



Ultrasound-Enhanced Green Synthesis of Silver Nanoparticles from *Embelia Drupacea* and *Embelia Schimperi* Extracts: Biological and Mathematical Investigations

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ABSTRACT: This study discusses the ultrasound-assisted green synthesis of silver nanoparticles (AgNPs) using the extracts of the plants *Embelia drupacea* and *Embelia schimperi*. The synthesized AgNPs demonstrated significant antioxidant potential and exhibited considerable cytotoxicity at higher concentrations. In the DPPH assays, AgNPs derived from *E. drupacea* were more active ($IC_{0,5} = 42.4(\mu g/mL)$) than those derived from *E. schimperi* ($IC_{5,0} = 46.47(\mu g/mL)$). The saline shrimp lethality assays returned $LC_{5,0}$: *E. drupacea* 74.4 ($\mu g/mL$) and *E. schimperi* 57.67 ($\mu g/mL$). A differential equations-based mathematical model was developed AgNP synthesis kinetics, providing estimates of the nucleation rate order, particle growth constants, and critical size thresholds. Compared to *E. schimperi*, AgNPs from *E. drupacea* had higher bioactivity, with the nano-assemblies showing superior performance in polar solvents. In comparative analysis, AgNPs from both sources of the plant were significantly more active than the crude extracts, and an increase in bioactivity supports this observation. This points to the possible use of *Embelia* AgNPs clinically, particularly for their plant sustainability, and to their role in antioxidant and antimicrobial therapy for the pharmaceutical and therapeutic industries. The inclusion of experimental and mathematical modeling approaches to understand the process of synthesis is distinctive in this work and increases the possibility of optimization subsequent biomedical uses.

Keywords: Silver nanoparticles, green synthesis, *Embelia drupacea*, *Embelia schimperi*, antioxidant activity, mathematical modeling, pharmaceutical applications.

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1. Introduction

Nanoparticles (NPs) offer remarkable possibilities in addressing various aspects in different fields, especially biomedical applications due to their distinctive properties. These include, but are not limited to, drug loading, delivering, antioxidants, antimicrobials, and many more. However, creating appropriate nanomaterials for certain applications remains a complex challenge [3]. The field of nanotechnology involves the control and manipulation of structures at the nanoscale [4]. Currently, the goal of the field is to synthesize materials of single dimensional sizes in the range of 1 to 100 nm, and for 1D materials, the range is 1-100 nm. The commercial applications of metal nanoparticles are in electronics, energy storage, and the pharmaceutical industry. Although there is a wide applications of NPs, the traditional method for NP synthesis still uses toxic chemicals which is dangerous for living organisms and the biosphere [5]. This is why more and more research is focused on the synthesis of NPs without the use of toxic materials. Emerging green nanotechnology highlights the use natural reducing agents for the reductive synthesis of metal nanoparticles [6]. This growing field of biotechnology focuses on the use plant extracts as replacement sources of reducing agents in the production of the biosynthesized nanoparticles [7]. Remedial applications, especially, of silver nanoparticles bio-extracted from the biosynthesized plant sources, are highly commendable, due to their silver biocompatibility. Aloe Vera [8], *Piper nigrum* [2] and Tasmanian blue gum [9] are a few of the many biosynthesizing plant sources used in eco-friendly production of the

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AgNPs. Admittedly, plants are especially interesting because over 60 percent of drugs used for defense against cancer are plant-based, demonstrating the strong legislative potential of these drugs as anticancer medications [10]. Thus, this work aims to benefit the growing body of literature on the synthesis of AgNPs derived from plants, in this case, comprising extracts of *Embelia drupacea* and *Embelia schimperi* for potential biomedical applications. This genus provides *Embelia* with a rich reservoir of biologically active Myrsinaceae family compounds. *Embelia drupacea* and *Embelia schimperi*, are prominent representatives, containing 25-30 and 750 species respectively [12]. While *Embelia schimperi* nanotechnology, a weak raspberry, thrives in moist, disturbed forests, has a much to offer, especially concerning the kinetics of silver nanoparticles derived from *Embelia* (ED/ES-AgNPs) releasing from nano-composite systems and the comprehensive mathematical modeling of it. For this purpose, we implemented a new specialized mechanistic mathematical model based on NP size distribution and electrostatic interaction data within given scenario boundaries [13]. This The model not only aids in the optimization and calibration of protein delivery parameters but also explains the accompanying phenomena. In this study, we describe the biosynthesis of AgNPs using the leaf extracts of *Embelia drupacea* and *Embelia schimperi*. The biosynthesized and properly characterized AgNPs underwent in vitro study with regard to biological activity and cytotoxicity [14]. The results indicated strong antioxidative activity with no cytotoxicity, pointing to possible medicinal value. Further, we optimized kinetic parameters using the Simple Green method based on concentration-dependent studies. One of the deterministic modelling approaches was synthesis and behaviour of the ED/ES-AgNPs and insights on the estimated differential equations model.

1.1 Mathematical Modeling of Nanoparticle Synthesis

Simplified models for the simulation of processes involved in the synthesis of nanoparticles can be created using differential equations. The equations in this instance would seek to find equilibrium amongst the complexity of the model and computational power available. In a cohesive document, the reaction mechanisms in a stepwise fashion would document the changes involved in the transformation of the reacting molecules to form the desired end product. Several parts of the reaction mechanism can be reviewed to glean the required information describing a chemical process. A distinctive feature of the proposed reaction would involve the sequence of molecular interactions, and that would ultimately define the reaction rate of the entire process. The sum of individual reaction steps would govern the overall reaction kinetics, and the molecules can be viewed as a mechanism that contributes to the aggregate of a given reaction. This mechanism would be the basis for forming the proposed differential equations. Thus, the rate equations and the rate of formation of the nanoparticles can be modeled successfully.

$$\frac{dE_s}{dt} = -c\{\delta_1[E_s n_H + E_s \zeta_A]\} - \mu E_s,$$

$$\frac{dE_d}{dt} = -c\{\eta_1[E_d n_H + E_d \zeta_A]\} - \mu E_d,$$

$$\frac{dE_{SNP}}{dt} = c\{\delta_1[E_s n_H + E_s \zeta_A]\},$$

$$\frac{dE_{dNP}}{dt} = c\{\eta_1[E_d n_H + E_d \zeta_A]\},$$

$$\frac{dn_H}{dt} = \delta_2[E_s n_H + E_s \zeta_A],$$

$$\frac{d\zeta_A}{dt} = \eta_2[E_d n_H + E_d \zeta_A],$$

$$E_s(0) \geq 0, E_d(0) \geq 0, n_H(0) \geq 0,$$

$$\zeta_A(0) \geq 0, E_{SNP}(0) \geq 0, E_{dNP}(0) \geq 0,$$

Where $c, n, \delta_1, \eta_1, \mu, \zeta_1$ are Positive constant.

2. Materials and Characterization

The present study utilized various chemicals, including silver nitrate (AgNO₃), deionized water, n-hexane, potassium ferricyanide, trichloroacetic acid, sodium phosphate buffer, FeCl₃, DPPH, ABTS,

potassium per sulfate, ascorbic acid, vincristine sulfate, biotin, etoposide, and potassium dichromate. *Embelia drupacea* and *Embelia schimperi* leaf extracts, collected from the Botanical Garden, Nilgiris District, Tamil Nadu, India, served as plant materials, while brine shrimp (*Artemia nauplii*) were employed for cytotoxicity assays.

2.1 Synthesis of Silver Nanoparticles (AgNPs) using Green Technology

With respect to prior work detailed (15), the preparation of silver nanoparticles (AgNPs) using the green reduction method has been employed. In summary, 0.17 grams of silver nitrate (AgNO₃) was dissolved in 1 litre of distilled water to prepare a 1 mM solution and 5 minutes of boiling was performed, and then in the presence of the plant extract which was also boiled continuously until a dark brown coloration indicating the reduction of silver and formation of AgNPs was achieved, the boiling was also performed for 5 minutes. The preparation was then centrifuged at 16,000 rpm for 15 minutes, and the resultant pellets were redispersed in diionized water and centrifuged again at 14,000 rpm for 10 minutes. This purification step was repeated 4 times. For the purpose of characterisation and biological studies, the purified nanoparticle suspension was diluted with diionized water to prepare stock dilute AgNPs of 15, 30, 40, 50 and 60 ppm for subsequent use.

2.2 Cytotoxicity Test

Cytotoxicity testing followed the brine shrimp lethality assay as described in (16). In brief, brine shrimp eggs (20 mg) were hatched in artificial seawater in a partitioned rectangular plastic container, where one half was kept in darkness and the other half was illuminated. After 48 hours, phototropic naive shrimp were collected and exposed to varying concentrations (10-1000 $\mu\text{g}/\text{mL}$) of plant extracts and 10-50 ppm AgNPs for shrimp to 10 nauplii, seawater 2 μL , and 500 μL of each concentration in glass vials. Vincristine sulphate, biotin, etoposide, and potassium dichromate served as positive controls and artificial seawater and DMSO were used as negative and blank controls, respectively. After 24 hours incubation per- room temperature, the total number of nauplii was tallied and used to determine the mortality rate by the equation percentage Mortality rate = $\left[\frac{pc-pt}{pc}\right] \times 100$ (where pt is treatment mortality and pc is control mortality). This assay allowed for the determination of the cytotoxicity and probable anticancer activity of the synthesized AgNPs and blended plant extracts.

$$\text{Percent Mortality rate} = \left[\frac{pc-pt}{pc}\right] \times 100 \quad (1)$$

Where: Percent Mortality rate = percentage of nauplii mortality pc = number of live nauplii in the control group pt = number of live nauplii in the treatment group.

2.3 Anti-Oxidant Assay

2.3.1 DPPH Free Radical Scavenging Assay

The DPPH free radical scavenging assay, adapted from Dhayalan et al. (2023)(17) was used to evaluate the antioxidant activity of silver nanoparticles (AgNPs) and plant extracts. A stock A solution was prepared by dissolving 24 mg of DPPH in 100 mL of n-hexane and left at 20°C. The working solution was prepared by diluting the stock solution in n-hexane to an absorbance of 0.916 at 519 nm and used in a sample flow photometer for the assay. To evaluate the radical scavenging activity of silver nanoparticles (AgNPs), 2 mL of AgNP suspension (10-50 ppm) or plant extract dilutions were placed into test tubes containing 3 mL of diluted DPPH solution. After 15 minutes at dark, the solution's absorbance was gauged at 517 nm. During this procedure, the positive control utilized was ascorbic acid. The radical scavenging activity was determined using the previously described equation-2. The constructed correlation curve of concentration versus scavenging activity was used for determining the IC_{50} values, or the concentration needed to scavenge 50 percentage of the DPPH radicals.

$$\text{Inhibition (percentage)} = \frac{(\text{Absorbancecontrol} - \text{absorbancesample})}{(\text{Absorbancecontrol})} \times 100 \quad (2)$$

2.4.1 ABTS Radical Cation Assay

2.4.2 ABTS Radical Cation Scavenging Assay

AgNPs and plant extracts ABTS radical cation scavenging activity was done using a modified procedure by Erenler, Ramazan, et al. (18) For the creation of the ABTS radical cation, 7 mM ABTS was com-

combined with 2.45 mM potassium per sulfate and, for the purpose of generating the radical, the solution was allowed to sit in darkness for an overnight incubation. The sample was then diluted with 50 percent methanol, to obtain an absorbance of approximately 0.700 at 745 nm.

In reference to the scavenging activity determined, 3 mL of ABTS solution was combined with the Ag-NPs and plant samples at different concentrations, and the absorbance was measured every minute for 6 minutes. Scavenging activity percentage was calculated using Equation 3. Ascorbic acid was utilized as a reference standard. The scavenging activity, plotted against different concentrations, made it possible to determine the IC_{50} value—the concentration required to scavenge 50 percent of the ABTS radicals. This gave the value of the antioxidant activity offered by the silver nanoparticles (AgNPs) and the plant extracts used.

$$\text{Scavenging activity (percentage)} = \frac{(\text{Absorbancecontrol} - \text{absorbance sample})}{(\text{Absorbancecontrol})} \times 100 \quad (3)$$

2.5 Reducing Power Assay

The Reducing power of the AgNPs was determined using a modified version of the technique described by Alavi, M et al. (19) 3 mL of n-hexane extract was combined with 2.5 mL of potassium ferricyanide, and then different concentrations of AgNPs were added. The sample was diluted with sodium phosphate buffer and heated to 50°C for 20 minutes. Trichloroacetic acid (TCA) was added (2.5 mL of a 10 percent (v/v) solution), and the sample was centrifuged for 10 minutes at 3000 rpm. TCA supernatants were mixed with 0.5 mL of 0.1percent FeCl3 and distilled water. The resultant solution's absorbance was measured at 710 nm, using ascorbic acid as the reducing agent. The reducing power was calculated using the equation provided:4 Reducing Power (percentage) = $\frac{(\text{Absorbancecontrol} - \text{absorbance sample})}{(\text{Absorbancecontrol})} \times 100$ (4)

Where: - Abscontrol = Absorbance of control - Absample = Absorbance of sample

This assay assessed the ability of AgNPs to reduce ferricyanide to ferriyanide, indicating their potential antioxidant activity.

3. Results and Discussion

3.1 Optical characterization of biosynthesized AgNPs (Fig. 1a)

The UV-Vis spectrum of the reaction mixture showed an unmistakably defined surface plasmon resonance (SPR) band with a peak of 434 nm, which indicates the formation of silver nanoparticles (AgNPs). (Fig. 1a). The presence of one symmetric peak suggests mostly spherical nanoparticles, while the peak width implies moderate polydispersion, which has been associated with SPR maxima in the range of 400-450 nm during the biosynthesis of nanoparticles of plant extracts (20, 21). The ultrasound-assisted method likely accelerated the nucleation stage of particle formation, and slowed down the uncontrolled growth which is the common cause of larger and less uniform particles — a finding which agrees with the sonochemical literature surrounding the formation of nanoparticles (22). The formation of nanoparticles during mild and rapid ultrasound exposure highlights the technique and method as being suitable for sustainable ultrasound-assisted nanomaterial creation.

Fig. 4. Antioxidant activity of biosynthesized AgNPs and plant extracts: (a) DPPH radical scavenging efficiency of biotin control versus AgNPs; (b) DPPH assay $IC_{5,0}$ values of crude extracts and AgNPs showing enhanced activity after nanoparticle formation; (c) AgNPs antioxidant assay concentration-absorbance relationship; (d) crude extracts and biotin comparative absorbance trending along with scavenging activity.

3.5 Absorbance trends and comparative profiles

In ABTS and DPPH assays, higher sample concentration led to greater radical quenching, as exemplified by decreasing absorbance. EdNPs absorbance dropped from 36.2 to 22.5, and EsNPs from 37.1 to 16.3 (Fig. 4c). The crude extracts contributed greater starting absorbance, and therefore, their scavenging (Fig. 4d) was weaker. These patterns confirm $IC_{5,0}$ values and demonstrate that the differences in antioxidant activity are meaningful and consistent across assays (28).

3.6 Mathematical sensitivity analysis

The sensitivity curves modeled showed a greater response for EsNPs than for crude extracts (Fig. 5). This indicates that the properties of the AgNPs respond more to the kinetic or surface-interaction components. This correlates with the observations made during the synthesis of the AgNPs, in which the

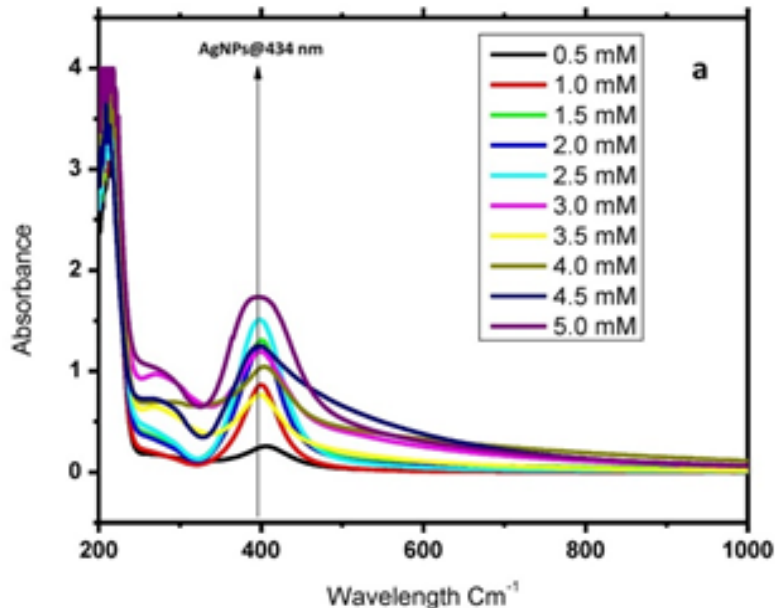


Figure 1: wavelength cm^{-1}

bioactivity was significantly altered with only a few changes to the synthesis parameters (sonication power, extract concentration). This kind of modeling can be used in a predictive capacity to optimize synthesis parameters for desired levels of cytotoxicity and antioxidant activity (29). In comparison to crude plant extracts, our ultrasound-assisted, green-synthesized AgNPs have \geq biocompatibility and greater antioxidant activity with adjustable levels of cytotoxicity. The mechanistic understanding that allows the control of bioactivity in synthesis and the combination of modeling and experimental data demonstrates the synthesis Embelia-derived AgNPs can be used in biomedicine and nutraceuticals.

Fig. 1. (a) UV-Vis absorption spectra of biosynthesized silver nanoparticles (AgNPs) derived from *Embelia drupacea* and *Embelia schimperi* leaf extracts, showing the characteristic surface plasmon resonance (SPR) band centered at ≈ 434 nm, indicative of successful nanoparticle formation under ultrasound-assisted green synthesis conditions.

3.2 Cytotoxicity: brine shrimp lethality and comparative LD_{50}

Brine shrimp lethality assays revealed a concentration-dependent mortality for both AgNPs and crude plant extracts (Fig. 2a–b). AgNPs from *E. drupacea* (EdNPs) and *E. schimperi* (EsNPs) had LD_{50} values of $74.40 \mu g.mL^{-1}$ and $57.67 \mu g.mL^{-1}$, respectively (Fig. 2c). Crude extracts were less toxic ($LD_{50} \approx 82\text{--}94 \mu g.mL^{-1}$), indicating that nanoparticle formation enhances lethality, a phenomenon also observed in other phytochemical AgNP systems(23). The higher toxicity of EsNPs may be due to differences in the phytochemical capping layer, which influences particle–cell interactions, ROS generation, and membrane disruption (24). Ultrasound-mediated synthesis may further alter surface chemistry, enhancing bioactivity. Since brine shrimp lethality correlates with preliminary cytotoxic potential, these findings justify targeted cancer cell line testing in follow-up studies(25). action which is likely surface-target interactions that occur at a defined concentration.(26). This suggests the concentration of the nanoparticles can be adjusted to a desired level maintaining a safe and bioactive level.

Fig. 2. Biological activity profiles of crude extracts and AgNPs: (a) concentration-dependent mortality rates for biosynthesized AgNPs in the brine shrimp lethality assay; (b) mortality rates for corresponding crude plant extracts; (c) comparison of LD_{50} values across extracts, fractions, and AgNPs, highlighting enhanced potency upon nanoparticle formation; (d) antioxidant activity expressed as per-

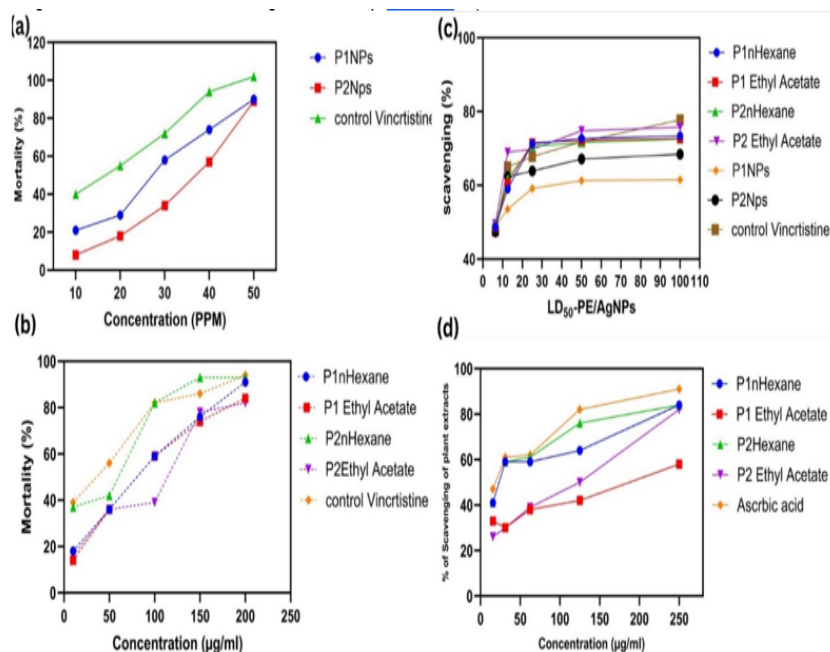


Figure 2:

centage DPPH scavenging for plant extracts.

3.3 Dose–response profiles and controls

AgNPs dose–response curves (Fig. 3a) showed monotonic increases in mortality with concentration, while the biotin control (Fig. 3b) which showed little to no mortality, confirmed that the effect was indeed caused by the nanoparticles. In a comparative LC_{50} analysis (Fig. 3c) the toxicity of most n-hexane extracts was found to be less than that of the corresponding AgNPs. The expected dose–response effect for the antioxidant assays (Fig. 3d) was the inverse relationship of radical signal intensity to sample concentration. The toxicity and antioxidant assays having a consistent dose–dependence suggests a similar underlying mechanism of Fig. 3. Cytotoxicity and antioxidant response patterns: (a) dose–response curves for AgNP–induced mortality; (b) biotin control profile confirming negligible background toxicity; (c) comparative LC_{50} analysis of AgNPs versus n-hexane extract fractions; (d) concentration–dependent antioxidant activity (percentage DPPH scavenging) for extracts, nanoparticles, and biotin control.

3.4 Antioxidant performance — DPPH and ABTS patterns

All samples showed (Fig. 4, Fig. 2d) concentration–dependent DPPH scavenging. For crude extracts, polar solvents yielded higher IC_{50} values (weaker activity), while *E. schimperi* n-hexane extract was most potent ($IC_{50} \approx 21.41 \mu g.mL^{-1}$). AgNPs from *E. drupacea* and *E. schimperi* displayed improved radical scavenging ($IC_{50} \approx 42.60$ and $46.47 \mu g.mL^{-1}$), outperforming most extracts but remaining less potent than ascorbic acid ($IC_{50} \approx 4.49 \mu g.mL^{-1}$). Enhanced scavenging by AgNPs likely stems from synergistic effects between phytochemical capping agents and the reactive AgNP surface, facilitating electron transfer (27). The similarity in performance between the two species' nanoparticles suggests that sonication preserves key antioxidant phytochemicals while producing smaller particles with higher surface reactivity.

Fig. 4. Antioxidant activity of biosynthesized AgNPs and plant extracts: (a) DPPH radical scavenging efficiency of biotin control versus AgNPs; (b) DPPH assay IC_{50} values of crude extracts and AgNPs showing enhanced activity after nanoparticle formation; (c) AgNPs antioxidant assay concentration–absorbance relationship; (d) crude extracts and biotin comparative absorbance trending along with scavenging activity

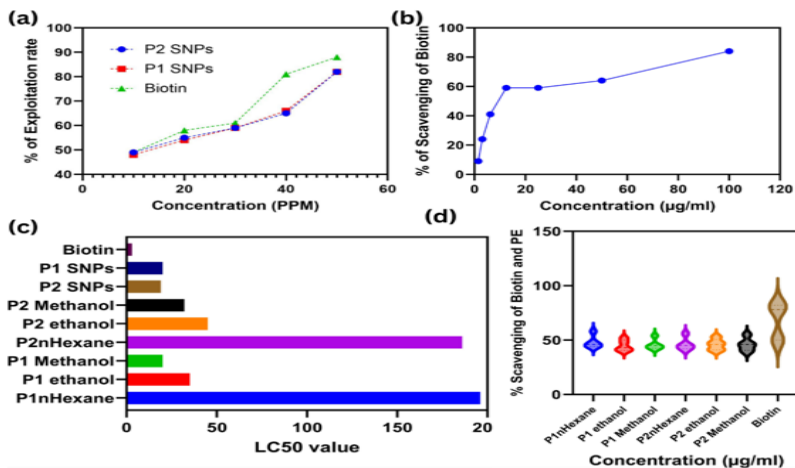


Figure 3:

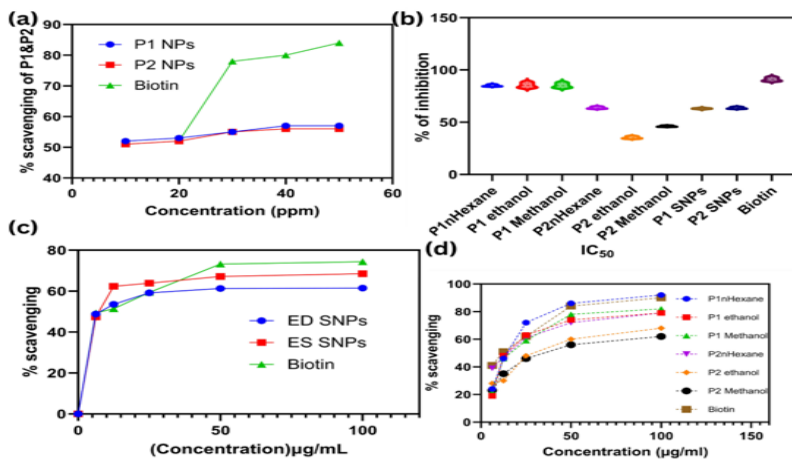


Figure 4:

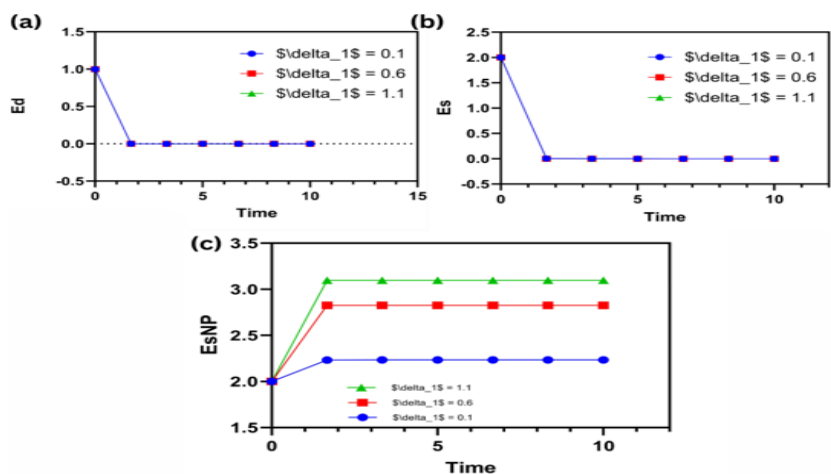


Figure 5:

3.5 Absorbance trends and comparative profiles

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3.6 Mathematical sensitivity analysis The sensitivity curves modeled showed a greater response for EsNPs than for crude extracts (Fig. 5). This indicates that the properties of the AgNPs respond more to the kinetic or surface-interaction components. This correlates with the observations made during the synthesis of the AgNPs, in which the bioactivity was significantly altered with only a few changes to the synthesis parameters (sonication power, extract concentration). This kind of modeling can be used in a predictive capacity to optimize synthesis parameters for desired levels of cytotoxicity and antioxidant activity (29). In comparison to crude plant extracts, our ultrasound-assisted, green-synthesized AgNPs have \geq biocompatibility and greater antioxidant activity with adjustable levels of cytotoxicity. The mechanistic understanding that allows the control of bioactivity in synthesis and the combination of modeling and experimental data demonstrates the synthesis Embelia-derived AgNPs can be used in biomedicine and nutraceuticals.

Fig. 5. Mathematical modeling of biosynthetic systems: sensitivity plots for parameter δ and modeled response for *E. drupacea* (Ed), *E. schimperi* (Es), and *E. schimperi*-derived AgNPs (EsNP). The δ response is steeper for EsNPs, suggesting that the AgNP systems have a greater response to kinetic and interaction parameters, as shown in the experimental trends.

4. Conclusions

This work is the first to demonstrate the ultrasound-assisted green synthesis of silver nanoparticles (AgNPs) using *Embelia drupacea* and *Embelia schimperi* leaf extracts. The extraction method was simple and eco-friendly. It was the first time a mathematical model was developed to simulate the biosynthetic process of AgNPs. The silver nanoparticles synthesized in this investigation exhibited excellent acryotoxicity and antioxidant properties, far superior to the crude extracts, and these were accomplished under mild reaction conditions, highlighting the biosafe synergy between the bioactive compounds and the nanoscaled properties. The pioneering model developed for this work was aimed at simulating the nucleation and growth kinetics of the biosynthesis to reaction optimise it within a certain biosynthetic framework. The gap between the model and actual synthesis speaks to the intricacy of the biosynthetic AgNPs synthesis,

and the model will be useful in future work for providing mechanistic control of synthesis AgNPs process parameters. Most importantly, this work presents AgNPs as novel Embelia-derived nanoparticles for potential pharmaceutical applications. Future research will focus on identifying and characterising the bioactive components responsible for nanoparticle formation, exploring the mechanisms behind their antioxidant and cytotoxic activities at a molecular level, and investigating the potential applications of the biosynthesized nanomaterials in clinical nanomedicine, including in vitro and in vivo correlation studies and formulation development.

Nomenclature:

1. AgNPs – Silver Nanoparticles
2. NPs – Nanoparticles
3. EdNPs – Embelia drupacea-derived Silver Nanoparticles
4. EsNPs – Embelia schimperi-derived Silver Nanoparticles
5. SPR – Surface Plasmon Resonance
6. IC_{50} – Half maximal inhibitory concentration ($\mu\text{g/mL}$)
7. LC_{50} / LD_{50} – Lethal concentration / dose for 50%. DPPH – 2,2-Diphenyl-1-picrylhydrazyl (free radical used in antioxidant assays)
9. ABTS – 2,2-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (radical cation scavenging assay)
10. ROS – Reactive Oxygen Species
11. Abscontrol – Absorbance of control sample
12. Abssample – Absorbance of test sample.

Data availability

All data generated or analyzed during this study are included in this published article.

Declaration of competing interest

The authors declare there is no competing of interest

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