Synthesis, characterization and antibacterial activity of silver nanoparticles using *Rhazya stricta*

Adeeb Shehzad 1, Munibah Qureshi 1, Saima Jabeen 1, Rizwan Ahmad 2, Amira H. Alabdalall 3, Meneerah Abdulrahman Aljafary 3, Ebtesam Al-Suhaimi 3, 4

1 Department of Biomedical Engineering and Sciences, School of Mechanical and Manufacturing Engineering, National University of Science and Technology, Islamabad, Pakistan
2 College of Clinical Pharmacy, Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia
3 Department of Biology, College of Sciences, Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia
4 Institute for Research and Medical Consultations, Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia

Corresponding Author: Ebtesam Al-Suhaimi
Email address: ealsuhaimi@iau.edu.sa

**Background** Green synthesis of metallic nanoparticles has gained significant attention in the field of nano-medicine due to environment-friendly and cost-effective alternative in comparison to other physical and chemical methods. Several metals such as silver, gold, iron, titanium, zinc, magnesium, and copper have been subjected to nano-formulation for a wide range of useful applications. Silver nanoparticles (AgNPs) are playing a major role in the field of nano-medicine and nanotechnology. They are widely used in diagnostics, therapeutic and pharmaceutical industries. Studies have shown potential inhibitory antimicrobial, anti-inflammatory and anti-angiogenesis activities of AgNPs. **Methods** AgNPs have been synthesized using silver nitrate (AgNO3) and methanolic root extract of *Rhazya stricta* that belongs to the *Apocynaceae* family. Stability and dispersion of nanoparticles were improved by adding xylitol. Synthesized nanoparticles were characterized by UV-vis spectroscopy, scanning electron microscopy (SEM), Energy dispersive spectroscopy (EDS), X-ray diffractometer (XRD), and Fourier transforms infrared spectroscopy (FTIR). Furthermore, the antibacterial effect of the plant extract and the nanoparticles were evaluated against gram-positive (*Bacillus subtilis*) and gram-negative (*Escherichia coli*) bacteria. **Results** The Average size of AgNPs synthesized, was 20 nm with the spherical shape. *Rhazya stricta* based nanoparticles exhibited improved antibacterial activity against both gram-positive and negative strains.
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Adeeb Shehzad¹, Munibah Qureshi¹, Saima Jabeen¹, Rizwan Ahmad², Amira H. Alabdalall³, Meneerah Abdulrahman Aljafary³, Ebtesam Al-Suhaimi³, ⁴*

The first two authors equally contributed to this work

¹Department of Biomedical Engineering and Sciences, School of Mechanical and Manufacturing Engineering, National University of Science and Technology, Islamabad, Pakistan

²College of Clinical Pharmacy, Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia

³Department of Biology, College of Sciences, Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia

⁴Institute for Research and Medical Consultations, Imam Abdulrahman Bin Faisal University, Saudi Arabia

**Correspondence***

Ebtesam A Al-Suhaimi, Department of Biology, College of Sciences & Institute for Research and Medical Consultations, Imam Abdulrahman Bin Faisal University, Saudi Arabia

Email: ealsuaimi@iau.edu.sa  Tel: +966133332444
Abstract

Background

Green synthesis of metallic nanoparticles has gained significant attention in the field of nanomedicine due to environment-friendly and cost-effective alternative in comparison to other physical and chemical methods. Several metals such as silver, gold, iron, titanium, zinc, magnesium, and copper have been subjected to nano-formulation for a wide range of useful applications. Silver nanoparticles (AgNPs) are playing a major role in the field of nano-medicine and nanotechnology. They are widely used in diagnostics, therapeutic and pharmaceutical industries. Studies have shown potential inhibitory antimicrobial, anti-inflammatory and anti-angiogenesis activities of AgNPs.

Methods

AgNPs have been synthesized using silver nitrate (AgNO3) and methanolic root extract of *Rhazya stricta* that belongs to the *Apocynaceae* family. Stability and dispersion of nanoparticles were improved by adding xylitol. Synthesized nanoparticles were characterized by UV–vis spectroscopy, scanning electron microscopy (SEM), Energy dispersive spectroscopy (EDS), X-ray diffractometer (XRD), and Fourier transforms infrared spectroscopy (FTIR). Furthermore, the antibacterial effect of the plant extract and the nanoparticles were evaluated against gram-positive (*Bacillus subtilis*) and gram-negative (*Escherichia coli*) bacteria.

Results

The Average size of AgNPs synthesized, was 20 nm with the spherical shape. *Rhazya stricta* based nanoparticles exhibited improved antibacterial activity against both gram-positive and negative strains.
1. Introduction

Nanotechnology is a new, fascinating and fast growing revolution in the interdisciplinary fields including biomedical, therapeutics, diagnostics and materials science. Nanotechnology involves creation and manipulation of materials at the nano scale by altering their molecular structure (Rajasekharreddy & Rani, 2014). Different metals such as silver, gold, iron, titanium, zinc, magnesium and copper have been subjected to nano-formulation for a wide range of beneficial applications by adopting various physical, chemical and biological techniques (Ponarulselvam et al., 2012). Green synthesis is an alternative approach for synthesizing nanoparticles (NPs) using natural resources such as microorganisms and medicinal plants as reducing agents. NPs synthesized by the green method are highly stable, environmental friendly, biocompatible, cost effective, less toxic and safe for diagnostic and therapeutic purpose (Ponarulselvam et al., 2012; Rajasekharreddy & Rani, 2014; Verma & Mehata, 2016).

Silver based nano-materials play a major role in the field of nano-medicine and nanotechnology (Logeswari & Abraham, 2015). Several studies have also proved antimicrobial activity of AgNPs against several pathogenic and multidrug-resistant microorganisms (Singh et al., 2016). Various chemical and physical techniques have been reported for the synthesis of AgNPs, however many of those techniques require costly chemicals which are also toxic (Ahmad et al., 2017). Therefore, the synthesis of NPs by using plant isolated compound could be an attractive strategy in the field of nanotechnology as it offers several advantages such as cost-effective, eco-friendly, rapid synthesis and high yield.

Owing to huge medicinal properties of plants, herein, it is reported green technique for the synthesis of silver nanoparticles by using roots of Rhazya stricta. It is a small, glabrous, erect shrub that contains alkaloids, flavonoids and phenolic compounds (Bukhari et al., 2017). Rhazya stricta
is also known as Harmal, widely distributed in Saudi Arabia and South Asia (Bukhari et al., 2017; Obaid et al., 2017). Seed oil from Harmal is considered a potential rich source of d-tocopherol, a major form of vitamin E (Nehdi et al., 2016). It is used for treating numerous diseases such as obesity, diabetes mellitus, sore throat, cardiovascular, metabolic, neurodegenerative diseases as well as cancers (Alagrafi et al., 2017). It exhibits anti-oxidant, anti-carcinogenic, antimicrobial, anti-dramatist, antihypertensive (VanBeek et al., 1985), antidepressant, anti-inflammatory, antipyretic, antifungal and herbicidal activities (Alagrafi et al., 2017; Ali et al., 2000; Baeshen et al., 2010; Lantero, 2014; Sarfaraz, 2012; Van Beek et al., 1985). Previously, gold nanoparticles (AuNPs) synthesized with extract of Rhazya stricta displayed better anticancer activity against MFC-7 cell lines (Baeshen, 2013). In this study, we have synthesized AgNPs by using root extract of Rhazya stricta plant as a reducing agent along with xylitol (sugar). The synthesized AgNPs was tested for antibacterial activity against gram-positive (Bacillus subtilis) and gram-negative bacteria (Escherichia coli).

2. Materials and Methods

2.1. Chemicals and reagents

AgNO3 and xylitol were purchased with \( \geq 99.0\% \) purity from Sigma-Aldrich. De-ionized water was used for reaction and Whatman filter papers (125mm) were used for the purification and filtration.

2.2. Preparation of root extract

Rhazya stricta was collected from southern part of Pakistan and identified in Taxonomy lab, Department of Botany at Arid Agriculture University Rawalpindi Pakistan. The voucher specimen number MAQ.2014.B313, was deposited in Department Herbarium of Botany, Arid Agriculture
University Rawalpindi. The roots of the plant were washed thrice with de-ionized water and shade dried. Extracts were prepared by soaking 147 g of dried roots in 80% methanol at room temperature for three days, and then filtered using Whatman filter paper (125mm). The solvent was evaporated using a rotary evaporator. The resulted extract after drying was 13g.

2.3. Biosynthesis of silver nanoparticles

For the synthesis of AgNPs, 10 g of dried extract was dissolved in 18mL methanol and placed at 4°C. 1mM solution of AgNO$_3$ was prepared in deionized water. 6mL methanol-extracted plant roots were added drop by drop in 100ml AgNO$_3$ (1mM) with continuous shaking. Another reaction was carried out using the same amount of extract and 100mL of AgNO$_3$ with 6mL xylitol (10$^{-2}$ M). Then both reactions were heated separately using a magnetic stirrer at 60 °C for two hours. The change in color was the indication for synthesis of NPs [Fig. 1].

For characterization of AgNPs, the sample was centrifuged for 20 minutes at 12000rpm. The supernatant was discarded to get rid of any uncoordinated material. The Sample was then washed thrice with distilled water to eliminate extra proteins or enzymes. After sonication, the sample was dried with a vacuum drier for an overnight at 50°C.

2.4. UV-Visible Absorbance spectroscopy

Bio-reduction of Ag$^+$ ions in AgNO$_3$ solution was observed by ultraviolet-visible (UV-Vis) spectroscopy (UV-2800, BMS). UV spectrums for 250μl, 350μL, 450μL, 550μL, 650μL concentrations of plant extract in 10mL of AgNO$_3$ solution were monitored. The UV-Vis absorption spectra for AgNPs was observed in a range of 300-600nm with UV-2800 spectrophotometer.
2.5. Fourier Transform Infrared Spectroscopy (FTIR)

Biomolecules responsible for reducing Ag\(^+\) were analyzed by Fourier transform infrared (FTIR) spectrometer. FTIR spectroscopy is an experimental technique used for analysis of functional groups, molecular structure and chemical bonding of organic and inorganic samples, by producing an infrared absorption spectrum (Perklin Elmer Spectrum 100) (Verma & Mehata, 2016).

2.6. X-ray Diffraction (XRD)

The structural characterization and crystalline nature of AgNPs were determined by X-ray diffractometer (D8 Advance, Bruker) for the vacuumed dried AgNPs (Ashraf et al., 2016).

2.7. Scanning Electron Microscopy (SEM)

The size and morphology of AgNPs were investigated using JEOL-6490A-JSMScanning Electron Microscope. Elemental composition of AgNPs was determined by Energy Dispersive Spectroscopy (EDS).

2.8. Antibacterial Activity

The antibacterial activity of the extract and AgNPs was evaluated against *Escherichia coli*, and *Bacillus subtilis* using disk diffusion method (Hu et al., 2017). Disks were soaked with distilled water (negative control), standard antibiotic cefipime (positive control) with the trade name of maxipime (glaxosmithkline), *Rhazya stricta* extract and the AgNPs solution with and without xylitol addition separately. 50\(\mu\)L concentration of AgNPs was used to ensure identification of antibacterial activity. Then the plates were incubated for 24-48 hours at 37°C and zone of inhibition was measured for both bacterial strains.
3. RESULTS

3.1. UV-VIS Spectroscopy

Synthesis of nanoparticles started after the addition of root extract of *Rhazya stricta* into an aqueous solution of AgNO3. The change in color from pale yellow to orange brown after 2 hours heating (60°C) on magnetic stirrer indicated the formation of reduced silver [Fig. 2A].

To examine further AgNPs in aqueous suspensions, UV-Vis spectroscopy was used. As shown in the Fig. 2B, the absorption peaks of AgNPs synthesized by *Rhazya stricta* root extract were detected at 380, 392, 402, 414 and 427nm wavelength. The increasing concentration of plant extracts also increased the broadening of the peak indicating the increased size of NPs. Previous studies indicated that the spherical AgNPs have absorption bands at around 400 nm in the UV visible spectra (Nasir, Mohammed, & Samir, 2016). These results indicate that the spherical AgNPs have absorption bands at around 402 nm in the UV visible spectra. Thus, it can be reported that particle size of AgNPs is highly subjective to the concentration of root extract.

3.2. SEM

For further confirmation, the size and shape of AgNPs, SEM was performed. Fig. 3A and B depict the formation of spherical shape NPs. It was noticed that AgNPs synthesized only with plant extract have shown more agglomeration [Fig.3A], while those synthesized with the addition of xylitol were not agglomerated, well dispersed and with average particle size of 20 nm.

3.3. Elemental analysis

The AgNPs synthesized using *Rhazya stricta* were also characterized by EDX analysis, which further confirmed the reduction of silver ion. The Optical absorption peak was recorded almost at
3 keV, which is obvious for silver nano-crystals absorption because of surface Plasmon resonance [Fig. 4]. The EDX spectrum has shown silver peak along with oxygen and chlorine peaks. The chlorine and oxygen peaks were may be attributed to glass biomolecules that were present on the surface of silver nanoparticles or to chlorine on the glass slides that were used for sample preparation [Fig. 4].

3.4. XRD

The dry powders of AgNPs synthesized with root extract of Rhazya stricta were subjected to XRD analysis. XRD patterns of synthesized AgNPs are shown in Fig.5. The synthesized NPs were of crystalline nature. The XRD pattern has shown the intense five peaks of NPs at 31.99, 45.5, 54.85, 57.52 and 67.24, were indexed to face-centered cubic structures of AgNPs. The XRD peaks revealed that AgNPs formed using Rhazya stricta root extract were crystalline and spherical in shape. The average nano size was confirmed by applying Debye–Scherrer formula $D = \frac{K \lambda}{\beta \cos \theta}$ which was found to be 20 nm.

3.5. FTIR

FTIR spectroscopy was used to investigate. FTIR analysis was done to identify biomolecules that are responsible for Ag$^+$ ions reduction and capping of biologically reduced AgNPs synthesized by Rhazya stricta root extract along with xylitol. Fig.6A shows the FTIR spectrum of xylitol displayed bands at 3362.59 (OH of alcohol) and 1417.88 (C-H bending of alkane). Fig.6B shows the FTIR spectrum of root extract of Rhazya stricta plant that is showing peaks at 724, 1024, 1455, 1687, 2123, 2921 and 3374 cm$^{-1}$. A peak at 3374 cm$^{-1}$ indicated stretching of the N–H bond of amino groups and presence of attached hydroxyl (-OH) group. The absorption peaks at 2921 cm$^{-1}$
could be due to stretching of –CH functional groups. The peak at 1687 cm\(^{-1}\) corresponds to C–O stretching in the carboxyl attached to the amide linkage in amide I. The absorption band at 1455 indicated the presence of C-N stretching in amide. The two bands present at 1024 and 742 cm\(^{-1}\) could be indicative of the –O– stretching vibrations of aromatic and aliphatic amines (Gou et al., 2015). Fig. 6C and Fig. 6D show FTIR spectra for AgNPs synthesized using only Rhazya stricta plant and along with xylitol.

3.6. Antimicrobial activity of AgNPs

Plant mediated synthesis of AgNPs exhibited tremendous antibacterial activity against various bacterial strains. For this purpose, the antibacterial activity of AgNPs was examined with various concentrations of root extract against Bacillus subtilis for gram-positive and Escherichia coli for gram-negative strains. As shown in the Fig. 7A, and C, three different concentrations (450μL, 550μL and 650μL) of plant extract in 10mL of AgNO3 were used to test the antibacterial activity. When concentration was increased from 450μL to 550μL, the zone of inhibition also increased, however, at 650μL, zone of inhibition was observed smaller than 550μL (Fig. 7A and C). The positive control 10 mM of cefipime exhibited maximum zone of inhibition, whereas distilled water, the negative control shown no inhibition on both strains (Fig. 7B and D). The AgNPs synthesized by Rhazya stricta root extract along with xylitol displayed better antibacterial activity as compared to plant extract only, because xylitol increased the stability and decreased the agglomeration of synthesized AgNPs. Thus, uniform distribution of NPs enhanced the antibacterial activity of synthesized AgNPs with root extract of Rhiza stricta.
3.7. Time-dependent effect of AgNPs

Once the concentration-dependent effect was confirmed, the effect of AgNPs synthesized using *Rhazya stricta* root extract was examined for its time dependent activity. The 550 µL of AgNPs with *Rhazya stricta* root extract was tested further with and without xylitol against *bacillus subtilis* (gram-positive) and *Escherichia coli* (gram-negative). As shown in Fig. 8A and B, AgNPs with *Rhazya stricta* root extract and xylitol have shown an increased zone of inhibition as compared to their individual zone of inhibition. These results confirm the enhanced antibacterial effect of AgNPs synthesized with *Rhazya stricta* root extract and xylitol.

3.8. Differential effect of AgNPs against gram-negative and positive pathogens

The synthesized silver nanoparticles had more inhibition effect on *Escherichia coli* as compared to *Bacillus subtilis* as shown in Fig. 9A and B. These results further confirmed that AgNPs synthesized with *Rhazya stricta* root extract have better bactericidal effects against gram-negative strains as compared to gram-positive strains.

4. DISCUSSION

Green synthesis of AgNPs could significantly overcome the problem of using chemical agents that cause various adverse effects. Therefore, green synthesis of NPs is environment-friendly approach (Gan & Li, 2012; Wang et al., 2017). *Rhazya stricta* plant roots possess antibacterial activity that is considerably enhanced after incorporation with NPs. The characterization techniques advocated the stability of the synthesized AgNPs. Experimentally, Xylitol has not the ability to reduce silver ions, but it is possibly involved in the capping of the synthesized AgNPs by electrostatic attraction or binding with the protein groups present in the extract through hydrogen bonding and enhanced
the silver nanoparticle’s stability. It also protected the NPs from agglomeration (Kaviya et al., 2011). Synthesis of the AgNPs starts with the change in color from pale yellow to orange brown upon the addition of *Rhazya stricta* root extract [Fig. 2A]. It has been reported that excitation of surface Plasmon resonance is responsible for yellowish brown color of AgNPs in aqueous solution (Kaviya et al., 2011). This surface Plasmon vibration confirmed the biotransformation from an ionic form to silver particles (Huang et al., 2007). Upon addition of *Rhazya stricta* root extract in the aqueous solution of AgNO3 and xylitol, the color changed from pale yellow to orange brown due to reduction of silver ion [Fig. 2A]. It was also noticed that increase in the concentration of root extract in the synthesis reaction, increases the particle size. Further increase in plant extract concentration shown noisy and broadened peak at UV-Vis spectrum. Current XRD results of synthesized AgNPs with root extract of *Rhazya stricta* confirmed the structure of AgNPs as face-centered cubic (fcc) with crystalline nature (Li et al., 2012). The EDX spectrum has shown clear silver signal along with weak oxygen and chlorine peak, which might be due to the interaction and binding of biomolecules to the surface of AgNPs (Kalainila et al., 2014).

FTIR analysis was performed to confirm that the bio-compounds involved in the reduction of ionic and capping of the reduced AgNPs synthesized by *Rhazya stricta* root extract along with xylitol. Fig. 6A shows FTIR spectrum of xylitol, it gave bands at 3362.59 (OH of alcohol) and 1417.88 (C-H bending of alkane). Fig. 6B represents the FTIR spectrum of root extract of *Rhazya stricta* plant; it gave peaks at 724, 1024, 1455, 1687, 2123, 2921 and 3374 cm\(^{-1}\). These peaks are expected to be related with amide I shift due to carbonyl stretch in proteins (1687 cm\(^{-1}\)), –C–H– stretch of amine (2921 cm\(^{-1}\)), C–N stretching vibration of amine (1455 cm\(^{-1}\)), and –O– stretching vibrations of aliphatic and aromatic amines (1024 and 742 cm\(^{-1}\)), respectively (Gou et al., 2015; Wang et al., 2017). A peak at 3374 cm\(^{-1}\) indicated the stretching of the N–H bond of amino groups and shown
the presence of bonded hydroxyl (-OH) group (Gou et al., 2015; Hu et al., 2017; Wang et al., 2017). After the formation of AgNPs, peaks were shifted to a little higher wavenumber as compared to plant extract as indicated by Fig. 6C. Xylitol did not reduce the silver ions in the solution, but it was used to cap/coat the synthesized AgNPs through electrostatic attraction or hydrogen bonding with protein groups that further aided in more stability and better dispersion of NPs Fig. 6D. FTIR spectrum of the *Rhazya stricta* roots extract confirmed the presence of carboxyl (-C=O), hydroxyl (-OH) and amine (N-H) groups of *Rhazya stricta* roots extract and further revealed their involvement in the reduction of silver ion to metallic silver nanoparticles (Nasir, Mohammed, & Samir, 2016). Plant extract contains proteins that cap the silver nanoparticles through carboxyl or amino group present in protein (Kaviya et al., 2011). It is believed that proteins of the root extract of *Rhazya stricta* could bind to AgNPs through either free amino or carboxyl groups (Nasir et al., 2016). The presence of the residual plant extract in the sample is due to the similarity of the spectra with little marginal shifts in peak position (Saiqa, 2015). Thus these results have shown that the functional groups of bio-compounds present in the root extract of *Rhazya stricta* have key role in the reduction as well as capping of AgNPs.

Previous studies have also reported the antibacterial activity of AgNPs against gram negative and gram positive strains of bacteria (Singh et al., 2008). It is well recognized that the antibacterial effect of AgNPs is size and dose dependent. The results were supported by previously reported studies (Sana & Dogiparthi, 2018), which have shown that AgNPs have more potential against gram-negative bacteria then gram positive bacteria (Fig. 7A and C). Increase in the size of NPs reduced their antibacterial activity (Kaviya et al., 2011). *Rhazya stricta*-AgNPs exhibited strong antioxidant and antibacterial effects. It is also concluded that the antibacterial activity of AgNPs
depends on the concentration of root extract and quantity of AgNPs in the medium (Rauf et al.,
2016).

Conclusion

AgNPs synthesized using Rhazya stricta root extract along with xylitol have shown potential
antimicrobial activity because they were mono dispersed, stable and have less aggregation. Plant
acted as a reducing agent in the synthesis of AgNPs and xylitol enhanced the dispersion of
nanoparticles. Smaller size of NPs displayed better antibacterial activity. Antibacterial activity of
Rhazya stricta plant root was enhanced when it was incorporated with AgNPs synthesis. Thus,
synthesized AgNPs increases the therapeutic efficacy, strengthen the medicinal values of Rhazya
stricta and provides a potent source of an antimicrobial agent against gram positive and gram
negative organisms. Future studies are needed to uncover the underlying mechanism of
bacteriostatic or bactericidal effects of green synthesized AgNPs.
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**Figure Legends**

**Figure 1**: Schematic presentation of methodology for synthesis of AgNPs with root extract of *Rhazya stricta*.

**Figure 2**: (A) Change in color after synthesis of AgNPs. (B) Concentration dependent UV-Vis Spectra of AgNPs of *Rhazya stricta*.

**Figure 3**: SEM showing spherical AgNPs. (A) AgNPs of plant extract only (B) AgNPs of plant extract with xylitol

**Figure 4**: Energy Dispersive X-Ray image of AgNPs synthesized using *Rhazya stricta*.

**Figure 5**: X-Ray Diffraction (XRD) image of the AgNPs synthesized using *Rhazya stricta*. 
Figure 6: (A) FTIR of Xylitol. (B) FTIR of medicinal plant Rhazya stricta. (C) FTIR of AgNPs using *Rhazya stricta*. (D) FTIR of AgNPs using *Rhazya stricta* along with xylitol.

Figure 7: (A) Concentration dependent effect of *Bacillus subtilis*. (B) Graphical presentation for concentration dependent effect of *Bacillus subtilis*. (C) Concentration dependent effect of *Escherichia coli*. (D) Graphical presentation for concentration dependent effect of *Escherichia coli*.

Figure 8: (A) Time dependent effect of *Bacillus subtilis*. (B) Graphical presentation for time dependent effect of *Bacillus subtilis*. (C) Time dependent effect of *Escherichia coli*. (D) Graphical presentation for time dependent effect of *Escherichia coli*.

Figure 9: (A) Difference between effect of silver nanoparticles of *R. stricta* along with xylitol on *Escherichia coli* and *Bacillus subtilis*. (B) Graphical presentation for comparative effect of AgNPs against *Escherichia coli* and *Bacillus subtilis*. 
Figure 1

Methodology for synthesis of AgNPs

Schematic presentation of methodology for synthesis of AgNPs with root extract of *Rhazya stricta*.
Figure 2

Characterization of AgNPs

(A) Change in color after synthesis of AgNPs. (B) Concentration dependent UV-Vis Spectra of AgNPs of *Rhazya stricta*.

Photo credit: Saima Jabeen
Figure 3

SEM images of AgNPs

(A) AgNPs of plant extract only (B) AgNPs of plant extract with xylitol
Figure 4

EDX spectrum

Energy Dispersive X-Ray image of AgNPs synthesized using *Rhazya stricta*
Figure 5

XRD patterns

X-Ray Diffraction (XRD) image of the AgNPs synthesized using *Rhazya stricta*
Figure 6

FTIR spectrum

(A) FTIR of Xylitol. (B) FTIR of medicinal plant R. stricta. (C) FTIR of AgNPs using Rhazya stricta. (D) FTIR of AgNPs using Rhazya stricta along with xylitol
Figure 7

Concentration dependent antimicrobial activity of Ag NPs

(A) Concentration dependent effect of Bacillus subtilis. (B) Graphical presentation for concentration dependent effect of Bacillus subtilis. (C) Concentration dependent effect of Escherichia Coli. (D) Graphical presentation for concentration dependent effect of Escherichia Coli.
Figure 8

Time dependent antimicrobial activity of AgNPs

(A) Time dependent effect of *Bacillus subtilis*. (B) Graphical presentation for time dependent effect of *Bacillus subtilis*. (C) Time dependent effect of *Escherichia Coli*. (D) Graphical presentation for time dependent effect of *Escherichia Coli*.
Figure 9

comparative effect of AgNPs against *Escherichia Coli* and *Bacillus subtilis*.

(A) Difference between effect of silver nanoparticles of *R. stricta* along with xylitol on *Escherichia Coli* and *Bacillus subtilis*. (B) Graphical presentation for comparative effect of AgNPs against *Escherichia Coli* and *Bacillus subtilis*. 