

Green synthesis of silver nanoparticles using *Trachyspermum ammi* fruit extract: Characterization and antibacterial activity

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Abstract

In recent years, bio-metallic nanoparticles have been considered in medical science due to their biomedical properties. This research aimed to develop an eco-friendly, simple and facile process to synthesize green silver nanoparticles (AgNPs) using *Trachyspermum ammi* fruit extract. The synthesized silver nanoparticles were characterized using FT-IR, UV-Vis spectroscopy, XRD, DLS, and TEM. The minimum inhibitory concentration (MIC) values of synthesized AgNPs and *T. ammi* extract for five standard bacteria strains were determined by the broth microdilution method. The obtained AgNPs exhibited Surface Plasmon Resonance centered at about 415 nm with an average particle size calculated to be 50 nm. The mean particle size and surface charge of biosynthesized AgNPs using *T. ammi* extract investigated by DLS and zeta potential were 26.78 ± 1.24 nm and -13.96 mV, respectively. Furthermore, green synthesized AgNPs show high and efficient antibacterial activity against *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *K. pneumonia* ATCC 9997, *S. aureus* ATCC 25923, and *E. faecalis* ATCC 29212 with MIC values of 19, 19.5, 75, 150, and 39 $\mu\text{g/ml}$, respectively. Noteworthy, the antibacterial results illustrated that green synthesized AgNPs possess significantly higher antibacterial potency than chemically produced silver nanoparticles. Our findings highlight the effective and efficient synthesis of silver nanoparticles by *T. ammi* fruit extract and its significant antibacterial activities.

1 Introduction

Infectious diseases are one of the leading causes of death worldwide, and multidrug-resistant bacterial strains as infectious agents are still a concern for human health. Silver has previously been used to heal wounds or treat infections, but its short effect time has made it unrecognizable as a good agent in medicine. With the development of nanotechnology, the synthesis of nanoscale metals with better antimicrobial properties has been explored to overcome this concern [1, 2].

Over the last few decades, nanomaterials have entered many fields of sciences such as engineering, medicine, environmental, textile, and so on due to their physical, chemical, and biological characteristics [3, 4]. Among various kinds of nanoparticles, silver nanoparticles (AgNPs) with a wide range of applications in different fields such as food preservatives, wound healing, water purification, biosensors, and drug delivery systems have been considered in medical science [5–8]. Much research has shown that AgNPs are redox catalysts with less activation energy and have a surface reactivity due to their high surface energy. Antibacterial potency and tumor cell targeting have made AgNPs an amazing nanomaterial in nanomedicine. The antibacterial activities of NPs are affected by their physicochemical characteristics, such as the size, morphology, distribution, chemical composition, and stability, as well as the type of microorganisms. The proposed antibacterial mechanism of NPs is related to the production of reactive oxygen species (ROS) [9–11].

Although various techniques of nanomaterial synthesis have been studied, life-threatening environmental pollution has led scientists to develop new approaches to natural substances such as plant extracts or microorganisms called "green synthesis". In the green synthesis of nanoparticles using plant extracts, the

present phytochemical compounds act as reducing, capping, and stabilizing agents [12, 13]. This method is simple, cost-effective, and time-consuming without toxic chemicals. Many studies have been done on synthesizing AgNPs with plant extracts such as *Trigonella foenum-graecum* [14], *Moringa oleifera* [15], *Ruellia tuberosa* [16], *Nardostachys jatamansi* [17], *Cornus mas* [18], and *Madhuca longifolia* [19]. *Trachyspermum ammi* is a medicinal plant that has antimicrobial, anti-inflammatory, antispasmodic, antipyretic, analgesic, anti-nociceptive, and antioxidant properties [20].

Given the high potential of plant extracts in the production of nanoparticles, we designed the green synthesis of AgNPs using *T. ammi* extract along with evaluating the parameters affecting the size and morphology of AgNPs and their antibacterial activity.

2 Materials And Methods

2.1 Material and Characterization

The fruits of *T. Ammi* (Fig. 1) were collected from the areas around Birjand (Iran) and approved by a botanist in the agriculture faculty at the University of Birjand. AgNO₃ (Silver nitrate) and Na₃C₆H₅O₇ (Sodium citrate) were obtained from Sigma-Aldrich Company (USA). Five standard bacteria strains including *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumonia* ATCC 9997, *Staphylococcus aureus* ATCC 25923, and *Enterococcus faecalis* ATCC 29212 were purchased from Pasteur Institute of Iran (Tehran, Iran). Fourier transform infrared spectroscopy (FT-IR) was employed for the identification of functional groups (Perkin-Elmer, USA), Transmission electron microscopy (TEM) for direct size and morphological observation of products (Zeiss, EM10C, Germany), X-ray diffraction (XRD) for determination of crystal structure of particles (Philips X'pert PRO, The Netherlands), and Dynamic light scattering (DLS) analysis for hydrodynamic diameter determination of silver nanoparticles (Nano-ZS, Malvern, UK).

2.2 Preparation of *T. ammi* Extract

Five gram of *T. ammi* fruits were collected and dried at room temperature conditions under shade. Then, the fruit powder was mixed with 50 ml of deionized water for 24 hours. Finally, the solution was filtered using Whitman and used for the synthesis of nanoparticles.

2.3 Synthesis of Silver Nanoparticles

Synthesis of AgNPs was carried out by two methods: (1) chemical route: 9 mg of AgNO₃ was dissolved at 50 ml of distilled water. Then, 1 ml of sodium citrate (1%) was added to the above solution under intense stirring at room temperature. Within 1 hour, the color of the solution changed from colorless to brown, indicating the formation of AgNPs. (2) Green synthesis: 1.5 ml of *T. ammi* fruit extract was added to the AgNO₃ (30 ml of AgNO₃ 1 mM) under intense stirring for 24 h. Finally, the solution was centrifuged (600 rpm), washed three times, and dried. The biosynthesis of AgNPs was monitored using UV-Vis absorption spectroscopy (300–500 nm).

2.4 Antibacterial Activity

In this study, the antibacterial activity of *T. ammi* aqueous extract, chemical AgNPs, and green AgNPs was investigated against *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *K. pneumonia* ATCC 9997, *S. aureus* ATCC 25923, and *E. faecalis* ATCC 29212. The minimum inhibitory concentration (MIC) was determined using the microdilution broth method according to Clinical and Laboratory Standards Institute (CLSI) guidelines [21]. Briefly, 100 μ l of two-fold serial dilutions of the test compounds were suspended in Muller Hinton broth (Merck, Germany) in sterile polystyrene 96-well microtiter plates, and then 100 μ l of the bacterial suspension with a final concentration of 5×10^5 CFU/ml was added to every well of the plate. Finally, the treated plates were incubated for 18–24 hours at 37°C in ambient air conditions. The bacterial suspension with medium and only the culture medium was used for the positive and negative control, respectively. The antibacterial activity of the test compounds was compared with Tetracycline as an effective antibacterial agent.

3 Results And Discussion

3.1 Determination of Nanoparticle Characterization

In our study, the following techniques were employed to determine the properties of synthesized AgNPs:

3.1.1 Dynamic Light Scattering (DLS) Analysis

The stability and average diameter of particle size of biosynthesized AgNPs using *T. ammi* extract were calculated using zeta potential and DLS. The average particle size and zeta potential of the synthesized AgNPs by chemical and green processes were about 51.73 ± 15.74 nm, 26.78 ± 1.24 nm, -16.35 mV, and -13.96 mV, respectively. The high electrostatic negative charges on the surface of nanomaterials due to the Van der Waals force interaction between the particles can play a crucial role in preventing the aggregation of nanomaterials [22]. The negative zeta potential of synthesized AgNPs using natural extracts was reported in previous studies [23–25]. So, the obtained zeta potential results confirm that the green synthesized AgNPs using *T. ammi* extract in our study have an adequate surface charge for electrostatic stability to prevent aggregation.

3.1.2 Fourier Transform Infrared Spectroscopy (FT-IR)

FT-IR spectroscopy was performed to investigate the functional groups present in the AgNPs samples synthesized using chemical (Fig. 2a) and green (Fig. 2b) routes. The strong broadband at about 3400 cm^{-1} was assigned to the O-H stretching vibration group of alcohols [26]. The band at about 2900 cm^{-1} can be related to the C-H stretching vibration band [27]. When AgNPs are synthesized using the extract, the extract is placed on the surface of the nanoparticles. This phenomenon was fully observed in the FT-IR spectrum of AgNPs synthesized using *T. ammi* extract (Fig. 2b). Several peaks at 1652, 1397, and 1076 cm^{-1} were related to -C = O, C-N stretching vibration of amines, and -C-O bending vibrations group,

respectively [28, 29]. All FT-IR results revealed that chemical compounds in *T. ammi* extract were the cause of biosynthesized of AgNPs.

3.1.3 Transmission Electron Microscopy (TEM)

TEM analysis was used to confirm the formation and morphological characteristics of the synthesized AgNPs using chemical (Fig. 3a) and green (Fig. 3b) processes. Oval and spherical morphologies and homogeneity were characteristics of AgNPs. These homogeneous structures may be due to the presence of biomolecules from *T. ammi* extract, which was the surface covering AgNPs. In addition, the various morphology and sizes of AgNPs might be due to the presence of reducing agents in the *T. ammi* extract [30, 31]. A closer look at the TEM images showed that the size of nanoparticles synthesized using the chemical and green methods was approximately 75 and 50 nm, respectively.

3.1.4 X-ray Diffraction (XRD)

XRD analysis was used to determine the crystallinity and structural phase of the samples. The XRD pattern of green synthesis of AgNPs using *T. ammi* extract is depicted in Fig. 4 and reveals the distinguished 2theta peaks with the values of 38°, 44°, 67°, and 77°. According to the XRD results, the cubic structure of AgNPs had respective planes (111), (200), (220), and (311), which were in good agreement with the standard JCPDS No: 01-087-0717. Furthermore, no impurities were observed in the XRD pattern of AgNPs, indicating that the crystal was a single-phase. Low-intensity peaks can be attributed to the crystallization of bioorganic phases that occur on the surface of the AgNPs [32]. These data are matched with the previous finding [33].

3.2 Antibacterial Activity

The MIC values for AgNPs synthesized using a chemical reagent and the *T. ammi* extract are shown in Tables 1 and 2. The results showed that chemical AgNPs and *T. ammi* extract had no antibacterial activity. Noteworthy, AgNPs synthesized using *T. ammi* extract showed very significant antibacterial activity compared to AgNPs synthesized with a chemical reagent. Also, the results showed a direct relationship between the concentration of nanoparticles and antibacterial activity, so with increasing the concentration of nanoparticles, antibacterial activity increases. As the concentration of AgNPs increased from 1–2%, the MIC value was reduced by almost half. Finally, in our study, the antibacterial activity of AgNPs synthesized using *T. ammi* extract seems to be higher against Gram-negative than Gram-positive bacteria. A similar result was obtained in previous studies for AgNPs biosynthesized with various plant extracts [34–36]. These results can be explained by the difference in the composition of the bacterial cell wall and the thickness of the peptidoglycan layer of Gram-positive bacteria, which protects against chemical agents. In addition, the higher electrostatic attraction of AgNPs with Gram-negative bacteria can lead to changes in cell wall composition by affecting its permeability [35, 37]. Although the exact mechanism of the antibacterial effect of AgNPs remains to be understood, the possible mechanism of their antibacterial activity is shown in Fig. 5.

Table 1
Antibacterial activity (MIC values in mg/ml) of chemically synthesized silver nanoparticles (AgNPs) and *Trachyspermum ammi* fruit extract.

Bacteria strains	<i>T. ammi</i> extract	Chemically synthesized AgNPs	
		Conc. 1%	Conc. 2%
<i>E. coli</i> ATCC 25922	>40	>20	>10
<i>P. aeruginosa</i> ATCC 27853	>40	20	10
<i>K. pneumonia</i> ATCC 9997	>40	>20	>10
<i>S. aureus</i> ATCC 25923	>40	>20	>10
<i>E. faecalis</i> ATCC 29212	>40	>20	>10

Table 2
Antibacterial activity (MIC values in µg/ml) of green synthesized silver nanoparticles (AgNPs) using *Trachyspermum ammi* fruit extract and Tetracycline.

Bacteria strains	Tetracycline	Green synthesized AgNPs	
		Conc. 1%	Conc. 2%
<i>E. coli</i> ATCC 25922	0.97	19	9
<i>P. aeruginosa</i> ATCC 27853	3.9	19.5	9.7
<i>K. pneumonia</i> ATCC 9997	7.8	75	37.5
<i>S. aureus</i> ATCC 25923	0.97	150	75
<i>E. faecalis</i> ATCC 29212	7.8	39	19.5

4 Conclusion

In this study, *T. ammi* fruit extract was introduced as an excellent reducing, capping, and stabilizing agent in the green synthesis of AgNPs. Our findings highlight the effective and efficient synthesis of AgNPs by *T. ammi* extract and its significant antibacterial activities. Based on the study results, it is concluded that AgNPs synthesized using *T. ammi* extract have potential antibacterial properties, especially against gram-negative bacteria.

Declarations

Ethical Approval

The Research Ethics Committee at Birjand University of Medical Sciences approved the study (IR.BUMS.REC.1398.288).

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

MYHB and MY: Designed the study, collected the data, and revised the manuscript; SH and EA: Collected the data; SM: Collected the data and wrote the draft manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

Not applicable

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Figures



Figure 1

Trachyspermum ammi fruit

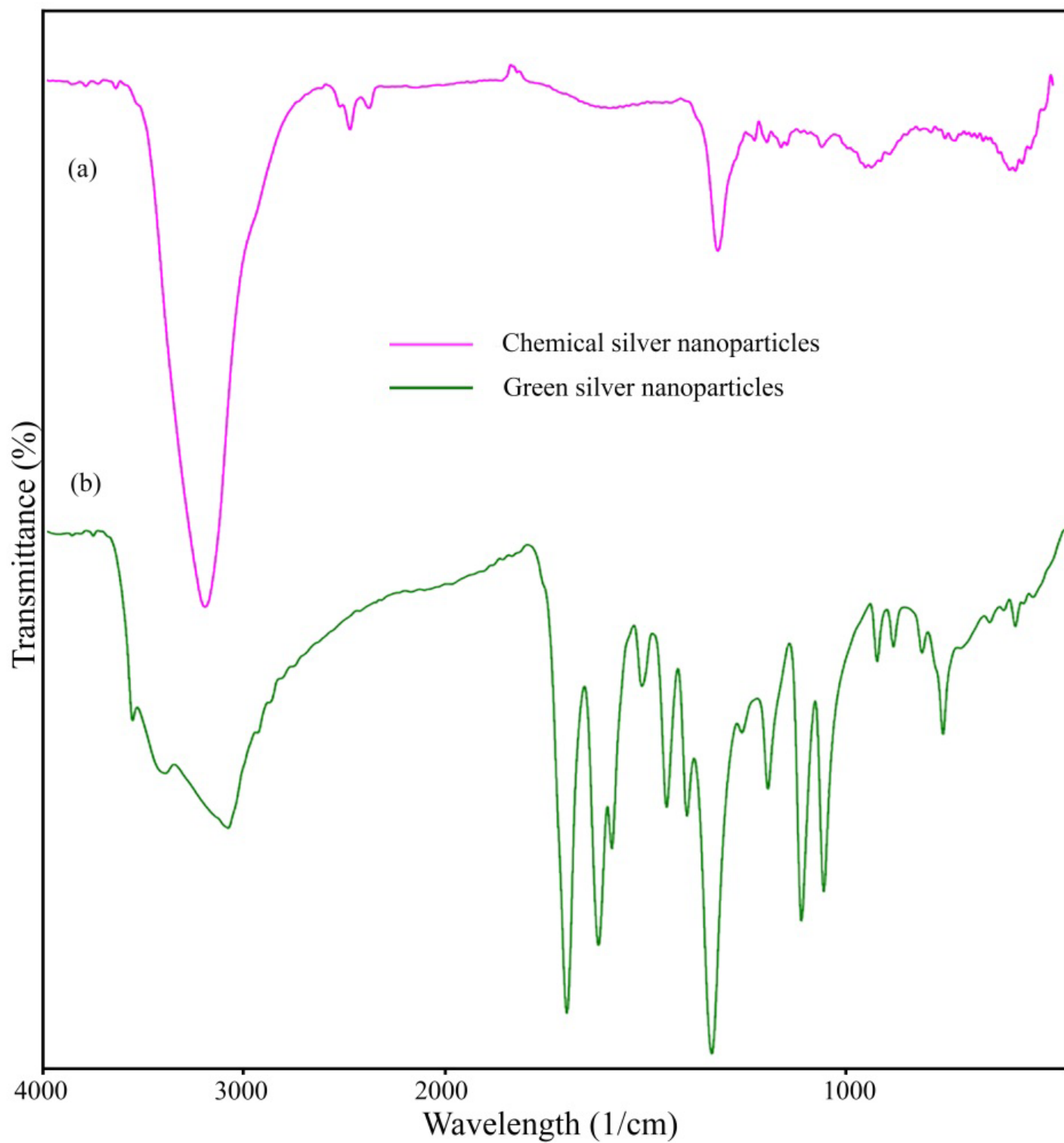


Figure 2

FT-IR spectra of (a) chemical synthesized silver nanoparticles and (b) green synthesized silver nanoparticles using *Trachyspermum ammi* fruit extract.

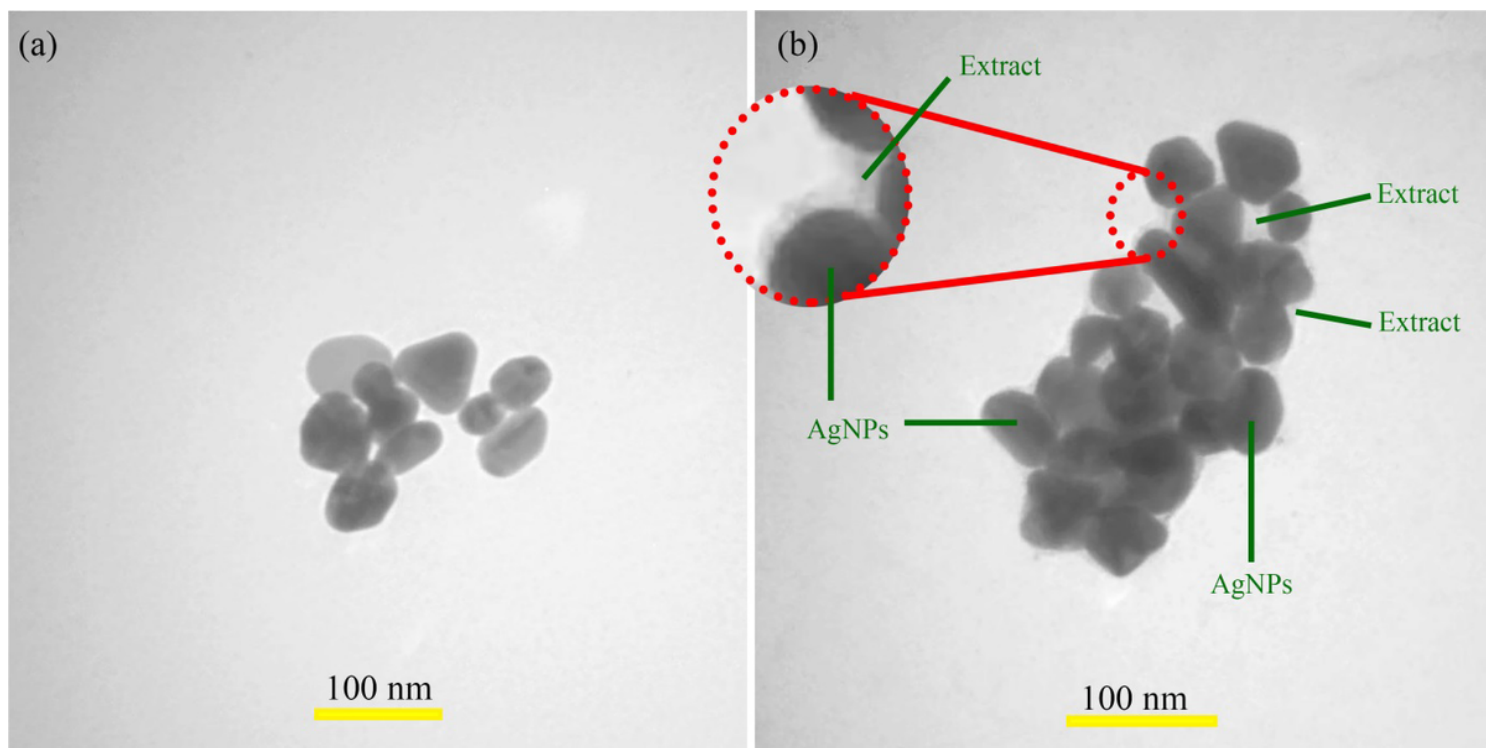


Figure 3

Transmission electron microscopy (TEM) image of (a) silver nanoparticles synthesized using chemical method and (b) synthesized silver nanoparticles using *Trachyspermum ammi* fruit extract.

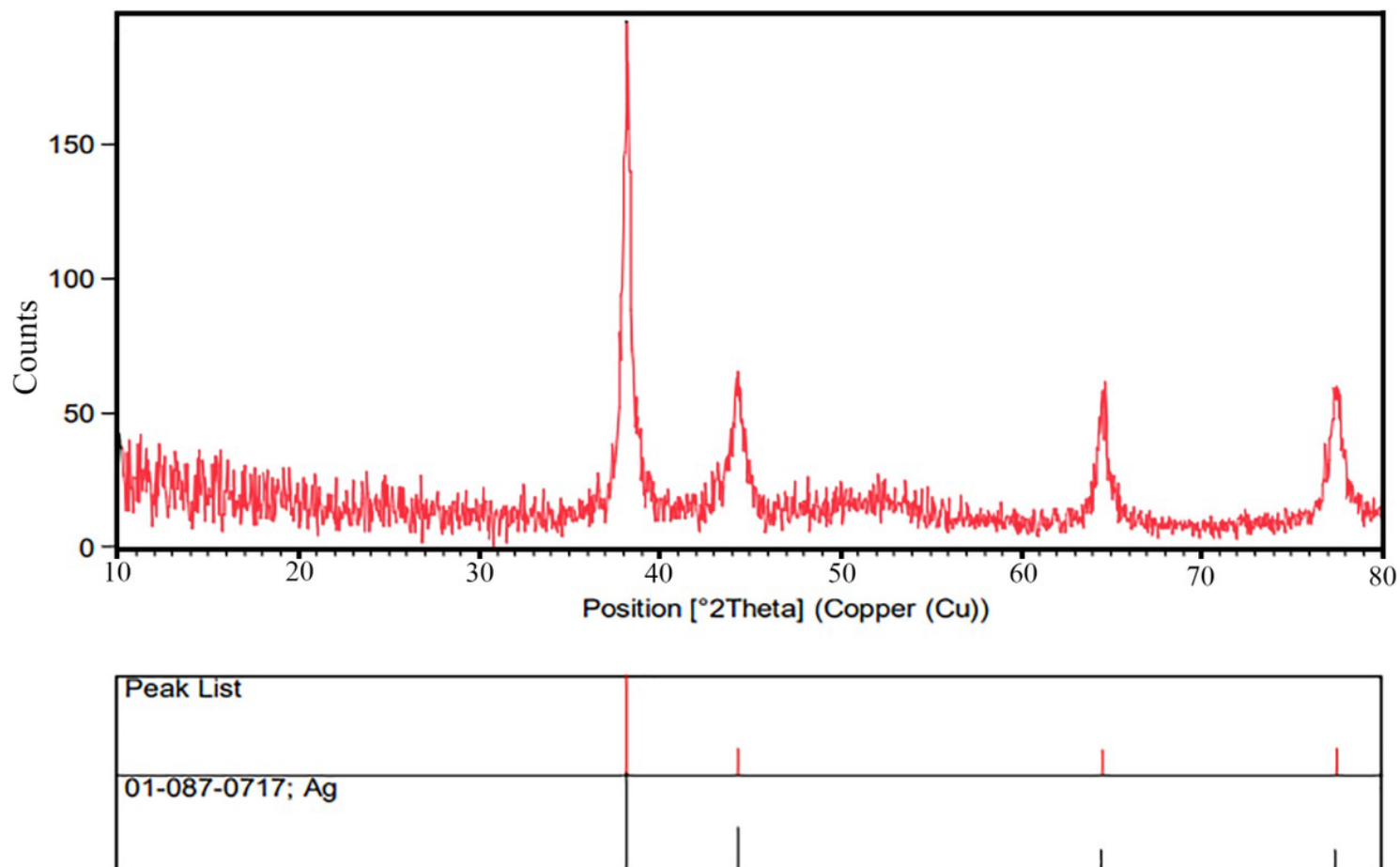


Figure 4

XRD pattern of biosynthesized AgNPs using *Trachyspermum ammi* fruit extract.

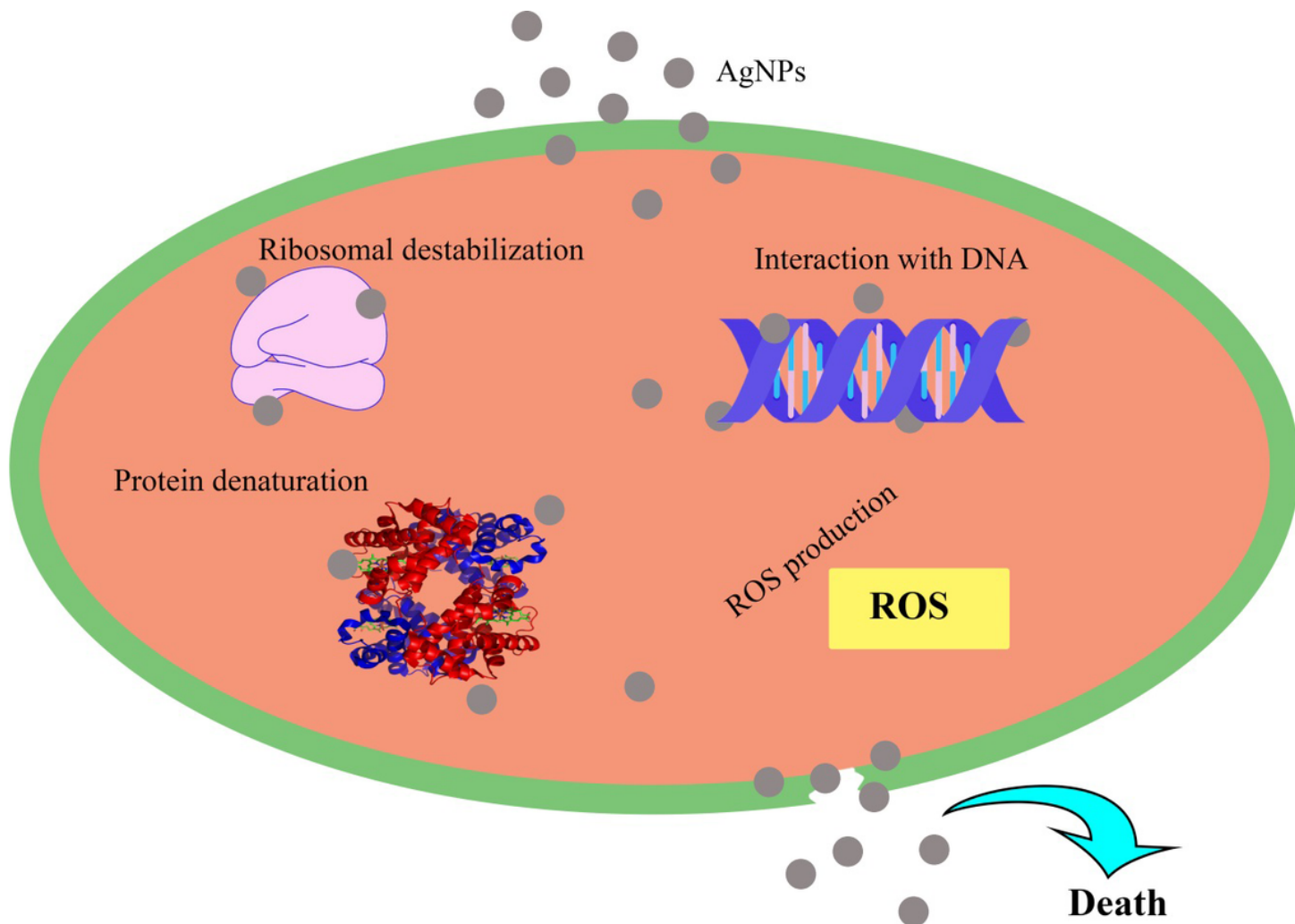


Figure 5

The antibacterial mechanism of silver nanoparticles.