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Research Article

Biogenic Synthesis of Silver Nanoparticles Using *Rhazya stricta* Extracts and Evaluation of Its Biological Activities

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Rhazya stricta is a well-known medicinal plant and source of numerous potential secondary metabolites including steroids, alkaloids, and tannins. *R. stricta* possesses multimedical applications and used for curing of various diseases such as inflammation, diabetes, sore throat, infectious, helminthiasis, arthritis, and cancer. The current investigation deals with synthesizing AgNPs using aqueous and ethanol extracts of *R. stricta*. The synthesized *R. stricta*-AgNPs were characterized through UV-visible, Fourier transform infrared (FTIR), and atomic force microscopy (AFM) methods. The UV-visible analysis exhibited a characteristic absorption λ_{max} at 475 nm in *R. stricta* ethanol AgNPs while this peak was absent in *R. stricta* aqueous crude extract. The thermal stability of *R. stricta*-AgNPs demonstrated that by increasing the reduction time and temperature, the absorption of AgNPs also increased, leading to more stable NPs formation. The FTIR spectra showed a broad peak at 450-550 cm^{-1} that confirmed the occurrence of AgNPs of *R. stricta*. The AFM study of the synthesized AgNPs revealed the spherical shape and size ranging from 30 nm to 90 nm. In antioxidant and antibacterial study, the *R. stricta*-AgNPs exhibited good antioxidant activity (87.94% and 88.37%) than the ethanol crude extract (50.00% and 56.81%) at 100 μg/mL using DPPH assay. Maximum antibacterial activity was recorded against Gram-positive bacteria (*Staphylococcus aureus*), which was 15 and 0 mm, while against Gram-negative bacteria (*Klebsiella pneumonia)* was found to be 16 and 14 mm, respectively, whereas against *Bacillus subtills*, a poor activity was recorded as 14 for extract and 0 mm for AgNPs, respectively. In the acetic acid-induced writhing model, the percent effect of extract (100 mg/kg) and AgNPs (15 mg/kg) was 79.98 and 83.23, respectively. The maximum muscle coordination effect of extracts in the inclined plan and traction test was 44% and 38% at

higher doses. A mild sedative effect was also recorded against extract and AgNPs. The significant ($p < 0.05$) effect of extract was noted at 100 mg/kg while AgNPs was more significant ($p < 0.01$) at the tested dose of 15 mg/kg. These findings have concluded that *R. stricta*-AgNPs is an effective bioreductant of AgNPs synthesis and exhibit several applications in distinctive biomedical and pharmaceutical industries.

1. Introduction

Nanobiotechnology is a promising field of material science leading to the production and development of a variety of nanomaterials. The nanomaterials are being used as bactericidal [1], catalytic, biological labelling, sensor technology [2], electronic, optical devices [3], anticancer therapies, and various other health ailments [4–6]. There are typical routes used for the development of nanoparticles (NPs) such as physical and chemical protocols which are toxic, noneconomic, noneco-friendly, and expensive. Therefore, recently, new biosynthetic methods have been explored for the synthesis of NPs using microorganisms and plants [7]. The study of literature reported that the biosynthesis of NPs with medicinal plant extracts as a source needs to be more thoroughly studied. Most importantly, green synthesis of NPs is a new field of nanotechnology research because of its many advantages, such as how easy it is to work with and how cheap it is. Currently, the development of cost-effective synthetic protocols is the main area of concern for researchers for the production of stable, biocompatible, and reproducible NPs. The world's key threat to mankind is antibiotic resistance. Silver NPs (AgNPs) as nanomedicines in nanobiotechnology play a vital role against drug-resistant bacteria [8, 9]. Oxidative stress is a complexity arising from imbalance between defensive mechanism of antioxidants and generation of free radicals [10]. This noxious phenomenon is responsible for deterioration of protein, lipid, DNA, and other vital elements that forms our body [11]. Oxidative stress may lead to neurological disorders, inflammatory diseases, cardiovascular diseases, and complications in immune system [12–15]. An antioxidant is an agent which can neutralize a free radical by donating an electron and diminish its capability to damage or cause harm to the body [16]. Apart from donation of electron, antioxidant also acts by deactivating the catalyst that initiates the formation of free radicals [17]. Among several antioxidants, the superoxide dismutase acts by initiating disintegration of superoxide anion, forming hydrogen peroxide and oxygen [18, 19] while the glutathione system causes deactivation of hydrogen peroxide which involves more than one chemical cofactors [20]. Ascorbic acid is a potential antioxidant which contingent upon reaction with glutathione to exert its free radical diminishing activity [21]. Medicinal plants shown to have promising stand to be a potential source of antioxidant agent [22–24]. Despite the promising mechanism of antimicrobials to protect the host, the resistance to antimicrobial is becoming a concerning issue nowadays. Inappropriate prescribing and overuse contributes the most in antimicrobial resistance. This demands antibiotic with developed mechanism of action to fight against this modern era crisis [25].

Insomnia is a complication related to lack of sleep due to various factors [26, 27]. The prevalence of insomnia varies between 10% and 30% of the world populations [28–32]. Anxiety, depression, diabetes, and hypertension can be influenced by insomnia [31]. Sedative shows potential outcome in the treatment of insomnia and depression [33]. Data from a survey report showed that 126.1 million people involved in pain related complications whereas 25.3 million people suffered every day in USA [34]. Yearly global statistics revealed that among every 5-adult people, at least 1 is suffering from pain. On the other hand, 1 in every 10 people is suffering from chronic pain [35]. In the management and treatment of pain analgesic drugs play the vital role which are divided into nonopioid and opioid class [36]. AgNPs are significantly used as antimicrobial agent and were already known for a variety of applications in textiles, antimicrobial, water treatment, paint coatings, HIV prevention, and treatment as well as in medical devices [37]. The plant materials are selected for biosynthesis due to the presence of reducing agents like ascorbic acid and phenolic compounds that may play a vital role in metal NP synthesis [38]. *Rhazya stricta* (family: Apocynaceae) is a traditional medicinal plant commonly found all through Western Asia, India, Afghanistan, and different places of Pakistan [39]. The plant has dense erect branches with smooth stem and overall appears to be glabrous shrub. It is traditionally used as a remedy [38], for fever, rheumatic pain, diabetes, syphilis, sore throat, inflammatory conditions, helminthiasis, and for the treatment of many other diseases [40]. In United Arab Emirates, the plant decoction is used to treat several health ailments, including diabetes mellitus, fever, sore throat, inflammatory conditions, and helminthiasis [41]. The leaves of *R. stricta* are traditionally used as a purported bitter tonic [42, 43] and cure for syphilis [42], chronic rheumatism [42], and associated forms of discomfort [41]. Branches are used as toothbrushes to alleviate toothaches [44]. Compelling evidence suggests that different fraction of this plant is abundant with alkaloids [45–48], which are mostly responsible for its pharmacological effect [40].

The current study aims to synthesize AgNPs from ethanol extract of *R. stricta* and evaluate several biological activities including antioxidant, antimicrobial, analgesic, and muscle relaxation; hence, proposing a potential source of NPs to be a potential medicinal active agent.

2. Materials and Methods

2.1. Plant Collection. Fresh leaves and stem of *R. stricta* were collected from (Khairabad) District Nowshera, Khyber Pakhtunkhwa, Pakistan. The plant identification was carried out by Dr. Barkath Ullah, Department of Botany, University of Peshawar, Peshawar, Pakistan. The voucher specimen Number UOP/Bot609 was kept in the herbarium of mention department.

2.1.1. Extraction. Fresh leaves of *R. stricta* were taken and washed twice with distilled water to make them free from dust and dried in shade at room temperature. The dried leaves (100 g) were ground into powder and soaked in ethanol and (1000 mL) and distilled water, respectively, in separate flasks and kept for three days. The resultant materials were filtered. The aqueous and ethanol (EtOH) filtrates were then concentrated by using low pressure rotary evaporator at 40°C to obtain a paste and were stored at 4°C for further use.

2.1.2. Phytochemical Screening. Phytochemical evaluation plays a major part in the presence of novel compounds and revelation of medications [48]. The presence of secondary metabolites present in aqueous and ethanol extract was carried out by using standard protocols of phytochemical screening [49]. Phytochemical screening of *R. stricta* ethanol extracts demonstrated the presence of tannins, saponins, flavonoids, terpenoids, steroids, coumarins, and emodines which may act as a major source of reducing agents during the AgNP synthesis [50] (Table 1).

Table 1: Phytochemical screening of ethanol, aqueous, and hexane extracts of *R. stricta*.

Chemical constituents	Ethanol extract	Aqueous extract	n-hexane extract
Anthraquinones	—	—	—
Reducing sugars	—	—	—
Flavonoids	—	—	—
Alkaloids	+	+	—
Steroids	+	—	—
Coumarins	+	+	—
Carbohydrates	—	—	—
Tannins	+	—	—
Anthocyanins and betacyanins	—	—	—
Terpenoids	+	+	+
Glycosides	—	—	—
Cardiac glycosides	—		—
Monosaccharides	—		—
Phlobatanins	—		—
Emodines	—	+	—
Saponins	—	+	—

Here, (+): present, (-): absent.

2.2. Synthesis of AgNPs

2.2.1. Preparation of Stock Solution and $AgNO_3$ Salt Solution. Stock solution was prepared by dissolving 1 g of plant crude alcoholic extract in 100 mL distilled water. While 1 mM silver salt solution of silver nitrate ($AgNO_3$) was prepared (17 mg $AgNO_3$/100 mL of de-ionized H_2O) for the biosynthesis of metallic NPs of plant extracts [48].

2.2.2. Synthesis of Silver NPs of EtOH Extract. NPs were synthesized by following simple method [51]. Ethanol extract and salt solution were mixed together in various ratios, i.e., 1 : 1, 1 : 2, 1 : 3, and 1 : 4 by keeping the silver nitrate solution concentration constant. The reaction mixture was first stirred for 1 hour at 70°C, and then, stirring was continued for 4 hours at room temperature. The color of reaction mixture was continuously changing during the stirring, and at last, brown color was developed. The color change indicated the synthesis of NPs by the reduction process. After this, the solution was centrifuged at 5000 rpm for 15 minutes. The pellet obtained after discarding the supernatant was air dried in the incubator. Later on, the abovementioned ratios were then heated to high temperatures (80°C), and UV-visible spectra were recorded for every step to monitor the stability of the synthesized AgNPs. The same procedure was followed with aqueous plant extract of *R. stricta* but no well-defined AgNPs were synthesized.

2.3. Characterization of AgNPs. AgNPs of *R. stricta* were characterized by using UV-visible spectrometer (Hitachi-U-3200, Japan). Atomic force microscopic characterization was done from HEJ, Karachi, where the sample solution was spread on a plate then plate was dried and the image was recorded by AFM Agilent 5500 Japan in dynamic mode.

Fourier transform infrared (FTIR) spectral analysis of *R. stricta* extracts was carried out to find the probable biomolecules which are responsible for reduction as well as capping the bio reduced AgNPs. IR spectrums were recorded on FTIR (Nicolet 380, Thermo Scientific, Japan) using KBr pallet method.

2.4. Biological Activities of Synthesized AgNPs

2.4.1. Antioxidant Activity. Free radical scavenging activity of *R. stricta* plant extract and their synthesized EtOH-AgNPs were measured by 2,2-diphenyl-1-picrylhydrazyl (DPPH). DPPH solution of 0.1 mM concentration was prepared in ethanol [52]. This solution (1 mL) was added to 5 mL of ethanol crude extract at different concentration (10, 20, 40, 80, 100, 150, and 250 μg/mL) prepared by dilution method. The solutions were mixed by vigorous shaking and then allowed to stand for 30 minutes at room temperature in dark. Later on, by using spectrophotometer at 517 nm, the absorbance was measured. Ascorbic acid was used as a reference standard, and the experiment was repeated thrice. An increase in the antioxidant activity is the measure of the decrease of the DPPH solution absorbance. Antioxidant activity as percent radical scavenging activities (%RSA) by DPPH was calculated as follows.

$$\%\text{DPPH} = \left(\frac{\text{OD}_{\text{Control}} - \text{OD}_{\text{Sample}}}{\text{OD}_{\text{Control}}}\right) \times 100, \quad (1)$$

where OD is control and OD sample is the absorbance of samples [49].

2.4.2. Antibacterial Activity. The antibacterial activity of *R. stricta* plant EtOH crude extract as well as their synthesized AgNPs was carried out against different bacterial strains, i.e., *Klebsiella pneumonia* (Gram-negative bacteria), *Bacillus subtills*,

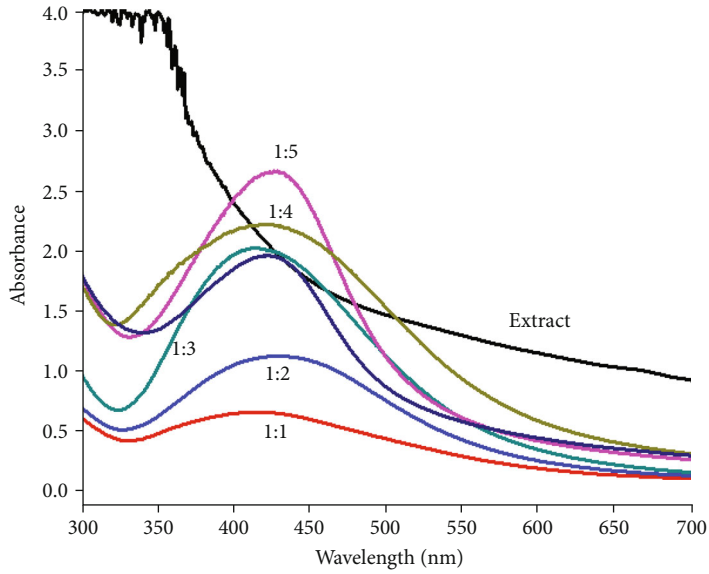

Figure 1: Optimization of AgNPs synthesis *R. stricta*.

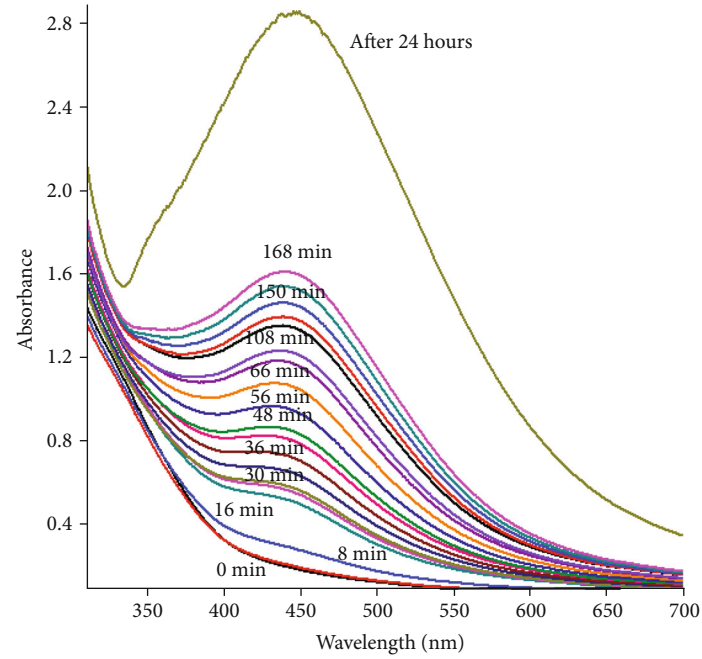

Figure 2: Time effect of green synthesized AgNPs of *R. stricta*.

and *Staphylococcus aureus* (Gram-positive bacteria), and to explore their medicinal values by using standard protocol. These bacterial strains were kept in Mueller-Hinton agar at 4°C in the refrigerator [53]. The antibacterial activity was performed by using modified agar well diffusion method where Mueller Hinton agar was used as medium. The culture taken in triplicate was cultivated in petri dish and later on equipped for 24-72 hours at 37°C. The petri dishes were first sterilized; then, 0.6 mL of prepared broth culture was added with the addition of 20 mL sterilized molten MHA to each Petri dish. Wells (size 6 mm) were bored in the agar medium by using sterilized borer and plants; EtOH crude extract at 0.2 mL by volume was poured into each well by the help of a micropipette whereas 2 mg/mL of synthesized AgNPs was used to study its antibacterial potential by using streptomycin (2 mg/mL) as a standard drug. The proper diffusion was carried out by keeping Petri dishes in laminar flow hood for 1 hour followed by the incubation of plates was done for 24 hours at 37°C. The zone of inhibition was measured next day.

Acetic acid induced writhing *in vivo* paradigm was used for assessment of analgesic potential of extract and AgNPs. Animals were classified in different groups ($n = 8$). The negative control group was treated with distilled water (10 mL/kg, i.p.). Positive control group received diclofenac sodium (10 mg/kg, i.p.), and the tested groups were treated with extract (10, 25, 50 and 100 mg/kg, p.o.) and AgNPs (2.5, 5,

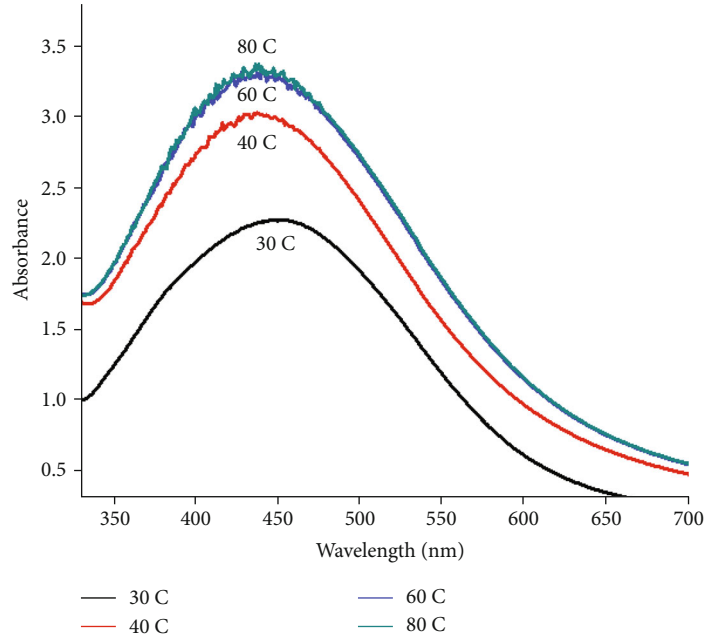

Figure 3: Temperature effect of green synthesized AgNPs of *R. stricta*.

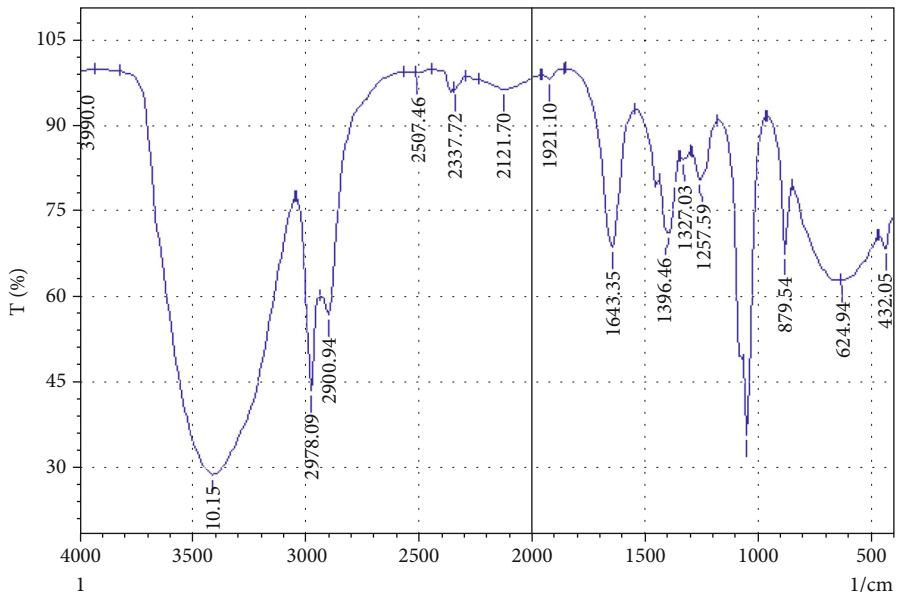

Figure 4: FTIR analysis of *R. strict* crude ethanol extract.

10 and 15 mg/kg, p.o.). All the animals were injected with 1% acetic acid solution (i.p.) after 30 min of the above treatments. After 10 min of the acetic acid injection, the number of abdominal contractions (writhing) was counted (for 10 min) for each group ($n = 8$) of animals. The percent effect was quantified using our published method [9].

2.5. Muscle Relaxant Activity

2.5.1. Inclined Plant Test. For the evaluation of fixed oil for muscle coordination effect, a plane of two woods was used in such a way that an angle of 65° was resulted from the connection. Animals after classification in various groups, the negative group was treated with distilled water (10 mL/kg), the positive group was injected with diazepam (1 mg/kg), and the tested groups were administered extract (10, 25, 50, and 100 mg/kg, p.o.) and AgNPs (2.5, 5, 10, and 15 mg/kg, p.o.). After 30, 60, and 90 min of the above treatment, animals were tested for the muscle coordination effect as, that animal was placed on the upper part of the inclined plane for 30 seconds to hang of fall. This method is the modified form of our published method [38].

2.6. Sedative Activity. For the evaluation of sedative effect of extract and AgNPs, a special box was used. The floor of the box was coated with white sheet (150 cm diameter) and was

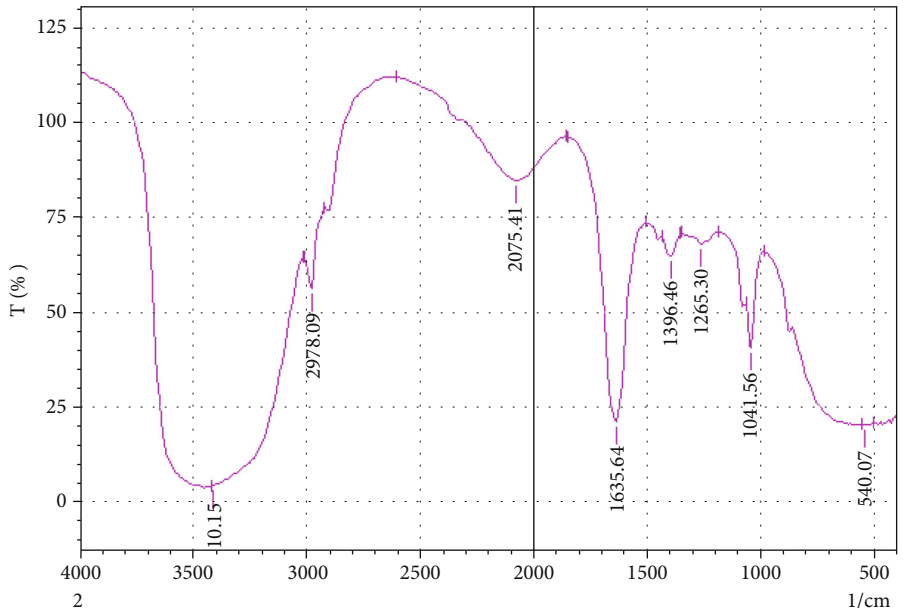

Figure 5: FTIR analysis of *R. stricta* AgNPs synthesized from crude ethanol extract.

divided with 20 squares by black lines. This open field box was placed in soundproof experimental room. Animals after classification in various groups, the negative group was treated with distilled water (10 mL/kg), the positive group was injected with diazepam (1 mg/kg), and the tested groups were administered extract (10, 25, 50, and 100 mg/kg, p.o.) and AgNPs (2.5, 5, 10, and 15 mg/kg, p.o.). After post-30 min of the above administration, each animal was tested for sedative effect by keeping in the center of box, and the number of lines crossed by animal was counted. The smaller number of lines crossed was meant for sedative effect.

2.7. Statistical Analysis. The data were expressed as mean ± standard error of the mean (SEM). Analysis of variance (ANOVA) was followed by Dunnett's test using GraphPad Prism 8.0 (GraphPad Software Inc., San Diego CA, USA).

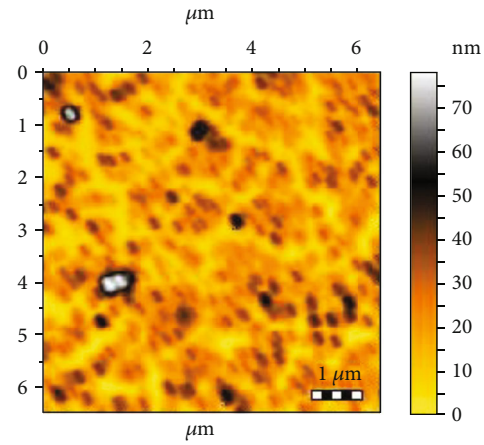

Figure 6: AFM image of ethanol AgNPs of *R. stricta*.

3. Results

3.1. Optimization of AgNPs. Silver nitrate was used in various ratios to a fixed amount of plant extract, including 1 : 1, 1 : 2, 1 : 3, 1 : 4, and 1 : 5. The plant extract was subjected to a reaction flask containing silver nitrate solution and stirred for one hour on high temperature, followed by four hours on low heat. The color of the solution changed from colorless to yellow to brown, and different ratios resulted in different absorbance values, but the optimal ratio was 1 : 5, which has the highest absorbance and sharpest peak. The sharper the peak, the more AgNPs of uniform size will be produced. The maximum absorption wavelength was between 420 and 440 nm (Figure 1).

Table 2: Antioxidant activity of ethanol crude extract and AgNPs of *R. stricta*.

Concentration (μg/mL)	Antioxidant activity	
	Ethanol crude extract	AgNPs
40	29.05	80.28
60	29.32	86.32
80	37.23	87.72
100	50.00	87.94
150	56.81	88.37

3.1.1. Time and Temperature Effect of AgNPs. A UV-visible spectrophotometer was used to monitor the synthesis of AgNPs in order to determine the effect of time. In this study, samples from reaction mixture were drawn at regular interval of time, and UV-visible spectra were noted. It was observed that with the passage of time, the number and uniformity of NPs were increased which was cleared from the increase in the absorbance of the graph (Figure 2).

Table 3: Antibacterial activity of crude extract and synthesized AgNPs.

Microorganisms	Zone of inhibition in mm		
	Crude ethanol extract	AgNPs	Streptomycin
Klebsiella pneumonia	16	14	26
Staphylococcus aureus	15	0	28
Bacillus subtilis	14	0	28

Table 4: Analgesic effect of extract and AgNPs.

Treatment	Dose (mg/kg)	Percent inhibition of writhing
Normal saline	10 mL/kg	—
Diclofenac sodium	10	$85.00 \pm 1.00^{***}$
Crude extract	10	40.09 ± 2.09
	25	54.32 ± 1.76
	50	67.09 ± 1.34
	100	79.98 ± 1.54
AgNPs	2.5	43.87 ± 1.09
	5	56.97 ± 1.07
	10	69.23 ± 1.03
	15	83.23 ± 1.00

The data collected are denoted as the mean ± for all animals, tolerance to thermal stimuli in sec. The level of significance was identified by ANOVA followed by Dunnett's screening model. Here $*** p < 0.01$.

For the effect of temperature on synthesized NPs, temperature variation was carried out during the synthesis. The NPs were synthesized at different temperature, and UV-visible spectra were obtained (Figure 3).

3.2. FTIR Analysis. FTIR analysis was used to confirm the synthesis of AgNPs in plant crude extract and the presence of different functional groups. FTIR spectra of the plant crude ethanol extract (Figure 4) showed the O-H bond stretching in the region of 3500-3700 cm^{-1} and C-H stretching in the range of 3500-3000 cm^{-1}. The band appeared in between 2500 and 3000 cm^{-1} showed =C-H bond stretching while the stretching for C-H bonds appeared from 1500 to 1700 cm^{-1}, and N-H bond stretching peak appeared from 1400 to 1450 cm^{-1}. The results of FTIR analysis for ethanol AgNPs were shown in Figure 5 where it was noticed that the bands present in the plant crude ethanol extract were found to be absent in the ethanol AgNP spectra. The absence of these bands showed the reduction of Ag^{+} ions and the development of silver complexes. The synthesis of ethanol AgNPs was confirmed by the appearance of a broad band in the range of 450-550 cm^{-1} [54].

3.3. AFM Analysis. The shape, morphology, and size of the synthesized AgNPs were studied by atomic force microscopy (AFM). The AFM image (Figure 6) of ethanol AgNPs showed that the NPs possess spherical shape with the calculated sizes 0.1-0.5 μm of AgNPs.

3.4. Biological Activities. R. stricta crude extracts and AgNPs of *R. stricta* were subjected to various biological activities to asses and explore their medicinal value.

3.4.1. Antioxidant Effect. The antioxidant activity of ethanol crude extract and AgNPs was determined by their free radical scavenging property against vitamin C (Table 2). The AgNPs displayed a promising effect against DPPH in a fixation subordinate way. The AgNPs showed significant movement of 88.37% and 87.94% at 100 μg/mL whereas crude ethanol extracts also exhibited excellent activity which was found to be increased from 26.44% to 56.81% along with the increase of concentration from 20 to 150 μg/mL. Generally, the NPs showed good activity than crude ethanol extract.

3.4.2. Antibacterial Activity. The tested samples (extract and AgNPs) against the bacterial strains demonstrated a little antibacterial effect as shown in Table 3. The maximum activity was noted against the Gram-positive bacteria (*Staphylococcus aureus)* was 15 and 0 mm while against Gram-negative bacteria (*Klebsiella pneumonia)* was found to be 16 and 14 mm, respectively, whereas against *Bacillus subtills*, a poor activity was recorded as 14 and 0 mm, respectively.

3.5. Analgesic Activity. Both the tested samples demonstrated a significant analgesic effect as shown in Table 4. The effect was dose dependent, and a higher attenuation in induced writhing was recorded against higher doses. The percent effect of extract (100 mg/kg) and AgNPs (15 mg/kg) was 79.98 and 83.23, respectively.

3.6. Muscle Relaxant Effect. A dose and time dependent effect was observed against the extract and AgNPs. The muscle coordination effect was not significant in both tested models. However, the maximum muscle coordination effect of extract in inclined plan and traction test was 44 and 38% at higher dose, respectively. A similar muscle relaxant effect was noted against AgNPs as shown in Table 5.

3.7. Sedative Effect. A mild sedative effect was also noted against extract and AgNPs. The significant ($p < 0.05$) effect of extract was noted at 100 mg/kg as shown in Table 6. The effect of AgNPs was more significant ($p < 0.01$) at the tested dose of 15 mg/kg.

4. Discussion

This multiindication, along with safety profile, attracts the researcher for the development and discovery of new, safe, and effective natural products as medicines. It is the confluence of biology and nanotechnology that is known as nanobiotechnology. Recent discoveries revealed novel and interesting biological processes for the production of nanosilver using microorganisms or botanic materials as possible bioreducers and biocappers, as well as new and fascinating biological techniques for the production of nanosilver [55, 56]. The phytochemical study of this plant revealed the accumulation of various constituents of various classes. These chemical constituents are the main biomolecules for the curing of various ailments [57, 58].

Table 5: Muscle relaxant activity of extract and AgNPs.

Group	Dose (mg/kg)	Inclined plan test (% activity)			Traction test (% activity)		
		30 min	60 min	90 min	30 min	60 min	90 min
Distilled water	10 mL	0.00 ± 0	0.00 ± 0	0.00 ± 0	0.00 ± 0	0.00 ± 0	0.00 ± 0
Diazepam	1	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.0	100 ± 0.00	100 ± 0.00
Crude extract	10	19.22 ± 1.45	25.86 ± 1.72	28.94 ± 1.09	19.34 ± 1.56	20.34 ± 1.45	19.91 ± 1.24
	25	23.77 ± 1.32	29.98 ± 1.83	34.09 ± 1.00	24.97 ± 1.37	25.77 ± 1.56	24.99 ± 1.44
	50	29.34 ± 1.09	34.90 ± 1.22	36.45 ± 1.34	29.44 ± 1.22	30.34 ± 1.60	32.00 ± 1.45
	100	37.28 ± 1.21	43.29 ± 1.45	44.40 ± 1.09	38.39 ± 1.66	38.99 ± 1.23	34.93 ± 1.98
AgNPs	2.5	24.09 ± 1.98	30.44 ± 1.23	31.49 ± 1.66	25.61 ± 1.61	26.32 ± 1.09	24.80 ± 1.98
	5	30.23 ± 1.84	34.51 ± 1.27	35.40 ± 1.89	30.81 ± 1.64	31.83 ± 1.56	30.77 ± 1.45
	10	35.17 ± 1.66	38.19 ± 1.32	43.11 ± 1.94	35.32 ± 1.33	36.82 ± 1.41	35.58 ± 1.09
	15	40.02 ± 1.30	44.43 ± 1.34	46.17 ± 1.33	40.09 ± 1.23	41.98 ± 1.39	40.14 ± 1.23

The data collected are denoted as the mean ± for all animals, tolerance to thermal stimuli in sec. The level of significance we identified by ANOVA followed by Dunnett's screening model.

Table 6: Sedative activity of extract and AgNPs in open field screening (locomotive activity).

Treatment	Dose (mg/kg)	No of line crossed in 10 min
Distilled water	10	125.00 ± 1.07
Diazepam	0.5	6.22 ± 1.05***
Crude extract	10	121.02 ± 2.67
	25	109.88 ± 2.44
	50	99.45 ± 2.45
	100	88.09 ± 1.60*
AgNPs	2.5	99.54 ± 1.43
	5	88.32 ± 1.43*
	10	76.09 ± 1.27*
	15	64.32 ± 1.30**

The data collected are denoted as the mean ± for all animals, tolerance to thermal stimuli in sec. The level of significance we identified by ANOVA following by Dunnett's screening model. Here, $***p < 0.01$ and $*p < 0.05$.

The extract and NPs of *R. stricta* inhibited various pathogenic bacteria which confirm the use of this plant in various infections. The present study showed that AgNPs and crude extract significantly inhibited the Gram-negative and Gram-positive bacteria while higher doses of AgNPs and crude extract exhibited analgesic effect bolstered by inhibition of writhing [59]. Additional experiments also revealed that both of the entities are of muscle relaxant and sedative potential. In most of the cases, infection is associated with fever and pain. Fortunately, this plant is found to have significant analgesic activity. This analgesic effect provides adjuvant to the antibacterial effect. The painkiller potential is always parallel to the antipyretic and anti-inflammatory effect [60, 61]. The reason behind it is the conversion of arachidonic acid into prostaglandins (PGs) which leads to the analgesic, pyrexia, and inflammatory condition. The current tested plant might be PGs blocker by inhibition of cyclooxygenase (COX) [40, 43, 44, 62]. To confirm the analgesic, antipyretic, and anti-inflammatory, further study is needed to check the COX inhibition by this plant extract/NPs. The mild muscle relaxation effect is also a very good indication along with analgesic property [46, 47, 63]. In most of the painful conditions, muscle relaxation is needed; therefore, if a plant extract is painkiller along with muscle relaxant effect, making it a best option to use as analgesic. The antioxidant effect is also worth mentioning. The free radical scavenging effect is responsible for the blockage of ample of diseases [64, 65].

AgNPs were synthesized from methanol extract of *R. stricta* root with the help of silver nitrate. The AgNPs were found to be 20 nm in size and of spherical shape having potential inhibitory activity against Gram-negative and Gram-positive bacteria [50]. Another study revealed that AgNPs from *R. stricta* are toxic to dengue and malaria containing mosquitos. In addition, it is capable of inhibiting bacteria that are capable to induce fatal diseases to the body [54]. Streptozotocin-induced Swiss albino mice were administered AgNPs from alkaloid extract of *R. stricta*. The dose containing the NPs significantly reduces the glucose in the blood stream as well as the reactive oxygen species to a greater extent [59, 66, 67].

5. Conclusions

To summarize, the biosynthesis of AgNPs using *Rhazya stricta* leaves extract is a simple, eco-friendly, convenient, and cost-effective technique of synthesis. The synthesized AgNPs were spherical in form and ranged in size from 1 to 10 m. The FTIR, UV-vis spectrometer, and AFM characterization techniques revealed that the larger of the NPs was found surrounded by a thin layer of proteins and metabolites such as terpenoids containing functional groups such as ketones, amines, aldehydes, and alcohols. The findings indicate that the form of

the NPs is regulated by the ratio of plant extract to metal ion concentration. Metal ion reduction is crucial in deciding the size of NPs at various concentrations. AgNPs and the crude extract of *Rhazya stricta* exhibited antioxidant, antibacterial, sedative, and analgesic activity. These pharmacological findings corroborate the folklore beliefs about this plant's efficacy in a variety of health ailments.

Data Availability

Available data are presented in the manuscript.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Sami Bawazeer, Abdur Rauf, and Talha Bin Emran did the conceptualization, investigation, supervision, formal analysis, methodology, validation, writing—original draft, writing—review and editing, and visualization. Ajmal Khan did the investigation, methodology, validation, and writing–review and editing. Abdullah S. M. Aljohani and Fahad A. Alhumaydhi did the project administration, visualization, and writing—review and editing. Zidan Khan and Laiba Ahmad did the formal analysis, validation, and writing—review and editing. Rohit Sharma did the formal analysis, validation, resources, and writing—review and editing. Naveed Muhammad and Aneela Maalik Ibrahim Khan did the data curation, formal analysis, validation, resources, writing—original draft, and editing.

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