

Herbal Nanotechnology Approach for the Management of Skin Disorders: Manjistha-Derived Silver Nanoparticles

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Abstract

Skin disorders are a major global health concern and are frequently associated with microbial infection, oxidative stress, and inflammation. There is a growing need for safer and more effective topical therapeutic strategies that can address these multifactorial pathologies while minimizing adverse effects. The present study describes the green synthesis, characterization, and biological evaluation of silver nanoparticles (AgNPs) using the ethanolic root extract of *Rubia cordifolia* Linn. (Manjistha). Preliminary phytochemical screening of the extract revealed the presence of glycosides, alkaloids, proteins, coumarins, flavonoids, steroids, tannins, phenolic compounds, and terpenoids, while Fourier-transform infrared (FTIR) spectroscopy confirmed functional groups consistent with phenolics, flavonoids, and anthraquinones that likely participate in the reduction and stabilization of silver nanoparticles. Silver nanoparticles were synthesized using varying concentrations of silver nitrate (1–5 mM), and the formulation prepared with 2 mM AgNO₃ at 45°C under continuous stirring (830–834 rpm) for 105–107 min was selected as the optimized batch on the basis of particle-size analysis. The synthesized nanoparticles were characterized by UV–Visible spectroscopy, scanning electron microscopy (SEM), and energy-dispersive X-ray (EDX) analysis. A characteristic surface plasmon resonance band was observed in the range of approximately 420–450 nm, and SEM micrographs revealed predominantly spherical nanoparticles with sizes ranging from approximately 74 to 102 nm. The optimized Manjistha-mediated silver nanoparticles exhibited superior antioxidant activity compared with the crude ethanolic extract in the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, showing 82.49% radical-scavenging activity at 100 µg/mL versus 70.04% for the extract. In agar well-diffusion assays, the nanoparticle formulation demonstrated enhanced antibacterial activity against *Escherichia coli*, producing a zone of inhibition of 20 mm at 100 µg/mL, compared with 16 mm for the extract. Against *Candida albicans*, the nanoparticles produced a 24-mm inhibition zone at 100 µg/mL, whereas the extract produced an 8-mm zone. In silico molecular docking studies further indicated strong predicted binding interactions between major Manjistha anthraquinone constituents (alizarin, purpurin, munjistin, and rubiadin) and selected microbial target proteins. Collectively, these findings suggest that *R. cordifolia*-mediated silver nanoparticles can enhance the antioxidant and antimicrobial potential of Manjistha and may represent a promising platform for the development of topical formulations for the management of bacterial and fungal skin infections. Nevertheless, further investigations including cytotoxicity, long-term stability, skin-permeation, and in vivo studies are warranted to establish their safety and clinical applicability.

Keywords: *Rubia cordifolia*; Manjistha; silver nanoparticles; green synthesis; nanotechnology; skin disorders; antioxidant activity; antimicrobial activity; molecular docking

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1. Introduction

Dermatological conditions are really health problems that affect a lot of people around the world. In fact, one-fourth of the world's population will have some kind of skin problem at some point in their lives. Skin conditions like acne, eczema and psoriasis can be really frustrating and painful. They can also lead to inflammation, oxidative stress and infections which can make people's lives miserable and expensive to treat. [1,2]

The problem is getting worse because some medicines are not working well as they used to. This is because of something called resistance. Also using medicines like corticosteroids and antibiotics for a long time can have bad side effects. So we need to find better ways to treat skin problems. [2]

Rubia cordifolia (Figure 1), a medicinal plant commonly known as Manjistha, has gained increased clinical attention because of its high therapeutic potential. [3,4,]

Globally, it has been used for the conventional treatment of skin problems/disorders, stimulation of lymphatic flow, and blood disorders [3,4,5]. The root of the plant has been used traditionally for the treatment of rheumatism, menstrual disorders, jaundice, urinary disorders, renal stones, skin disorders, and blood detoxification. Previous studies have reported its antioxidant activity and enhanced production of antioxidant markers such as superoxide dismutase, catalase, and glutathione [3,4], along with the anti-inflammatory activity of Manjistha extract (MJE) [5,6]. Besides its medicinal value, the plant is used in food, syrups, and medicated oils as a natural colorant. Moreover, the leaf extract is widely used for cataracts, eczema, psoriasis, and in the treatment of chikungunya fever. Herbal extracts containing flavonoids, tannins, and terpenoids are soluble in water but the drawback is

low absorption, which affects the bioavailability and efficacy [6,7]. Nanotherapeutics is a desired drug-targeted goal to provide high safety and efficacy over the conventional system. [6,7,8] People have been using plants to treat skin problems for a time. These plants are often cheap. Do not have many bad side effects. One of these plants is called *Rubia cordifolia* Linn or Manjistha. It is used in Ayurveda to clean the blood and skin. The roots of the Manjistha plant have good things in them like antioxidants and anti-inflammatory compounds. These compounds can help with wounds, bacteria and fungi. [7,8]

Manjistha is a candidate for treating skin problems because it has many good properties. However, using the extract of Manjistha is not very practical. This is because it does not dissolve well in water or fat and it has molecules that are hard to absorb. As a result, the good stuff in Manjistha does not get to where it needs to go. It does not work very well. [8]

Green synthesis of AgNPs using plant extracts offers an environmentally

friendly and cost-effective alternative to conventional chemical methods. In this approach, phytochemicals present in the plant extract act as both reducing and stabilizing agents, eliminating the need for toxic chemicals while simultaneously incorporating therapeutic phytoconstituents onto the nanoparticle surface [8,9].



Figure 1. Schematic representation of the overall workflow of the present study, including extraction of *Rubia cordifolia*, phytochemical screening, green synthesis of silver nanoparticles, characterization, and biological evaluation for the management of skin disorders. [10]

This study is about making nanoparticles with Manjistha. The process uses a method Figure 1 shows how it works. Silver nanoparticles are made using *Rubia cordifolia* extract. The extract acts as both a reducer and a protector. The goal is to get the particle size. [5]

The nanoparticles are tested using:

UV- spectroscopy Fourier, Transform Infrared spectroscopy, Scanning Electron Microscopy, their antioxidant and antimicrobial properties are checked., The tests are against skin pathogens. The results are compared to medicines using computer simulations. The idea is that silver nanoparticles with Manjistha will work better, then the extract. This is because they are more stable and can be absorbed by the skin easily. [18]

The combination of nanoparticles and Manjistha chemicals is expected to have better therapeutic benefits. Silver nanoparticles and Manjistha are used together to make it more effective. The study focuses on nanoparticles loaded with Manjistha. [6]

2. Materials and Methods

2.1. In Silico Molecular Docking

Molecular docking studies were performed using PyRx software incorporating the AutoDock Vina docking engine to evaluate the binding interactions of selected phytoconstituents with target microbial proteins. The three-dimensional structures of the target proteins from *Candida albicans* (PDB ID: 2WV2) and a representative bacterial species (PDB ID: 8Q5I) were retrieved from the Protein Data Bank (PDB). Selected phytochemical constituents of *Rubia cordifolia* (Manjistha), including alizarin, purpurin, munjistin, and rubiadin, along with the standard antimicrobial agents fluconazole and ciprofloxacin, were subjected to molecular docking analysis to assess their binding affinities and interaction profiles. The docking results were further analyzed, and ligand–protein interactions were visualized using BIOVIA Discovery Studio Visualizer. [11,12].

2.2. Substances

Manjistha extract was procured from the Citspray Aroma Sciences. Pvt. Ltd. Nagpur India. The chemicals Silver Nitrate (AgNO_3) was of analytical grade.

2.1.2. Compounds and Substances

Various analytical-grade chemicals and reagents were utilized in the present study, including absolute ethanol, silver nitrate (AgNO_3), deionized water, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin–Ciocalteu reagent, and aluminum chloride (AlCl_3). Microbiological culture media such as nutrient agar and Sabouraud dextrose agar were employed for microbial growth and analysis. Standard antimicrobial agents, including ciprofloxacin,

streptomycin, and other relevant antibiotics, were used for antimicrobial activity assessments. All chemicals, reagents, culture media, and antibiotics used in this investigation were of analytical grade and procured from reputable commercial sources. [13,14].

A range of laboratory instruments and equipment were employed throughout the study. These included a magnetic hotplate stirrer for sample preparation, a UV–Visible spectrophotometer for absorbance measurements, a Fourier Transform Infrared (FTIR) spectrometer for functional group analysis, a particle size analyzer for determining nanoparticle size distribution, and a zeta potential analyzer for surface charge characterization. Morphological analysis was performed using a Scanning Electron Microscope (SEM). Additional equipment included an analytical balance for precise weighing, an incubator for controlled-temperature cultivation, an autoclave for sterilization purposes, and a digital pH meter for pH measurements. Other standard microbiological laboratory instruments and accessories were used as required during the experimental procedures. [15,16].

2.3. Preparation of Ethanolic Extract of Manjistha

The ethanolic extract of *Rubia cordifolia* (Manjistha) roots was prepared using the maceration method. Briefly, the powdered root material was mixed with ethanol in a conical flask at an appropriate solvent-to-sample ratio. The flask was covered with aluminum foil to prevent light exposure and solvent evaporation. The mixture was continuously stirred using a magnetic hotplate stirrer and maintained under controlled conditions for seven days to facilitate efficient extraction of bioactive constituents. Following the extraction period, the mixture was filtered to remove the solid residue, yielding a brown-colored filtrate. The obtained extract was collected in sterile amber-colored bottles and stored under refrigerated conditions (4°C) until further use. [3,15,16].

Figure 2. Preparation of the Manjistha (*Rubia cordifolia*) ethanolic extract. (a) Initial mixing of Manjistha root powder with ethanol. Maceration of the extract in a borosilicate beaker covered with aluminium foil; (b) Magnetic stirring at 45°C using an Equiptronics EQ-725 magnetic hotplate stirrer. Continuous extraction under optimized conditions for seven days prior to filtration.

2.1. Preliminary Phytochemical Screening

The ethanolic extract was subjected to preliminary qualitative phytochemical screening using standard analytical procedures to identify the presence of major phytoconstituents. The extract was evaluated for carbohydrates, proteins, alkaloids, glycosides, flavonoids, tannins, terpenoids, steroids, coumarins, and phenolic compounds using their respective qualitative assays. These included the Molisch test for carbohydrates, Biuret test for proteins, Dragendorff's test for alkaloids, Keller–Killiani test for glycosides, Shinoda test for flavonoids, ferric chloride test for phenolic compounds and tannins, Salkowski test for terpenoids, Liebermann–Burchard test for steroids, Ninhydrin test for amino acids and proteins, and UV fluorescence analysis for coumarins. The results were recorded based on the characteristic color changes or precipitate formation observed during each test. [3,15].

2.2. FTIR Analysis of Manjistha Extract

Fourier Transform Infrared (FTIR) spectroscopy was employed to identify the functional groups present in the ethanolic extract. The FTIR spectrum was recorded in the range of $4000\text{--}400\text{ cm}^{-1}$ using an Attenuated Total Reflectance (ATR) accessory at a spectral resolution of 4 cm^{-1} . The observed absorption bands were analyzed and the corresponding functional groups were assigned by comparing the characteristic peak positions with standard infrared correlation charts and published reference data. [15].

2.3. Green Synthesis of Silver Nanoparticles

Silver nanoparticles (AgNPs) were synthesized through a green synthesis approach employing the ethanolic extract of *Rubia cordifolia* (Manjistha) as a reducing and stabilizing agent. This method was selected to minimize the use of hazardous chemicals and to exploit the phytoconstituents present in the plant extract for the eco-friendly production of nanoparticles. [13]

For the synthesis process, aqueous silver nitrate (AgNO_3) solutions were prepared at five different concentrations, namely 1, 2, 3, 4, and 5 mM. Each AgNO_3 solution was separately mixed with a predetermined volume of the ethanolic extract of *Rubia cordifolia* under aseptic conditions. The reaction mixtures were subjected to continuous magnetic stirring at a speed ranging from 830 to 834 rpm to ensure uniform mixing and effective interaction between silver ions and the bioactive constituents present in the plant extract. [13,14]

The synthesis reactions were carried out at a controlled temperature of 45°C throughout the process. The elevated temperature facilitated the reduction of Ag^+ ions to elemental silver (Ag^0) nanoparticles and enhanced the nucleation and growth processes. During the reaction, the formation of silver nanoparticles was initially indicated by a visible colour change in the reaction mixture, resulting from the surface plasmon resonance phenomenon characteristic of AgNPs. The reaction mixtures were maintained under the specified conditions until complete reduction of silver ions was achieved. [16,19]

To determine the optimal concentration of silver nitrate for nanoparticle synthesis, the AgNPs produced from each formulation (1–5 mM AgNO_3) were characterized for particle size distribution using Dynamic Light Scattering (DLS) analysis. DLS measurements were performed to evaluate the hydrodynamic diameter and size distribution profile of the synthesized nanoparticles. The mean particle size obtained from each formulation was recorded and compared (Table 1). The formulation exhibiting the smallest mean particle size with a narrow size distribution and satisfactory stability was considered the optimized formulation and selected for subsequent characterization and biological evaluation. [17,18]

The optimization study enabled the identification of the most suitable AgNO_3 concentration for the synthesis of stable and uniformly distributed Manjistha-mediated silver nanoparticles, thereby ensuring reproducibility and enhanced physicochemical properties for further pharmaceutical and dermatological investigations. [19,20,21]

Table 1. Batch formulation for green synthesis of Manjistha-silver nanoparticles.

Batch No.	AgNO_3 Concentrate	Concentration of Manjistha Extract
1	1 mM	1mg/ml
2	2 mM	1mg/ml
3	3 mM	1mg/ml
4	4 mM	1mg/ml
5	5 mM	1mg/ml

2.4. UV-Visible Spectroscopic Analysis

The synthesized silver nanoparticles (AgNPs) were characterized using UV-Visible spectroscopy to confirm their formation and optical properties. The absorption spectra were recorded over a wavelength range of 300–700 nm. Successful synthesis of silver nanoparticles was confirmed by the appearance of a characteristic surface plasmon resonance (SPR) absorption peak in the range of approximately 420–450 nm, which is indicative of the formation of stable silver nanoparticles. [18,19,20].

2.5. Antioxidant Activity

The antioxidant activity of the *Rubia cordifolia* (Manjistha) ethanolic extract and the synthesized silver nanoparticles was

evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay. [22,23] Samples at different concentrations were prepared and mixed with 0.1 mM DPPH solution, followed by incubation in the dark for 30 minutes at room temperature. After incubation, the absorbance of each reaction mixture was measured at 517 nm using a UV-Visible spectrophotometer. Ascorbic acid was employed as the reference standard for comparison. The antioxidant activity was expressed as percentage free radical inhibition, and the half-maximal inhibitory concentration (IC_{50}) values were calculated to assess the free radical scavenging potential of the extract and synthesized nanoparticles. [24].

2.6. Antibacterial Activity

The antibacterial activity of the *Rubia cordifolia* (Manjistha) ethanolic extract and synthesized silver nanoparticles was evaluated using the agar well-diffusion method against *Escherichia coli*. Sterile nutrient agar plates were inoculated with standardized bacterial suspensions, and wells were aseptically prepared and loaded with the respective test samples. Ciprofloxacin was used as the positive control. The inoculated plates were incubated at 37°C for 18–24 hours, after which the antibacterial activity was assessed by measuring the diameter of the zones of inhibition surrounding each well. The extent of inhibition was used as an indicator of the antibacterial efficacy of the test samples. [25].

2.10. Fungal Inhibition Effects

The antifungal activity of the *Rubia cordifolia* (Manjistha) ethanolic extract was evaluated against *Candida albicans* using the agar well-diffusion method on Potato Dextrose Agar (PDA) plates. A standardized inoculum of *C. albicans* was uniformly spread onto the surface of sterile PDA plates. Wells were aseptically prepared in the agar medium, and different volumes of the extract (25 μL , 50 μL , and 100 μL) were introduced into the respective wells. The plates were allowed to stand at room temperature for approximately 1 hour to facilitate diffusion of the extract into the agar medium. [26,27]

Subsequently, the plates were incubated at 30°C for 72 hours to promote fungal growth. Following incubation, the antifungal activity was assessed by measuring the diameter of the inhibition zones surrounding the wells. The size of the inhibition zone was considered an indicator of the antifungal efficacy of the extract. Standard antifungal drugs, Itraconazole (30 μg) and fluconazole (25 μg), were used as positive controls for comparative evaluation. The antifungal activity exhibited by the extract was compared with that of the standard drugs to determine its effectiveness against *Candida albicans*. [28,29,30]

3. Results

3.1. *In Silico* Molecular Docking

The molecular docking analysis demonstrated significant antimicrobial potential of the selected anthraquinone phytoconstituents against both fungal and bacterial target proteins. For the *Candida albicans* target protein (Table 2), munjistin exhibited the highest binding affinity with a docking score of -8.4 kcal/mol, followed by alizarin (-7.6 kcal/mol), purpurin (-7.5 kcal/mol), and rubiadin (-7.3 kcal/mol). Notably, all tested anthraquinone compounds showed stronger binding affinities than the reference antifungal drug fluconazole, which exhibited a docking score of -6.7 kcal/mol.

Similarly, docking studies against the bacterial target protein (Table 3) revealed that rubiadin possessed the strongest binding affinity, with a docking score of -8.8 kcal/mol, followed by munjistin (-8.6 kcal/mol), alizarin (-8.5 kcal/mol), and purpurin. All investigated anthraquinone compounds demonstrated higher binding affinities than the standard antibacterial drug ciprofloxacin, which exhibited a docking score of -7.2 kcal/mol.

Overall, the selected anthraquinone phytoconstituents exhibited stronger predicted interactions with both fungal and bacterial target proteins compared to the respective standard antimicrobial agents. These findings suggest that the anthraquinone compounds present in *Rubia cordifolia* may possess considerable antimicrobial potential and could serve as promising candidates for the development of novel antimicrobial therapeutics.

Table 2. *In silico* molecular docking analysis of selected Manjistha phytoconstituents against the antifungal target protein of *Candida albicans* (PDB: 2WV2), showing binding affinity together with the corresponding 3D and 2D ligand–protein interaction profiles.

Table 3. *In silico* molecular docking analysis of selected Manjistha phytoconstituents against the bacterial target protein (PDB: 8Q51), showing binding affinity values together with the corresponding 3D and 2D ligand–protein interaction profiles.

3.2. First Look at The Phytochemicals in *Rubia cordifolia*

Preliminary phytochemical screening of the ethanolic extract of *Rubia cordifolia* (Manjistha) revealed the presence of several bioactive secondary metabolites, including glycosides, alkaloids, proteins, coumarins, flavonoids, steroids, tannins, phenolic compounds, and terpenoids. However, carbohydrates and amino acids were not detected in the extract. The abundance of phenolic compounds and flavonoids suggests a strong reducing and stabilizing potential, which may facilitate the green synthesis of silver nanoparticles by acting as natural reducing and capping agents. Furthermore, the presence of anthraquinone glycosides, characteristic constituents of *R. cordifolia*, is of particular significance due to their well-documented antioxidant, anti-inflammatory, and antimicrobial activities. These phytoconstituents may contribute substantially to the biological properties exhibited by the extract and the synthesized nanoparticles.

Phytochemical	Result
Carbohydrates	–
Amino acids	–
Glycosides	+
Alkaloids	+
Proteins	+
Coumarins	+
Flavonoids	+
Steroids	+
Tannins and Phenolics	+

Table 4. Preliminary phytochemical screening of Manjistha ethanolic extract.

3.3. What The FTIR Spectroscopic Examination Showed

FTIR analysis of the *Rubia cordifolia* (Manjistha) ethanolic extract revealed several characteristic absorption peaks corresponding to hydroxyl ($-\text{OH}$), carbonyl ($\text{C}=\text{O}$), aromatic ($\text{C}=\text{C}$), and aliphatic ($\text{C}-\text{H}$) functional groups. The presence of these functional groups indicates the occurrence of bioactive phytoconstituents such as polyphenols, flavonoids, tannins, and anthraquinones within the extract. These compounds are known to play a crucial role in the green synthesis of silver nanoparticles by acting as reducing agents that facilitate the conversion of silver ions (Ag^+) into silver nanoparticles (AgNPs). In addition, they serve as natural capping and stabilizing agents, thereby enhancing the stability and preventing the aggregation of the synthesized nanoparticle.

3.2. Green Synthesis of Silver Nanoparticles

The successful synthesis of silver nanoparticles was initially confirmed by the gradual color change of the reaction mixture from pale yellow to dark brown during the course of the reaction (Figure 4). This characteristic color transformation is attributed to the surface plasmon resonance (SPR) phenomenon of silver nanoparticles and indicates the reduction of silver ions (Ag^+) to metallic silver (Ag^0) by the bioactive phytochemicals present in the *Rubia cordifolia* (Manjistha) extract.

Optimization of the synthesis parameters (Table 5) revealed that a silver nitrate concentration of 2 mM, reaction temperature of 45°C , stirring speed of approximately 830–834 rpm, and reaction duration of 105–107 minutes constituted the optimal conditions for nanoparticle synthesis. Under these conditions, the synthesized silver nanoparticles exhibited the smallest mean particle size of 171 nm, indicating efficient nanoparticle formation and enhanced colloidal stability.

Figure 4. Time-dependent colour development of the Manjistha-silver nanoparticle reaction mixture, reflecting progressive reduction of Ag^+ to Ag^0 by the extract phytoconstituents. (a) After 1 h, showing the initial pale-yellow reaction mixture; (b) After 6 h, showing an intermediate reddish-brown colour; (c) After 24 h, showing the fully developed dark reddish-brown colloidal suspension characteristic of mature silver nanoparticle formation.

Table 5. Optimization of AgNO_3 concentration for green synthesis of Manjistha-silver nanoparticles.

Batch No.	AgNO_3 Concentrate	Mean Particle Size (nm)
1	1 mM	382
2	2 mM	171
3	3 mM	175
4	4 mM	233
5	5 mM	245

3.3. UV-Visible Spectroscopic Analysis

The UV-Visible absorption spectrum of the synthesized silver nanoparticles (Figure 5) displayed a characteristic surface plasmon resonance (SPR) absorption band in the range of 420–450 nm, confirming the successful formation of silver nanoparticles. The presence of this distinct SPR peak is a well-

established indicator of silver nanoparticle synthesis. Furthermore, the sharp and well-defined nature of the absorption band suggested the formation of relatively stable nanoparticles with a uniform size distribution and good dispersion within the colloidal system.

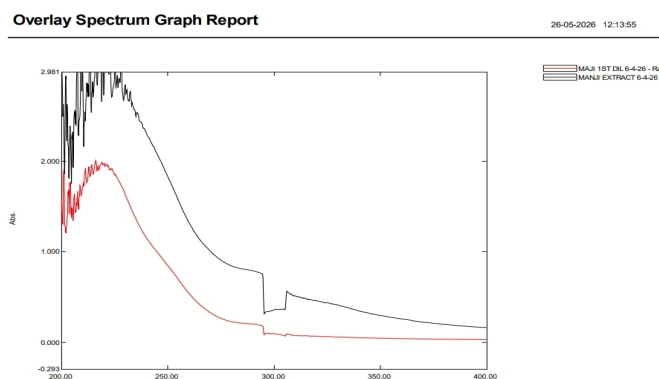


Figure 5. UV–Visible absorption spectrum of the Manjistha-silver nanoparticle colloidal suspension, showing the characteristic surface plasmon resonance (SPR) band that confirms reduction of Ag^+ ions by the phytoconstituents present in the Manjistha extract.

3.4. SEM Analysis

Scanning Electron Microscopy (SEM) analysis confirmed the successful synthesis of silver nanoparticles (Figure 8). The SEM micrographs revealed predominantly spherical nanoparticles with particle sizes ranging from approximately 74 to 102 nm. The nanoparticles exhibited a relatively uniform size distribution with minimal aggregation, indicating effective stabilization during the synthesis process.

Furthermore, Energy-Dispersive X-ray (EDX) analysis of the selected regions confirmed the presence of elemental silver within the synthesized nanoparticles. The EDX spectra also indicated the presence of organic constituents associated with the phytochemical capping layer surrounding the nanoparticles. These findings validate

Figure 9a EXD Spectrum Showing Elemental composition
Figure No. 9b EXD Spectrum Showing Elemental composition

Figure 9. FE-SEM micrograph showing the size and surface morphology of the green-synthesized Manjistha-silver nanoparticles, illustrating predominantly spherical particles of approximately 74–102 nm with minimal agglomeration.

3.5. Antioxidant Activity

The DPPH free radical scavenging assay revealed a concentration-dependent increase in antioxidant activity for both the *Rubia cordifolia* (Manjistha) ethanolic extract and the synthesized silver nanoparticles (Table 5). However, the nanoparticle formulation consistently exhibited greater free radical scavenging activity than the crude extract across all tested concentrations. At a concentration of 100 $\mu\text{g/mL}$, the synthesized nanoparticles achieved 82.49% inhibition, whereas the crude extract exhibited 70.04% inhibition. The enhanced antioxidant activity of the nanoparticle formulation may be attributed to its increased surface area, improved bioavailability, and effective interaction with free radicals at the nanoscale. These findings indicate that nanoparticle synthesis significantly improved the antioxidant potential of the Manjistha extract.

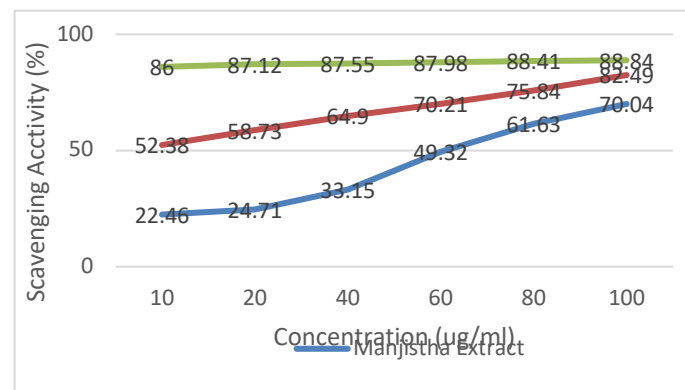


Table 6. DPPH free-radical-scavenging activity of Manjistha extract and Manjistha-silver nanoparticles.

Concentration ($\mu\text{g/mL}$)	Extract (%)	AgNPs (%)	Ascorbic Acid (%)
10	22.46	52.38	86.00
20	24.71	58.73	87.12
40	33.15	64.90	87.55
60	49.32	70.21	87.98
80	61.63	75.84	88.41
100	70.04	82.49	88.84

3.6. Antibacterial Activity

The antibacterial activity of the *Rubia cordifolia* (Manjistha) formulations against *Escherichia coli* is presented in (Table 7). The synthesized silver nanoparticle formulation exhibited superior antibacterial activity compared to the crude ethanolic extract at all tested concentrations. At a concentration of 100 $\mu\text{g/mL}$, the nanoparticle formulation produced a zone of inhibition of 20 mm, whereas the crude extract exhibited a zone of inhibition of 16 mm. The antibacterial efficacy of the nanoparticle formulation approached that of the standard antibiotic ciprofloxacin, which produced a zone of inhibition of 27 mm. Representative agar plates showing the zones of inhibition are presented in (Figure 7). These results suggest that the incorporation of silver nanoparticles significantly enhanced the antibacterial activity of the Manjistha extract, likely due to improved bioavailability, increased surface area, and the intrinsic antimicrobial properties of silver nanoparticles.

Table 7. Antibacterial activity against *E. coli* at a test concentration of 100 $\mu\text{g/mL}$.

Sample	Zone of Inhibition (mm)
Ciprofloxacin (standard)	27
Manjistha extract	16
Manjistha-silver nanoparticles	20

Figure 7. Zone-of-inhibition plates showing antibacterial activity against *E. coli*. (a) Standard drug (ciprofloxacin) and crude Manjistha extract; (b) Manjistha-silver nanoparticle

3.7. Antifungal Activity

The antifungal activity of the synthesized silver nanoparticle formulation against *Candida albicans* was significantly greater than that of the crude *Rubia cordifolia* (Manjistha) extract, as presented in Table 8 and Figure 8. At a concentration of 100 $\mu\text{g/mL}$, the nanoparticle formulation produced a zone of inhibition of 24 mm, indicating enhanced antifungal efficacy compared to the crude extract. When compared with the standard antifungal drug fluconazole, which exhibited a zone of inhibition of 31 mm, the nanoparticle formulation demonstrated substantial antifungal activity.

The improved antifungal performance of the nanoparticle formulation may be attributed to the increased surface area, enhanced cellular interaction, and synergistic effects between

the bioactive phytoconstituents of Manjistha and the antimicrobial properties of silver nanoparticles. These findings suggest that nanoparticle-based delivery significantly enhances the antifungal potential of Manjistha and may represent a promising strategy for combating fungal infections, particularly in the context of the growing prevalence of azole-resistant *Candida* species.

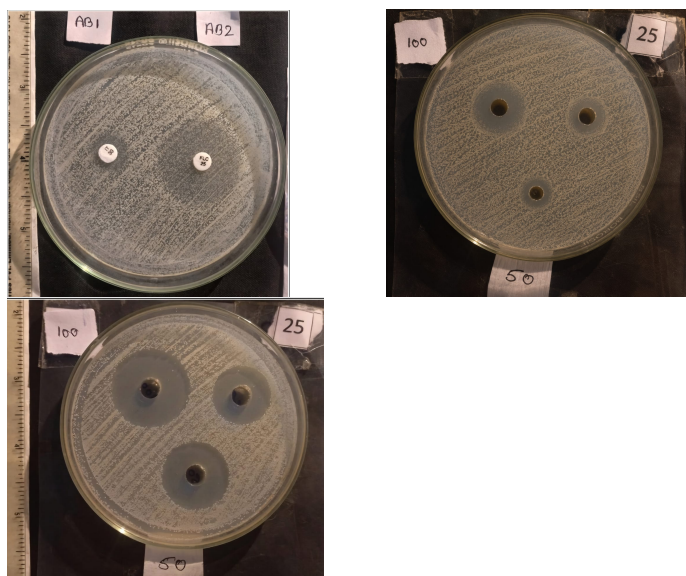


Figure 8. Zone-of-inhibition plates showing antifungal activity against *Candida albicans*.

(a) Standard antifungal drugs (Itraconazole and fluconazole);
(b) Manjistha ethanolic extract

Table 8. Antifungal activity (zone of inhibition) against *Candida albicans*.

Formulation	Concentration	Zone of Inhibition (mm)
Itraconazole (standard)	30 mcg	14
Fluconazole (standard)	25 mcg	31
Manjistha extract	25 µg/mL	2
Manjistha extract	60 µg/mL	5
Manjistha extract	100 µg/mL	8
Manjistha-silver nanoparticles	25 µg/mL	18
Manjistha-silver nanoparticles	50 µg/mL	21
Manjistha-silver nanoparticles	100 µg/mL	24

3.8 Discussion

The findings of the present study demonstrate that *Rubia cordifolia* (Manjistha) extract can be effectively utilized for the green synthesis of silver nanoparticles through an environmentally friendly and sustainable approach. Phytochemical screening and FTIR analysis confirmed the presence of bioactive constituents, including phenolic compounds, flavonoids, tannins, and anthraquinones, which play a crucial role in the reduction and stabilization of silver nanoparticles during the synthesis process.

The successful formation of silver nanoparticles was confirmed through UV–Visible spectroscopy, SEM, and EDX analyses, which demonstrated the characteristic physicochemical properties, morphology, and elemental composition of the synthesized nanoparticles. The nanoparticles exhibited enhanced biological activity compared to the crude extract, including superior antioxidant, antibacterial, and antifungal effects. Notably, the synthesized nanoparticles showed significant inhibitory activity against *Escherichia coli* and

Candida albicans, approaching the efficacy of standard antimicrobial agents.

Furthermore, molecular docking studies revealed strong binding affinities of the major anthraquinone constituents toward selected microbial target proteins, supporting their potential role in the observed antimicrobial activity. Collectively, these findings suggest that Manjistha-mediated silver nanoparticles represent a promising natural Nano therapeutic system with potential applications in the development of novel antimicrobial, antioxidant, anti-inflammatory, and dermatological formulations. The study highlights the potential of integrating traditional medicinal plants with nanotechnology to develop effective and eco-friendly therapeutic agents.

4. Conclusions

The present study successfully demonstrated the green synthesis, characterization, and biological evaluation of *Rubia cordifolia* (Manjistha)-mediated silver nanoparticles with potential applications in dermatological therapeutics. Preliminary phytochemical screening confirmed the presence of several bioactive constituents, including glycosides, flavonoids, tannins, phenolic compounds, alkaloids, steroids, terpenoids, and coumarins. FTIR analysis further verified the presence of functional groups associated with anthraquinones, polyphenols, and other phytoconstituents that are responsible for the reduction, stabilization, and capping of silver nanoparticles during the synthesis process.

Green synthesis of silver nanoparticles was successfully achieved using the Manjistha extract as both a reducing and stabilizing agent under optimized reaction conditions, namely a temperature of 45°C, stirring speed of approximately 830–834 rpm, reaction time of 105–107 minutes, and a silver nitrate concentration of 2 mM, selected through a five-point optimization study. The optimized formulation yielded predominantly spherical nanoparticles with particle sizes ranging from 74 to 102 nm.

The synthesized nanoparticles exhibited significantly enhanced antioxidant activity compared with the crude Manjistha extract, indicating improved free radical scavenging potential following nanoscale formulation. In addition, the nanoparticle formulations demonstrated superior antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*, with the optimized formulation exhibiting efficacy approaching that of the standard antibiotic control. Antifungal evaluation against *Candida albicans* revealed markedly enhanced activity of the nanoparticle formulation relative to the crude extract, suggesting a synergistic interaction between the bioactive phytoconstituents of Manjistha and the antimicrobial properties of silver nanoparticles.

Overall, the findings of this study highlight the successful integration of traditional herbal medicine with green nanotechnology as an environmentally sustainable and effective approach for enhancing the therapeutic potential of Manjistha. While further investigations, including zeta potential analysis, long-term stability studies, cytotoxicity assessment, and in vitro skin permeation studies, are required to establish clinical applicability, the present work provides a strong scientific foundation for the development of Manjistha-based silver nanoparticle formulations as promising candidates for the topical management of bacterial and fungal skin infections.

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