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RESEARCH ARTICLE

GREEN SYNTHESIS AND PHYSICOCHEMICAL CHARACTERISATION OF SILVER NANOPARTICLES USING A ROSE-JASMINE FORMULATION: EVALUATION OF CYTOTOXIC POTENTIAL

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Abstract: The changing lifestyle as well as the food habits has resulted in the introduction of numerous diseases in the past few decades. Moreover, by the passing day, we are becoming more aware about the drug resistance that is developed in most of the disease-causing pathogens. In this scenario, bringing more herbal medicines into the market is the only way to improve the situation. Moreover, Rose and Jasmine already have proven their medicinal properties were taken to check the cytotoxic effects along with the help of Ag- NPs. The aim of this study was to assess the cytotoxic effects of Rose and Jasmine formulated silver nanoparticles (Ag-NPs). The Rose and Jasmine for the synthesis of Ag-NPs and analysed the cytotoxic effects of Ag-NPs using brine shrimp lethality assay. The Ag-NPs can be synthesized using herbal formulation using Rose and Jasmine. The Ag-NPs synthesized using herbal formulation of Rose and Jasmine have cytotoxic effects.

Keywords: Green Synthesis, Nanomedicine, Phytochemicals, Antimicrobial resistance, Cytotoxicity, Eco-friendly Formulation

INTRODUCTION

The rapid increase in antimicrobial resistance, along with the growing burden of chronic and lifestyle-associated diseases, has necessitated the development of novel and sustainable therapeutic strategies [1]. In this context, plant-based nanotechnology has emerged as a promising interdisciplinary approach, combining the advantages of phytochemistry and nanoscience.

Nanoparticles, particularly those within the size range of 1–100 nm, exhibit unique physicochemical properties such as enhanced surface area, reactivity, and bioavailability, making them highly suitable for biomedical applications including drug delivery, diagnostics, and anticancer therapy [2]. Among various metallic nanoparticles, silver nanoparticles (AgNPs) have attracted significant attention due to their potent antimicrobial, antioxidant, and cytotoxic properties, along with their broad-spectrum biomedical applicability [3].

Conventional methods for nanoparticle synthesis, including physical and chemical approaches, are often associated with high cost, energy consumption, and environmental toxicity [4]. In contrast, green synthesis using plant extracts offers a simple, cost-effective, and eco-friendly alternative, wherein phytochemicals such as flavonoids, phenolics, alkaloids, and terpenoids act as both reducing and stabilizing agents [5]. These bioactive compounds not only facilitate nanoparticle formation but also enhance their biological activity through synergistic interactions.

Rosa canina (rose) is well documented for its antioxidant, anti-inflammatory, and antimicrobial properties, primarily attributed to its rich polyphenolic and flavonoid content [6]. Similarly, *Jasminum officinale* (jasmine) contains a wide spectrum of bioactive compounds, including alkaloids, glycosides, and essential oils, which contribute to its pharmacological potential [7–9]. The combination of these two botanicals may enhance the efficiency of nanoparticle synthesis while imparting improved biological activity.

Recent studies have demonstrated that plant-mediated AgNPs exhibit enhanced therapeutic efficacy, including antimicrobial, anticancer, antiviral, and anti-inflammatory activities, due to their ability to interact at the molecular level with biological systems [10]. Furthermore, the size, morphology, and surface chemistry of nanoparticles strongly influenced by synthesis parameters play a critical role in determining their biological interactions and cytotoxic potential [11]. Therefore, the present study aims to synthesize silver nanoparticles using a rose–jasmine formulation via a green synthesis approach and to evaluate their cytotoxic potential using a brine shrimp lethality assay as a preliminary screening model.

2. MATERIALS AND METHODS

2.1. PREPARATION OF PLANT EXTRACT

Fresh rose (*Rosa canina*) and jasmine (*Jasminum officinale*) flowers were collected and thoroughly washed. The plant materials were boiled in distilled water to obtain an aqueous extract, which was filtered and stored for further use. (Figure 1A,1B)

2.2. GREEN SYNTHESIS OF SILVER NANOPARTICLES

Silver nanoparticles were synthesized by adding the prepared plant extract to an aqueous solution of silver nitrate ($AgNO_3$). The reaction mixture was incubated at room temperature. Formation of AgNPs was confirmed by a visible color change from pale yellow to brownish-yellow, indicating reduction of silver ions. (Figure 1C)

2.3. BRINE SHRIMP LETHALITY ASSAY

Cytotoxic activity was evaluated using the brine shrimp lethality assay. (Figure 2)

- **Saline preparation:** 2 g of iodine-free salt was dissolved in 200 mL of distilled water.
- **Experimental setup:** Approximately 10–12 mL of saline solution was added to each well of a 6-well ELISA plate.
- **Inoculation:** Ten brine shrimp nauplii were introduced into each well.
- **Treatment:** AgNPs were added at concentrations of 5, 10, 20, 40, and 80 µL. A control group without treatment was maintained.
- **Incubation:** Plates were incubated for 24 hours.
- **Assessment:** The number of surviving nauplii was counted, and percentage mortality was calculated using:

Total number of nauplii
Percentage mortality = -- x 100
Number of dead nauplii

RESULTS

3.1. VISUAL CONFIRMATION OF SILVER NANOPARTICLES SYNTHESIS

The formation of silver nanoparticles (AgNPs) using the rose–jasmine formulation was initially confirmed by a distinct color change in the reaction mixture. Upon addition of the plant extract to the silver nitrate solution, the solution gradually transitioned from a pale yellow to a brownish-yellow color. This visible transformation is indicative of the reduction of silver ions (Ag^+) into metallic silver nanoparticles (Ag^0), mediated by phytochemicals present in the extract. The color change is attributed to surface plasmon resonance, a characteristic optical property of AgNPs. (Figure 1C)

3.2. CYTOTOXIC ACTIVITY ASSESSED BY BRINE SHRIMP LETHALITY ASSAY

The cytotoxic potential of the synthesized AgNPs was evaluated using the brine shrimp lethality assay across a range of concentrations (5, 10, 20, 40, and 80 µL). The results demonstrated a concentration-dependent response in nauplii survival. At lower concentrations (5–40 µL), minimal cytotoxicity was observed, with nearly all nauplii remaining viable after 24 hours of incubation. However, at the highest concentration tested (80 µL), a reduction in survival was noted, with the number of live nauplii decreasing from 10 (control) to 9, indicating the onset of cytotoxic effects. (Table 1)

3.3. QUANTITATIVE ANALYSIS OF MORTALITY

The percentage mortality of brine shrimp nauplii was calculated based on the number of dead larvae after 24 hours. The control group exhibited 0% mortality, confirming the suitability of the experimental conditions.
A slight increase in mortality was observed with increasing concentration of AgNPs. The highest concentration (80 µL) showed approximately 10% mortality, whereas all lower concentrations exhibited negligible or no mortality. This suggests that the synthesized nanoparticles possess low to moderate cytotoxicity under the tested conditions. (Figure 3)

3.4. DOSE RESPONSE RELATIONSHIP

The observed trend indicates a positive correlation between AgNP concentration and cytotoxic effect. Although the increase in mortality was not statistically steep, the results suggest that higher concentrations may enhance cytotoxic activity. The relatively low mortality observed in this study indicates that the synthesized AgNPs may exhibit controlled or mild cytotoxic effects, which could be advantageous for biomedical applications where selective toxicity is desired.

CYTOTOXIC EFFECT MICROLITRE	ROSE AND JASMINE
5	10
10	10

20	10
40	10
80	9
CONTROL	10

Table 1: Cytotoxic Effect of Rose-Jasmine Synthesized AgNPs on Brine Shrimp (Artemia salina) This table displays the survival count of brine shrimp larvae (nauplii) after 24 hours of exposure to varying microliter concentrations of the herbal silver nanoparticle formulation. It compares five treatment concentrations (5 µL to 80 µL) against a control group to determine dosage-dependent mortality.

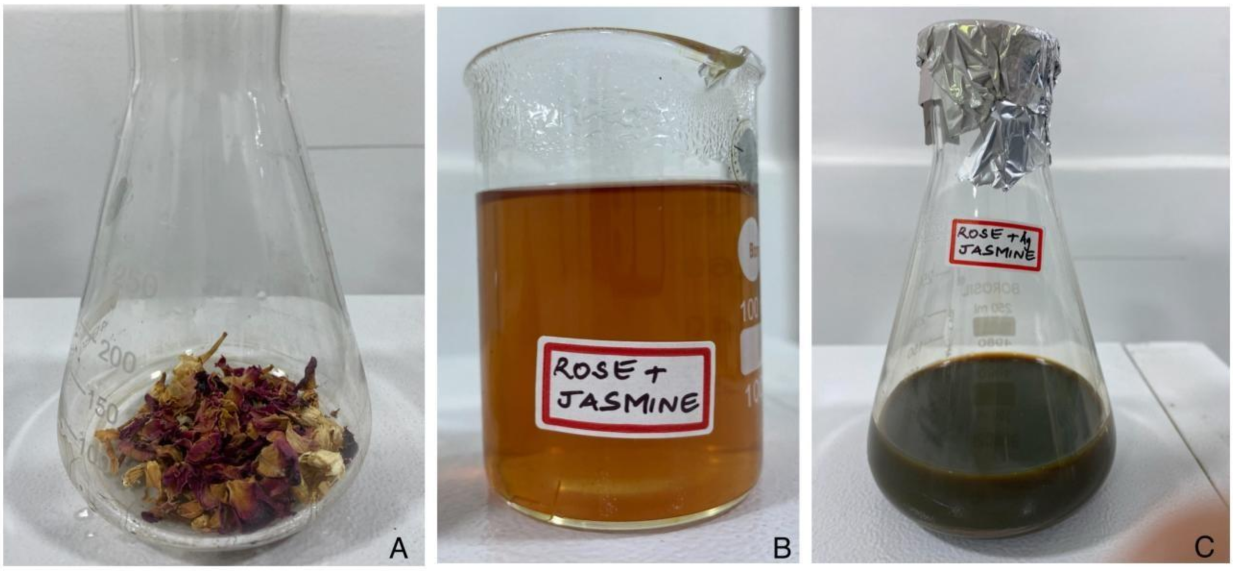

Figure 1A: Dried Floral Raw Materials - A visual representation of the starting herbal components, consisting of a mixture of dried Rosa canina (Rose) and Jasminum officinale (Jasmine) petals prior to aqueous extraction.
Figure 1B: Aqueous Herbal Extract - The filtered liquid obtained after boiling the rose and jasmine formulation. This extract serves as the natural reducing and capping agent for the synthesis of silver nanoparticles.
Figure 1C: Visual Confirmation of Nanoparticle Synthesis - The reaction mixture following the addition of silver nitrate ($AgNO_3$). The transition to a distinct brownish-yellow tint indicates the successful reduction of silver ions into silver nanoparticles (AgNPs).

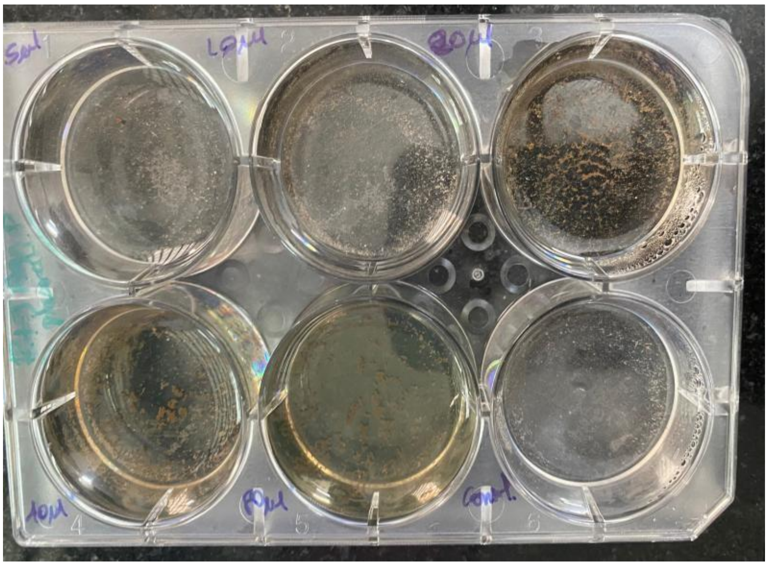

Figure 2: Brine Shrimp Lethality Assay Experimental Setup - A 6-well ELISA plate containing ten brine shrimp nauplii per well in a saline environment. The wells are labeled for 5 different treatment concentrations (5 µL, 10 µL, 20 µL, 40 µL, and 80 µL) and a control to observe cytotoxic effects.

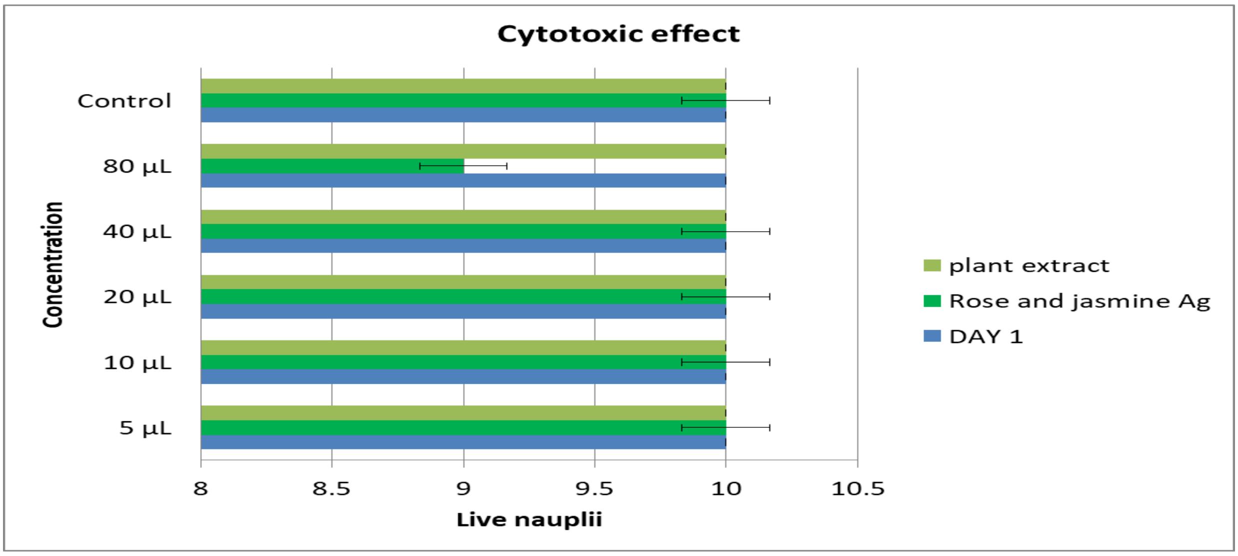

Figure 3: Comparative Cytotoxicity Analysis of Plant Extract vs. AgNPs A bar graph illustrating the number of live nauplii remaining after 24 hours (Day 1). The chart compares the safety profile of the raw plant extract against the synthesized Rose-Jasmine AgNPs, highlighting a slight decrease in viability specifically at the 80 µL concentration

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DISCUSSION

The present study demonstrates the successful green synthesis of silver nanoparticles using a rose–jasmine formulation, with cytotoxic activity confirmed through a brine shrimp lethality assay. The observed color change during synthesis indicates the reduction of silver ions (Ag^+) into metallic silver (Ag^0), mediated by phytochemicals present in the plant extracts [12].

Green synthesis of AgNPs has gained considerable attention due to its eco-friendly nature and the dual role of plant metabolites as reducing and stabilizing agents. Phytochemicals such as flavonoids, terpenoids, and phenolic compounds facilitate electron transfer, leading to nanoparticle formation while simultaneously capping and stabilizing the particles [13]. This process eliminates the need for toxic chemicals and enhances the biocompatibility of the synthesized nanoparticles.

The cytotoxic activity observed in this study is consistent with previous reports indicating that AgNPs exert biological effects primarily through the generation of reactive oxygen species (ROS), leading to oxidative stress, mitochondrial dysfunction, and eventual cell death [14]. Additionally, AgNPs can interact with cellular proteins, DNA, and membranes, disrupting normal cellular function and inducing apoptosis. The nanoscale size and large surface area further enhance their cellular uptake and biological interactions [15].

The concentration dependent cytotoxicity observed in the brine shrimp assay aligns with earlier findings, where increased nanoparticle concentration resulted in higher mortality rates. This suggests that the biological activity of AgNPs is dose-dependent and influenced by nanoparticle characteristics such as size, morphology, and surface chemistry [16].

Furthermore, the synergistic interaction between silver ions and plant derived phytoconstituents may contribute to the enhanced cytotoxic effects [17]. Studies have shown that plant mediated AgNPs often exhibit greater biological activity compared to crude plant extracts alone, due to the combined effects of metallic nanoparticles and bioactive surface compounds [18].

Despite these promising findings, the brine shrimp lethality assay provides only a preliminary indication of cytotoxic potential. While it is widely used as a rapid and cost effective screening tool, it does not fully replicate complex cellular mechanisms observed in mammalian systems [19]. Therefore, further studies involving in vitro cell line models and in vivo investigations are essential to validate the therapeutic potential and safety profile of these nanoparticles [20].

Overall, the findings of this study support the growing body of evidence that plant-mediated silver nanoparticles represent a promising avenue for the development of novel biomedical agents, particularly in anticancer and antimicrobial applications [21–23].

5. LIMITATIONS

The present study has several limitations that should be acknowledged. First, the cytotoxic evaluation was performed using the brine shrimp lethality assay, which serves as a preliminary screening model and does not fully represent complex mammalian cellular responses. Second, detailed physicochemical characterization techniques such as UV–Visible spectroscopy, Fourier-transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), or transmission electron microscopy (TEM) were not employed to confirm nanoparticle size, morphology, and surface properties. Third, quantitative analysis including determination of half-maximal lethal concentration (LC_{50}) was not performed, limiting the precision of cytotoxic assessment. Additionally, the study did not compare the activity of the synthesized nanoparticles with standard cytotoxic agents or with individual plant extracts to evaluate synergistic effects. Finally, the absence of in vitro cell line studies and in vivo validation restricts the direct translational applicability of the findings.

6. CONCLUSION

In conclusion, silver nanoparticles were successfully synthesized using a green approach with a rose jasmine formulation, demonstrating an eco-friendly and cost-effective method of nanoparticle production. The synthesized nanoparticles exhibited concentration-dependent cytotoxic activity in the brine shrimp lethality assay, indicating their potential as bioactive agents. The findings suggest that plant-mediated silver nanoparticles may serve as promising candidates for future biomedical applications, particularly in the development of anticancer and antimicrobial therapies. However, further comprehensive studies involving advanced characterization techniques, mechanistic evaluations, and in vitro and in vivo models are essential to validate their efficacy and safety for clinical applications.

7. ACKNOWLEDGEMENT

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8. CONFLICT OF INTEREST

All the authors declare that there was no conflict of interest in the present study.

9. SOURCE OF FUNDING

NIL

10. AUTHORS CONTRIBUTION

Arun Ganesh M K: Literature search, data collection, analysis, manuscript drafting
Abirami Arthanari: Data verification, manuscript drafting.

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