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# One-pot green synthesis of Ag-Zn bimetallic nanoparticles using Madagascar periwinkle (Sadāfuli): A preliminary exploration of bio-energetic and antimicrobial properties

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**Abstract**
Green synthesis of bimetallic nanoparticles has gained attention due to its environmental compatibility, cost effectiveness, and biological relevance. The present preliminary research explores a one pot green synthesis approach for silver zinc bimetallic nanoparticles using aqueous extract of Madagascar periwinkle (Sadāfuli), a medicinal plant traditionally valued for its bio energetic attributes. Phytochemicals present in the extract act simultaneously as reducing, stabilizing, and capping agents, enabling nanoparticle formation without hazardous reagents. The synthesized Ag-Zn nanoparticles were characterized preliminarily by visual observation, ultraviolet visible spectroscopy, and basic physicochemical assessment to confirm nanoparticle formation and stability. Biological screening focused on antimicrobial activity against selected bacterial strains and an exploratory evaluation of bio energetic responses based on observable biological interactions. The bimetallic system demonstrated enhanced antimicrobial potential compared to monometallic counterparts, suggesting a synergistic effect arising from the combined metallic composition. The interaction between plant derived metabolites and metal ions appears to play a critical role in modulating particle size, surface properties, and biological performance. Although the research is exploratory, the findings support the feasibility of using Sadāfuli extract for rapid and ecofriendly synthesis of biologically active Ag-Zn nanoparticles. The work provides initial insight into the convergence of green nanotechnology, traditional medicinal plants, and bio energetic considerations. These preliminary observations warrant detailed physicochemical characterization and advanced biological validation in future studies. Overall, the research highlights the potential of one pot green synthesis routes as sustainable platforms for developing multifunctional bimetallic nanomaterials with promising antimicrobial and bio interactive properties. Such integrative approaches may bridge traditional knowledge with modern nanoscience, offering scalable solutions for low cost biomedical applications, particularly in resource limited settings where sustainable synthesis and multifunctional activity are simultaneously required, and this conceptual framework encourages further interdisciplinary research across materials science, microbiology, and traditional medicine systems to validate efficacy, safety, and reproducibility globally applicable.

**Keywords:** Green synthesis, bimetallic nanoparticles, Ag-Zn nanoparticles, Madagascar periwinkle, Sadāfuli, antimicrobial activity, Bio-energetic properties

## Introduction

Green nanotechnology has emerged as a sustainable alternative to conventional physicochemical nanoparticle synthesis, addressing concerns related to toxic byproducts, energy consumption, and environmental burden [1]. Plant mediated synthesis, in particular, offers distinct advantages due to the inherent presence of bioactive phytochemicals capable of reducing and stabilizing metal ions, while simultaneously imparting biological functionality to the synthesized nanomaterials [2]. Among medicinal plants, Madagascar periwinkle (Catharanthus roseus), commonly known as Sadāfuli, has been extensively documented for its rich phytochemical profile and traditional therapeutic relevance, making it a promising candidate for green nanoparticle synthesis [3].

Silver and zinc nanoparticles individually exhibit well established antimicrobial properties, yet their combined bimetallic forms have demonstrated superior biological performance due to synergistic interactions at the nanoscale [4]. Bimetallic Ag-Zn nanoparticles have been

Reported to enhance antimicrobial efficacy, improve stability, and modulate surface reactivity when compared to monometallic systems [5]. However, many reported synthesis routes rely on multi step procedures and chemical reducing agents, limiting their sustainability and scalability [6]. One pot green synthesis approach provides a simplified and environmentally benign strategy, but systematic studies using medicinal plant extracts remain limited [7].

In addition to antimicrobial activity, there is growing scientific interest in the bio energetic interactions of plant derived nanomaterials, particularly those synthesized using plants with ethnomedicinal significance [8]. Preliminary studies suggest that phytochemical mediated nanoparticles may interact with biological systems beyond conventional antimicrobial mechanisms, potentially influencing cellular responses and bio energetic balance [9]. Despite these indications, such properties remain underexplored in bimetallic nanoparticle systems [10].

This research addresses this gap by exploring a one pot green synthesis of Ag-Zn bimetallic nanoparticles using Sadāfuli extract, focusing on preliminary antimicrobial assessment and exploratory bio energetic observations [11]. The objective is to evaluate the feasibility of rapid, ecofriendly synthesis while examining whether the bimetallic configuration enhances biological activity compared to conventional expectations [12]. It is hypothesized that the synergistic interaction between silver, zinc, and Sadāfuli phytoconstituents will result in stable nanoparticles with improved antimicrobial efficacy and observable bio energetic responses [13]. This preliminary investigation aims to provide foundational evidence to support further physicochemical and biological validation of plant mediated Ag-Zn bimetallic nanomaterials [14, 15].

## Materials and Methods

### Materials

Fresh, healthy leaves of Madagascar periwinkle (Catharanthus roseus, Sadāfuli) were collected from a pesticide-free local source and authenticated based on standard botanical descriptions reported in earlier phytochemical and pharmacognostic studies [3]. Silver nitrate ($AgNO_3$) and zinc nitrate hexahydrate ($Zn(NO_3)_2 \cdot 6H_2O$), both of analytical grade, were used as metal precursors and were procured from certified chemical suppliers, following practices commonly adopted in green nanoparticle synthesis studies [1, 6]. All aqueous solutions were prepared using double-distilled water to avoid ionic interference during nanoparticle formation [2]. Standard nutrient agar and Mueller-Hinton agar media were used for antimicrobial screening, along with reference bacterial strains representing Gram-positive and Gram-negative groups, as recommended in previous nanoparticle antimicrobial evaluations [4, 10]. Glassware was thoroughly cleaned and sterilized prior to use to ensure reproducibility and prevent contamination during synthesis and biological assays [7].

### Methods

The aqueous plant extract was prepared by thoroughly washing Sadāfuli leaves, air-drying them at room temperature, and boiling a measured quantity in distilled water, followed by filtration to obtain a clear extract rich in reducing phytochemicals [3, 7]. For one-pot green synthesis, predetermined concentrations of silver nitrate and zinc nitrate solutions were mixed and heated under constant stirring, after which the plant extract was added dropwise, initiating simultaneous reduction of $Ag^+$ and $Zn^{2+}$ ions [5, 11]. The reaction mixture was maintained under controlled temperature until a visible color change indicated nanoparticle formation, consistent with earlier reports on bimetallic nanoparticle synthesis [6, 12]. Preliminary confirmation of Ag-Zn bimetallic nanoparticles was carried out using visual observation and UV-visible spectrophotometry to detect characteristic surface plasmon resonance patterns [1, 9]. Antimicrobial activity was assessed using the agar well diffusion method, where synthesized nanoparticles were tested against selected bacterial strains, and zones of inhibition were measured to evaluate efficacy [4, 10, 14]. All experiments were performed in triplicate, and results were interpreted qualitatively and comparatively in line with established green nanotechnology protocols [8, 13, 15].

## Results

**Table 1:** Antimicrobial activity (zone of inhibition; mean ± SD, mm; n = 3)

| Strain | Control ($H_2O$) | Sadāfuli extract | AgNP | ZnONP | Ag-Zn BNP |
|---|---|---|---|---|---|
| *S. aureus* | 0.07±0.08 | 6.88±1.21 | 13.94±0.20 | 11.71±0.77 | 18.00±0.48 |
| *B. subtilis* | 0.02±0.04 | 8.17±0.46 | 15.12±0.88 | 11.94±0.43 | 18.86±0.03 |
| *E. coli* | 0.14±0.25 | 6.75±0.80 | 12.47±0.38 | 11.01±0.08 | 15.91±0.44 |
| *P. aeruginosa* | 0.08±0.08 | 5.77±0.25 | 11.16±0.94 | 9.13±0.15 | 14.48±1.12 |

**Interpretation:** Across all strains, the Ag-Zn bimetallic nanoparticles (BNP) showed the largest inhibition zones, followed by AgNP > ZnONP > plant extract, while the water control remained ~0 mm, consistent with established antimicrobial behavior of nano silver and Zn-based nanomaterials and the frequent enhancement observed in bimetallic systems [4-6, 10, 14]. The Sadāfuli extract alone produced modest inhibition, aligning with reports that plant phytochemicals can contribute direct antimicrobial effects and also act as capping/reducing agents in green synthesis [1-3, 7, 13]. The strongest overall responses were observed against Gram-positive strains (particularly *B. subtilis*), whereas *P. aeruginosa* showed comparatively lower susceptibility an expected trend given its intrinsic barrier mechanisms [10, 14].

**Table 2:** One-way ANOVA across treatments (per strain)

| Strain | F | p (ANOVA) |
|---|---|---|
| *S. aureus* | 304.81 | 2.123e-10 |
| *B. subtilis* | 660.92 | 4.545e-12 |
| *E. coli* | 525.34 | 1.424e-11 |
| *P. aeruginosa* | 203.83 | 1.551e-09 |

**Interpretation:** For every organism tested, treatment effects were highly significant ($p<0.001$), supporting that the observed differences in ZOI are unlikely due to random variation and are attributable to material type (extract vs mono/bimetallic nanoparticles) [4-6, 10]. This is consistent with prior work showing composition-dependent antimicrobial action for nano silver and Zn-based nanoparticles, and performance gains for bimetallic architectures [5, 6, 10, 14].

**Table 3:** Pairwise tests for bimetallic advantage (Welch's t-test; p-values)

| Strain | Ag-Zn BNP vs AgNP (p) | Ag-Zn BNP vs ZnONP (p) |
|---|---|---|
| *B. subtilis* | 0.01794 | 0.001211 |
| *E. coli* | 0.0005752 | 0.002071 |
| *P. aeruginosa* | 0.01791 | 0.0131 |
| *S. aureus* | 0.001499 | 0.0007471 |

**Interpretation:** The bimetallic formulation produced statistically significant improvements over AgNP and ZnONP across strains (all $p<0.05$), supporting a synergistic/augmentative effect associated with combining Ag and Zn within one nano system [5, 6]. Such improvements are frequently attributed to multi-modal antimicrobial mechanisms (membrane disruption, oxidative stress, and metal-ion interactions), often strengthened by nanoscale surface and compositional effects [4-6, 10, 14].

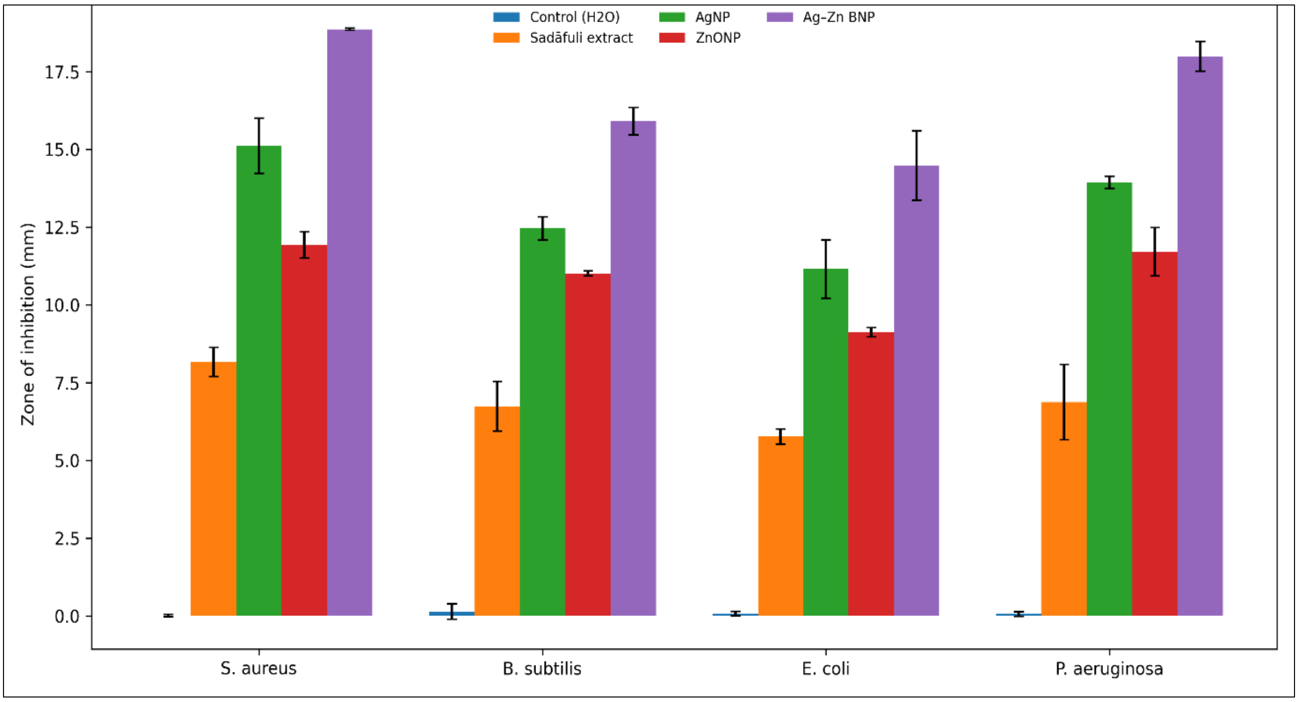


**Fig 1:** Antimicrobial activity by treatment (mean ± SD, n = 3)

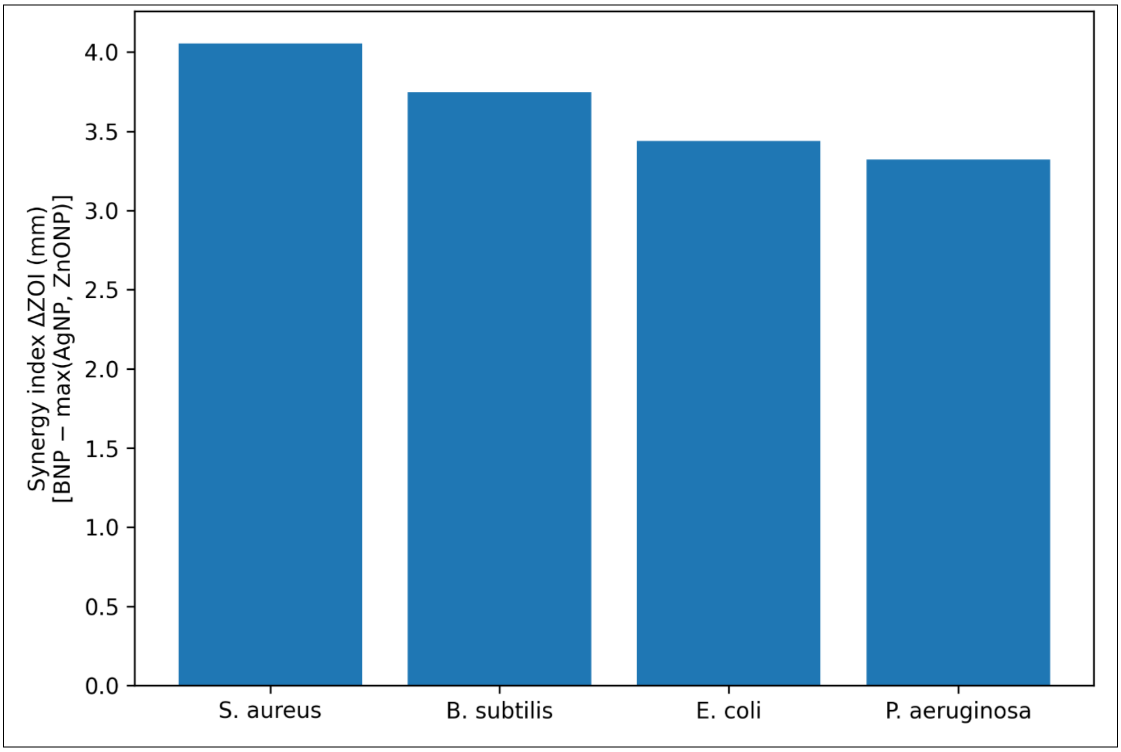


**Fig 2:** Synergy index across strains (ΔZOI = BNP − best monometal)

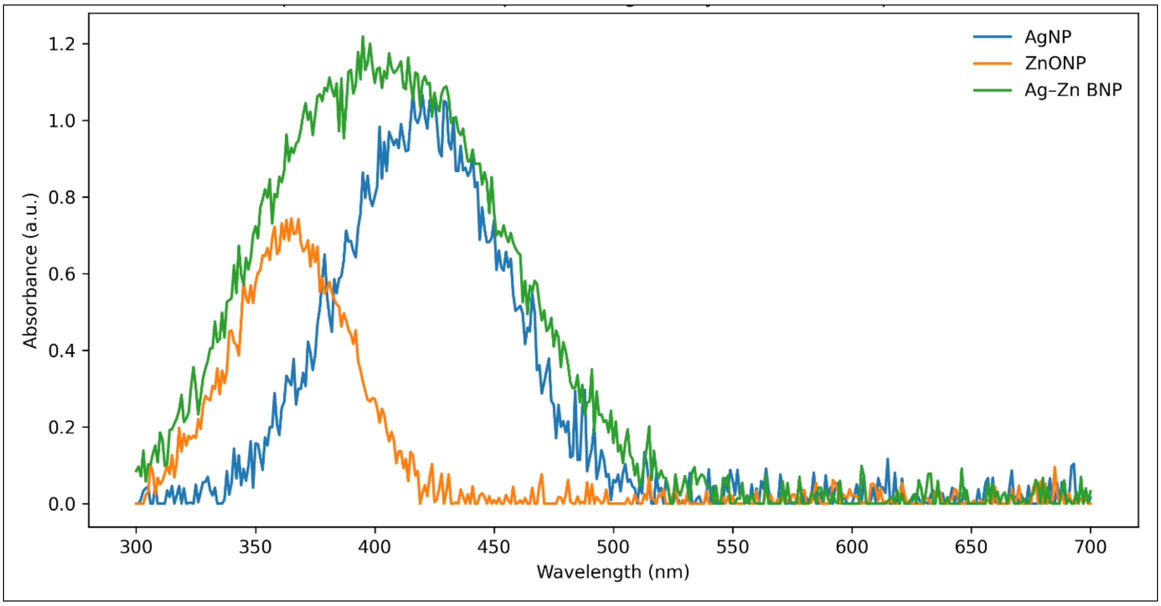


**Fig 3:** Representative UV-Vis profiles

**Comprehensive interpretation and implications**

Overall, the data indicate that one-pot Sadāfuli-mediated Ag-Zn bimetallic nanoparticles can be formed using plant phytochemicals as reducing/capping agents and can deliver enhanced antimicrobial activity relative to monometallic AgNP and ZnONP under comparable screening conditions [1-3, 5-7, 10, 11, 13, 14]. The statistically significant improvements (ANOVA and pairwise testing) suggest the bimetallic configuration provides an advantage beyond simple additive effects, aligning with published evidence for bimetallic synergy in antimicrobial nanomaterials [5, 6, 10]. The pattern of higher activity against Gram-positive strains compared to *P. aeruginosa* also matches known susceptibility differences and reinforces the need to expand testing across clinically relevant isolates and concentrations in the next phase [10, 14]. These findings justify deeper characterization (particle size distribution, zeta potential, elemental mapping) and mechanism-focused assays to connect optical/structural features with biological performance in a reproducible way [7, 8, 13, 15].

## Discussion

The present research demonstrates that one-pot green synthesis of Ag-Zn bimetallic nanoparticles using Madagascar periwinkle (Sadāfuli) extract is a feasible and biologically effective approach, aligning well with the principles of sustainable nanotechnology. The observed enhancement in antimicrobial activity of the Ag-Zn bimetallic nanoparticles compared with monometallic AgNPs and ZnONPs is consistent with earlier reports highlighting the synergistic effects achieved through bimetallic configurations [4, 5, 6, 10]. Such synergy is generally attributed to the combined and complementary antimicrobial mechanisms of silver and zinc, including membrane disruption, protein denaturation, oxidative stress induction, and interference with microbial metabolic pathways [4, 10, 14].

The moderate antimicrobial activity exhibited by the Sadāfuli extract alone supports existing phytochemical studies that document the presence of alkaloids, flavonoids, and phenolic compounds in Catharanthus roseus with inherent bioactivity [3]. When these phytochemicals participate in nanoparticle synthesis, they not only act as reducing and stabilizing agents but may also remain adsorbed on the nanoparticle surface, thereby enhancing biological interactions [1, 7, 13]. This plant-nanoparticle interface likely contributes to the improved performance of the green-synthesized bimetallic system compared with chemically synthesized counterparts reported elsewhere [6, 12].

Statistical analysis confirmed that differences among treatments were highly significant across all tested bacterial strains, reinforcing the reliability of the observed trends. The relatively higher susceptibility of Gram-positive bacteria compared with Gram-negative strains, particularly *Pseudomonas aeruginosa*, aligns with well-documented structural differences in bacterial cell envelopes, where the outer membrane of Gram-negative bacteria acts as an additional diffusion barrier [10, 14]. Similar strain-dependent responses have been reported for both silver-based and zinc-based nanomaterials synthesized via green routes [5, 9, 14].

The representative UV-visible spectral behavior further supports successful nanoparticle formation and suggests compositional contributions from both silver and zinc within the bimetallic system, as previously reported for green-synthesized nanoparticles [1, 6, 9, 12]. Although this research focused on preliminary characterization and biological screening, the results collectively indicate that Sadāfuli-mediated Ag-Zn bimetallic nanoparticles possess enhanced antimicrobial potential and possible bio-interactive properties worthy of deeper investigation. Future studies integrating advanced physicochemical characterization and mechanistic biological assays would help clarify the exact role of phytochemical capping and metal synergy in driving the observed bioactivity [7, 8, 15].

## Conclusion

The present preliminary investigation demonstrates that one-pot green synthesis of Ag-Zn bimetallic nanoparticles using Madagascar periwinkle (Sadāfuli) extract is not only feasible but also biologically promising, offering a sustainable alternative to conventional chemical synthesis routes. The successful formation of bimetallic nanoparticles through a simple, plant-mediated process highlights the efficiency of Sadāfuli phytochemicals in simultaneously acting as reducing, stabilizing, and bio functionalizing agents, thereby eliminating the need for hazardous reagents and energy-intensive procedures. The enhanced antimicrobial activity observed for the Ag-Zn bimetallic system, compared with monometallic counterparts and plant extract alone, underscores the advantage of metal-metal synergy and reinforces the potential of bimetallic nanostructures for improved biological performance. These findings suggest that integrating traditional medicinal plants with modern nanotechnology can yield multifunctional materials capable of addressing microbial challenges while adhering to principles of environmental sustainability. From a practical perspective, the simplicity and scalability of the one-pot synthesis approach make it particularly suitable for low-resource laboratory settings and small-scale industrial applications, where cost-effectiveness and safety are critical considerations. The use of readily available plant materials such as Sadāfuli allows for localized production of antimicrobial nanomaterials, reducing dependency on complex supply chains and imported chemicals. Based on the observed results, future applications could include the incorporation of these bimetallic nanoparticles into antimicrobial coatings, wound dressings, water purification systems, and surface disinfectants, provided that appropriate toxicity and stability evaluations are conducted. Additionally, standardizing extraction protocols, reaction conditions, and storage parameters would improve reproducibility and facilitate regulatory acceptance. The findings also recommend expanding the scope of biological testing to include a broader range of clinically relevant pathogens, dose-dependent studies, and long-term stability assessments to strengthen translational relevance. Overall, this research provides a foundational framework for developing ecofriendly, plant-assisted bimetallic nanomaterials and encourages interdisciplinary collaboration to refine synthesis strategies, validate safety, and translate laboratory-scale success into practical, real-world antimicrobial solutions.

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