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Research Article

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Green synthesis of silver nanoparticles using *Eugenia uniflora* extract, its characterization, antioxidant, and anti-inflammatory activities

Ayodeji O. Oriola[1]*, Pallab Kar[2]*, Adebola O. Oyedeji[1,2]

[1]Department of Chemical and Physical Sciences, Walter Sisulu University, Nelson Mandela Drive, P/Bag X1, Mthatha 5117, South Africa, [2]African Medicinal Flora and Fauna Research Niche Area, Walter Sisulu University Nelson Mandela Drive, P/Bag X1, Mthatha 5117, South Africa

ABSTRACT

Nowadays, the use of environmentally benign raw materials, especially medicinal plants, is preferred in the preparation of nano substances known as nanoparticles (NPs), for use in various biological applications. *Eugenia uniflora* is an edible plant with enormous medicinal uses owing to its rich phytochemical content. Therefore, this study was carried out to obtain an *E. uniflora* extract-based silver nanoparticles (Eu-Ag NPs), and to elucidate its antioxidant and anti-inflammatory potential. The NPs were prepared by a conventional green synthesis method. The ultraviolet visible (UV-Vis) and energy-dispersive X-ray (EDX) spectroscopy, X-ray diffraction (XRD) method, and field emission scanning electron microscopy (FESEM), assisted with characterization of the NPs. *In vitro* antioxidant analysis was based on hydrogen peroxide (H_2O_2), nitric oxide (NO), superoxide, and hydroxyl radical scavenging assay methods. The inhibitory effect of the NPs on egg albumin denaturation (EAD) was used for the *in vitro* anti-inflammatory screening. Morphological assessment of the Eu-Ag NPs by FESEM revealed the shape to be spherical to cubical. The maximum absorption peak at 410 nm suggests the formation of AgNPs, while the conversion of metallic silver into elemental silver was confirmed by EDX with 99.50%. The FESEM showed a median size of 45.35 nm for the NPs. The Eu-Ag NPs had good antioxidant and anti-inflammatory potentials, exhibiting an IC_{50} of 48.91±0.12 and 157.41±0.90 µg/mL against H_2O_2 radical and EAD, respectively. This study has shown that *E. uniflora* leaf aqueous extract-mediated silver nanoparticles possess unique structural attributes with considerable antioxidant and anti-inflammatory properties.

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***Corresponding Authors:**
Ayodeji O. Oriola
E-mail: aooriola@gmail.com;
Pallab Kar
E-mail: pallabkar.bio@gmail.com

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INTRODUCTION

Nanotechnology is fast becoming a leading technology, traversing physical, material, chemical, biological, agricultural, pharmaceutical, and medical sciences (Biswas *et al.*, 2022). The unique properties of nanoparticles (NPs) can be influenced by their size, shape, and distribution (Khan *et al.*, 2019). They have a greater surface area than macro-sized materials because of their minute size (Said *et al.*, 2024). Thermal decomposition, chemical reduction, laser ablation, ultrasonication, electrochemical-assisted synthesis, and other physical and chemical processes can all be used to create NPs, but many of the processes involved are expensive and require the use of dangerous chemicals that could cause serious environmental hazards and negatively impact human health (Iravani *et al.*, 2014). Biological methods have emerged as the favoured synthesis approach for creating NPs in recent years because of their relative safety, affordability, and eco-friendliness (Jafarzadeh *et al.*, 2025). The various metals used in nano-synthesis include aluminium, gold, copper, iron, cobalt, zinc, and silver. However, silver is the most widely preferred metal because of its higher thermal stability, better electrical conductivity, and reduced oxidative resistance (Duman *et al.*, 2024). Silver nanoparticles (AgNPs) have also shown promising biological properties including antioxidant and anti-inflammatory activities (Almatroudi *et al.*, 2020).

Recently, plant extracts have become a viable biogenic material in nano-formulations, due to their availability, cost-effectiveness, efficacy, and fewer side effects when compared to synthetic chemicals (Singh *et al.*, 2023). Plants contain fatty acids, terpenoids, flavonoids, alkaloids, phenolics, glycosides and other secondary metabolites, functioning as capping agents in NPs, reducing metal salts into their nanocrystalline form (Santhosh *et al.*, 2022). By squelching reactive oxygen species

(ROS) and free radicals, these bioactive substances mitigate oxidative damage and exhibit potent antioxidant effects (Patil & Kim, 2017).

Eugenia uniflora L. (family Myrtaceae) is small tree that produces whitish inflorescence and greenish berries, with its fruits becoming dark red at full maturity (Burkill, 1985). An infusion of the plant is used ethnomedicinally as a remedy for rheumatism and other inflammation-related ailments (Consolini & Sarubbio, 2002). The plant has several pharmacological activities linked to it, such as antimicrobial, antifungal, antiviral, anti-helminth, insecticidal, anti-diarrhoeal, anti-hypertensive, antitumor, and anti-rheumatic effects (Matsumura *et al.*, 2000; Ogunwande *et al.*, 2005; Amorim *et al.*, 2009). Considering the enormous medicinal value of this plant, the study synthesized *E. uniflora* leaf aqueous extract-based AgNPs and evaluated the antioxidant and ant-inflammatory activities.

MATERIALS AND METHODS

Plant Collection and Processing

Eugenia uniflora leaves were collected from the town of Ile-Ife in Osun State, Nigeria during early rainy season. Plant authentication was done at Ife Herbarium. An herbarium specimen having a voucher number, IFE 16589, was submitted for future reference. The leaves were separated from their branches and air-dried away from direct sunlight inside the screen house with frequent turning. The dried leaves were crushed and milled into fine powder.

Biogenic Synthesis of AgNPs using *E. uniflora*

Biogenic synthesis of NPs was performed using standard procedure reported by Kar *et al.* (2025). The steps include adding 3 mg/mL aqueous solution of *E. uniflora* leaf extract (EU) to $AgNO_3$ solution (0.10 M of $AgNO_3$ in 0.09 L of distilled H_2O). The solution was magnetically stirred at 80±1 °C for 6 h until it turned from colourless to greyish brown. After 1 h of cooling, residues (NPs) were separated from the supernatant using a centrifuge set at 6000 revolutions per minute (rpm) for a period of 20 min, affording *E. uniflora* aqueous extract-based silver nanoparticles (Eu-Ag NPs) (Kar *et al.*, 2022, 2025).

Characterization of *E. uniflora* Extract-based Silver Nanoparticles (Eu-Ag NPs)

The Eu-Ag NPs were analysed on a scanning electron microscope (German manufacturer Carl Zeiss, Jena, Germany) operating at a 3 kV accelerating voltage at 20,000 × magnification. The UV-Vis spectrum was recorded at 200-700 nm wavelength on a PerkinElmer Lambda 25 UV/Vis spectrometer (PerkinElmer, Shelton, CT06484, USA). The weight percentage of elemental silver (Ag) in the NPs was determined on a JEOLJSM-IT100InTouchScope™ scanning electron microscope (Tokyo, Japan) with an in-built Oxford-EDX software package (Oxford Instruments, Abingdon, Oxfordshire, England, UK). An EMPYREAN X-ray diffractometer (Make PAN analytical, The Netherlands), operating at CuK_α radiation of 1.54584 Å, was used to determine NP's crystallinity.

In vitro Antioxidant Analysis of Eu-Ag NPs

NO radical inhibition test

An aqueous solution of Eu-Ag NPs (0-200 µg/mL), phosphate-buffered saline (PBS, pH 7.2±2.0), and 10 mM of sodium nitroprusside were mixed and thoroughly vortexed. The reaction mixture was incubated in the dark at room temperature (≈25 °C) for 2½ h. Thereafter, 0.5 mL of the pre-incubated reaction mixture was dissolved in CH_3COOH (20%) and added to 1 mL of sulfanilamide (0.33%). Finally, 1 mL of N-(1-Naphthyl) ethylenediamine dihydrochloride (0.1%) was added to the reaction mixture and incubated at ≈25 °C for half an hour. The experiment was performed in three replicates, with the optical density recorded at 540 nm wavelength (Unuofin *et al.*, 2017). The percentage inhibition of NO was calculated according to Equation 1:

$$Percentage\,inhibition = \frac{OD0 - OD1}{OD0} \times 100 \quad (1)$$

Where, *OD0* = optical density of negative control (distilled water), and *OD1* = optical density of samples/standard.

H_2O_2 radical scavenging test

The H_2O_2 scavenging capacity of the Eu-Ag NPs was elucidated using the standard method reported by Long *et al.* (1999). The test samples comprising Eu-Ag NPs (0-200 µg/mL), silver nitrate (SN, 0-200 µg/mL) and H_2O_2 (50 mM) were combined, vortexed, and incubated at ≈25 °C for 30 min. Thereafter, H_2O_2-methanol-FOX reagent (100:11:1) were introduced into the reaction mixture in three replicates, and gently vortexed. It was allowed to settle for half an hour before the optical density was recorded at 560 nm. *L*-Ascorbic acid served as the standard antioxidant drug.

Hydroxyl ion radical scavenging test

Here, the standard method reported by Kunchandy and Rao (1990) was used. This entailed combining 2.8 mM of 2-deoxy-2-ribose, 20 mM of KH_2PO_4-KOH (pH 7.4), 100 µM of $FeCl_3$, 100 µM of ethylene diamine tetraacetic acid, 1.0 mM of H_2O_2, 100 mM of *L*-Ascorbic acid, and test samples in three replicates to make 1.0 mL final volume. The reaction mixture was gently vortexed and incubated at 37 °C for 1 h. The mixture, 2.8% trichloroacetic and 1% thiobarbituric acid were combined in ratio 1:2:2. It was incubated for 20 mins at ≈90 °C, while the optical density was recorded at 532 nm after few minutes of cooling. *L*-Ascorbic acid was the standard antioxidant drug.

Superoxide radical anion scavenging test

The superoxide radical anion test was carried out using standard protocol reported by Fontana *et al.* (2001). A reaction mixture

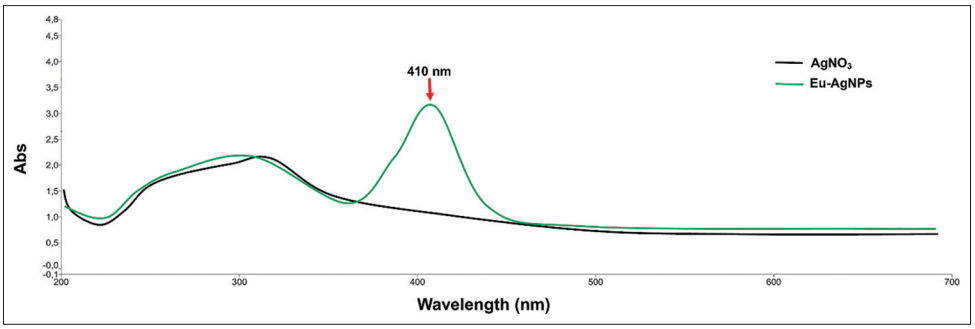

Figure 1: Stacked UV-Vis spectra of $AgNO_3$ and *Eugenia uniflora* extract-mediated AgNPs

comprising 20 mM of PBS, 50 µM of nitro-blue tetrazolium, 15 µM of phenazine methosulphate, 73 µM of Nicotinamide-adenine dinucleotide, and test samples, was prepared to a total volume of 1 mL. Thereafter, it was gently vortexed and incubated for 5-10 min at room temperature. The experiment was performed in three replicates, and the optical density was read at 562 nm.

In vitro Anti-inflammatory Screening of Eu-Ag NPs by Egg Albumin Denaturation Assay

The inhibition of egg albumin denaturation (EAD) by Eu-Ag NPs was determined using standard procedure reported by Oriola *et al.* (2023). The experiment entailed combining fresh chicken egg albumin with PBS (pH 7.2±2.0), and test samples (silver nitrate (SN) and Eu-Ag NPs and Diclofenac) in the ratio 1:14:10. The test samples were serially diluted from 200 to 12.5 µg/mL in three replicates. Thereafter, it was gently vortexed and incubated away from direct daylight at 37 °C for 15 min. Then, it was boiled in a thermostatic water bath at 70 °C for 5 min, and the optical density was recorded at 655 nm. The anti-inflammatory activity of the Eu-Ag NPs was expressed in terms of its inhibitory effect on EAD, using Equation II:

$$\%Inhibition\,of\,denaturation = \frac{ABScontrol - ABSsample}{ABScontrol} x100 \quad (2)$$

Statistical Analysis

The acquired antioxidant and anti-inflammatory data on the Eu-Ag NPs were subjected to One-way analysis of variance (ANOVA), followed by Student's t-test on the KyPlot program (version 5.0).

RESULTS AND DISCUSSION

UV-Visible Spectroscopy

The UV-Vis spectrum of Eu-Ag NPs presented in Figure 1 showed a maximum absorption peak at 410 nm, which aligns with previous report on the maximum absorption peak of biogenic AgNPs around 420 nm (Mandal *et al.*, 2024).

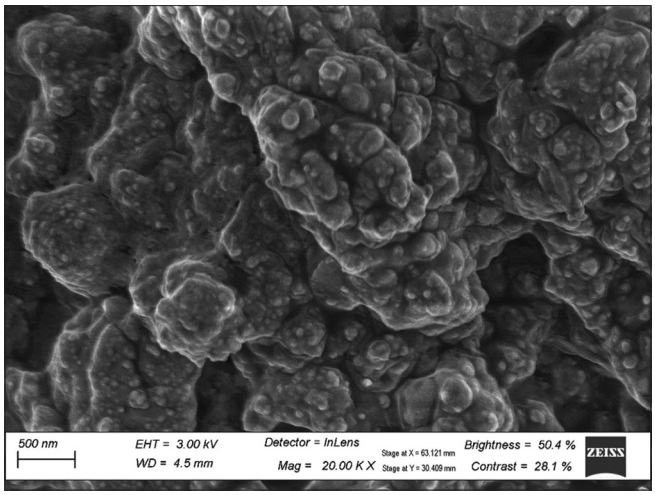

Figure 2: FESEM micrograph analysis of biosynthesized silver nanoparticles

Shape and Size Distribution of Eu-Ag NPs

The Eu-Ag NPs exhibited a spherical to cubical shape, as shown by the FESEM imaging (Figure 2), which predominantly determined the size and surface morphology of the particles. The study showed that the of Eu-Ag NPs exhibit nano-sized particles with <100 nm. According to Das *et al.* (2019), there are instances where the bulking of nanoparticles can occur as a result of solvent evaporating or crosslinking during sample preparation.

Elemental Composition of Eu-Ag NPs based on EDX Spectroscopy

The EDX spectrum presented in Figure 3 showed the presence of elemental Ag having a major weight percentage composition of 99.50%. Despite multiple elements, including N (0.00%) and P (0.50%), the presence of a single acute curve at ~3 Kev suggests the presence of biogenic AgNPs (Bhakya *et al.*, 2016).

Crystallinity of Eu-Ag NPs based on XRD Analysis

The X-ray diffractogram of Eu-AgNPs is presented in Figure 4. The result revealed various peaks at 38.13°, 44.33°, 64.51°, and 77.35°, indicating metallic silver reflection at (111), (200), (220), and (311), respectively. Based on Bragg's law and a comparison

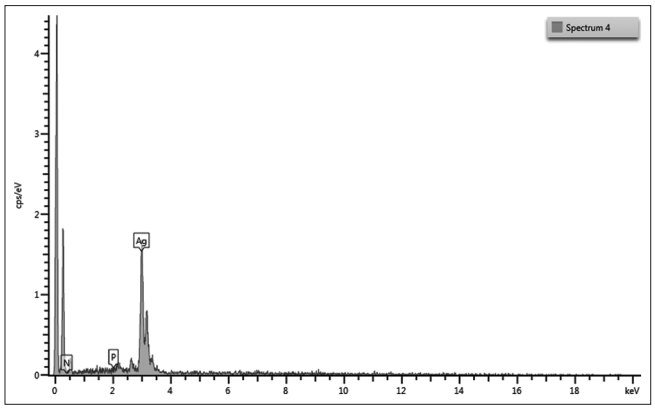

Figure 3: EDX spectrum of Eu-Ag NPs showing the elemental profile

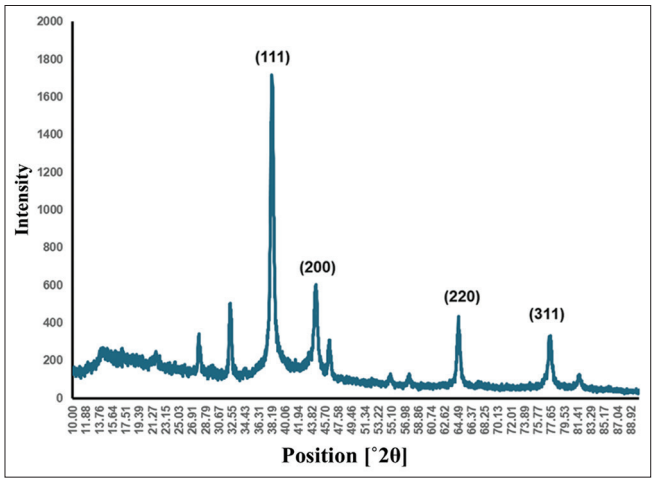

Figure 4: X-ray diffractogram of Eu-Ag NPs

of this data with the Debye-Scherrer formula, the median size of the Eu-Ag NPs is 45.35 nm. The study findings corroborate with previous report on the crystalline nature and nano-size of biogenic AgNPs (Figure 4) (Bhakya *et al*., 2016).

In vitro Antioxidant Activity of Eu-Ag NPs

Biological macromolecules, such as DNA and proteins, are harmed by superoxide anions ($O_2^{\cdot-}$). The superoxide anion is a less reactive free radical that can lead to the creation of more reactive species when oxidative stress is prevalent (Martemucci *et al*., 2022). Based on the result presented in Figure 5a, at the highest concentration, Eu-Ag NPs exhibited 69.13±1.22% inhibition. AgNPs and *L*-Ascorbic acid exhibited IC_{50} values of 160.24±6.29, and 94.5±3.7 µg/mL, respectively. These findings demonstrated the considerable antioxidant capacity of the optimized AgNPs produced with *E. uniflora* leaf extract, which might be attributed to the putative phytochemicals. These substances shield cells from oxidative harm by acting as scavengers of free radicals. According to Banerjee *et al*. (2022), the neutralization of hydroxyl radicals by Eu-Ag NPs (75.43±0.60% at 200 µg/mL) safeguards cells and contributes to the formation of a stable cellular environment (Figure 5b). Nitric oxide is an essential signaling molecule, but too much of it can damage cells and tissues by acting as reactive oxygen species (ROS). In comparison to SN (silver nitrate), Eu-Ag NPs demonstrated nitric oxide scavenging activity (54.37±1.90% at 200 µg/mL) that reacted in a dose-dependent manner. In living organisms, nitric oxide and silver nanoparticles work together to prevent excessive nitric oxide accumulation that might damage membranes (Figure 5c). One kind of oxygen that is not a radical is hydrogen peroxide. However, occasionally it can turn toxic

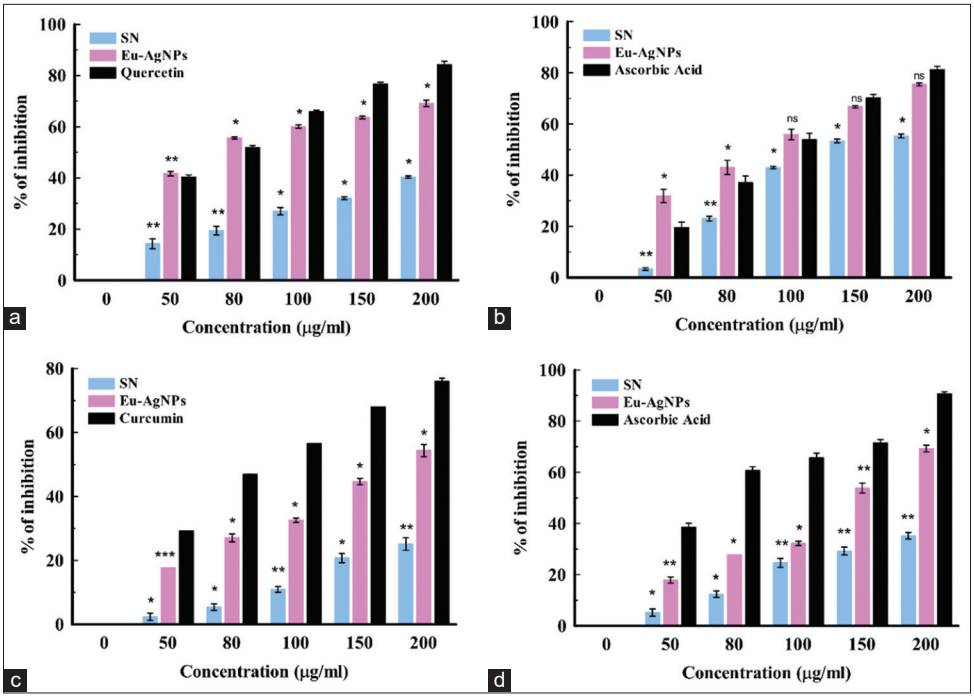

Figure 5: Antioxidant activity of synthesized Eu-AgNPs based on a) superoxide, b) Hydroxyl, c) Nitric oxide, and d) Hydrogen peroxide radical scavenging activities (Data expressed as mean±S.D. *p<0.05; **p<0.01; ***p<0.001; NS-Non significant when compared to standard antioxidant drug)

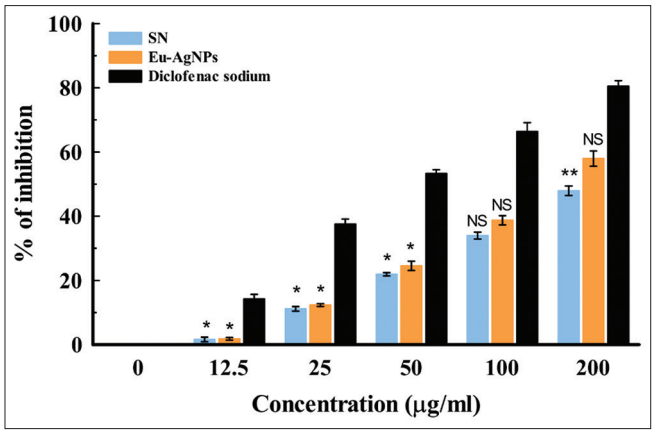

Figure 6: *In vitro* anti-inflammatory activity based on mean percentage inhibition of the biosynthesized Eu-Ag NPs against EAD (Results are given as mean±S.D. *p<0.05; **p<0.01; NS-Non-significant when compared to Diclofenac sodium)

and release hydroxyl (OH^-) radicals, which can harm cells (Kumar *et al.*, 2012). Consequently, the antioxidant defense system depends on its elimination. The Eu-Ag NPs showed substantial concentration-dependent H_2O_2 radical scavenging action (Figure 5d). The IC_{50} values were 57.76±0.41 and 48.91±0.12 µg/mL for ascorbic acid and AgNPs, respectively.

In vitro Anti-inflammatory Effect of Eu-AgNPs

At low concentrations, the biogenic NPs significantly inhibited albumin denaturation when compared to Diclofenac, the standard anti-inflammatory drug (Figure 6). Eu-AgNP and Diclofenac exhibited IC_{50} values of 157.41±0.90 and 48.08±1.64 µg/mL, respectively. According to Das *et al.* (2019), *A. belladonna*-synthesised AgNPs inhibited albumin denaturation with an IC_{50} value of 84 µM.

CONCLUSION

In this study, a biogenic silver nanoparticle (AgNP) was synthesized using an aqueous extract of *E. uniflora* (Eu). Successful nanoparticle synthesis was confirmed by the FESEM, UV-Vis, EDX and XRD analyses. Based on microscopic evaluation from FESEM imaging, the morphology of the Eu-Ag NPs was observed to be ultrafine, spherical-to-cubical-shaped particles <100 nm size, with a median size of 45.35 nm. The maximum absorption peak at 410 nm supports elucidation of the NPs. The conversion of metallic silver into elemental silver was confirmed with an EDX analysis. Biologically, the Eu-AgNPs showed considerable concentration-dependent H_2O_2, NO, superoxide, and hydroxyl radical scavenging activities as promising antioxidant agents. Additionally, it exhibited notable anti-inflammatory properties by inhibiting albumin denaturation in a dose-dependent manner. This study has shown that *Eugenia uniflora* leaf aqueous extract-mediated silver nanoparticles possess unique structural attributes with promising biological properties as a free radical scavenger and anti-inflammatory agent. Future studies may include determining the in vivo anti-inflammatory and toxicological profiles of the green synthesized NPs.

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