

# Biogenic silver nanoparticles using *Rhinacanthus nasutus* leaf extract: synthesis, spectral analysis, and antimicrobial studies

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**Abstract:** Nanotechnology is gaining momentum due to its ability to transform metals into nanoparticles. The synthesis, characterization, and applications of biologically synthesized nanomaterials have become an important branch of nanotechnology. Plant extracts are a cost-effective, ecologically friendly, and efficient alternative for the large-scale synthesis of nanoparticles. In this study, silver nanoparticles (AgNps) were synthesized using *Rhinacanthus nasutus* leaf extract. After exposing the silver ions to the leaf extract, the rapid reduction of silver ions led to the formation of AgNps in solution. The synthesis was confirmed by ultraviolet-visible spectroscopy, Fourier transform infrared spectroscopy, and transmission electron microscopy. The in vitro antimicrobial activity of the AgNps synthesized using *R. nasutus* leaf extract was investigated against *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli*, *Aspergillus niger*, and *Aspergillus flavus* using a disc diffusion method. The AgNps showed potential activity against all of the bacterial strains and fungal species, indicating that *R. nasutus* has the potential to be used in the development of value-added products in the biomedical and nanotechnology-based industries.

**Keywords:** *Rhinacanthus nasutus*, silver nanoparticles, TEM, antimicrobial activities

## Introduction

Nanomaterials have extensive applications for improving human health and the environment. The first reported use of nanomaterials for human health was over 5,000 years ago in the Indian system of Ayurveda medicine, in which nanoscience technology was applied before the term “nano” was even coined.<sup>1</sup> It was only in the 21st century<sup>2</sup> that modern science initiated nanoscience research, and development in this field has been rapidly growing throughout the world. A major outcome of this research is the development of new materials at the nanometer scale, including the development of nanoparticles.

Nanoparticles are particulate materials that consist of at least one dimension that is less than 100 nanometers (nm). In the case of quantum dots, nanoparticles can even consist of zero dimensions. Due to their small size, surface (interface), and quantum tunnel effects, nanomaterials have different characteristics compared to non-nanomaterials composed of similar components. With the development of novel chemical and physical production methods, there is the increasing concern of environmental contamination due to the large amounts of hazardous by-products often generated by the chemical procedures utilized for the synthesis of nanomaterials. There is a strong need for “green methods,” methods that are clean, nontoxic, and environmentally friendly, for the synthesis of nanoparticles.<sup>2</sup>

Metal nanoparticles have a high specific surface area and a small fraction of surface atoms. They have been extensively studied due to their unique physicochemical characteristics, which include improved optical, electronic, antibacterial, and magnetic properties, as well as their catalytic activities.<sup>3</sup> The synthesis of noble nanoparticles for electronics and environmental and biotechnology applications is an area of continued interest.<sup>4</sup> In general, metal nanoparticles are synthesized and stabilized using chemical methods such as chemical reduction,<sup>5,6</sup> electrochemical techniques,<sup>7</sup> and microwave-assisted process,<sup>8</sup> while more recent methods utilize green technology.<sup>9</sup> The use of plants for the synthesis of nanoparticles is novel and provides a cost-effective and environmentally friendly alternative to chemical and physical synthesis. In addition, the use of plants can be easily scaled up for large-scale synthesis without the use of toxic chemicals or the need for high pressures, energy, and temperatures.

Although both bacteria and fungi can be used for the synthesis of nanoparticles,<sup>10,11</sup> the use of leaf extract<sup>12</sup> is inexpensive and eliminates the requirement for special culture preparations and isolation techniques. Plants may play a major role in the synthesis of nanoparticles because their use would eliminate the formation of toxic by-products formed during the synthesis process.<sup>13</sup> In recent years, the biosynthesis of nanoparticles using plant extracts has achieved much attention when compared to physical and chemical methods, with even higher attention received when compared to using microbes since maintaining aseptic conditions are not required.<sup>14</sup> Furthermore, all plant parts such as the leaves, stems, seeds, roots, and fruits can be used in the synthesis, replacing the need to use potentially hazardous chemicals such as sodium borohydride ( $\text{NaBH}_4$ ).<sup>15</sup> Nanoparticles are usually prepared from plant extracts since they can act not only as reducing agents but also as capping agents.<sup>16,17</sup> Silver (Ag) has long been recognized for its inhibitory effect on microbes that may be present in medical and industrial processes.<sup>18,19</sup> Silver nanoparticles (AgNps) may be used in more applications compared to other metal nanoparticles, including nonlinear optical, spectrally selective coating for solar energy absorption, biolabeling, intercalation materials as optical receptors for electrical batteries, antibacterial agents, and catalysts in chemical reactions.<sup>20</sup> The most important application of Ag and AgNps in the medical industry is their use in topical ointments that prevent infections in burns and open wounds.<sup>21</sup> Jayaseelan reported the activity of synthesized AgNps against *Hippobosca maculata* larvae by using an aqueous leaf extract of *Musa paradisiaca*.<sup>22</sup> Other recent reports include the efficacies of anti-parasitic activities of

AgNps using the aqueous extract of *Cissus quadrangularis* stem against the adult hematophagous fly *H. maculata* (Diptera: Hippoboscidae) and the larvae of the cattle tick, *Rhipicephalus (Boophilus) microplus* (Acari: Ixodidae).<sup>23,24</sup> Earlier reports on AgNps include its properties as potential antifungal,<sup>25</sup> antibacterial,<sup>26</sup> and antiviral activities.<sup>27</sup> In this scenario, we have selected *Rhinacanthus nasutus* leaf extract for this study to prepare the AgNps and test the antimicrobial activities.

*R. nasutus*, commonly known as snake jasmine, is an erect, small-branched shrub from the *Acanthaceae* family. It is a medicinal plant found in some regions of India, the People's Republic of China, and in areas of Southeast Asia including Thailand.<sup>28</sup> Traditional medicinal preparations from the roots, stems, and leaves of this shrub have long been used in Thai traditional medicine for the treatment of various diseases.<sup>29</sup> Previously, we reported that *R. nasutus* exhibits potential antimicrobial properties and can kill a variety of infecting organisms, in addition to revealed anti-diabetic effects, amelioration of mitochondrial and cytosolic enzymes,<sup>30</sup> hypolipidemic activity,<sup>31</sup> and significant in vitro and in vivo antioxidant activities.<sup>32</sup>

We report for the first time, the synthesis and characterization of AgNps generated by the reduction of *R. nasutus* aqueous leaf extract. The biologically synthesized nanoparticles were analyzed and tested against several different pathogenic microorganisms.

## Material and methods

### Collection of the plant material

Fresh *R. nasutus* (Linn.) leaves (500 g) were collected from Seshachala Hills, Tirumala and Andhra Pradesh. They were authenticated by a botanist, Dr Madhava Chetty, from the Botany department, Sri Venkateswara University, Tirupati and Andhra Pradesh.

### Preparation of the plant extract and AgNps

Dried leaf powder (10 g) was mixed with 100 mL of deionized water in an Erlenmeyer flask (500 mL) - 4060024 (Borosil Glass Works Ltd., Thadani Marg, Worli, Mumbai, India), and was boiled for 20 minutes. For the reduction of Ag ions ( $\text{Ag}^+$ ), 5, 10, and 15 mL of the aqueous leaf extract were carefully added to 90 mL of 1 mM aqueous Ag nitrate and chloroauric acid solution in 250 mL flasks. For this process, the aqueous leaf extract of *R. nasutus* was used.

For the synthesis of AgNps using *R. nasutus*, 5, 10, and 15 mL of the aqueous leaf extract were carefully added to 90 mL of 1 mM aqueous Ag nitrate and chloroauric acid

solution in 250 mL flasks. The mixture was then heated for 20 minutes at a temperature ranging between 60°C and 80°C. The color of the solution changes from brown to red to confirm the synthesis of AgNps.

### Fourier transform infrared spectroscopy (FTIR) and ultraviolet-visible (UV-Vis) spectral analysis of AgNps

To remove compounds that did not function as the capping ligand of the nanoparticles as well as any unbound biomass residue, the residual solution (100 mL) was centrifuged at 5,000 rpm for 10 minutes, and the supernatant was resuspended in 10 mL of sterile distilled water. The centrifugation and resuspension process was repeated three times. The purified suspension was then freeze dried to obtain the dried powder, and the dried nanoparticles were analyzed using FTIR (Tensor 37; Bruker Optik GmbH, Ettlingen, Germany).

The reduction of the pure Ag<sup>+</sup> ions was monitored by measuring the UV-Vis spectrum of the reaction medium at 5 hours following the dilution of a small aliquot of the sample in distilled water. The UV-Vis spectral analysis was conducted using a UV-Vis spectrophotometer between 200 and 800 nm (Cary 4000 UV-Vis spectrophotometer; Avantes Technologies, Santa Clara, CA, USA) with specific monitoring at 437 nm.

### Transmission electron microscopy (TEM)

TEM (H-7500; Hitachi Ltd, Tokyo, Japan) is a microscopy technique in which a beam of electrons is transmitted through an ultra-thin specimen. The ultra-thin film was prepared on a carbon-coated copper grid by placing a small amount of *R. nasutus* nanoparticles on the grid and then drying the particles under a lamp. An image is formed as a result of the interaction of the transmitted electrons with the specimen.

### Particle size and zeta potential measurement – dynamic light scattering (DLS)

Particle size distribution was studied using a DLS technique (Nanopartica SZ-100; HORIBA Ltd, Kyoto, Japan). The scattered light from the particles present in the sample was collected either at 90 or 173 degrees, which was automatically selected by the instrument as the optimum scattering angle based on sample concentration. The zeta potential, which is an indicator of dispersion and stability of the prepared nanoparticles was also measured.



Figure 1 Photograph of *Rhinacanthus nasutus*.

### X-ray diffraction (XRD) analysis of AgNps

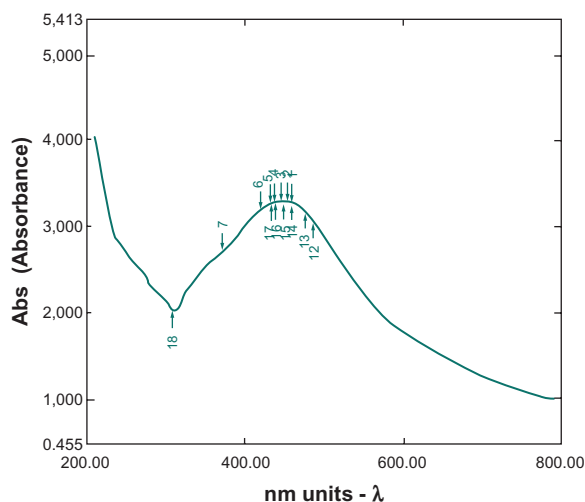
The silver nanoparticle solution obtained above was purified by conducting repeated centrifugation at 5,000 rpm for 20 minutes followed by redispersion of AgNps pellet into 10 mL of deionized water. After freeze drying of the purified Ag particles, the structure and composition was analyzed by using an XRD machine (RINT 2100 series; Rigaku Corporation, Tokyo, Japan). The dried mixture of AgNps was collected for the determination of the formation of AgNps by X'Pert Pro X-ray diffractometer (PANalytical BV, Almelo, The Netherlands) operating at a voltage of 40 kV and a running current of 30 mA with Cu K $\alpha$  radiation in a  $\theta$ -2 $\theta$  configuration.

### Antibacterial activity

The antimicrobial activity of *R. nasutus* and the AgNps prepared from *R. nasutus* was evaluated using the disc diffusion method. Ciprofloxacin (10  $\mu$ g/disc) was used as a standard for comparison. Filter paper discs were soaked in the extract (50  $\mu$ g/mL), and the ciprofloxacin discs were aseptically placed on seeded agar medium (Hi-Media Laboratories Pvt Ltd, Mumbai, India).



Figure 2 Color change of *Rhinacanthus nasutus* leaf extract containing silver before (left) and after (right) synthesis of silver nanoparticles.



**Figure 3** Ultraviolet-visible spectra of the silver nanoparticles synthesized from *Rhinacanthus nasutus* leaf extract.

The medium was incubated with the test organisms at 37°C for 24 hours,<sup>33</sup> and the antimicrobial activity was assessed based on the inhibition zones formed around the discs by the plant extracts compared to the zones around the ciprofloxacin.

### Antifungal activity

The antifungal activity of *R. nasutus* and the AgNps prepared from *R. nasutus* was determined against *Aspergillus niger* and *Aspergillus flavus* using the disc diffusion method. Prior to the experiment, the filter paper discs were individually

saturated with the extracts (50 µg/mL) and then aseptically placed on Sabouraud Dextrose Agar - M063 (Himedia Laboratories, L.B.S. Marg, Mumbai, India), medium that had been incubated with the culture. The plates were then incubated at 37°C for 48 hours<sup>33</sup> since the incubation time for microbial growth varies between fungi and bacteria. The zone of inhibition was measured (in millimeters), and the means of triplicate samples were recorded.

### Statistical analysis

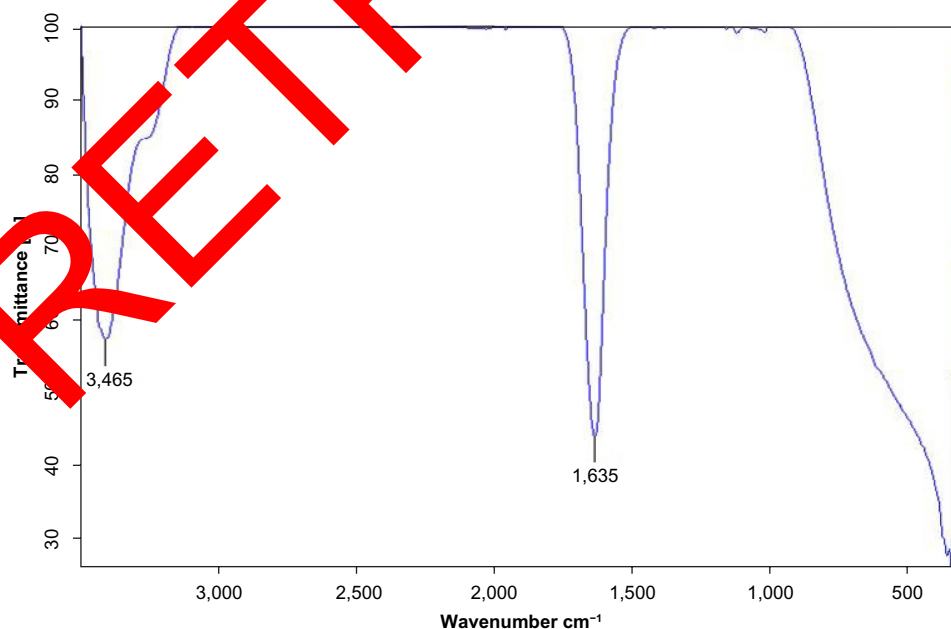
The results were expressed as the mean ± standard deviation of triplicates. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's test.  $P < 0.05$  was considered statistically significant.

### Results and discussion

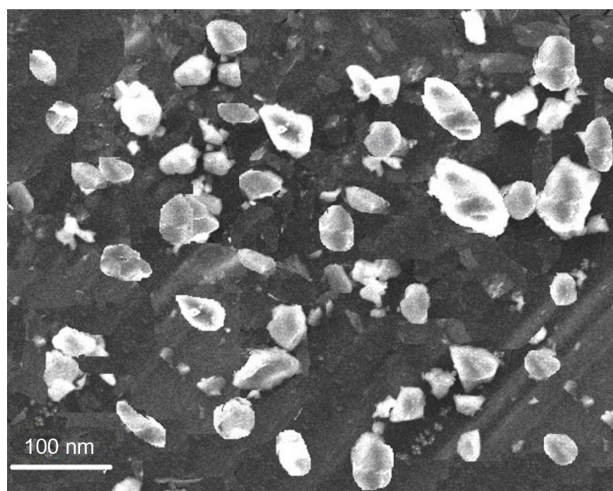
The development of biologically inspired experimental processes for the synthesis of nanoparticles is evolving into an important branch of nanotechnology. In this study, we successfully synthesized AgNps using *R. nasutus* leaf extract (Figure 1).

### UV-Vis spectral analysis of AgNps

The formation and stability of AgNps in an aqueous colloidal solution was investigated using UV-Vis spectral analysis. As expected, AgNps turned yellowish brown in the aqueous solution, which has been attributed to the excitation of surface plasmon vibrations in AgNps (Figure 2).<sup>34,35</sup>



**Figure 4** Fourier transform infrared spectroscopy spectra of the silver nanoparticles synthesized from *Rhinacanthus nasutus* leaf extract after 24 hours.



**Figure 5** Transmission electron microscopy image of the silver nanoparticles synthesized from *Rhinacanthus nasutus* leaf extract.

Following the addition of *R. nasutus* leaf extract to the  $\text{AgNO}_3$  aqueous solution, the color changed from yellow to reddish brown as a result of the reduction of the  $\text{Ag}^+$  ions into AgNps.

UV-Vis spectroscopy is utilized to analyze the size and shape of nanoparticles in aqueous suspensions,<sup>36</sup> and the UV-Vis spectra was recorded after the solution was heated at  $80^\circ\text{C}$  for 15 minutes. The absorption spectra of the AgNps had an absorbance peak at 437 nm, and a broadening of the peak indicated that the particles were polydispersed (Figure 3).

### FTIR analysis of AgNps

The FTIR spectra were recorded to identify potential biomolecules that contributed to the reduction of the  $\text{Ag}^+$

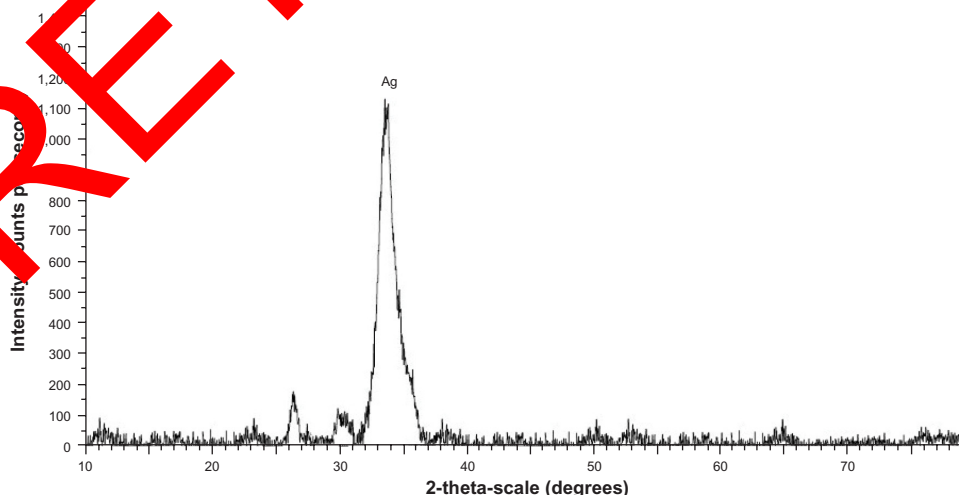
ions and to the capping of the bioreduced AgNps. The FTIR spectrum was recorded 1 day following the formation of the AgNps. A band observed at  $1,635\text{ cm}^{-1}$  may be attributed to the carbonyl groups in the  $\alpha$ -helices present in the plant extract. The amide I band primarily consisted of the carbonyl ( $\text{C}=\text{O}$ ) stretching of the peptide backbone at  $1,635\text{ cm}^{-1}$ . The energy at this vibration is sensitive to the secondary and tertiary structure of the proteins. The band observed at  $3,465\text{ cm}^{-1}$  was characteristic of  $-\text{NH}$  stretching of the amide (II) band. Several bands between  $2,200\text{ cm}^{-1}$  to  $3,400\text{ cm}^{-1}$  were absent, which could be attributed to protein precipitation occurring during the reduction and stabilization of the AgNps (Figure 4).

### Morphological analysis of the AgNps using TEM

The applications for AgNps are highly dependent on the chemical composition, shape, size, and monodispersity of the particles.<sup>37</sup> To broaden the potential scope of applications, the AgNps were characterized using TEM. The samples resulted in a narrow particle size distribution with a mean size for all of the synthesized AgNps of less than 22 nm and a small size ( $\sim 11.5\text{ nm}$ ) that appeared to be spherical (Figure 5).

### Particle size and zeta potential measurement

The distribution of the particle size and the zeta potential were measured using a Nanopartica SZ-100 (HORIBA Ltd). The mean particle size was 329 nm. It was evident



**Figure 6** X-ray diffraction pattern of silver nanoparticles synthesized from *Rhinacanthus nasutus* leaf extract.

**Table 1** Antimicrobial activity of *Rhinacanthus nasutus* extract, silver nanoparticles of *R. nasutus* and ciprofloxacin as the zone of inhibition (mm)

Test organisms	Zone of inhibition (mm)		
	<i>R. nasutus</i> extract	Silver nanoparticles of <i>R. nasutus</i> extract	Ciprofloxacin
<i>Staphylococcus aureus</i>	8.33 ± 0.57	17.66 ± 0.57	18.66 ± 1.52
<i>Bacillus subtilis</i>	10.33 ± 0.57	15.66 ± 1.15	18.33 ± 1.52
<i>Pseudomonas aeruginosa</i>	12.66 ± 2.51	17.33 ± 1.52	18.66 ± 1.52
<i>Escherichia coli</i>	11.33 ± 1.52	17.33 ± 1.52	17.66 ± 2.51
<i>Klebsiella pneumonia</i>	13.33 ± 1.52	17.66 ± 1.52	19.66 ± 0.57
<i>Aspergillus niger</i>	9.66 ± 2.08	17.66 ± 1.52	16.66 ± 1.52
<i>Aspergillus flavus</i>	12.66 ± 2.51	18.66 ± 1.52	18.66 ± 0.57

from the TEM micrograph that the particles were agglomerated and the size measured by the DLS technique may be the size of the cluster rather than an individual particle. This was further confirmed from the low zeta potential (−18.1V).

Data is provided for both the zeta potential and the particle size in Figures S1 and S2.

### XRD analysis of AgNPs

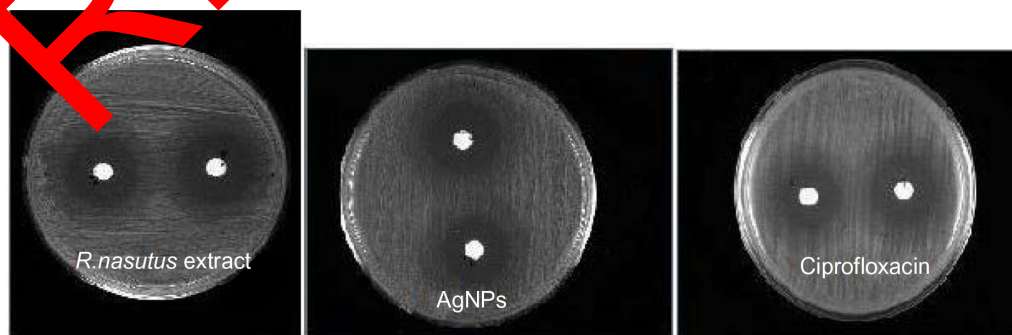
The XRD confirmed the presence of Ag colloids in the sample. The Bragg reflections were observed in the XRD pattern at  $2\theta = 26.3$  and  $30.01$ . A strong diffraction peak located at  $34.50$  was ascribed to the (111) facets of Ag. The XRD pattern thus clearly indicated that the AgNPs formed in the present synthesis were crystalline in nature. No impurity peaks were observed in the XRD pattern indicating that the investigated AgNPs were pure (Figure 6).

### Antimicrobial activity

In this study, *R. nasutus* leaf extract and the AgNPs synthesized with *R. nasutus* leaf extract were tested for their antimicrobial activities against *Staphylococcus aureus*,

*Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumonia* and the two fungal cultures *A. niger* and *A. flavus*. In general, the AgNPs potentiated the antimicrobial activity of *R. nasutus*. The antimicrobial activity was performed against several Gram-positive bacteria, Gram-negative bacteria, and fungi also. The data presented in (Table 1) indicates that the AgNPs from *R. nasutus* inhibited the growth of all tested microorganisms to various levels. The AgNPs showed highest activity against the gram positive bacteria *S. aureus* ( $17.66 \pm 0.57$  mm), gram negative *K. pneumonia* ( $17.66 \pm 1.52$  mm), as well as against the types of fungi: *A. flavus* ( $18.66 \pm 1.52$ ) and *A. niger* ( $17.66 \pm 1.52$  mm).

AgNPs are extensively used in the pharmaceutical industry and have inhibitory activities on various microorganisms. They have also been used in balms and ointments to avert infections following burns and wounds.<sup>13</sup> The maximum inhibitory activity was shown against *A. flavus*, while the lowest activity was observed against the bacteria *B. subtilis* ( $15.66 \pm 1.15$  mm). When compared to ciprofloxacin, the AgNPs showed comparable activities against fungal cultures (Figure 7).

**Figure 7** Antibacterial activity of *Rhinacanthus nasutus* leaf extract (control), silver nanoparticles, and ciprofloxacin against *Bacillus subtilis*.

**Abbreviation:** AgNPs, silver nanoparticles.

## Conclusion

The *R. nasutus* leaf extract reduced Ag<sup>+</sup> metal ions and led to the formation of AgNps with fairly well-defined dimensions. This “green chemistry” approach for the synthesis of AgNps has many advantages, such as the ease with which the process can be scaled up and its economic viability. Applications for these eco-friendly nanoparticles in bactericidal, wound healing, and other medical and electronic applications signifies that this method has the potential for the large-scale synthesis of other inorganic nanomaterials. The antimicrobial screening demonstrated that the synthesized AgNps had a high inhibitory effect on bacteria. These observations may serve as a guide for studying the controlled release of these synthesized AgNps, which has potential in the field of infectious diseases. These AgNps may be explored as an option for decreasing the pathogenic potential of infectious bacterial and fungal species.

## Disclosure

The authors report no conflicts of interest in this work.

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## Supplementary materials

### Measurement results

**Figure S1** Zeta potential micrograph of silver nanoparticles synthesized using *R.nasutus* leaf extract.

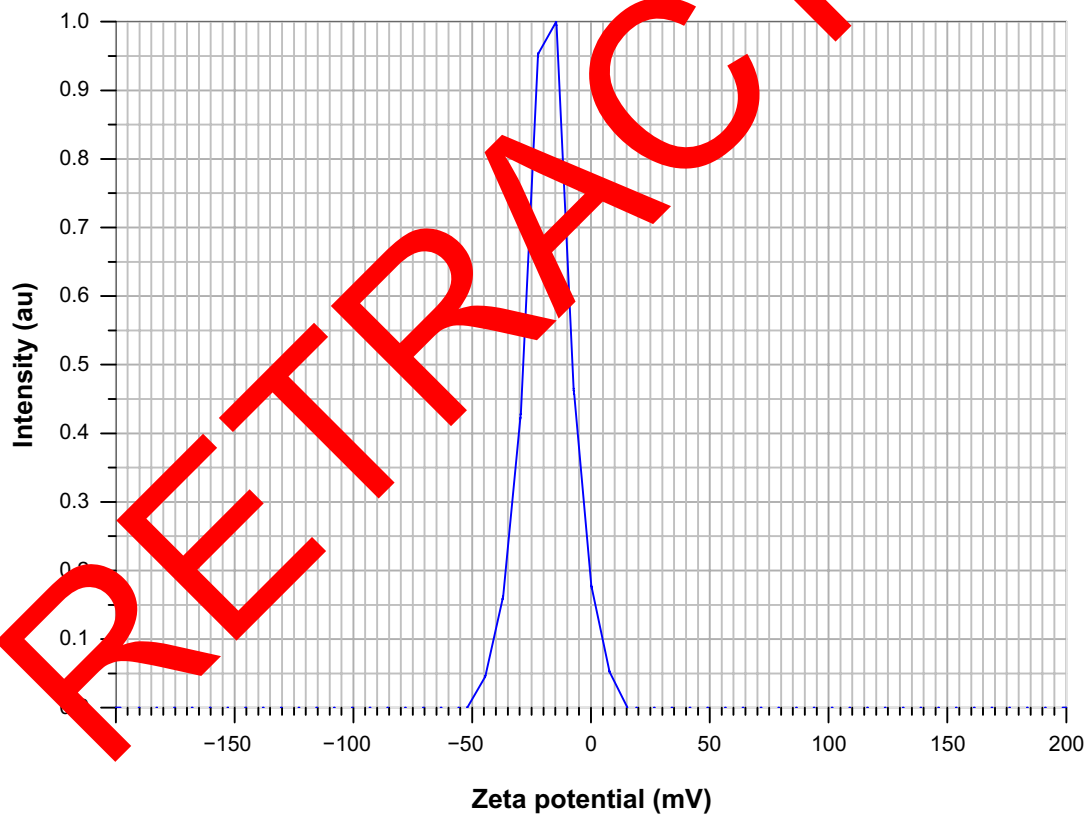
#### Measurement results

Date : Sunday, January 27, 2013 5:26:22 PM  
Measurement type : Zeta potential  
Sample name : 7  
Temperature of the holder : 25.0 °C  
Viscosity of the dispersion medium : 0.894mPa·s  
Conductivity : 0.260 mS/cm  
Electrode voltage : 3.3 V

#### Calculation results

Peak Number	Zeta potential	Electrophoretic mobility
1	- 18.1 mV	-0.000140 cm <sup>2</sup> /Vs
2	- mV	- cm <sup>2</sup> /Vs
3	- mV	- cm <sup>2</sup> /Vs

Zeta potential (mean) : -18.1 mV  
Electrophoretic mobility mean : -0.000140 cm<sup>2</sup>/Vs



**Figure S2** Particle size distribution of silver nanoparticles synthesized using *R.nasutus* leaf extract.

**Measurement results**

Date : Sunday, January 27, 2013 6:20:49 PM  
 Measurement type : Particle size  
 Sample name : 7  
 Scattering angle : 173  
 Temperature of the holder : 25.0 °C  
 Transmission percent (T%) before measure : 1715  
 Viscosity of the dispersion medium : 0.894 mPas  
 Form of distribution : Standard  
 Representation of result : Scattering light intensity  
 Count rate : 1355 kCPS

**Calculation results**

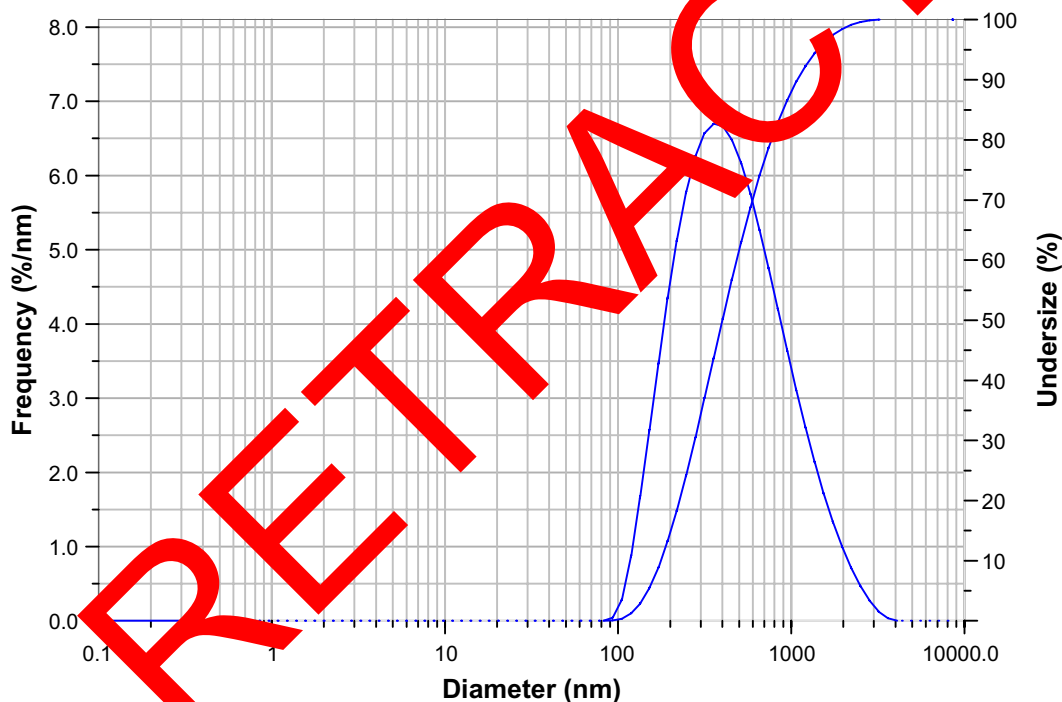
Peak Number	Specific surface area ratio	Mean	Standard deviation	
1	1.00	538.6 nm	427.8 nm	335.4 nm
2	–	– nm	– nm	– nm
3	–	– nm	– nm	– nm
Total	1.00	538.6 nm	427.8 nm	335.4 nm

**Cumulant operations**

Z-average : 329.4 nm  
 Polydispersity index : 0.694

**Molecular weight measurement**

Molecular weight : –  
 Mark–Houwink–Sakurada parameters : –



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