

## Green Synthesis of Selenium Nanoparticles from Okra (*Abelmoschus esculentus*) Extract with Characterization and Antioxidant Activity

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**Abstract:** Selenium nanoparticles (SeNPs) have gained attention in the medical field because of their enhanced bioactive properties and bioavailability. The green synthesis of nanoparticles offers an eco-friendly synthesis method. In this study, *Abelmoschus esculentus* (okra) extract was used for the green synthesis of SeNPs. The synthesized SeNPs were characterized using UV-visible (UV-vis), scanning electron microscopy-energy dispersive X-ray (SEM-EDX), Fourier-transform infrared spectroscopy (FTIR), and particle size analyzer (PSA). UV-vis analysis showed a peak at a wavelength of 288 nm, and the SeNPs demonstrated stability at low temperatures for up to 30 days. FTIR analysis indicated interactions between the pectin functional groups on okra and the surface of SeNPs. The SeNPs had a 6.48 nm diameter and polydispersity index of 0.13. A spherical morphology was observed in the nanoparticles. The antioxidant activity of SeNPs was evaluated using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) methods. The results showed a dose-dependent increase in free radical inhibition with 31.44 and 15.37% by DPPH and ABTS tests, respectively. The green synthesis of SeNPs using okra extract produced nanoparticles with unique properties based on several characterization results. In conclusion, SeNPs exhibited promising antioxidant activity, highlighting their potential for biomedical applications.

**Keywords:** green synthesis; nanoparticle; okra; selenium; antioxidant

## ■ INTRODUCTION

Synthesis of nanoparticles has been applied in many fields, such as diagnostics, electronics, biomedicine, and the environment [1]. This material can be synthesized using physical, chemical, and biological methods [2]. The use of nanomaterials in physical and chemical methods often results in issues such as high cost, high toxicity, and complex methods [3]. Green synthesis is becoming a new

solution due to its low cost, low toxicity, fewer side effects, and eco-friendliness. This method uses non-hazardous reagents, simple processes, and light reactions [4]. This can significantly reduce toxic precursors related to conventional physical and chemical methods [5]. Green synthesis uses plants, fungi, algae, and bacteria because of their bioactive compounds that can act as reductors to stabilize nanoparticles [6]. Several natural bacterial

strains, such as *Pseudomonas putida*, *Bacillus thuringiensis*, *Oscillatoria limnetica*, *Bacillus haynesii*, *Lactobacillus casei*, *Aeromonas hydrophila*, and *Streptomyces* spp produce proteins, enzymes, and carbohydrates, which also act as reductors [7]. Some plants, including *Morinda citrifolia* L leaves, *Nymphae odorata* leaves, *Capparis zeylanica* leaves, *Jatropha curcas* latex, *Tribulus terrestris* fruit, Cinnamon bark, *Curcuma pseudomontana* rhizome, *Rosa hybrid* rose petals, *Curcuma longa* root, *Citrus sinensis* fruit peel, and *Beta vulgaris* root, have various biomolecules such as proteins, flavonoids, terpenoids, saponins, enzymes, organic acids, vitamins, and polysaccharides, which act as reducing agents and stabilizers to inhibit aggregation and support continuous nanoparticle synthesis [8].

Selenium (Se) is a well-known metalloid or semimetal with significant physiological roles in the body [9]. It is also recognized as an essential microelement contributing to antioxidant activity in the body, along with zinc, copper, and iron [10]. This element serves as a cofactor in the formation of selenoproteins, while catalyzing the hydroperoxide by glutathione peroxidase (GPx) [11]. Se is also recognized as a protective agent against reactive oxygen species, which can lead to degenerative diseases such as diabetes, cancer, and inflammation [12]. Se nanoparticles (SeNPs) are popular despite their optical characteristics, catalytic, antiviral, anti-inflammatory, antibacterial, and anticancer properties [13]. SeNPs have been proven to reduce toxicity and improve the bioavailability of Se compared to their inorganic (selenate and selenite) and organic forms (selenomethionine and selenocysteine) [14]. Several plants, such as broccoli, lemons, almonds, green tea, and roselle, have been used as stabilizers for the synthesis of SeNPs [15]. Plant extracts can control nanoparticle size, shape, and stability without toxic chemical agents. Several factors, such as pH, extract percentage, temperature, and reaction time, play a role in the nanoparticle topology [16]. During green synthesis, metals can interact with proteins and bioactive compounds from plants and bacteria [17]. However, research on the green synthesis of SeNPs remains inadequate and requires further exploration, particularly in the biomedical field.

*Abelmoschus esculentus* (okra) belongs to the Malvaceae family [18]. Okra grows first in Ethiopia and Africa and then widely spreads across the world, especially in tropical and subtropical regions. It is well-known in several countries, such as Thailand, Japan, Malaysia, China, and India. Recently, okra has gained popularity and is being cultivated extensively in Asian and Middle Eastern countries [19]. Okra contains numerous bioactive compounds, such as quercetin, saponin, linoleic acid, and pectin, which are derived from various parts of plants, including fruits, seeds, leaves, and stems [20]. These compounds contribute to the diverse bioactivities of plants, such as anti-obesity, antioxidant, immunomodulatory, hepatoprotective, and wound-healing activities [21]. Pectin in okra comprises several components, such as *D*-galactose (25%), galacturonate acid (27%), and *L*-ramosa (22%) [18]. Some okra compounds can facilitate green synthesis of nanoparticles, including gold, silver, copper, and iron nanoparticles [22-25]. However, there is still a lack of information in the green synthesis of SeNPs with okra extract.

Antioxidants have become essential for a healthy human life. Some studies have shown that antioxidants are necessary for maintaining a good body fit. Every year, antioxidants have become a new issue regarding the elevation of free radicals from several sources such as cigarettes, UV light, vehicle smoke, industry waste, late sleep, and fast food [26]. Moreover, some disruption processes in body metabolism also contribute to increasing free radicals involved in exercise, inflammation, and ischemia [27]. Antioxidants necessary more become elevate every day endogenous antioxidant from the body such as glutathione, superoxide dismutase (SOD), catalase, and GPx always neutralize free radicals and try to protect cells from oxidation [28]. Oxidative stress is an imbalance condition between antioxidants and free radicals. Endogenous antioxidants often become exhausted against free radicals, especially when the body's condition is not fit. The body must be able to take exogenous antioxidants from the outside body, including natural food from animals and plants [29]. Alternative supplements are also needed by the body to boost the

antioxidant. Nanoparticles are known to have the potential for antioxidant activity. However, multiple nanoparticle variations are still necessary as alternative supplements for the body [15]. More variation of nanoparticles can be an option for humans to consume, surely with no toxicity, high antioxidant activity, and safety when consumed in the body. Unique nanoparticle properties can enhance Se functionality in the body as a primary cofactor for antioxidant enzymes. Se has several weaknesses in the body, such as being regarded as a toxic material and having low bioavailability, which requires a nanoparticle state. Moreover, flavonoids from the extract have a limited time in the body, decreasing their effectiveness in antioxidant activity over time. SeNPs can become a new solution for elevating antioxidant activity in the body [25].

Based on this information, bioactive compounds from plants, especially okra extracts, can be used for the green synthesis of SeNPs. These nanoparticles were characterized by particle size analysis (PSA) to measure the size of SeNPs, ultraviolet-visible (UV-vis) spectrophotometry to analyze wavelength and absorbance from SeNPs, Fourier-transform infrared spectroscopy (FTIR) to analyze functional group of SeNPs, and scanning electron microscope-energy dispersive X-ray (SEM-EDX) to analyze morphology shape, and main components from SeNPs. Additionally, antioxidant activities were evaluated using 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) to explore antioxidant activity values of SeNPs by a green synthesis from okra extract.

## ■ EXPERIMENTAL SECTION

### Materials

Okra fruits were purchased from a local garden in Malang, East Java, Indonesia. Selenious acid ( $\text{H}_2\text{SeO}_3$ ) was purchased from Sigma-Aldrich (USA). DPPH, ABTS, potassium persulfate ( $\text{K}_2\text{S}_2\text{O}_8$ ), and dimethyl sulfoxide (DMSO) were obtained from Merck, Germany.

### Instrumentation

This study used several instruments such as a UV-vis spectrophotometer (GENESYS 40/50, ThermoScientific,

USA), X-ray diffraction (XRD) (Miniflex600, Rigaku, Japan), FTIR (Nicolet iS 10, ThermoScientific, USA), SEM-EDX (Phenom ProX, Thermo Fisher-Scientific, USA), PSA (Beckman Coulter, Delsa Nano C), and enzyme-linked immunosorbent assay (ELISA) reader (800 TS, Agilent Biotek, USA).

### Procedure

#### *Okra pod extraction*

The okra was chopped into small pieces and dried at 80 °C for 12 h. The dried parts were ground into powder for extraction. The extraction method was performed according to Alagesan and Venugopal with slight modifications [30]. Briefly, 10 g of okra powder was mixed with 500 mL of deionized water to obtain a 2% (b/v) okra extract. The mixture was boiled at 85 °C for 1 h until it turned yellow. To remove unnecessary residues, the solution was filtered through Whatman filter paper (Cytiva, UK) with 0.45–90 µm. The filtrate was centrifuged three times at 2000 rpm for 20 min to purify the okra extract and subsequently stored at 4 °C for use in the green synthesis of SeNPs.

#### *SeNPs preparation*

Green synthesis of SeNPs with okra extract was conducted by carefully adding 50 mM  $\text{H}_2\text{SeO}_3$  solution to the okra extract at a ratio of 1:4 v/v. Sodium hydroxide (NaOH) was added to adjust the pH level to 10. The solution was magnetically stirred at 400 rpm at room temperature for 24 h. The green synthesis process was indicated by a change in color from no color to brick red, and the nanoparticles were characterized using UV-vis and PSA. The resulting solution of SeNPs was lyophilized using a freeze-dryer to obtain a powdered sample. This powder was further characterized using FTIR and SEM. The lyophilization method was chosen over high-temperature drying to minimize the deformation of the bioactive compounds.

#### *UV-vis spectroscopy analysis*

The analysis was conducted using UV-vis spectroscopy within a wavelength range of 250–700 nm, employing a fast scan with a 2.0 nm interval. The SeNPs suspension was diluted 30 times and placed on a sample holder for the initial analysis. Prior to this, a blank

solution was measured for calibration. The results of the analysis are presented as a UV-vis spectroscopy graph.

The stability of the SeNPs was assessed using UV-vis spectroscopy and visual observation based on the period times and storage conditions. The samples were stored in vial bottles at 4 °C (low temperature) and 27 °C (room temperature) for 30 days to measure their stability. The conditions after green synthesis were set as day 0. A stability analysis was conducted on days 1, 5, 21, and 30. The sample was subjected to UV-vis spectroscopy and color changes.

#### PSA analysis

The SeNPs solution was placed in a sample holder with a volume of 3.0–3.5 mL for measurement. Each sample was diluted 100 times before analysis. The solution was then sonicated for 10 min using an ultrasonicator. The suspension was analyzed to determine the nanoparticle size and PDI.

#### FTIR analysis

Sample preparation was conducted by creating a powdered sample of SeNPs to form a thin pellet along with the KBr matrix. The sample was placed on an infrared radiation track. The pellet was then placed in an infrared radiation track. This analysis used a spectrum range of 500–4000  $\text{cm}^{-1}$ , 4–8  $\text{cm}^{-1}$  resolution, and time accumulation of 40 s.

#### SEM-EDX analysis

The analysis was conducted by placing the sample powder of the SeNPs on carbon tape. The SeNPs were coated with gold using a sputter coater. This analysis produced figures with different magnifications for the 15 kV mode. EDX analysis was also performed in 15 kV mode to determine the elemental composition of the samples.

#### DPPH test

The DPPH assay was performed to measure the antioxidant activity of SeNPs. Preparing test samples involved preparing stock solutions of SeNPs at a concentration of 4 mg/mL. Dilutions were performed to prepare different concentrations of SeNPs (2000, 1500, 1000, 800, 400, 200, 100, and 50  $\mu\text{g/mL}$ ). Each concentration was mixed with DPPH 50  $\mu\text{g/mL}$  in a ratio

of 1:2. The final mixture was allowed to react in a dark room for 1 h, and the absorbance of the sample was measured at 490 nm using an ELISA reader. The experiments were performed in triplicate. The percentage of antioxidant activity after the DPPH test was calculated using Eq. (1);

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

where  $A_{\text{control}}$  and  $A_{\text{sample}}$  are the absorbance of the control and sample, respectively.

#### ABTS test

The method begins by preparing the ABTS reagent. First, 7 mM solution of ABTS was mixed in a 1:1 ratio with 2.45 mM  $\text{K}_2\text{S}_2\text{O}_8$ . The mixture was then allowed to react in the dark for 12–16 h to form ABTS cation radicals. Subsequently, the mixture was diluted with ethanol until the absorbance value reached approximately 0.7 at a wavelength of 734 nm. The subsequent procedure followed that of the DPPH assay. Briefly, different concentrations (ranging from 50 to 2000  $\mu\text{g/mL}$ ) of SeNPs and okra extract were mixed with the ABTS solution prepared in a 1:40 ratio. The mixture was agitated on a shaker for 5 min and incubated in the dark for 10 min. The degree of decolorization was measured at 630 nm wavelength. The percentage of  $\text{ABTS}^+$  inhibition was also calculated using Eq. (1).

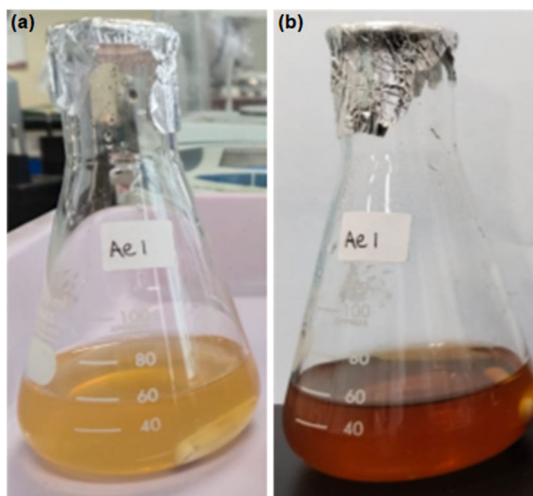
#### Data analysis

The antioxidant data was displayed as mean  $\pm$  SD. OriginPro 2024 software was used to analyze the UV-vis, stability, FTIR, and PSA results. Antioxidant activity was determined using Excel and GraphPad Prism 10 for the DPPH and ABTS assays.

## ■ RESULTS AND DISCUSSION

### Visual Observation

The visual observation was conducted for 24 h at room temperature for early screening of the green synthesis process of SeNPs. In addition of  $\text{H}_2\text{SeO}_3$  (0 h), the sample had brown with yellowish color in the liquid sample (Fig. 1(a)). After 24 h, the sample showed different colors to become reddish, as shown in Fig. 1(b). Color changes that happened from sample indicated a



**Fig 1.** Visual observation of SeNPs (a) before and (b) after 24 h

successful selenium ion reduction process in okra extract solution, especially from surface plasmon resonance (SPR) by SeNPs adsorption in light [17,31].  $\text{Se}^{4+}$  ion, which is colorless, becomes a  $\text{Se}^0$ , which has a brick red color after being reduced by active compounds from okra extracts. Reduced Se faced nucleation, which was stopped by a capping agent from okra's phytochemicals [7]. Several studies have similar results, but more phytochemicals from the extract were affected by color alteration, too. This alteration represents nanoparticle stabilization from the metal solution after being reduced by several bioactive compounds [4,25,28].

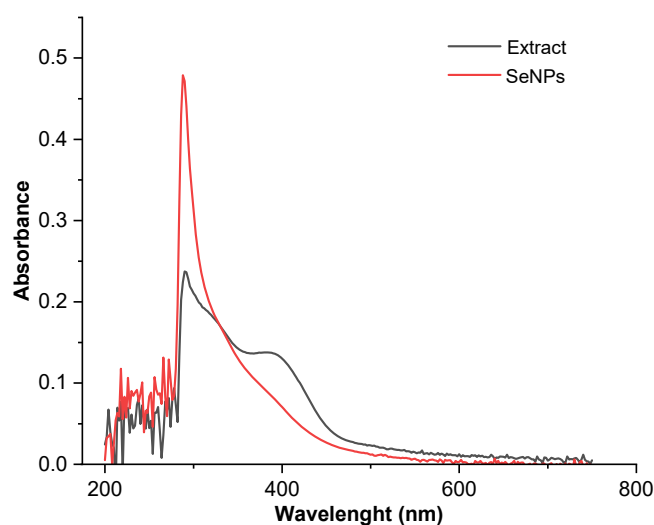
### Characterization of UV-vis Spectroscopy

UV-vis spectroscopy was characterized to confirm nanoparticle formation by identifying their characteristic absorption, known as SPR [32]. As shown in Fig. 2, the UV-vis absorption peak of SeNPs was observed after 24 h, with a distinct peak at a wavelength of 288 nm. SeNPs synthesized using plant extracts exhibit distinct absorption peaks within the wavelength range of 250–290 nm [33–34]. Additionally, the absorption peak of okra extract was included as a reference for comparison with SeNPs. The comparison between SeNPs and okra extract revealed similar absorption patterns but different absorbance values. The okra extracts also exhibited a peak at 382 nm. An increase in the absorbance values demonstrated the formation of SeNPs [35]. This peak also

indicated that after 24 h of incubation, the extract reacted with selenium precursors to form SeNPs optimally and replaced all traces of okra extract absorption with SeNPs absorption at 288 nm [36]. UV-vis data provided information related to the optical properties of the SeNPs. The sharp absorption peak observed suggests that the SeNPs likely had a spherical shape without aggregation, which also determines the full light absorbed by SeNPs. Otherwise, in comparison with a peak from the extract that is not fully spherical due to many aggregations was formed which could disrupt light absorption [37–39].

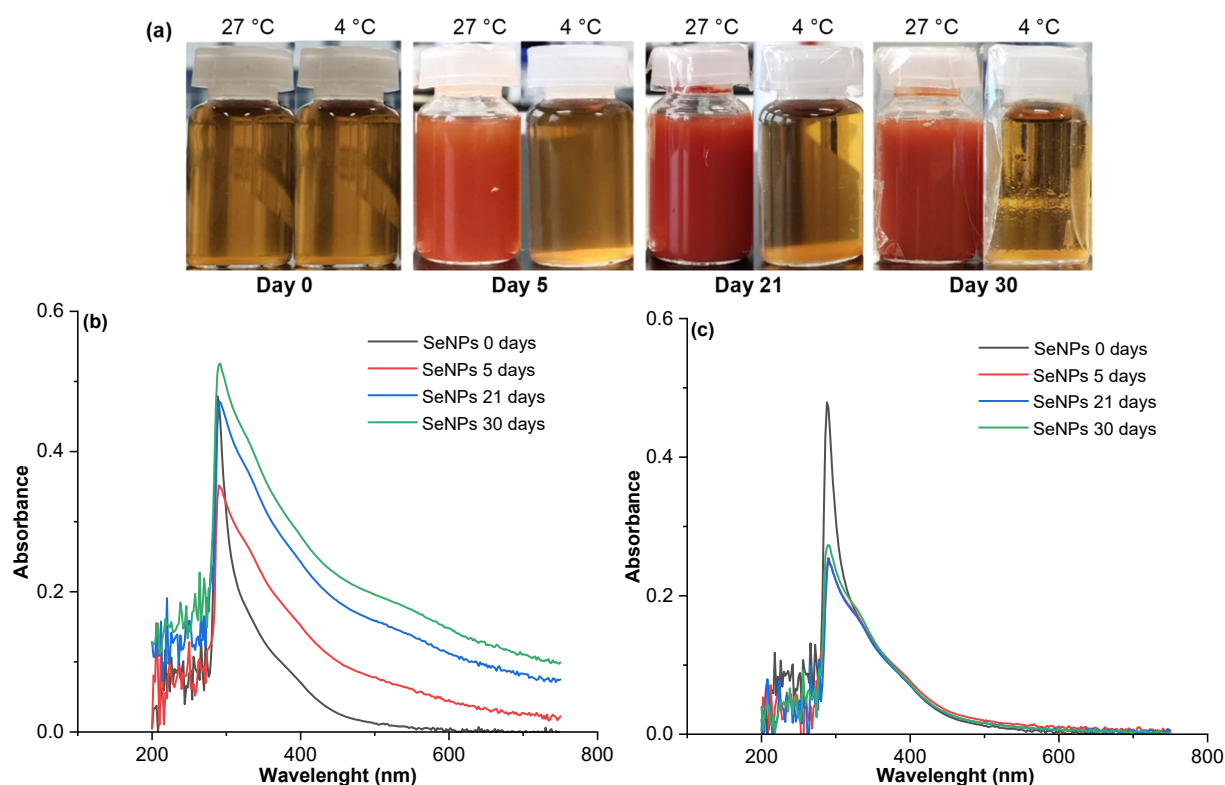
### Stability Test

The stability of SeNPs was investigated under two distinct storage conditions: room temperature (27 °C) and low temperature (4 °C) for 30 days. Observations were conducted on days 5, 21, and 30, encompassing visual assessments and UV-vis absorption measurements based on days and temperatures. Visually, significant color changes and precipitation occurred in samples stored at room temperature (Fig. 3(a)). The SeNPs solution transitioned from brownish to brick-red, becoming more concentrated over time. Conversely, no color change or precipitation was observed under low-temperature storage (4 °C), while in the same intensity of light lamp at room temperature. This color change resulted from the reduction process by



**Fig 2.** UV-vis spectroscopy analysis of SeNPs





**Fig 3.** Stability test of SeNPs where (a) visual observation of SeNPs on day 0, 5, 21, and 30 and UV-vis spectroscopy analysis for 0, 5, 21, and 30 days at (b) 27 °C and (c) 4 °C

okra extract, which affected the SPR effect of SeNPs. Meanwhile, deposition processes can be attributed to nanoparticle agglomeration along with time, which is consistent with the theory that higher temperatures enhance particle kinetics and collisions. Moreover, long-term storage of SeNPs affected by pH declined after faster degradation of a capping agent from the okra compound at room temperature than at low temperature. This phenomenon could increase the nucleation process in the green synthesis of SeNPs, which produces more particles. Some SeNPs also aggregate to form stability between particles [16,40-41]. Additionally, SeNPs exhibited rapid changes at room temperature (on day 5), possibly owing to excess reducing agents from okra. Okra extracts contain many bioactive compounds that take the role of bio-reduction, such as quercetin, pectin, epigallocatechin, and rutin. This excess may accelerate continuous nanoparticle formation, limiting particle size control by the encapsulating or capping agent [19,42]. The results of the UV-vis analysis are shown in Fig. 3(b), which shows that room-temperature storage led to increased

absorbance, peak broadening, shifts, and new absorption features, consistent with the agglomeration and high deformation levels observed visually [36]. In contrast, low-temperature storage maintained stable peak absorption until day 30, as shown in Fig. 3(c). This condition reveals that the nucleation process from green synthesis is affected by temperature. Increasing temperature denatures capping agents from the bioactive compounds, which accelerates the nucleation process in the green synthesis of SeNPs [43-44].

### FTIR Analysis

Based on the FTIR analysis shown in Fig. 4, this observation aimed to identify compound contents and investigate reactions based on their functional groups. Okra extract is known to be rich in polysaccharides, including pectin, and several flavonoids, such as quercetin, epigallocatechin, rutin, and catechin [18]. Pectin is a complex polysaccharide with a galacturonate chain structure [45]. The FTIR spectrum SeNPs revealed several functional group signals at 1626, 1410, 1259,

1078, and  $742\text{ cm}^{-1}$ . Furthermore, the spectrum of okra extract demonstrated characteristic polysaccharide and flavonoid absorption bands, including a broad and strong hydroxyl ( $\text{-OH}$ ) band at  $3398\text{ cm}^{-1}$ , a weak stretching ( $\text{C-H}$ ) band at  $2939\text{ cm}^{-1}$ , and absorption bands at  $1259$  and  $1078\text{ cm}^{-1}$  related to  $\text{C-O}$  and  $\text{C-O-C}$  stretching. Furthermore, the intense and sharp bands at  $1626\text{ cm}^{-1}$  and the absorption band at  $1410\text{ cm}^{-1}$  correspond to the asymmetric and symmetric stretching of the carbonyl group ( $\text{C=O}$ ) in carboxylate anion ( $\text{-COO}^-$ ), confirming the presence of uronic acid which displayed characteristic of pectin polysaccharides [46-49]. In this study, SeNPs had several functional groups similar to the structural components of okra extract, containing pectin and flavonoids. These functional groups come from okra extract, which proves that there is interaction between Se and the okra phytocompound. This interaction is important for green synthesis in order to prevent aggregation and increase long-term stability [50]. These transmittances are likely influenced by oxidation-reduction reactions involving  $\text{C=O}$  and  $\text{-OH}$  components, acting as natural reducers and encapsulating agents [50]. Additionally, a significant increase was observed at  $742\text{ cm}^{-1}$ , associated with vibrational stretching of metal-oxygen bonds, indicating SeNPs binding to the  $\text{-OH}$  groups in the okra extract, forming  $\text{Se-O}$  coordination bonds [51-54].

### PSA Analysis

PSA analysis was conducted to examine the effect of the extract concentration on the size distribution and PDI of the synthesized SeNPs. The size distribution of SeNPs is depicted in Fig. 5, where the average diameter of the SeNPs was found to be  $6.48\text{ nm}$ . The SeNPs exhibited a uniform size distribution with a PDI of  $0.1$ , indicating that the extract concentration significantly influenced the particle size of the SeNPs. The small PDI explained that uniformity from SeNPs is vital to prevent agglomeration between particles [35]. The green synthesis of SeNPs involves the reduction of  $\text{Se}^{4+}$  to  $\text{Se}^0$ . This process occurs through tautomerization, transferring electrons to Se ions [55]. The extract contains active compounds, including potent reducing agents and stabilizers, that facilitate the formation of stable selenium nanoparticles [56].

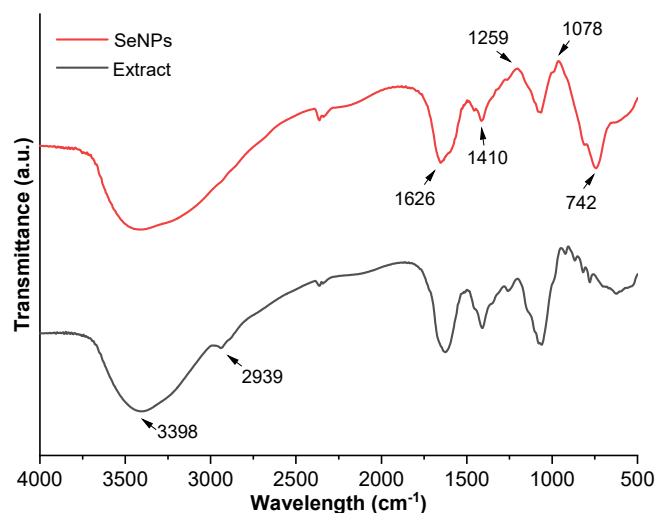


Fig 4. FTIR analysis of SeNPs and extract

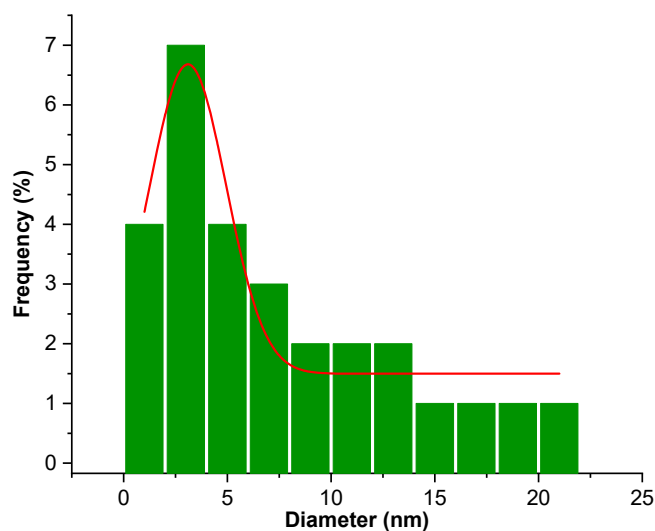


Fig 5. PSA analysis of SeNPs

Pectin compounds present in okra extract exhibit distinct interactions during the reduction of  $\text{Se}^{4+}$  ions [57]. In this process, not only do the  $\text{-OH}$  play a role in the SeNPs formation through a series of oxidation-reduction reactions, but other functional groups, such as  $\text{-COOH}$ , also contribute to SeNPs stability [58]. During the SeNPs green synthesis using okra extract, adding  $\text{NaOH}$  facilitated de-esterification, converting methoxy ester groups ( $\text{-OCH}_3$ ) into  $\text{-OH}$  groups within the pectin chain. This process is crucial for enriching  $\text{-COOH}$  groups [59]. Subsequently, an advanced mechanism occurred by deprotonating the  $\text{-COOH}$  groups into  $\text{-COO}^-$ . This condition was affected by the alkaline pH of the solution, which increased the

interaction between Se and phytochemicals from okra extract. These carboxylate anions, along with other functional groups, such as  $-OH$  from pectin and flavonoids, are known to cover and stabilize the nanoparticle surface through steric and electrostatic interactions [60]. Steric interactions involve the functional groups surrounding the formed nanoparticles, creating physical barriers that prevent particle aggregation. Electrostatic interactions occur between  $-COO^-$  and Se ions, further enhancing nanoparticle stability [16].

### SEM-EDX Analysis

SEM-EDX analysis was conducted to investigate the morphology and elemental composition of SeNPs. Fig. 6(a) shows an SEM image of the SeNPs at a magnification of 35,000 $\times$ . The SeNPs exhibited spherical shapes and were distributed as uniform particles, consistent with the findings of other studies on SeNPs [61]. Nanoparticle aggregation can be attributed to nucleation and growth processes mediated by phytochemical compounds in the extract and physical interactions that may occur during green synthesis [62]. Typically, nanoparticles have a tiny size and tend not to aggregate rapidly. Smaller sizes have a higher chance of aggregating between particles. In this study, besides SeNPs having a tiny size, they also showed a low uniformity index by PDI. It was confirmed by SEM analysis with homogeneous size, meaning that bioactive compounds from okra extract also act as a capping agent [2]. EDX analysis was performed to determine the elemental composition of the samples. Fig. 6(b) displays a high Se content after EDX, belonging to oxygen (O) and

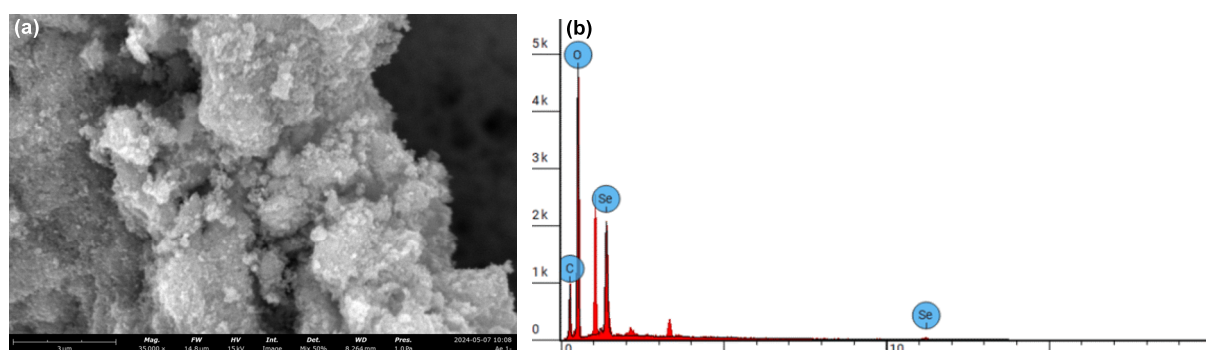
carbon (C) elements. The detailed elemental composition of the sample is presented in Table 1, indicating a Se weight percentage of 19.7%, along with O and C contents of 58.8 and 21.5%, respectively. This composition displayed that SeNPs had three main components, same as the FTIR result, in which Se becomes a key element in this nanoparticle besides O and C. The higher percentages of O and C are closely related to the abundance of polyphenolic components in the okra extract [63].

### Antioxidant Activity

This study examined the antioxidant activity of SeNPs using DPPH and ABTS methods. These two methods represent different mechanisms for scavenging free radicals with hydrogen atom transfer (HAT) and single-electron transfer (SET), respectively [64]. The samples were mixed with either DPPH or ABTS reagent, and their antioxidant capacity was determined by the decrease in absorbance at various sample concentrations [65]. The inhibitory activities against free radicals were shown in Fig. 7. These results indicate that SeNPs have antioxidant activity that increases dose-dependently. In the DPPH test, SeNPs displayed an inhibition activity of up to 31.44%. This result displayed the ability of SeNPs to scavenge DPPH radicals. This process was proven by

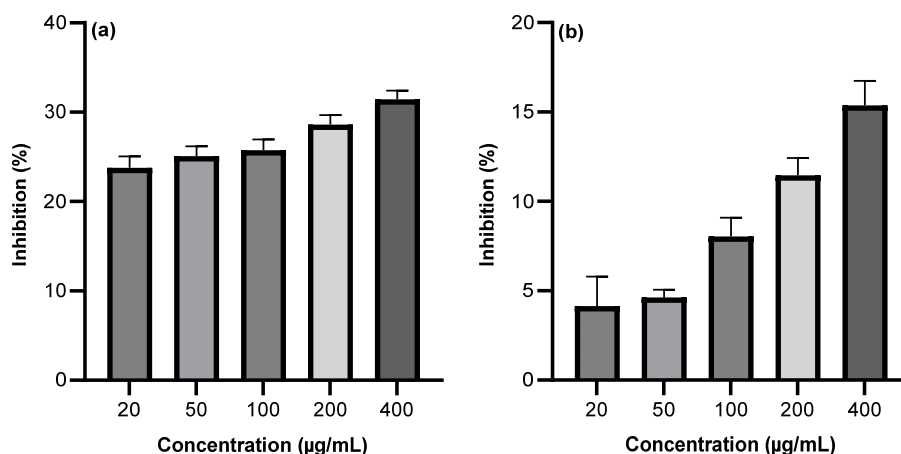
**Table 1.** Elemental composition from SeNPs

Element	Weight (%)
Se	19.7
O	58.8
C	21.5



**Fig 6.** (a) SEM image of SeNPs and (b) EDX graphic of SeNPs





**Fig 7.** Antioxidant activity of SeNPs analyzed by (a) DPPH and (b) ABTS tests

a color change from violet to yellow, which showed a reaction between SeNPs and DPPH radicals [36]. In the ABTS assay, ABTS radicals come from the reaction between ABTS and  $K_2S_2O_8$ . In this study, SeNPs inhibited activity by 15.37% against ABTS radical. In ABTS, the reagent displayed a color change from blue to soft blue to colorless. This means that SeNPs can scavenge against free radicals. The SeNPs displayed could play the role of antioxidants by giving hydrogen atoms to scavenge ABTS radicals. Antioxidant capacity is attributed to the active compounds available during the biosynthesis process using okra. These antioxidant tests rely on the scavenging ability of the sample, which was conducted by SeNPs in this study. The SeNPs have antioxidant activity based on their OH groups, which use hydrogen atoms to scavenge free radicals from DPPH and ABTS. This finding showed that SeNPs had potential for application related to an antioxidant, such as in food, supplements, and cosmetics, absolutely with proper dosage to prevent toxicity from this material [66]. These findings align with the EDX analysis, which revealed a high O composition (over 50%) in SeNPs, signifying the presence of phytochemical components. These components play a crucial role in enhancing the antioxidant activity of SeNPs [28].

## ■ CONCLUSION

The green synthesis of SeNPs using okra extract produces larger quantities of nanoparticles, as evidenced by the high absorbance values observed in UV-vis data. This method also results in nanoparticles with a small

diameter and homogeneous PDI. FTIR analysis confirmed that green synthesis occurred through interactions between the functional groups of pectin and flavonoids in the okra extract. Furthermore, SeNPs exhibited good colloidal stability, maintaining their properties when stored at low temperatures for up to 30 days. The antioxidant activity of SeNPs increased with dosage, highlighting that okra extract not only facilitates nanoparticle synthesis and stabilization but also enhances the antioxidant activity of SeNPs.

## ■ ACKNOWLEDGMENTS

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## ■ CONFLICT OF INTEREST

There is no conflict of interest between authors.

## ■ AUTHOR CONTRIBUTIONS

Raden Joko Kuncoroningrat Susilo, Retna Apsari, and Suhailah Hayaza conceptualized the study. Raden Joko Kuncoroningrat Susilo, Yeremia Budi Cristian, Vania Griselda Prasetyo, Imanda Widiyanti, and Anastasia Alin Palmasih conducted experiments. Raden Joko Kuncoroningrat Susilo, Retna Apsari, and Suhailah Hayaza analyzed the data. Raden Joko Kuncoroningrat Susilo, Retna Apsari, Khairunadwa Jemon, and Elma Sakinatus Sajidah drafted and revised the manuscript accordingly. All the authors have agreed to the final version of the manuscript.

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