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Green Synthesis of Silver Nanoparticles Using *Rhazya stricta* Decne Extracts and Their Anti-Microbial and Anti-Oxidant Activities

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Abstract: The present study shows the synthesis of silver nanoparticles (Ag NPs) using a methanolic and aqueous extract of *R. stricta*. UV–visible spectroscopy, energy-dispersive X-ray diffraction (EDX), field emission scanning electron microscopy (FESEM), and Fourier transform infrared spectroscopy (FTIR) techniques were used to further characterize the Ag NPs. UV–visible spectra give surface Plasmon resonance (SPR) at 490–560 nm for Ag NPs. The existence of various functional groups existing in biomolecules capping the nanoparticles is indicated by the FTIR spectrum. The average size of Ag NPs is 20–35 nm, while the shape is spherical, as confirmed by FESEM. The plant extract and Ag NPs were evaluated against their antioxidant, antibacterial (*Staphylococcus aureus*, *E. coli*, and *Salmonella typhi*), and antifungal activities (*Trichophyton longifusis*, *Candida albican*, and *Fusarium solani*), where the Ag NPs exhibited superior activity versus the plant extract. The inhibitory effect of NPs against the tested strain was more effective as compared to the crude extract of *R. stricta*.

Keywords: *Rhazya stricta*; phytochemical screening; silver nanoparticles; anti-fungal; antioxidant; anti-bacterial activities

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1. Introduction

Throughout the world, nanotechnology research and development are growing rapidly [1–4]. Nanotechnology is a field of science and engineering that involves the manipulation of matter on a nanoscale. This is typically done by engineering and manipulating matter at the atomic and molecular levels, typically on a nanometer scale. Nanotechnology has applications in a wide range of fields, including medicine, energy, materials, and computing. It is used to develop new materials and products such as sensors, drug delivery, and medical implants. It is also used to improve existing products, such as improving the efficiency of solar cells and batteries. Nanotechnology has grown in importance in the fields of medicine and health over the last few decades [5,6]. Noteworthy interest has been in the research of NPs during the recent decade, particularly for biomedical applications [7–9]. Nanoparticles (NPs) are particles in the nanometer range (i.e., 1–100 nm), and they have unique physical, chemical, and biological properties that set them apart from larger particles. Nanoparticles have been used for medical applications, such as drug delivery systems, diagnosis, cancer therapy, and tissue engineering. Nanoparticles have unique properties that allow them to interact with biological systems in a different way than traditional drug molecules. For example, NPs can act as drug carriers, targeting specific cells and tissues for the delivery of therapeutic agents such as drugs, genes, or proteins. Additionally, NPs can be used as imaging agents for diagnosis, enabling doctors to detect diseases such as cancer at

an early stage. Nanoparticles can also be used for cancer therapy, as they can selectively target and destroy cancer cells. Furthermore, NPs are being used in tissue engineering, where they can be used to create three-dimensional scaffolds for the growth of new tissues and organs. The field of medical sciences has entered a new era with the integration of nanotechnology. As a result, there is the potential to provide novel methods for the handling of various diseases that were formerly challenging to mark due to their size limits. For many research groups, the production of bifunctional NPs is crucial for biomedical applications [10]. The conventional methods used to synthesize nanoparticles have significant drawbacks, including the use of hazardous chemicals and toxic solvents, which pose a threat to both the environment and human health. Green synthesis of nanoparticles has emerged as an environmentally friendly and sustainable alternative. Green synthesis is a novel approach to synthesizing nanoparticles using natural materials such as plants, fruits, and microorganisms as reducing agents and stabilizing agents. This approach not only reduces the environmental impact of nanoparticle production but also results in the production of biocompatible and non-toxic nanoparticles [11–14]. The use of natural materials in green synthesis has several advantages over traditional synthetic methods. For example, natural materials are often less toxic and more environmentally friendly, making the process safer for both the environment and human health. Additionally, using natural materials can also produce nanoparticles with unique properties, such as improved biocompatibility and low toxicity, making them useful for various biomedical applications [15–19].

There are various methods for the green synthesis of nanoparticles, including green synthesis, phytochemical synthesis, and microbial synthesis. These methods involve the use of microorganisms, such as bacteria and fungi, as reducing agents to synthesize nanoparticles. This method is attractive because microorganisms can synthesize nanoparticles in a controlled and reproducible manner, making it possible to produce large quantities of nanoparticles with consistent quality. Similarly, plant extracts such as leaves, stems, and flowers can be used as reducing agents and stabilizing agents. This method is attractive because plants are widely available and can be easily harvested, making the synthesis process both economical and environmentally friendly. The use of plant extracts in green synthesis has been shown to result in the production of nanoparticles with improved biocompatibility and low toxicity, making them useful for various biomedical applications. Finally, microbial synthesis involves the use of microorganisms as both reducing agents and stabilizing agents in the synthesis process. This method is attractive because microorganisms can synthesize nanoparticles with improved biocompatibility and low toxicity, making them useful for various biomedical applications. Additionally, microbial synthesis is often less expensive and more environmentally friendly than traditional synthetic methods, making it a promising alternative for the production of nanoparticles [20,21]. In conclusion, green synthesis of nanoparticles is a novel and environmentally friendly approach to synthesizing nanoparticles. By using natural materials such as plants, fruits, and microorganisms as reducing agents and stabilizing agents, this method reduces the environmental impact of nanoparticle production and results in the production of biocompatible and non-toxic nanoparticles. With the increasing demand for sustainable and environmentally friendly products, green synthesis of nanoparticles is expected to play a crucial role in the development of new and innovative materials and technologies [22–28].

The genus *Rhazya* (family Apocynaceae) consists of two species, namely *R. stricta* Decaisne and *R. orientalis* [29]. The genus *Rhazya* belongs to the order Gentianales, family Apocynaeae, and subfamily Rauwolfoideae [30–32]. The *Rhazya* species was coined after the name of the renowned Muslim scientist Abu Bakar Muhammad bin Zakariya Ar-Raze. The species of this plant in European countries is still Latinized as Rhazes [33]. *Rhazya stricta* is an evergreen, small, glabrous, erect, poisonous shrub that is mostly found in the Middle East and the Indian subcontinent. It has been used as a folk medicine and for a variety of treatments, including fever and chronic rheumatism [34]. This plant is very important in health care and is used in many herbal drugs for the treatment of various diseases in a variety of countries, including Iran, Afghanistan, the UAE, Iraq, Saudi Arabia, Pakistan, India, and Qatar [32]. Different portions of the *R. stricta* plant are used in medicines for

curing different health problems like gastric pain, burning of the skin, diabetes mellitus, skin diseases, cancer, inflammation, throat diseases, and rheumatism [35–37]. The leaves of *R. stricta* are used in folk medicines for the treatment of body aches, and fresh leaves are kept in shoes for underfoot burning, while branches are used for curing tooth pain [38].

The crude extracts of the plant have bioactive phytochemicals, which are significant in healthcare medicines. The secondary metabolites produced by plants are considered to be responsible for the antimicrobial activity of plants as well as for the reduction and stabilization of metallic nanoparticles [39,40]. The bioactive secondary metabolites have been proven by phytochemical screening [41]. The present study is about screening the phytoconstituents of *R. stricta*, developing silver nanoparticles, and exploring the antimicrobial activities of crude extract and Ag NPs.

The synthesis of silver nanoparticles (AgNPs) using plant extracts of *R. stricta* offers several advantages over other biological methods. One advantage is cost-effectiveness, as plant extracts eliminate the need for expensive synthetic reagents. This makes the synthesis of AgNPs using plant extracts a cost-effective alternative to other biological methods. Another advantage is scalability, as the plant extract can be easily scaled up for large-scale production of AgNPs. In addition, the use of plant extracts for AgNP synthesis is environmentally friendly, as it minimizes the environmental impact associated with synthetic methods by avoiding the use of toxic chemicals and solvents. Furthermore, plant extracts contain phytochemicals that serve as reducing and stabilizing agents for the formation of AgNPs, resulting in the formation of stable and uniform AgNPs with improved biological properties. In contrast, other biological methods often require the use of toxic chemicals and solvents, which can have negative environmental impacts. Overall, the synthesis of AgNPs using plant extracts is cost-effective, making it a valuable subject for further research in the field of nanotechnology and phytomedicine.

The study on the green synthesis of silver nanoparticles using *Rhazya stricta* Decne extracts and their anti-microbial and anti-oxidant activities are an original and important contribution to the field of nanotechnology. The use of plant extracts as a reducing and stabilizing agent for the synthesis of silver nanoparticles is a novel approach and adds to the growing body of research on green synthesis methods. The evaluation of the anti-microbial and anti-oxidant properties of the synthesized nanoparticles highlights their potential applications in various fields. The anti-microbial and anti-oxidant activities of the synthesized silver nanoparticles are of clinical significance, as they can potentially be used in the development of new antimicrobial agents to combat drug-resistant bacterial infections and in the treatment of oxidative-stress-related diseases. More research is needed to determine the safety and efficacy of using silver nanoparticles in a clinical setting. Further studies are needed to evaluate the toxicity of silver nanoparticles and to determine the optimal methods for their use in the medical field. This study not only contributes to the advancement of nanotechnology but also highlights the potential of plant extracts as sustainable and eco-friendly alternatives to the conventional chemical.

2. Experimental

2.1. Collection of Plant Materials and Preparation of Aqueous and Methanolic Extract

The fresh plant of *R. stricta* was collected from district Mohmand, Khyber Pakhtunkhwa, Pakistan. The *R. stricta* plant was shade-dried for fifteen days to evaporate the water contents. Then, the plant was ground by the local grinder. The ground plant was soaked in distilled water as well as in methanol by dipping 400 g of ground plant material in water and methanol separately for one week. The plant was filtered three times from distilled water and methanol. The final filtered product of methanol was concentrated by a Rotary evaporator, while the aqueous sample was heated at 70 °C in a water bath.

2.2. Phytochemical Screening

Phytochemical screening is an essential step in the study of medicinal plants, as it helps to identify the presence or absence of secondary metabolites. Secondary metabolites

are chemical compounds produced by plants, which are not involved in primary metabolic pathways such as growth and development but have various biological assays such as microbicides, anti-inflammatory, and radical scavenging activities. In this study, the phytochemical screening was performed on the plant extract obtained using water and methanol as solvents. The use of different solvents helps to extract a different range of secondary metabolites. Methanol is known to extract polar and semi-polar compounds, while water is used to extract hydrophilic compounds. The screening was carried out using specific reagents that react with different secondary metabolites, providing a characteristic color change [42].

2.3. Synthesis of Ag NPs

The synthesis of Ag NPs was performed in three different sets, each with 1.0, 0.7, and 0.5 mM $AgNO_3$ in a 1% plant extract solution in different proportions, with continuous stirring at 70 °C for 15 min. The formation of the Ag NPs was observed by a change in the color of the reaction mixture and by scanning the UV–vis spectra of it from 250 to 750 nm using a UV–vis spectrophotometer. After analyzing the UV data, we selected a 0.7 mM concentration for further studies.

2.4. Instrumentation

The characterization of the Ag NPs was performed using a combination of techniques to gain a comprehensive understanding of their morphological features, elemental composition, and chemical interactions. The FESEM (Field Emission Scanning Electron Microscopy) instrument (JEM 2100, a Jeol CRL) was utilized to visualize the morphology of the Ag NPs and the EDS (Energy-Dispersive X-ray Spectroscopy) instrument (ADX-8000 MINI) was used to perform elemental analysis.

To identify the functional groups present in the extract and their interaction with silver ions during green synthesis, the ATR FTIR spectrophotometer (Attenuated Total Reflectance Fourier Transform Infrared Spectrophotometer) (Shimadzu FTIR—8400-S) was employed. The formation of the Ag NPs was studied over time through time-dependent UV–vis spectroscopy (300 Plus Optima Japan), which allowed for the measurement of changes in the optical properties of the NPs as a result of the synthesis process.

These instruments provided crucial information on the structural, compositional, and chemical properties of the Ag NPs, which was essential in understanding the green synthesis process and the formation of the NPs.

2.5. Biological Assays

The minimum inhibitory concentration and minimum bactericidal concentration were used in determining the microbial activity. The bacteria 25923 and *E. coli* ATCC6633 were used in the microbial assay. The selected strains were obtained from the stock culture in the PNRL Lab, University of Peshawar, Pakistan. Microdilutions of the NPs were prepared. The bacterial culture was prepared using nutrient agar and incubated for 24 h at a temperature of 37 °C. Dilutions of NPs were also used in a specific area to determine the MIC of NPs. The NPs containing Petri dishes were also incubated at a temperature of 37 °C for 24 h. The MBC, i.e., minimum bactericidal concentration was determined by using the lowest dosage of Ag NPs and that the area of the zone of inhibition was measured. The process was repeated thrice to obtain accurate results. Similarly, the microdilution plate assay method was used to determine anti-fungal activity. The *Trichophyton longifusis*, *Candida albican*, and *Fusarium solani* strands were used in the assay. The 20 mM buffer solution of sodium phosphate was mixed with 20 mL of Ag NPs. Water dilutions of 5, 2.5, and 1.25 mg/mL of the sample were incubated at a temperature of 37 °C for 2 h. Similarly, the biosynthesized AgNPs and the methanolic extract were evaluated for their antioxidant activity by the DPPH method. The percent inhibition of activity was calculated as $[(Ao - Ae)/Ao] \times 100$ (Ao = absorbance without extract; Ae = absorbance with extract).

3. Results and Discussion

3.1. Phytochemical Analysis

The phytochemical screening was conducted using various standard tests to detect the presence of various phytoconstituents such as flavonoids, steroids, tannins, anthraquinones, saponins, phlorotannins, terpenoids, glycosides, coumarins, beta-cyanins, and anthocyanins.

The methanol extract of *R. stricta* showed positive results for the presence of tannins, flavonoids, saponins, glycosides, steroids, terpenoids, coumarins, and beta-cyanins. On the other hand, the aqueous extract confirmed the presence of phlorotannins, flavonoids, saponins, glycosides, steroids, and terpenoids. The results obtained are tabulated in Table 1.

Table 1. Phytochemical screenings (qualitative) of *R. stricta* plant.

Phytochemicals	Methanol Extract	Aqueous Extract
Phlobatanins	−	+
Tannins	+	−
Flavonoids	+	+
Saponins	+	+
Glycosides	+	+
Steroids	+	+
Terpenoids	+	+
Caumarine	+	−
Betacyanins	+	−
Anthocyanins	−	−

Positive (+) sign represents the presence of and negative (−) sign indicates the absence of selected metabolites.

The results of this study suggest that *R. stricta* is a rich source of various phytoconstituents including flavonoids, steroids, tannins, saponins, glycosides, terpenoids, coumarins, and beta-cyanins. These phytoconstituents have been reported to possess various pharmacological activities such as antioxidant, anti-inflammatory, and anti-cancer properties. Further studies are required to isolate and identify these phytoconstituents and to evaluate their biological activities.

Therefore, the phytochemical screening of *R. stricta* revealed a rich composition of various phytoconstituents with potential pharmacological activities. This study lays the foundation for further research aimed at exploring the biological properties of these phytoconstituents.

3.2. Characterizations of Ag NPs

3.2.1. UV–Visible Spectroscopy

Initially, the NPs were characterized by a UV–visible spectrophotometer in the wavelength ranging from 200 to 800 nm. The spectroscopic analysis for Ag NPs was carried out by using freshly prepared fractions at 37–38 °C and by using an optical path 1 cm length of quartz cuvettes using a spectrometer (300 Plus Optima Japan). The Ag NPs solution gave an absorption maximum of 420–450 nm. We recorded the UV spectra of the bio-synthesized Ag NPs in a time-dependent spectrophotometer. We observed that as time increases, the intensity of the peak related to the silver nanoparticles increases to 305 nm wavelength. The formation of silver NPs shows peaks from 300 nm to 700 nm by UV–visible spectrophotometer [43]. The UV spectra of the biosynthesized Ag NPs are provided in the inset of Figure 1a.

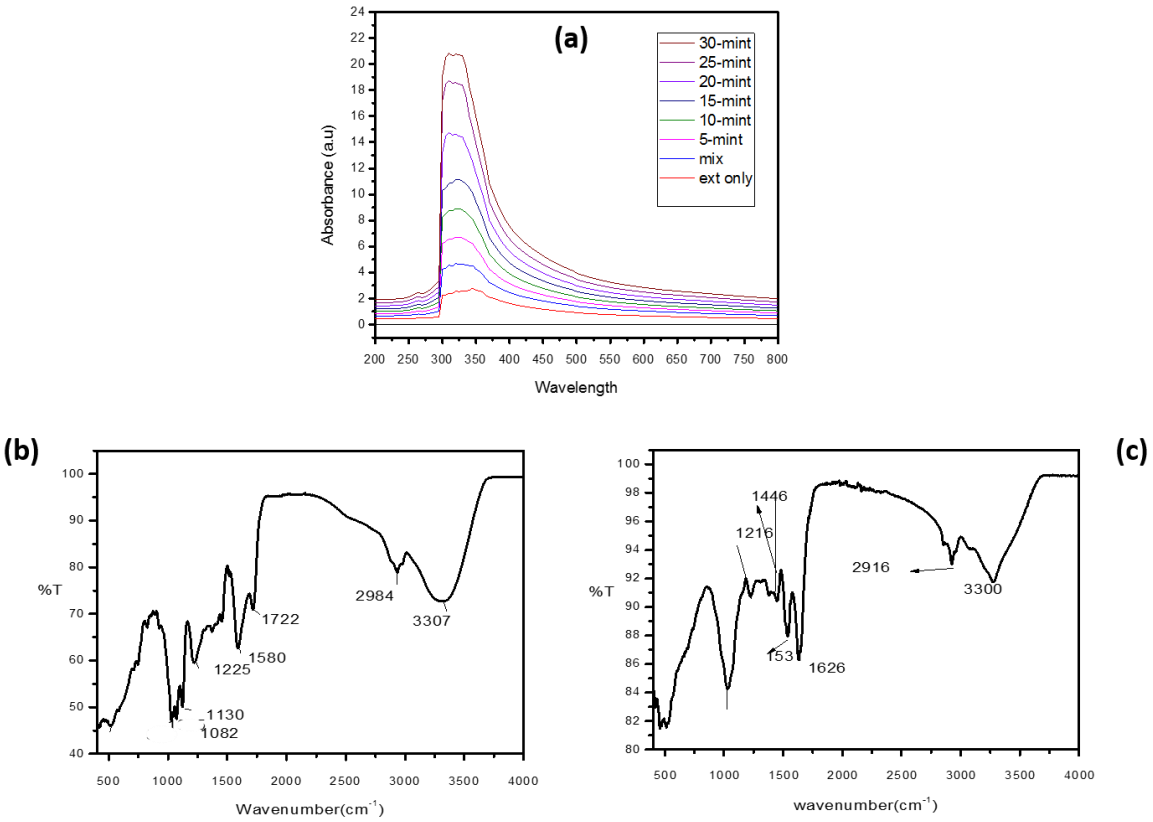

Figure 1. UV-vis spectra of Ag NPs (**a**), FTIR spectrum of plant ext. (**b**), and biosynthesized Ag NPs (**c**).

3.2.2. FTIR

The functional group involved in the formation of Ag NPs was evaluated using Shimadzu FTIR—8400-S, a Fourier transform spectrometer. The samples were prepared using the powdered sample. The powdered samples were placed in NaCl cells and were placed in pellet cells of KBr. The bands detected on the computer showed the results. The range of 4000–400 cm^{-1} was used. The FTIR spectrum of the plant extract shows characteristics peaks at 3307 cm^{-1} for the OH functional group, 2984 cm^{-1} for the carboxyl (COOH) functional group, and 1782 cm^{-1} for the carbonyl (C=O) functional group of saturated aldehyde. The peak at 1580 cm^{-1} is often associated with the amide I band, which can indicate the presence of proteins, amides, and/or peptides in the plant tissue.

The peak at 1225 cm^{-1} can be assigned to the stretching vibrations of the C-O-C (carbonyl) group in aldehydes, ketones, and/or carboxylic acids. The peak at 1130 cm^{-1} is typically seen in spectra and is related to the asymmetric stretching vibrations of C-O in esters, alcohols, and/or ethers. The peak at 1080 cm^{-1} is usually associated with the stretching vibrations of the C-O bonds in ethers, alcohols, and/or phenols [44,45]. Figure 1b.

In Figure 1c, there are prominent shifts in the functional group region of the FTIR spectrum from 3307, 2984, and 1782 cm^{-1} to 3300, 2916, and 1626 cm^{-1}. The shifts in the various functional groups of plant extract confirm that it acts as a reducing and capping agent in the synthesis of AgNPs. The results of the current study match with a previous study that evaluate that biological molecule act as capping and reducing agents in the synthesis of NPs [44].

3.2.3. EDX

For EDX analysis of AgNPs, the ADX-8000 MINI was used. Throughout the EDX analysis of biosynthesized AgNPs, the energy of the electron beam was kept at 15 keV. The EDX spectrum of AgNPs is given in the inset of Figure 2a. The EDX analysis revealed that

Ag was the most abundant element in the precipitate. The energy bands for the signals of Ag were in the range of 1.5, 1.75, 2.00, 9.00, and 11.5 keV. All results of the EDX analysis for the current study of Ag were found to be similar to the previous study evaluated for the AgNPs [46]. The EDX analysis also confirmed the presence of other elements including aluminum, carbon, and oxygen in the precipitate. The presence of aluminum is associated with silver salts as an oxide or hydroxide. The carbon and oxygen bands in the EDX spectrum are thought to be associated with carbon and oxygen atoms found in plant extract phytochemicals that capped the AgNPs [44].

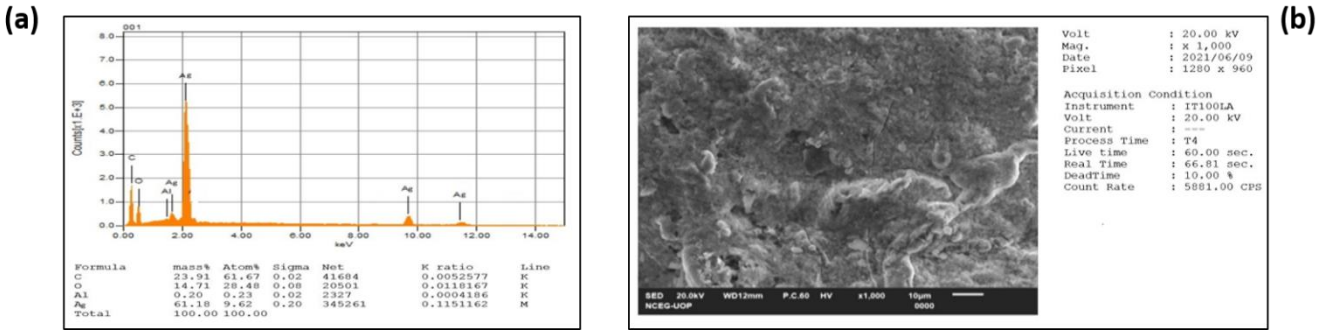

Figure 2. EDX spectrum (**a**) and FESEM image of the biosynthesized Ag NPs (**b**).

3.2.4. FESEM

The size and shape of NPs were confirmed by using the JEM 2100, a Jeol CRL field scanning electron microscope. For this purpose, a layer of AgNPs thin sediment was placed under a vacuum pressure of 5–8 Torr. From the FSEM analysis, it was concluded that the synthesized AgNPs were agglomerated and spherical in shape. From the FSEM analysis, it was concluded that AgNPs are effectively produced at an average size of 20–35 nm by this method. Figure 2b shows the FSEM analysis of synthesized AgNPs.

3.3. Biological Assays

3.3.1. Anti-Bacterial Activities

The ager-well diffusion process was applied to find the antibacterial profile of *R. stricta* plant extract and its AgNPs against the selected strains [47]. First, 1% DMSO was used as a negative control plate, as shown in Table 2. The antibacterial activity of *R. stricta* plant extract was studied. The zones of inhibition (in millimeters) against *S. aureus*, *E. coli*, and *S. typhi* were 9.8 ± 0.6 mm, 16 ± 0.45 mm, and 11.6 ± 0.3 mm, respectively. The zones of inhibition for the antibacterial activity of AgNPs against *S. aureus*, *E. coli*, and *S. typhi* were 20 ± 0.73 mm, 22 ± 0.37 mm, and 18 ± 0.5 mm, respectively. The plant extract has very mild antibacterial activity, while that of AgNPs showed good antibacterial activity compared to the standard control, cefixime. The greatest zone of inhibition against *E. coli* was 22 0.37 mm.

Table 2. Antibacterial activity of the synthesized NPs and plant extract.

Sample	Zone of Inhibition (n = 3)		
	S. aureus	***E. coli***	***S. typhi***
Cefixime	19 ± 0.5 mm	24 ± 0.76 mm	19 ± 0.3 mm
DMSO	NA	NA	NA
R. stricta plant extract	9.8 ± 0.6 mm	16± 0.45 mm	11.6 ± 0.3 mm
AgNPs	20 ± 0.73 mm	22 ± 0.37 mm	18 ± 0.5 mm

3.3.2. Anti-Fungal Activities

The antifungal potential of the biosynthesized AgNPs was investigated by the microdilution plate assay method [48]. The antifungal activity of *R. stricta* plant extract was studied. The zones of inhibition (in millimeters) against *Trichophyton longifusis*, *Candida albican*, and *Fusarium solani* were 21 ± 0.6, 20 ± 0.45, and 16 ± 0.3 mm, respectively. The zones of inhibition for antifungal activity of AgNPs against *Trichophyton longifusis*, *Candida albicans*, and *Fusarium solani* were 31 ± 0.23, 29 ± 0.37, and 26 ± 0.5 mm, respectively. The plant extract and AgNPs showed good antifungal activities compared to the standard control, amphotericin. AgNPs had the greatest zone of inhibition (31 ± 0.23 mm) against *Trichophyton longifusis*, as shown in Table 3.

Table 3. Antifungal activity of *R. stricta* plant extract and its silver nanoparticles (inhibition zone in mm).

Sample	Zone of Inhibition (*n* = 3)		
	Trichophyton longifusis	***Candida albican***	***Fusarium solani***
Amphotericin B	26 ± 0.5 mm	31 ± 0.76 mm	32 ± 0.3 mm
DMSO	NA	NA	NA
R. stricta plant extract	21 ± 0.6 mm	20 ± 0.45 mm	16 ± 0.3 mm
AgNPs	31 ± 0.23 mm	29 ± 0.37 mm	26 ± 0.5 mm

3.3.3. Anti-Oxidant Activities

The biosynthesized AgNPs and the methanolic extract were evaluated for their antioxidant activity by the DPPH method [49,50]. The percent inhibition of activity was calculated as [(Ao − Ae)/Ao] × 100 (Ao = absorbance without extract; Ae = absorbance with extract). According to our findings, both the raw extract and the biosynthesized AgNPs are capable of scavenging free radicals. The AgNPs, however, had more DPPH scavenging efficacy than the raw extract, which can be attributed to silver ions acting as powerful antioxidant agents. These ions are known to interact with free radicals, thus neutralizing them and preventing oxidative damage to biological molecules. On the other hand, the raw extract was found to contain a mixture of various phytochemicals such as polyphenols, flavonoids, and terpenoids, which have been shown to act as antioxidants and scavenge free radicals. The antioxidant activates of extract and Ag NPs has been tabulated in Table 4.

Table 4. Antioxidant activity of *R. stricta* plant extract and AgNPs.

S. No.	Sample Name	Solvent	Percent DPPH Scavenging Effect
1	*R. stricta* plant extract	Methanol	43.12% ± 2.1
2	AgNPs	Methanol	75.16% ± 0.04

In conclusion, these findings provide evidence that the biosynthesized AgNPs and the methanolic extract of the studied plant have significant antioxidant properties, making them potential candidates for the development of new natural, antioxidant-based products. Further research is needed to investigate the long-term effects and toxicity of these substances and their potential applications in the prevention of oxidative-stress-related diseases.

4. Conclusions

The current study demonstrates the potential of *Rhazya stricta* extract as a green method for the synthesis of silver nanoparticles (AgNPs). The biosynthesized AgNPs were characterized using UV–visible, FTIR, EDX, and FESEM analyses, confirming their synthesis and characteristics. Phytochemical examination revealed the presence of secondary metabolites that may act as reducing and capping agents for AgNP synthesis. Compared to the extract

alone, the plant-AgNPs exhibited enhanced antioxidant, antibacterial, and antifungal activities, suggesting their potential for various applications in the field of biomedicine and biotechnology. This study provides valuable insights into the green synthesis of AgNPs using *Rhazya stricta* extract and opens up new avenues for exploring the applications of green nanoparticles.

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