ECO-FRIENDLY SYNTHESIS AND ANTIOXIDANT POTENTIAL OF IRON NANOPARTICLES MEDIATED by AVOCADO SEED EXTRACT

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ABSTRACT

The contribution of oxidative stress to chronic diseases has prompted the exploration of sustainable antioxidant agents derived from natural sources. This study aimed to develop and characterize iron nanoparticles (FeNPs-A) synthesized through a green approach using aqueous avocado (Persea americana) seed extract as a biogenic reducing and stabilizing agent. The formation of FeNPs-A was initially identified through visual observation of the colloidal color, followed by UV-Visible spectrophotometric analysis to determine the absorption wavelength. Changes in the hydroxyl functional groups of the extract and FeNPs-A were further examined using FTIR spectroscopy, and the antioxidant activity was evaluated using the DPPH assay. The synthesis process was visually indicated by a distinct color transition from brownish yellow to deep black, confirming the reduction of Fe^{3+} ions. UV-Vis analysis exhibited a characteristic absorption peak at 282 nm, validating nanoparticle formation through surface plasmon resonance (SPR). FTIR spectra showed significant shifts in O-H, C=O, and C-O stretching vibrations and the appearance of a Fe-O band at 399-433 cm^{-1}, indicating the participation of hydroxyl and carbonyl groups in nanoparticle nucleation and stabilization. Antioxidant assessment revealed an IC_{50} value of 78.20 ± 12.44 µg/mL, demonstrating substantial free radical scavenging capacity, with inhibition percentages increasing from 65.83 ± 4.08% to 91.55 ± 0.92% as the concentration increased from 312.5 to 5000 ppm.

Keywords: Antioxidant activity, DPPH assay, Green synthesis, Iron nanoparticles, Persea americana,

INTRODUCTION

Oxidative stress, arising from the excessive accumulation of free radicals, plays a pivotal role in the pathogenesis of numerous chronic diseases, including cardiovascular, neurodegenerative, and malignant disorders (Jomova et al., 2023). In response to this challenge, the identification and development of effective and safe antioxidant agents have become a major focus of pharmaceutical and biotechnological research aimed at preventing or alleviating oxidative damage in biological systems. Alongside these efforts, the rapid advancement of nanotechnology has opened new avenues for enhancing the biological performance of bioactive compounds through nanoparticle-based formulations, which offer a high surface-to-volume ratio and unique physicochemical properties that can improve their reactivity and bioavailability (Nahari et al., 2022).

In this context, green synthesis strategies utilizing plant extracts as both reducing and stabilizing agents have gained increasing attention as environmentally sustainable alternatives to conventional chemical synthesis methods, which frequently involve toxic reagents. Plant-derived secondary metabolites, such as phenolics, flavonoids, tannins, and alkaloids, facilitate the reduction of metal ions into nanoparticles while simultaneously acting as natural capping agents that regulate particle size, morphology, and stability. This approach offers several advantages, including the use of environmentally benign solvents, reduced hazardous waste

generation, and enhanced biocompatibility of the synthesized nanomaterials (Haider et al., 2024; Lee et al., 2015; Yasmin et al., 2014).

Among various plant-based resources, avocado seeds (Persea americana) are an abundant and underutilized agro-industrial by-product rich in bioactive compounds, such as polyphenols, flavonoids, tannins, and lipophilic constituents, with well-documented antioxidant and anti-inflammatory properties. The utilization of avocado seed extract as a bioactive precursor for nanoparticle synthesis not only contributes to the valorization of agricultural waste but also provides an effective natural system for the reduction and stabilization of iron nanoparticles (FeNPs). Previous investigations into the phytochemical composition and antioxidant potential of avocado seeds support their suitability for application in functional and biomedical formulations (Bhuyan et al., 2019; Shabatina et al., 2020; Yan et al., 2021).

In parallel, the biogenic synthesis of iron-based nanoparticles, including FeNPs and iron oxide nanoparticles, has been reported to yield materials with favorable magnetic properties, improved surface stability, and tunable biological activity, which are largely influenced by the phenolic constituents present in plant extracts. Accumulating evidence indicates that green-synthesized iron nanoparticles exhibit notable antioxidant activity and hold promise for diverse biomedical applications, such as drug delivery systems, magnetic resonance imaging (MRI) contrast agents, and therapeutic modulation of oxidative stress through radical scavenging or controlled redox processes (Laurent et al., 2008; Nahari et al., 2022). Therefore, the present study aimed to develop a green synthesis method for iron nanoparticles using aqueous avocado seed extract and evaluate their in vitro antioxidant activity using the DPPH radical scavenging assay.

MATERIALS AND METHODS

Materials and Equipment

Wavelength measurements were performed using a Microplate Reader (Spectrostar Nano) and a Thermo Scientific Multiskan GO spectrophotometer. The materials used included avocado seeds (*Persea americana*) collected from Malang, East Java, Indonesia, ferrous sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$); distilled water (aquadest), methanol, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) reagent.

Preparation of Avocado Seed Extract

Fresh avocado seeds were washed, chopped, and finely ground to obtain a uniform paste. The ground material was then macerated in distilled water for 30 minutes at a ratio of 1:5 (w/v) (avocado seeds: water). The mixture was filtered, and the resulting filtrate was used as a reducing and stabilizing agent for the green synthesis of iron nanoparticles.

Green Synthesis of Iron Nanoparticles (FeNPs-A)

The iron nanoparticles were synthesized by mixing 25 mL of avocado seed extract with 5 mL of 0.01 M $FeSO_4 \cdot 7H_2O$ solution. The reaction mixture was then incubated for 30 minutes at room temperature. After incubation, the mixture was centrifuged at 10,000 rpm for 10 minutes at 4°C. The supernatant was discarded, and the pellet containing the synthesized nanoparticles was collected and stored at 4°C for subsequent analyses.

Observation of FeNPs-A Formation

The formation of iron nanoparticles was visually confirmed by the color change of the reaction mixture from yellow (extract) to black (FeNPs), indicating nanoparticle formation. The optical characteristics of FeNPs-A were analyzed using a UV-Visible spectrophotometer within a wavelength range of 200-400 nm, where characteristic absorption peaks signified successful synthesis.

Antioxidant Activity Assay (DPPH Method)

The antioxidant activity of FeNPs-A was evaluated using a DPPH radical scavenging assay. A 0.2 mM DPPH solution was prepared using methanol. The FeNPs-A samples were tested at concentrations of 5000, 2500, 1250, 625, and 312.5 ppm. The FeNPs-A and DPPH solutions were mixed in a 1:1 (v/v) ratio and incubated for 30 minutes at room temperature in the dark. The absorbance was measured at 517 nm using a spectrophotometer, following a modified method of (Çalışkan et al., 2020).

Radical scavenging activity was calculated using the following equation:

$$Scavenging\ activity\ (\%) = \frac{(A\ control - A\ sample)}{A\ control}\ x\ 100\%$$

where (A-{control}) is the absorbance of the DPPH solution without the sample and (A-{sample}) is the absorbance of the DPPH solution mixed with FeNPs-A.

Data Analysis

All experiments were performed in triplicate, and the results are expressed as the mean ± standard deviation (SD). The percentage of DPPH radical scavenging activity plotted against the sample concentration was calculated using Excel.

RESULTS AND DISCUSSION

Synthesis and Characterization of FeNPs-A Using UV-Visible Spectrophotometry

The synthesis of iron nanoparticles (FeNPs-A) using aqueous avocado seed extract (Ext-A) as a reducing and stabilizing agent was visually confirmed by the distinct color changes during the reaction process. As shown in **Figure 1**, bottle (a) contains a pure iron(III) solution (Fe-Sol), which appears colorless, indicating that the iron ions remain in their dissolved ionic form without undergoing reduction. Bottle (b) represents the aqueous extract of avocado seeds (Ext-A), which is yellowish-brown owing to the presence of phytochemicals such as polyphenols, flavonoids, and tannins, which act as electron-donating biomolecules. Upon mixing Fe-Sol with Ext-A and incubating the reaction mixture, a gradual color transition from brownish-yellow to dark brown and eventually to black was observed, as shown in bottle (c). This visual change strongly suggests the successful reduction of Fe^{3+} ions to zero-valent iron nanoparticles (Fe^0), facilitated by the bioactive compounds present in the extract. This color transformation is a well-established visual indicator of the successful green synthesis of metal nanoparticles, reflecting both the reduction of metal ions and the formation of nanoscale metallic cores. The emergence of a darker color corresponds to surface plasmon resonance (SPR) phenomena, which are typically associated with nanosized metallic particles that exhibit unique optical properties (Haider et al., 2024; Kumar et al., 2023).

These observations are consistent with previous reports that plant-derived phenolic compounds act as dual-function agents, serving as reducing agents that donate electrons to convert metal ions into their nanoparticle form and as capping or stabilizing agents that prevent particle aggregation during synthesis (Nahari et al., 2022; Ong et al., 2022). Therefore, the macroscopic color change observed in this study provides preliminary evidence for the successful green synthesis of iron nanoparticles using avocado seed extract.

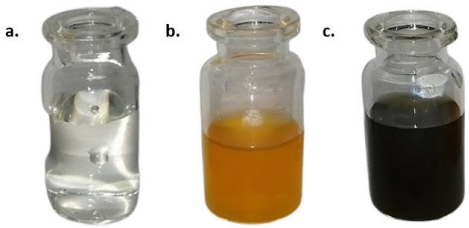

Figure 1. *A visual transformation was observed during the green synthesis of iron nanoparticles (FeNPs-A) using aqueous avocado seed extract (Ext-A) as a reducing and stabilizing agent. a.* **Iron solution b. Avocado extract c. FeNPs-A**

The Fe-Sol spectrum exhibited a relatively low absorption intensity. It lacked any distinct peak within the 200-400 nm range, suggesting that Fe^{3+} ions remained in the dissolved state without significant interaction with organic molecules. In contrast, the Ext-A spectrum exhibited strong absorption bands in the 200-300 nm region (Fig. 2), corresponding to $\pi \rightarrow \pi^*$ electronic transitions in aromatic rings and conjugated double bonds of phenolic and flavonoid compounds naturally present in the avocado seed extract (Bhuyan et al., 2019; Ong et al., 2022).

The FeNPs-A spectrum displayed a distinct absorption peak at approximately 282 nm (Fig.2), which is characteristic of iron nanoparticles formed via the reduction of Fe^{3+} ions by bioactive components in the extract. The shift in the peak position and changes in the absorption intensity compared with Ext-A indicate electronic interactions between iron atoms and organic functional groups such as -OH, -COOH, and -C=O from secondary metabolites, which act as both reducing and capping agents (Haider et al., 2024; Kumar et al., 2023).This observation aligns with previous studies on plant-mediated green synthesis of metallic nanoparticles, where the appearance of absorption bands in the 270-300 nm region corresponds to surface plasmon resonance (SPR) phenomena typical of nanoscale iron particles (Shojaee & Shahri, 2016).

The spectral profile transformation from Ext-A to FeNPs-A provides optical evidence supporting the successful reduction of Fe^{3+} ions into Fe^0 or Fe_2O_3 nanoparticles, facilitated by the phytochemical constituents of the avocado seed extract. These differences in absorption characteristics confirm that the green synthesis method effectively produced stable iron nanoparticles. Moreover, the presence of phenolic compounds adsorbed on the nanoparticle surface may further enhance their biological functionality, particularly their antioxidant activity.

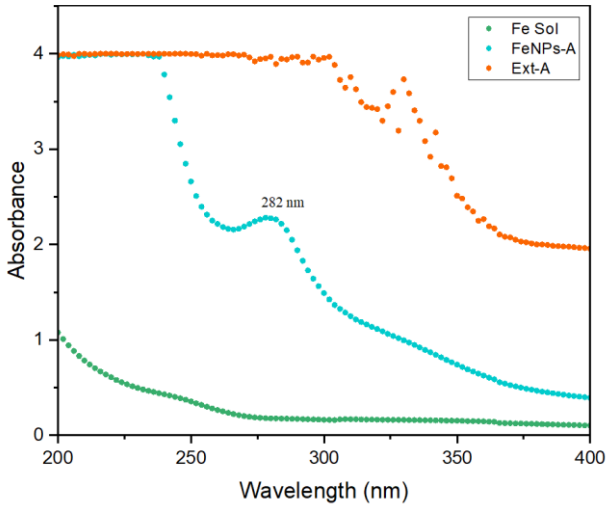

Figure 2. Wavelength Of Fe Solution, Iron Nanoparticles Synthesized With Avocado Seed Extract And Avocado Seed Extract

FT-IR analysis of extracts and FeNPs-A

The FTIR spectra of *Persea americana* extract (Ext-A) and synthesized FeNPs-A exhibited characteristic alterations in functional group vibrations, consistent with a green bioreduction and stabilization mechanism (Fig.3). A broad band observed at 3,300-3,780 cm^{-1} corresponds to the hydrogen-bonded O-H stretching vibrations. Following nanoparticle formation, this band exhibited a blue shift, suggesting the involvement of hydroxyl and phenolic groups in the reduction of Fe^{2+} ions and the formation of intermolecular interactions on the nanoparticle surface. Additionally, the absorption band at approximately 1,637 cm^{-1},

attributable to C=O stretching (amide I or conjugated carbonyl) or aromatic C=C vibrations, decreased in intensity from ext (%T= 68.12) to FeNPs-A (% T= 67.97), implying the participation of carbonyl-containing compounds as electron donors or coordinating sites during particle nucleation and growth. Similar spectral behavior has been reported in other plant-mediated syntheses of metal nanoparticles, where carbonyl and phenolic groups act simultaneously as reducing and capping agents (Çalışkan et al., 2020; Ullah et al., 2025)

In the fingerprint region (1,400-1,000 cm^{-1}), bands near 1,378, 1,252, and 1,008 cm^{-1} correspond to C-H bending, C-O stretching, and aromatic skeletal vibrations, respectively. The redshifts and changes in these bands after FeNPs-A formation indicate the adsorption of oxygenated functional groups on the nanoparticle surface, contributing to the colloidal stabilization. The emergence of a distinct absorption band at 399–433 cm^{-1} in the FeNPs-A spectrum was assigned to Fe-O stretching, confirming the formation of Fe-based nanostructures during the bioreduction of $FeSO_4 \cdot 7H_2O$. The concurrent modifications in the O-H, C-O, and Fe-O regions support the dual functional role of the extract molecules as reducing agents converting Fe^{2+} to Fe-based species and as surface-capping ligands stabilizing the nanostructures. These findings align with those of prior studies on the green synthesis of iron oxide nanoparticles using *Persea americana* and other plant extracts, which reported analogous spectral transformations (Elkhateeb et al., 2024; Tran et al., 2022).

Table I. FTIR Characteristic Peak Positions And Intensity Variations Of Avocado Seed Extract And FeNPs-A

No.	Extract		FeNPs-A	
	Peak	**Intensity**	**Peak**	**Intensity**
1	433.71	27.68	399.24	28.41
2	1212.08	90.31	1008.15	87.46
3	1381.54	89.70	152.29	87.91
4	1456.21	89.70	1378.67	89.26
5	1637.16	68.12	1637.16	67.97
6	2099,59	94.98	2119.70	94.98
7	2352.35	95.13	2352.36	96.57
8	3331.77	45.93	3334.65	46.13
9	3776.97	98.52	3776.97	98.66

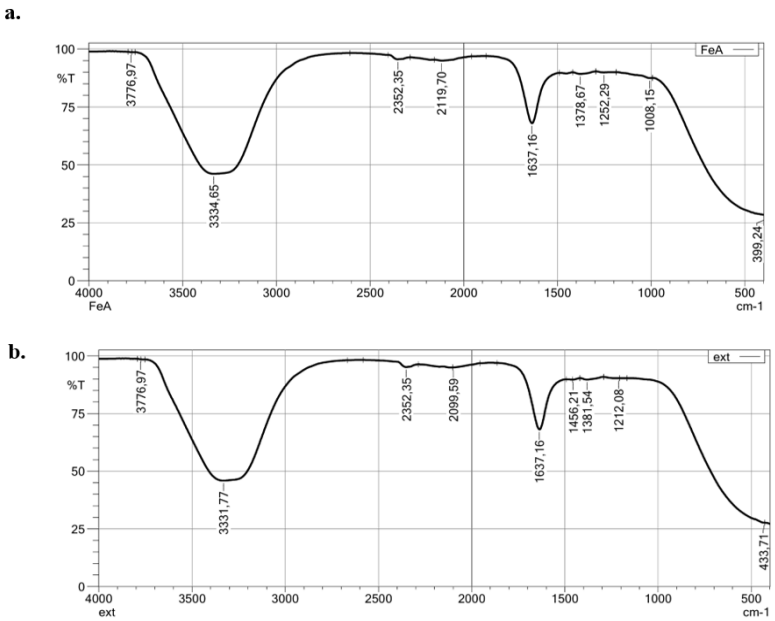

Figure 3. FT-IR Spectra Of A. Synthesized FeNPs Using Avocado Seed Extract And B. Avocado Seed Extract

Antioxidant Activity of Iron Nanoparticles Synthesized Using Avocado Seed Extract (FeNPs-A)

The antioxidant activity of iron nanoparticles synthesized with avocado seed extract (FeNPs-A) was evaluated using the DPPH radical scavenging assay, and the results are presented in **Table I**. The data revealed a concentration-dependent increase in free radical inhibition, indicating that the antioxidant activity of FeNPs-A increased with increasing sample concentration. At the lowest concentration (312.5 µg/mL), FeNPs-A exhibited a radical scavenging activity of 65.83 ± 4.08%. Increasing the concentration to 1250 µg/mL resulted in a gradual rise in inhibition percentage to 71.84 ± 1.46%, suggesting a positive correlation between FeNPs-A concentration and its DPPH scavenging efficiency.

A pronounced increase in antioxidant activity was observed at 2500 µg/mL, where the inhibition reached 91.36 ± 1.01%. This level remained relatively stable at 91.55 ± 0.92% for 5000 µg/mL. This plateau phenomenon indicates that the maximum scavenging capacity of FeNPs-A was achieved at higher concentrations, reflecting the saturation of the active antioxidant sites. The relatively low standard deviation values (≤5%) across all concentrations confirmed the reproducibility and stability of the antioxidant performance of the synthesized nanoparticles. Furthermore, the IC_{50} value derived from the DPPH inhibition curve was determined to be 78.20 ± 12.44 µg/mL, indicating that FeNPs-A exhibits substantial antioxidant efficacy by achieving 50% scavenging of DPPH radicals at a relatively low concentration.

The ability of FeNPs-A to neutralize DPPH radicals is attributed to a dual contribution mechanism. First, the phenolic and carboxylic functional groups from avocado seed phytochemicals adsorbed on the nanoparticle surface can donate hydrogen atoms or electrons to reduce the DPPH• radical to its non-radical form (DPPH-H). Second, the redox-active iron core may participate in additional electron transfer reactions, thereby enhancing the overall antioxidant efficiency. The synergistic interaction between the organic capping molecules and iron nanoparticle core strengthens the radical scavenging mechanism through improved electron transfer and hydrogen donation (Bhuyan et al., 2019; Haider et al., 2024). These findings confirm that FeNPs-A possesses a strong antioxidant capacity, supporting its potential application as an eco-friendly, nanoparticle-based antioxidant agent for biomedical or functional formulations.

Table II. Percentage Inhibition Of Iron Nanoparticles Synthesized Using Avocado Seed Extract (FeNPs-A)

Concentration (µg/mL)	DPPH Inhibition (%) ± SD
312.5	65.83 ± 4.08
625	68.80 ± 2.61
1250	71.84 ± 1.46
2500	91.36 ± 1.01
5000	91.55 ± 0.92

CONCLUSION

In conclusion, iron nanoparticles (FeNPs-A) were successfully synthesized via a green approach using aqueous avocado seed extract as a reducing and stabilizing agent. Spectroscopic analyses confirmed the formation of Fe-based nanoparticles mediated by phytochemicals, with functional groups contributing to particle stabilization. FeNPs-A exhibited notable antioxidant activity, as indicated by an IC_{50} value of 78.20 ± 12.44 µg/mL. These findings highlight the potential of avocado seed extract as a sustainable and cost-effective resource for producing environmentally friendly iron nanoparticles with antioxidant applications. Nevertheless, further characterization using transmission electron microscopy (TEM) is required to confirm the nanoparticle size and morphology, and comprehensive toxicity assessments are necessary to evaluate the safety of FeNPs-A.

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