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RESEARCH

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Biosensing and anti-inflammatory effects of silver, copper and iron nanoparticles from the leaf extract of *Catharanthus roseus*

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Abstract

Background In this study, we present a low-cost, environmentally friendly method for producing silver, copper, and iron nanoparticles using fresh *Catharanthus roseus* leaf extract. The biomolecules found in the plant extract play a crucial role as stabilizing and reducing agents. The spectral profile of the UV–visible spectrophotometer was measured to confirm and identify the biosynthesized nanoparticles. The synthesized nanoparticles were tested for biosensing activities and anti-inflammatory effects.

Result UV–visible spectra showed a prominent surface resonance peak of 415 nm, 300 nm, and 400 nm, corresponding to the formation of silver, copper, and iron nanoparticles, respectively. The in vitro anti-inflammatory properties of the synthesized AgNPs, CuNPs, and FeNPs showed the maximum inhibition of protein denaturation at 58%, 54.15%, and 44.26% at a concentration of 400 µg/ml, respectively. Furthermore, at a 400 µg/ml concentration, Diclofenac, utilized as a control, showed a maximal inhibition of 93.37%. According to the biosensing activity, these nanoparticles are also a good source for biosensing hazardous heavy salts. So, this article provides the first description of the silver, copper, and iron nanoparticles from *Catharanthus roseus* leave biosensing capabilities and anti-inflammatory characteristics.

Conclusion Overall, this study revealed that due to their biocompatibility, silver, copper, and iron nanoparticles could be appealing and environmentally acceptable options that could be used as innovative therapeutic agents for the prevention and treatment of inflammation. The primary outcome of the research will be the development of potential pharmaceutical uses for the *C. roseus* medicinal plant in the biomedical and nanotechnology-based industries.

Keywords Nanoparticles, Medicinal plant, Biosensing activity, Anti-inflammatory, *Catharanthus roseus*

1 Background

Nanotechnology is a burgeoning field of research in modern materials science and engineering, in which phenomena occurring at nanometer scales are employed in the design, characterization, manufacture, and application of materials, structures, devices, and systems. Metal nanoparticles have a wide range of uses due to their identical physical and chemical features. According to several research studies, nanoparticles consisting of several noble metals, such as Ag, Cu, and Fe, can be applied in antiplasmodial, antibacterial activities [1–3]. Chemical reduction [4], physical [5], photochemical [6, 7], electrochemical [8–10], and biological techniques [11] have all been documented in the literature as strategies for the synthesis of nanoparticles. However, biological approaches involving microbes [12, 13], enzymes [14], fungi [15], and plants or plant extracts [16–20] have been proposed as environmentally beneficial alternatives. Plants or components

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of plants can be used to synthesize nanoparticles, which can be advantageous over other biological processes by removing the difficult processes [16].

Green synthesized metal nanoparticles have been studied by researchers because of their unique characteristics. The mechanism of nanoparticle formation consists of mainly three stages: reduction of ions, clustering, and further nanoparticle growth. The biomolecules in the plant extract play an important role as stabilizing and reducing agents. The features of each stage depend upon the nature of the reducing agent, and its concentration.

Catharanthus roseus is a medicinal plant belonging to the Apocynaceae family used in traditional medicine. *C. roseus* thrives in tropical and subtropical climates around the world. A vast spectrum of bioactive chemicals with therapeutic uses can be found in plants. *C. roseus* has meaningful biological actions, including antibacterial, antiviral, antifungal, antioxidant, and anticancer activities [21, 22]. Silver has been shown to have an inhibiting effect on microbes found in medical and industrial processes [23, 24]. The most common uses of silver and silver nanoparticles are in the medical field, such as preventing infection in burns and wounds [25]. Against multidrug-resistant human infections, it is also extremely hazardous.

Because copper is very conductive and cheaper than silver and gold, the green production of copper nanoparticles is of great interest [26]. Copper oxide nanoparticles are useful as antimicrobials and in gas sensors, batteries, high-temperature superconductors, solar energy conversion tools, and other applications [27–30]. Copper (Cu) and copper complexes have been used by humans for a variety of reasons, including water purification, fungicides, and antibacterial agents. The biosynthesis of metal nanoparticles utilizing inactivated plant tissue, plant extracts, and other live plant parts is a popular current method [31]. It's a very cost-effective process, making it a viable commercial option for large-scale manufacturing. In the literature review, microorganisms, such as bacteria, fungi, and yeast have been used for the biosynthesis of copper oxide nanoparticles [32–34].

Because of its numerous applications in various science sectors, nanoscale iron is essential [35]. The biological synthesis of FeNPs from plants is an important technology because it does not involve hazardous chemicals, pressure, or temperature [36]. This study was designed using a simple, cost-effective and environmentally friendly synthesis method for silver, copper, and iron nanoparticles using *C. roseus* leaf extract as a reducing agent. The biosensing activities and anti-inflammatory properties of silver, copper, and iron nanoparticles from *Catharanthus roseus* leaves are described for the first time in this publication.

2 Methods

2.1 Reagents and equipments

Distilled water, Silver nitrate(Merck KGaA, Germany, purity 99.5%), Copper sulfate (Merck, Mumbai, purity 99%), Ferric chloride (Loba Chemie, Mumbai, purity 98%), Ferrous sulfate (PT.SMART LAB, Indonesia, purity 98.5%), Lead acetate (Merck KGaA, Germany, purity 99.5%), Zinc sulfate (PT.SMART LAB, Indonesia, purity 98%) purity), Diclofenac sodium (Clofenac 50, Square Pharmaceuticals Ltd., Bangladesh). T60 UV–visible Spectrophotometer (PG Instruments Limited), Hotplate Stirrer (Daihan Labtech Co.LTD), Tabletopcentrifuge (Digisystem Laboratory Instruments Inc.), Water Bath (JSWB-11T).

2.2 Collection and identification of plant sample

Catharanthus roseus plant was collected from a local area of Rajshahi University, Bangladesh. The plant was identified and verified by Professor Dr. AHM Mahbubur Rahman (Taxonomist), Department of Botany, University of Rajshahi, Bangladesh. The voucher specimen (no. RR 266) was preserved and deposited in the herbarium of the Department of Botany, University of Rajshahi, Bangladesh. *Catharanthus roseus* is an evergreen subshrub or herbaceous plant growing 1 m tall. The leaves are oval to oblong, 2.5–9 cm long and 1–3.5 cm wide.

2.3 Preparation of plant extraction

To eliminate soil, grime, and other contaminants, the leaves were thoroughly washed with tap water and then distilled water. Eight grams of leaves were broken into little pieces and placed in a 100 ml beaker with sterile distilled water, which was heated to 90 °C for 10 min. To obtain the aqueous plant extracts, the extract was filtered via filter paper.

2.4 Green synthesis of silver nanoparticles

50 ml of the 0.01 M working standard silver nitrate was placed in a beaker and stirred with a magnetic stirrer at 45–50 °C for 15 min. Drop by drop, about 6 ml of plant extract was added to the solution. The synthesis of nanoparticles is indicated by the formation of black particles. To prevent nanoparticle aggregation, those were stored in the dark at room temperature in an airtight container [37].

2.5 Green synthesis of copper nanoparticles

50 ml of the working standard copper sulfate (0.01 M) was placed in a beaker and stirred in a magnetic stirrer at 45–50 °C for 15 min. Drop by drop, about 5 ml of plant extract was added to the solution. The presence of green implies the formation of copper nanoparticles. Before the analysis, it was kept in the dark for roughly 8–12 h [38].

2.6 Green synthesis of iron nanoparticles

50 ml of ferric chloride (0.01 M) working standard solution was placed in a beaker and stirred with a magnetic stirrer at 45–50 °C for 15 min. The plant extract was progressively added to the solution, about 8 ml at a time. After 15 to 30 min of stirring, the development of a blackish-green tint signals the formation of iron nanoparticles. It was incubated for 12–24 h in the dark at ambient temperature [39, 40].

2.7 Characterization of synthesized nanoparticles

2.7.1 UV–visible absorption spectroscopy

The silver, copper and iron nanoparticles synthesized using *Catharanthus roseus* plant leaf extracts were characterized using a T60 UV–visible spectrophotometer (PG Instruments Limited) in the wavelength range of 250–850 nm. The absorption curve was generated once the results were recorded.

2.7.2 Biosensing activity

Silver, iron, and copper nanoparticles are used in heavy metal biosensing activities. Heavy metal salt solutions containing 0.01 M are used to treat them. Heavy metal salts such as ferrous sulfate, ferric chloride, lead acetate, and zinc sulfate were synthesized as 0.01 M solutions. The test tube was filled with 0.1 ml fluid and 1 ml nanoparticles. 0.1 ml of generated AgNPs in 1 ml of distilled water was used as a control. The results were observed after 24 h incubation period [41]. The results were visually assessed by comparing the color of the test solution to that of the control.

2.7.3 In vitro anti-inflammatory effects

The greenly synthesized AgNPs, CuNPs, and FeNPs from *Catharanthus roseus* leaves were employed to treat inflammation using the protein denaturation method [42]. 0.2 ml of egg albumin (from fresh hen's eggs), 2.8 ml of phosphate buffered saline (PBS), and 2 ml of various quantities of the test extracts (Ag, Cu, and Fe nanoparticles) made up the entire reaction mixture, which had final concentrations of 100, 200, 300, 400 µg/ml. As a control, the same volume of double-distilled water was used. The mixes were then incubated for 15 min at 37 °C and heated for 5 min at 70 °C. After cooling, their absorbance at 660 nm was measured using a vehicle as a blank with a T60 visible spectrophotometer (PG Instruments Limited). Diclofenac sodium was used as a reference medication and handled similarly to determine the absorbance at a final concentration of (100, 200, 300, and 400 µg/ml).

The following equation was used to determine the percentage inhibition for protein denaturation:

$$\text{Protein denaturation inhibition percentage} = 100 - \left(\frac{A1 - A2}{A0}\right) \times 100$$

A_1 denotes the sample's absorbance, A_2 the product control's absorbance, and A_0 the positive control's absorbance [43].

3 Results

3.1 Synthesis of nanoparticles

The reduction of ions and the emergence of color validated nanoparticle creation. The color development of black with the addition of silver nitrate confirmed the reduction of silver ions. It confirmed that silver nanoparticles were present [44]. The formation of a green hue with the addition of copper sulfate indicated the decrease of copper ions [38]. It demonstrated the presence of copper nanoparticles. The formation of a greenish-black

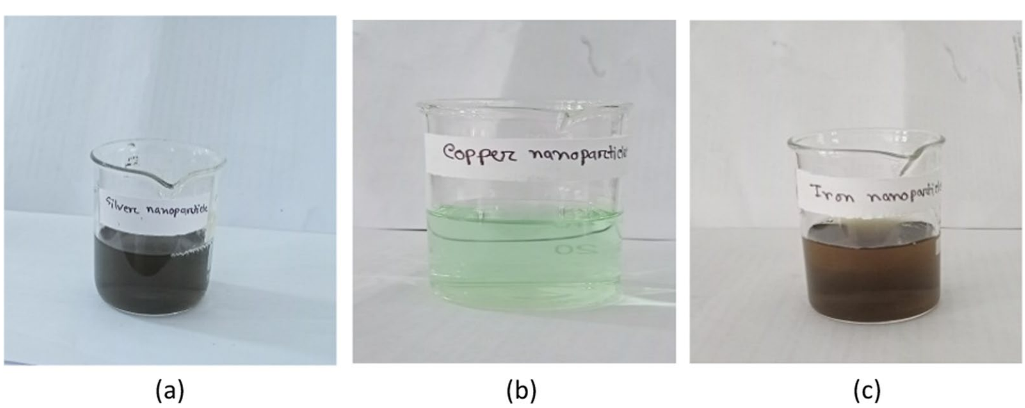

Fig. 1 Synthesis of nanoparticles **a** silver, **b** copper and **c** iron

color in the solution with the addition of ferric chloride, suggesting the reduction of ferric ions, confirmed the presence of iron nanoparticles [40]. Figure 1 shows the color development of the solution. The essential compounds in the plant extract are hydroxyl and carbonyl groups. The plant extract was able to act as a reducing and stabilizing agent due to the presence of both functional groups [45].

3.2 UV–visible spectroscopy

The preliminary characterization of nanoparticles is done with a UV–visible spectrophotometer. The bioreduction of the ions was observed by analyzing the solution with UV–Vis spectroscopy. All the produced, including silver, copper, and iron nanoparticles, were monitored between 250 and 850 nm. Figure 2a shows the most significant absorbance peak of the generated silver nanoparticle at 415 nm, which is similar to earlier literature for silver nanoparticles from *Oedera genistifolia* leaf extract, which showed the peak of 290 to 360 nm [44].

The absorbance peak maximum of the generated copper nanoparticles was 310 nm [2], which is similar to the 300 nm peak of copper nanoparticles synthesized from *Catharanthus roseus* leaf extract shown in Fig. 2b. Figure 2c shows the maximum absorbance peak of the iron nanoparticles synthesized from *Catharanthus roseus* leaf extract was 400 nm, which is extremely close to the maximum peak of the iron nanoparticles generated from *Aesculus hippocastanum* leaf extract, which was 300 nm [40]. The absorption spectra of all produced nanoparticles are shown in Fig. 2.

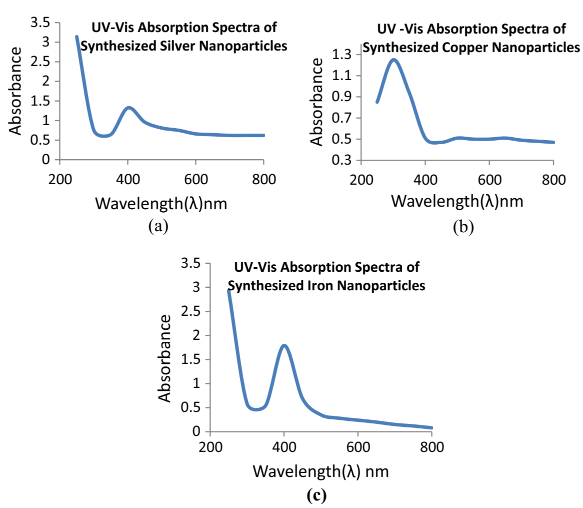

Fig. 2 UV–Vis Absorption spectra of synthesized **a** silver, **b** copper and **c** iron nanoparticles

3.3 Biosensing assay

The following are the results of the biosensing activity: the silver and copper nanoparticles are positive for all four heavy metal salts. There is a precipitate formation with ferrous sulfate, lead acetate, zinc sulfate, and ferric chloride. The iron nanoparticles demonstrate negative biosensing with ferrous sulfate, lead acetate, zinc sulfate, and ferric chloride since there is no precipitate formation or color changes as described in Table 1.

3.4 Evaluation of anti-inflammatory effects

3.4.1 Inhibition of protein denaturation activity of synthesized silver nanoparticles

Silver nanoparticles made from *Catharanthus roseus* extracts inhibited the heat-induced denaturation of egg albumin. At all of the measured concentrations, there was significant resistance to egg albumin denaturation in a concentration-dependent manner. At the highest measured concentration (400 µg/ml), the maximum percentage of inhibition was 58%, as shown in Fig. 3. Furthermore, at a concentration of 400 µg/ml, Diclofenac, which was utilized as a control medication, showed a maximal inhibition of 93.37%.

3.4.2 Inhibition of protein denaturation activity of synthesized copper nanoparticles

Copper nanoparticles made from *Catharanthus roseus* extracts effectively prevented heat-induced denaturation

Table 1 Biosensing activity of silver, copper and iron nanoparticles from *Catharanthus roseus* leaves extract

Heavy metal salts	Silver	Copper	Iron
Ferrous sulphate	+	+	−
Lead acetate	+	+	−
Zinc sulphate	+	+	−
Ferric chloride	+	+	−

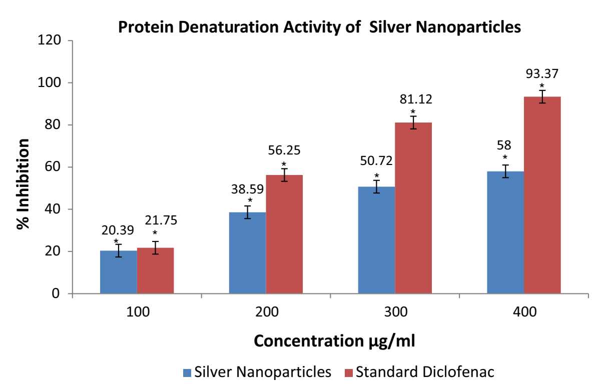

Fig. 3 Inhibition of protein denaturation activity of synthesized silver nanoparticles. Data are expressed in mean ± SEM (*$p < 0.05$)

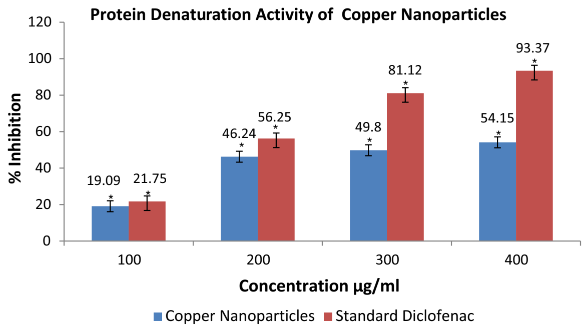

Fig. 4 Inhibition of protein denaturation activity of synthesized copper nanoparticles. Data are expressed in mean ± SEM (*$p < 0.05$)

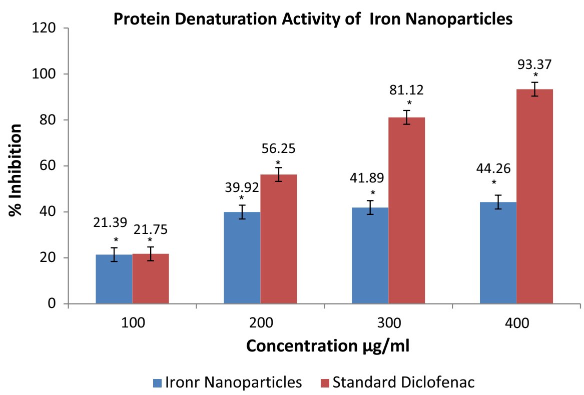

Fig. 5 Inhibition of protein denaturation activity of synthesized iron nanoparticles. Data are expressed in mean ± SEM (*$p < 0.05$)

of egg albumin. All of the measured concentrations significantly decreased the denaturation of egg albumin in a concentration-dependent manner. At the highest measured concentration (400 µg/ml), the maximum percentage of inhibition was 54.15%, as shown in Fig. 4. At a concentration of 400 µg/ml, diclofenac, which was utilized as a control, showed a maximal inhibition of 93.37%.

3.4.3 Inhibition of protein denaturation activity of synthesized iron nanoparticles

Egg albumin denaturation caused by heat was successfully inhibited by synthesized iron nanoparticles derived from *Catharanthus roseus* extracts. At all measured concentrations, the denaturation of egg albumin was significantly and concentration-dependently reduced. At the highest measured concentration (400 µg/ml), the maximum percentage of inhibition was 44.26%. Additionally, as shown in Fig. 5, diclofenac utilized as a standard medication demonstrated a maximal inhibition of 93.37% at a concentration of 400 µg /ml.

4 Discussions

Silver, copper, and iron nanoparticles were successfully obtained utilizing *Catharanthus roseus* leaf extracts in the bioreduction of silver nitrate, copper sulfate, and ferric chloride. Visual observations and UV–Vis spectroscopic techniques demonstrate that *Catharanthus roseus* leaf extracts generate silver, copper, and iron nanoparticles. The most significant absorbance peak of the generated silver nanoparticle was at 415 nm, copper nanoparticles were at 300 nm, and iron nanoparticles were at 400 nm, according to the current study. This result was very significant when compared with synthesized silver nanoparticles from *Tectona grandis* seed extracts, which showed the maximum absorbance at 440 nm [46], *Jatropha curcas* CuNPs, which showed absorption peaks at 337 and 266 nm [47] and iron nanoparticles (FeNPs) synthesized from *Bauhinia tomentosa* leaf extract, which showed absorption peaks at 328 nm [48]. On the other hand AgNPs synthesized from several plant extract have reported absorption peaks around 446, 456, 443, and 347 nm for neem, aloe vera, Indian mint, and guava leaves, respectively [49]. Another example indicated that Ag-NPs exhibited UV–Vis absorption spectra at 424 nm [50].

The biosensing activity demonstrates that these nanoparticles are a promising source for harmful heavy salt biosensing. Silver, copper, and iron nanoparticles were biogenically created without the use of any materials other than the precursor in an entirely green process. The potency of anti-inflammatory compounds was tested, and the results revealed that they were efficient against protein denaturation. In the present study, protein denaturation was discovered to be the primary source of inflammation. The capacity of the extract to reduce protein denaturation was investigated as part of the inquiry into the mechanism of the anti-inflammatory effect. Heat-induced albumin denaturation was effectively inhibited by certain extracts. *Catharanthus roseus* showed 58%, 54.15%, and 44.26% inhibition for silver, copper, and iron nanoparticles, respectively. As indicated in Figs.3, 4, and 5, Diclofenac sodium was utilized as a standard anti-inflammation medicine. At the same time another experiment was made to study the anti-inflammatory activity of silver nanoparticles from *Cotyledon orbiculata* plant extract [51]. *A. marina*, on the other hand, demonstrated anti-inflammatory activity with a crude extraction of 68.92% and synthesized AgNPs was 72.1%, respectively. Aspirin was used as a standard anti-inflammation drug [43]. This is an effort to raise awareness of the potential therapeutic use of iron, copper, and silver nanoparticles. Future anti-inflammatory drugs can be created using an inventive, efficient, and secure method provided by *Catharanthus* *roseus*. More in vivo research, preclinical testing, and clinical trials are required even though these medications appear to be promising.

5 Conclusion

Based on the findings of this study, it can be inferred that silver, copper, and iron nanoparticles green synthesized from *Catharanthus roseus* leaf has an anti-inflammatory effect in vitro, which could be linked to numerous phytochemicals in the extract. The production of silver, copper, and iron nanoparticles using *Catharanthus roseus* leaf extract could be a source of anti-inflammatory effects. One of the properties of various non-steroidal anti-inflammatory medicines is their ability to stabilize and prevent denaturation. Furthermore, the biosensing activity demonstrates that these nanoparticles are a viable source for harmful heavy salt biosensing. As a result, our research suggests that the synthesized nanoparticles from *Catharanthus roseus* could be employed as a lead chemical in developing an effective anti-inflammatory medicine to treat inflammation. As a result, more research is required to achieve this goal.

Abbreviations

AgNPs	Silver nanoparticles
CuNPs	Copper nanoparticles
FeNPs	Iron nanoparticles
PBS	Phosphate buffered saline

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Author contributions
FRSA provided the conception and designed the research. MJS and SN carried out the experiments. MJS analyzed the data and drafted the manuscript. MJS and FRSA reviewed and edited the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
Not applicable.

Declarations

Ethics approval and consent to participate
No any animal or human were used during this experimental study.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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