-polar phenolic compounds, while the leaves contain polar phenolic compounds. Table 2 showed the total phenolic content of Noni's fruits and leaves extract as a function of different samples and solvent polarity, respectively.

The results were in accordance with Ramamoorthy and Bono [16], who reported that Noni (*Morinda citrifolia* L.) fruits contain a higher quantity of semi-polar antioxidant compounds, as evidenced by higher total phenolic content when extracted with ethyl acetate than with ethanol (polar solvent).

Meanwhile, leaves contain higher levels of polar compounds, as supported by the study of Rivera *et al.* [17], which found that leaves contain larger polar compounds. In contrast, the phenolic content extracted with hexane was not significantly different. Hexane resulted in almost no phenolic content, as most phenolic compounds were polar and semi-polar. Furthermore, usually, hexane extracts waxes and fats.

According to the statistical analysis of the total phenolic content, different solvent polarities gave a significant effect on the phenolic content of fruits and leaves of Noni (*Morinda citrifolia* L.) ($p < 0.05$). However, there was no significant effect on the total phenolic content between fruit and leaves.

Table 2. Total phenolic content of Noni (*Morinda citrifolia*) fruit and leaves extracts

Type of solvent	Total phenolic content (mgGAE/g sample)	
	Fruit extract	Leaves extract
Water	37.237 ± 0.417^{b}	57.17 ± 2.802^{c}
Ethyl acetate	60.184 ± 1.285^{c}	46.507 ± 0.391^{b}
Hexane	3.095 ± 0.173^{a}	LOD

Values with different superscript letters within the same column are significantly different ($p < 0.05$).
LOD = below the limit of detection

3.1.3. Total Flavonoid of Noni (*Morinda citrifolia* L.) Plant Extracts

Generally, the result of the total flavonoid showed that most of the flavonoid compounds of Noni (*Morinda citrifolia* L.) were semi-polar. Followed by water and hexane as the least. As has been explained earlier, Ramamoorthy and Bono [16] stated that fruits have higher semi-polar antioxidant compounds and showed in their research that Noni (*Morinda citrifolia* L.), when extracted using ethyl acetate, has higher total phenolic, total flavonoid, and free radical scavenging activity compared to ethanol (polar solvent). This statement supported the result of the flavonoid content that fruit extracted with ethyl acetate has the highest flavonoid content among others. However, for the leaves, if following Rivera *et al.* [17], the fruit should have higher levels of polar compounds. However, this might be due to the assay used for flavonoid content determination attracting higher levels of semi-polar flavonoid compounds. Meanwhile, for the hexane, the flavonoid content was much lower than that of the others. In fact, it can be seen that the leaf has a non-polar flavonoid compound, which is higher than in the fruits.

The total flavonoid content of the fruit extract and the leaves extract can be seen in Table 3. According to the statistical analysis of total flavonoid content, different polarities of solvent resulted in a significant effect on the total flavonoid content of Noni (*Morinda citrifolia* L.)'s fruits and leaves extract ($p < 0.05$). However, the t-test indicated that fruits and leaves had a significant effect on total phenolic content. The total phenolic content of fruits was significantly higher than that of leaves ($p < 0.05$).

3.2. Reduction of ZnO-NPs

The reduction process of ZnO-NPs by Noni (*Morinda citrifolia* L.) plant extract occurred because zinc acetate possesses high reduction potentials, as metals (Zinc) are attached to the anionic parts and have the tendency to donate electrons. Thus, Zinc in its ionic form rapidly dissociates from the anionic groups and is reduced using plant extracts. In this case, the polyphenols and flavonoids in the Noni (*Morinda citrifolia* L.) plant extract act as chelating agents by binding to the cationic portion of Zinc and reducing it to the zero-valent state. Resulting in the formation and stabilization of ZnO-NPs.

Table 3. Total flavonoid content of Noni (*Morinda citrifolia*) fruit and leaves extracts

Type of solvent	Total flavonoid content (mgQE/g sample)	
	Fruit extract	Leaves extract
Water	20.191 ± 0.583^{b}	21.88 ± 0.700^{b}
Ethyl acetate	38.155 ± 2.081^{c}	34.558 ± 0.524^{c}
Hexane	1.91 ± 0.171^{a}	9.66 ± 1.943^{a}

Values with different superscript letters within the same column are significantly different ($p < 0.05$).

In the reduction process of ZnO-NPs, 0.5 g of dry matter and 50 mL of 0.6 M Zinc acetate were used. All samples were standardized to a common dry matter content to ensure consistency. Standardizing the amount of plant extract used to reduce ZnO-NPs ensured that all reactions contained the same amount of reactant (phytochemicals). Thus, it could yield a fair and comparable analysis of the synthesized ZnO-NPs. Even though the same amount of reactant, the kinds of phytochemicals extracted from different solvents resulted in different types of phytochemicals. During the reduction of ZnO-NPs, after mixing the plant extract with a zinc acetate solution, the mixture was adjusted to pH 8 with 1 M NaOH. According to Pai *et al.* [10], the pH of the mixture affected the morphology of the synthesized ZnO-NPs.

3.2.1. Amount of ZnO-NPs

The highest reducing power to synthesize the ZnO-NPs from fruit extract resulted from the fruits extracted using ethyl acetate solvent. For the leaves, the highest reducing power was observed in those extracted with deionized water. This means that fruits have higher phenolic content than leaves, while leaves have higher flavonoid content, such as rutin and quercetin. The higher yield in ethyl acetate is attributed to the concentration of semi-polar phenolics, which possess superior electron-donating capacity compared to the bulk polar matter in water. The synthesis proceeds via the coordination of zinc ions (Zn^{2+}) with the hydroxyl groups of these phenolics, forming a complex that facilitates the reduction and nucleation of ZnO-NPs. Those components were responsible for reducing ZnO-NPs.

Phenolic and flavonoid compounds that were analyzed in the previous section showed that fruits extracted using ethyl acetate resulted in the highest total phenolic and flavonoid content. For leaves, the highest total phenolic content was observed in extracts obtained with deionized water. The highest total flavonoid of leaf extracts resulted from ethyl acetate. The result was supported by the study of Kuppusamy *et al.* [18], which stated that phytochemicals such as phenolics, flavonoids, and alkaloids were responsible for the reduction of nanoparticle formation. Ramamoorthy and Bono [16] stated a stronger result, since it was stated that fruit contains largely semi-polar solvents. Moreover, Rivera *et al.* [17] also stated that leaves are composed of highly polar compounds.

Table 4 shows the amount of ZnO-NPs synthesized using fruit and leaves extract of Noni (*Morinda citrifolia* L.). According to statistical analysis conducted using SPSS software, different solvent polarities have a significant effect on the reducing power of the plant extract, as measured by the amount of ZnO-NPs in either fruits or leaves ($p < 0.05$). In addition, based on the t-test, the different samples also show a significant effect on the reducing power of the extract, as measured by the amount of ZnO-NPs. The results for the highest amount of ZnO-NPs between fruits and leaves are shown in Table 4.

Table 4. The amount of ZnO-NPs synthesized using fruit and leaves extract of Noni (*Morinda citrifolia* L.)

Type of solvent	Amount of ZnO-NPs (mg)	
	Fruit extract	Leaves extract
Water	1.579 ± 0.029^b	2.247 ± 0.084^c
Ethyl acetate	2.883 ± 0.110^c	1.836 ± 0.076^b
Hexane	0.779 ± 0.026^a	0.698 ± 0.009^a

Values with different superscript letters within the same column are significantly different ($p < 0.05$).

3.2.2. Free Radical Scavenging Activity

The synthesized ZnO-NPs were evaluated for their free radical scavenging activity using the DPPH method. The results of the free radical scavenging activity of ZnO-NPs powder are shown in Table 5. Corresponding to the amount of ZnO-NPs, the free radical scavenging activity of the synthesized ZnO-NPs was also exhibited by ZnO-NPs synthesized from fruit using ethyl acetate and from leaves using deionized water. The result of the free radical scavenging activity was based on the % inhibition/mg of ZnO-NPs. The result was not expressed in the inhibition concentration 50% (IC_{50}) because of the insolubility of the ZnO-NPs powder in water. The free radical scavenging activity of the Zinc acetate (metallic bulk ions) was also evaluated, and the result shows that the metallic bulk ions do not have any free radical scavenging activity. The mechanism of the free radical scavenging activity was explained by Kumar *et al.* [1]. The cationic parts of the zinc oxide nanoparticles are electrostatically attracted to the bioactive compounds in the plant extract, resulting in a reducing effect, while the phytochemicals cap the ZnO-NPs, and their bioactivity increases synergistically.

The statistical analysis of the free radical scavenging activity shows that the solvent significantly affects the free radical scavenging activity of the ZnO-NPs that were synthesized from fruits and leaves ($p < 0.05$). Furthermore, variations in the sample also yield significantly different results for free radical scavenging activity, as indicated by the t-test. The result of the highest free radical scavenging activity between fruits and leaves is shown in Table 5.

Table 5. Free radical scavenging activity of ZnO-NPs from fruit and leaves extract of Noni (*Morinda citrifolia* L.)

Type of solvent	Free radical scavenging activity (% Inhibition/mg ZnO-NPs)	
	Fruit extract	Leaves extract
Water	5.02 ± 0.475^b	6.89 ± 0.545^c
Ethyl acetate	10.63 ± 0.651^c	5.79 ± 0.548^b
Hexane	2.77 ± 0.863^a	1.57 ± 0.515^a

Values with different superscript letters within the same column are significantly different ($p < 0.05$).

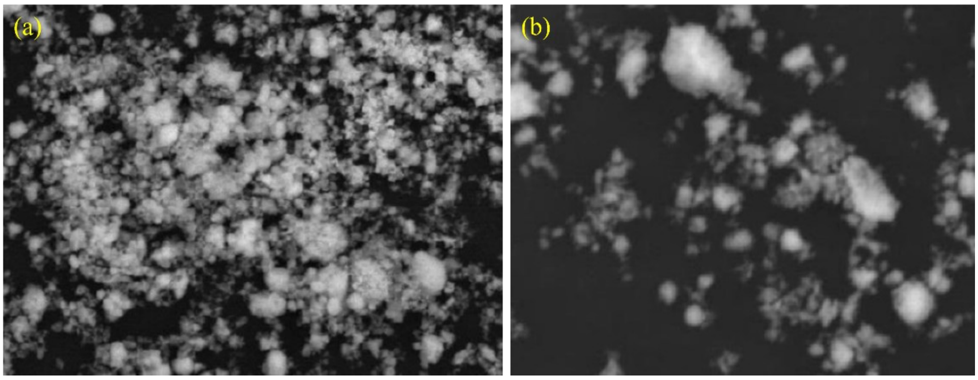

Figure 1. SEM images of the ZnO-NPs (a) before and (b) after calcination

3.2.3. Calcination

Calcination was performed to assess its effect on the characteristics of ZnO-NPs (size and morphology). The size of the synthesized ZnO-NPs was observed using a PSA, while the morphology was evaluated using images from SEM. The ZnO-NPs that were synthesized from fruit-ethyl acetate had the highest free radical scavenging activity. Thus, only that sample undergoes the calcination process. According to Kleitz *et al.* [22], during the calcination, some physical and chemical formations were formed. Moisture, carbon dioxide, and other volatile compounds were evaporated. Moreover, calcination might oxidize part or all of the substance, altering its chemical composition.

3.2.4. Characterization of ZnO-NPs

Calcination was performed to assess its effect on the characteristics of ZnO-NPs (size and morphology). The size of the synthesized ZnO-NPs was observed using a PSA, while the morphology was evaluated using images from SEM. The SEM results are shown in Figure 1. The ZnO-NPs that were synthesized from fruit-ethyl acetate had the highest free radical scavenging activity. Thus, only that sample undergoes the calcination process. According to Kleitz *et al.* [22], during the calcination, some physical and chemical formations were formed. Moisture, carbon dioxide, and other volatile compounds were evaporated. Moreover, calcination might oxidize part or all of the substance, altering its chemical composition.

The size of the ZnO-NPs increased during the calcination process. The crystallite sizes increased from 115.9 nm to 313.6 nm. According to Parra and Haque [12], the increasing sizes of the ZnO-NPs were due to the nucleation-aggregation phenomenon. Nucleation-aggregation occurred due to the rapid formation of a crystal nucleus. The increase in size of the ZnO-NPs also happened due to the loss of phytochemicals of the plant extract due to high temperature, since the phytochemicals also act as a capping agent for the ZnO-NPs [11].

In conclusion, Ravikumar and Kumar [23] also reported that calcination at 100°C was essential for complete water removal and for achieving higher crystallinity in the nanoparticles, leading to larger particle sizes. The increase in particle size and loss of phytochemical capping during calcination present a trade-off between structural stability and functional efficacy. While higher crystallinity improves purity, the reduced surface-area-to-volume ratio and the degradation of bioactive capping agents may lower the total antioxidant potency. Consequently, uncalcined biogenic particles may be more suitable for applications requiring maximum radical scavenging, whereas calcined particles offer better structural integrity.

4. Conclusion

In this study, some variations of the sample (fruits and leaves) and solvent polarities have been applied for the synthesis of ZnO-NPs. Different solvent polarities and sample types significantly affect the reducing power and free radical scavenging activity of ZnO-NPs. The best combination was Noni (*Morinda citrifolia* L.) fruit extracted with ethyl acetate (a semi-polar solvent). It has the highest reducing power to synthesize ZnO-NPs in terms of their amount (2.8828±0.11 mg), the highest free radical scavenging activity (10.63 ±0.651 % inhibition/mg of dissolved ZnO-NPs), and resulted in particles with a mean size of 115.9 nm. These findings suggest that *Morinda citrifolia*-mediated ZnO-NPs could be utilized as effective bioactive additives in the development of active food packaging systems to prevent oxidative degradation.

Acknowledgement

The authors acknowledge the Department of Food Technology, Faculty of Science and Technology, Universitas Pelita Harapan, Indonesia, for providing laboratory facilities to this study.

References

[1] Brajesh Kumar, Kumari Smita, Luis Cumbal, Alexis Debut, Green Approach for Fabrication and Application of Zinc Oxide Nanoparticles, *Bioinorganic Chemistry and Applications*, 2014, (2014), 523869 https://doi.org/10.1155/2014/523869

[2] Abdulaziz A. Al-Askar, Amr H. Hashem, Nadeem I. Elhussieny, Ebrahim Saied, Green Biosynthesis of Zinc Oxide Nanoparticles Using *Pluchea indica* Leaf Extract: Antimicrobial and Photocatalytic Activities, *Molecules*, 28, 12, (2023), 4679 https://doi.org/10.3390/molecules28124679

[3] Sekar Vijayakumar, Baskaralingam Vaseeharan, Balasubramanian Malaikozhundan, Malaikkarasu Shobiya, *Laurus nobilis* leaf extract mediated green synthesis of ZnO nanoparticles: Characterization and biomedical applications, *Biomedicine and*

Pharmacotherapy, 84, (2016), 1213-1222 https://doi.org/10.1016/j.biopha.2016.10.038

[4] Gaurav Pal, Priya Rai, Anjana Pandey, Chapter 1 - Green synthesis of nanoparticles: A greener approach for a cleaner future, in: A.K. Shukla, S. Iravani (Eds.) *Green Synthesis, Characterization and Applications of Nanoparticles*, Elsevier, 2019, https://doi.org/10.1016/B978-0-08-102579-6.00001-0

[5] Agnieszka Kołodziejczak-Radzimska, Teofil Jesionowski, Zinc Oxide-Form Synthesis to Application: A Review, *Materials*, 7, 4, (2014), 2833-2888 https://doi.org/10.3390/ma7042833

[6] Dušan Nohavica, Petar Gladkov, ZnO Nanoparticles and Their Applications - New Achievements, *2nd International Conference NANOCON 2010 Proceedings*, Czech Republic, 2010

[7] Th. Abhishek Singh, Anirudh Sharma, Neeraj Tejwan, Noyel Ghosh, Joydeep Das, Parames C. Sil, A state of the art review on the synthesis, antibacterial, antioxidant, antidiabetic and tissue regeneration activities of zinc oxide nanoparticles, *Advances in Colloid and Interface Science*, 295, (2021), 102495 https://doi.org/10.1016/j.cis.2021.102495

[8] D. Rehana, D. Mahendiran, R. S. Kumar, A. K. Rahiman, In vitro antioxidant and antidiabetic activities of zinc oxide nanoparticles synthesized using different plant extracts, *Bioprocess and Biosystems Engineering*, 40, (2017), 943-957 https://doi.org/10.1007/s00449-017-1758-2

[9] Duduku Krishnaiah, Rajesh Nithyanandam, Rosalam Sarbatly, Phytochemical Constituents and Activities of *Morinda citrifolia* L., in: V. Rao (Ed.) *Phytochemicals – A Global Perspective of Their Role in Nutrition and Health*, IntechOpen, 2012, https://doi.org/10.5772/26094

[10] Asha R. Pai, Kavitha S, Shweta Raj S., Priyanka P., Vrinda A., Vivin T. S., Silpa Sasidharan, Green Synthesis and Characterization of Silver Nanoparticles Using Fresh Leaf Extract of *Morinda citrifolia* and Its Anti-Microbial Activity Studies, *International Journal of Pharmacy and Pharmaceutical Sciences*, 7, 3, (2015), 459-461

[11] Parth Malik, Ravi Shankar, Vibhuti Malik, Nitin Sharma, Tapan Kumar Mukherjee, Green Chemistry-Based Benign Routes for Nanoparticle Synthesis, *Journal of Nanoparticles*, 2014, (2014), 302429

[12] Mohammad Ramzan Parra, Fozia Z. Haque, Aqueous chemical route synthesis and the effect of calcination temperature on the structural and optical properties of ZnO nanoparticles, *Journal of Materials Research and Technology*, 3, 4, (2014), 363-369 https://doi.org/10.1016/j.jmrt.2014.07.001

[13] Paini Sri Widyawati, Tarsisius Dwi Wibawa Budianta, Fenny Anggraeni Kusuma, Evelyn Livia Wijaya, Difference of Solvent Polarity to Phytochemical Content and Antioxidant Activity of *Pluchea indica* Less Leaves Extracts, *International Journal of Pharmacognosy and Phytochemical Research*, 6, 4, (2014), 850-855

[14] Vijay D. Tambe, Rajendra S. Bhambar, Estimation of Total Phenol, Tannin, Alkaloid and Flavonoid *Hibiscus tiliaceus* Linn. Wood Extracts, *Research and Reviews: Journal of Pharmacognosy and Phytochemistry*, 2, 4, (2014), 41-47

[15] J. Yang, R. Paulino, S. Janke-Stedronsky, F. Abawi, Free-Radical-Scavenging Activity and Total Phenols of Noni (*Morinda citrifolia* L.) Juice and Powder in Processing and Storage, *Food Chemistry*, 102, 1, (2007), 302-308 https://doi.org/10.1016/j.foodchem.2006.05.020

[16] Praveen. K. Ramamoorthy, Awang Bono, Antioxidant Activity, Total Phenolic and Flavonoid Content of *Morinda citrifolia* Fruit Extract from Various Extraction Processes, *Journal of Engineering Science and Technology*, 2, 1, (2007), 70-80

[17] A. Rivera, L. Cedillo, F. Hernández, V. Castillo, A. Sánchez, D. Castañeda, Bioactive Constituents in Ethanolic Extract Leaves and Fruit Juice of *Morinda citrifolia*, *Annals of Biological Research*, 3, 2, (2012), 1044-1049

[18] Palaniselvam Kuppusamy, Mashitah M. Yusoff, Gaanty Pragas Maniam, Natanamurugaraj Govindan, Biosynthesis of metallic nanoparticles using plant derivatives and their new avenues in pharmacological applications – An updated report, *Saudi Pharmaceutical Journal*, 24, 4, (2016), 473-484 https://doi.org/10.1016/j.jsps.2014.11.013

[19] A. Sirelkhatim, S. Mahmud, A. Seeni, N. H. M. Kaus, L. C. Ann, S. K. Mohd Bakhori, H. Hasan, D. Mohamad, Review on Zinc Oxide Nanoparticles: Antibacterial Activity and Toxicity Mechanism, *Nano-Micro Letters*, 7, (2015), 219-242 https://doi.org/10.1007/s40820-015-0040-x

[20] Sathia Lingam Valan, Alice Escalante De Cruz, Patricia Jayshree Jacob, Sinouvassane Djearamane, Sustainable Synthesis of Copper Oxide Nanoparticles Using *Aquilaria malaccensis* (Agarwood) Leaf Extract as Reducing Agent, *International Journal of Technology*, 13, 5, (2022), 1115-1125 https://doi.org/10.14716/ijtech.v13i5.5845

[21] Yanti Puspita Sari, Amanda Qory Suchi, Rudy Agung Nugroho, Biosynthesis and Characterization of Silver Nanoparticles Using Single Garlic Callus Extract (*Allium sativum* L.), *Malaysian Journal of Fundamental and Applied Sciences*, 19, 4, (2023), 563-572 https://doi.org/10.11113/mjfas.v19n4.2944

[22] Freddy Kleitz, Wolfgang Schmidt, Ferdi Schüth, Evolution of mesoporous materials during the calcination process: structural and chemical behavior, *Microporous and Mesoporous Materials*, 44-45, (2001), 95-109 https://doi.org/10.1016/S1387-1811(01)00173-1

[23] P. Ravikumar, S. Sathish Kumar, Antifungal Activity of Extracellularly Synthesized Silver Nanoparticles from *Morinda citrifolia* L., *International Journal of Technical Research and Applications*, 2, 4, (2014), 108-111