Antibacterial activity of green gold and silver nanoparticles using ginger root extract

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Abstract
Recent studies demonstrated that the speed of synthesis, biocompatibility, and antimicrobial activity of gold (Au) and silver (Ag) metals is enhanced when biosynthesized in nano-sized particles. In the present study, Au- and Ag-based nanoparticles (NPs) were synthesized via a biological process using aqueous Ginger root extract and characterized by various spectroscopic methods. The NPs have hexagonal and spherical shapes. The average particle size for Au and Ag NPs was 20 and 15 nm, respectively. The dynamic light scattering (DLS) technique has shown that the zeta potential values of synthesized NPs were 4.8 and −7.11 mv, respectively. Gas chromatography–mass spectrometry (GC–MS) analysis of Ginger root extract revealed 25 compounds. The synthesized NPs showed significant activity against *Staphylococcus aureus* and *Escherichia (E). coli* in vitro, with IC50 and IC90 values for Au and Ag NPs, respectively, noted to be 7.5 and 7.3 µg/ml and 15 and 15.2 µg/ml for both bacterial strains. The protein leakage level was tremendous and morphological changes occurred in bacteria treated with biosynthesized NPs. These results suggest that the biosynthesized metallic NPs have the suitable potential for application as antibacterial agents with enhanced activities.

Keywords Green synthesis · Antibacterial agent · Ginger extract · Metallic nanoparticle

Abbreviations

| Abbreviation | Description |
|--------------|-------------|
| FTIR         | Fourier-transform infrared spectroscopy |
| MIC          | Minimum inhibitory concentration |
| NPs          | Nanoparticles |
| ROS          | Reactive oxygen species |
| TEM          | Transmission electron microscopy |

Introduction

Biological methods are low-cost, ecologically friendly, and straightforward strategies for synthesizing, manipulating, and using materials at 1–100 nm known as green nanotechnology [1]. In addition, the ability of nature to reduce possible dangers to the environment, human health, and preparation expenses for nanoparticles is referred to as green nanotechnology. Plants have garnered much attention from various biological sources to create nanomaterials. In plant extracts, alkaloids, terpenes, saponins, phenols, alcohols, and proteins are examples of phytochemicals that act as reducing and capping agents. The separated phytochemicals aid in improving the repeatability of nanomaterials with controlled size and morphology. These nanoparticles are bioactive and have several uses in pharmacological and biomedical research [2, 3].

Infectious diseases are one of the health threats to human society. Antibacterial drugs to control and treat infectious diseases are used everywhere. However, continued antibacterial drug using can lead to drug resistance [4]. In this condition, using the antibacterial drug to control and treat infectious diseases is not practical, so eliminating this problem...
requires developing new therapeutic agents [5]. Indeed, some problems with traditional antibiotics, such as low efficacy and drug resistance, are the most significant challenges. This urgent need led to considerable research efforts to develop, improve and design new antibacterial elements with a unique mechanism of action for use in clinical [4]. In the last decades, pharmaceutical companies identifying a few antibacterial agents to replace traditional antibacterial agents. Indeed, pharmaceutical science has been coping with this problem by modifying the existing antibacterial agents [6]. The metallic NPs have good antibacterial properties that can originate from their large surface area to volume ratio. Due to their extensive surface area, some metallic nanoparticles, such as Au and Ag, can better contact microorganisms and show effective antimicrobial properties compared to other NPs. Those nanoparticles are attached to the bacteria cell membrane and penetrate it. The bacterial membrane has sulfur-containing proteins, and the Au and Ag nanoparticles can interact with these proteins and the phosphorus-containing compounds in the cell. The nanoparticles’ attack the cell division and respiratory chain and finally lead to bacteria cell death. Those nanoparticles’ release cationic ions into the bacterial cells enhancing bactericidal activity [7].

There are several methods to synthesize Ag and Au NPs, including laser ablation, gamma irradiation, and the chemical agent as a reducing and capping agent [4]. The critical problems of these methods are expensive and the use of toxic chemical agents that are not safe for human health and the environment [8]. The green synthesis of NPs is one of the emerging fields in nanotechnology. Green synthesis NPs have shown high activity against the primary biofilm. Plants are used to synthesize NPs and have advantages over physical and chemical processes [9]. In recent years, NP synthesis using plant extracts has been increasing because these are available, environmentally friendly, easy to use, and have a wide range of secondary metabolites that act as a reducing agent [10].

Ginger (Zingiber officinale), Roscoe belonging to the family Zingiberaceae, is a perennial herb with thick tuberous rhizomes. Ginger extracts have antibacterial activity. Malu and co-workers show that materials such as n-hexane, ethyl acetate, and soxhlet in the ginger extract solution have antibacterial effects. In addition to having bactericidal activity, it can also inhibit bacterial growth [11]. E. coli and S. aureus are Gram-negative and Gram-positive bacteria, respectively, that E. coli causes can penetrate lymphocytes by certain bacteriophage and induces an inflammatory reaction with the host, causing bloody diarrhea [12]. S. aureus is the leading cause of food poisoning and surgical wound infection, which, together with epidermis syndrome, causes infections associated with medical equipment [13].

Gold and silver nanoparticles were synthesized with green and chemical synthesis methods to develop new antibacterial agents. For this purpose, Ginger root extract and citrate were used as reducing agents, respectively. The synthesized metallic NPs were characterized by dynamic light scattering (DLS), transmission electron microscope (TEM), Ultraviolet–Visible spectroscopy UV–Vis, Atomic absorption spectroscopy (AAS), and Fourier-transform infrared spectroscopy (FTIR). The antibacterial activity of synthesized NPs investigated with E. coli and S. aureus strains in vitro.

Materials and methods

Microorganisms

Standard strains of S. aureus (ATTC 25,923) and E. coli (ATTC 25,922) were purchased from the Iranian Research Organization for Science and Technology (IROST). These strains were cultivated in the nutrient broth medium and were incubated at 37 °C for 24 h. For further experiments, a small number of bacterial colonies were stored in Trypticase soy broth containing glycerol at − 70 °C.

Preparation of ginger extracts

Roots of ginger were purchased from the local market, the Islamic Republic of Iran, and frequently washed with ultra-pure deionized water. After shredding the ginger root, it dried and crashed into powder with the steel hammer. After that, 2 g of powder was mixed with 80 ml of ethanol and incubated at 40 °C for 24 h. Then for obtaining ginger extract, the solution was filtered with Whatman No.1 filter paper.

Green and chemical synthesis of gold and silver nanoparticles

For the green synthesis of Au NPs, 1 mL ginger root extract was added to a 50 mL boiling solution of Tetrachloroauric (III) acid trihydrate (HAuCl₄·3H₂O, 1 mM), and the boiling continued for 5 min. Then, the solution was kept undisturbed at room temperature until the colorless solution converted to a wine red color, which indicated the formation of Au NPs. For the chemical synthesis of Au NPs, HAuCl₄ solution was boiled, and then trisodium citrate dehydrate was added slowly into the boiling solution under stirring. A few minutes later, the color of the solution from light yellow converted to wine red.

For the green synthesis of Ag NPs, 1 ml of ginger extract was added with 100 silver nitrates (2 mM) boiling solution. The reaction mixture was kept undisturbed at room temperature until the colorless solution converted to reddish-brown color, which indicated the formation of Ag NPs. For the
chemical synthesis of silver nanoparticles, silver nitrate solution and trisodium citrate was used as a metal ion source and reduction agent, respectively. When the citrate sodium was added to the silver nitrate, the color of the solution converted from pale yellow to pale brown color. And finally, nanoparticles were purified by centrifugation.

**Gas chromatography–mass spectrometry analysis (GC–MS)**

Ginger extract was analyzed by a mass scientific trace 2200 (gas chromatography) system with a thermal Saturn mass selective detector (Varian Company). The machine was equipped with a TG-5MS (mass spectrometry) column (30 * 0.25 mm (5% phenyl) -methylpolysiloxane capillary column, film thickness * 0.25 µm, 220 °C temperature injector, and 250 °C temperature transfer line. The oven temperature was programmed as follows: initial temperature; 50 °C for 5 min and then increase four °C/min up to 250 °C. The gas carrier was He at a 1.0 ml/min flow rate. 1 µl sample was injected, and the ionization energy was 70 eV. Identifying individual components was based on their retention time and comparing their mass spectral pattern with standard library data. (National institute of standards and technology).

**Characterization of gold and silver nanoparticles**

A UV–Vis spectrophotometer (SPECTOR 250, Analytic Jena) monitored the reduction of gold and silver ions in the 350–800 nm wavelength range. Due to the evaluation of the concentration of green and chemical synthesized NPs, a solution of synthesized NP was diluted (1:10). Then, the amount of Au and Ag NPs was measured by atomic absorption spectroscopy (AAS) (NovaAA400, Analytic Jena Co). Also, transmission electron microscopy (TEM) (Zeiss Leo q06) operating at 200 kV accelerating voltage was used to determine the shape and size of the nanoparticles. For the sample preparation, ten microliters of aliquots of NPs solution were drop-casting onto a carbon-coated copper grid and then placed on a piece of paper to get rid of excess solvent. The average particle size, distribution, and stability of the gold and silver NPs were measured by the ELSZ-1000 zeta potential and particle sizer (Mastersizer 2000, Malvern, USA). For FTIR analysis, the powdered gold and silver NPs were recorded by FTIR spectrometer (Tensor 27, Bruker Co) over the 4000 – 400 cm⁻¹ frequency with 4 cm⁻¹ resolutions using a KBr pellet method.

**Antibacterial assay**

The antibacterial activity of chemical and green synthesized Au, and Ag NPs were performed by the good diffusion agar method. Standard strains of bacteria were subcultured on plates containing Muller Hinton agar using the pour plate method. Then the wells, which have 6 mm diameter, were punctured onto the agar plates, and 25 µg/ ml of Au and Ag NPs’ solution and aqueous plant extract were loaded into the wells. After 24 h of incubation, the inhibition of zone diameter around wells was measured. To compare the effectiveness of Au and Ag NPs and ginger extract against tested bacteria, we used Streptomycin (30 µg/ml). For evaluation, the minimum inhibitory concentration (MIC) was studied using a two-fold dilution method with the first test concentration of 30 µg/ml [14]. The minimum inhibitory concentration was calculated as the minimum dose of the NPs inhibiting the visual growth of the test cultures on the agar plates. The culture tests were conducted in triplicates.

**Intracellular protein leakage**

For this purpose, 30 µg/ml of NPs for 8 h at 37 °C were used to treat the bacteria cultures. After incubation, the bacteria were centrifuged at 5000 rpm for 10 min, and then supernatants were collected. The intracellular protein leakage was evaluated by assayed according to the method of Bradford (1976). The assay consisted of 1 ml of supernatant.

| Table 1 | Compounds of ginger extract were analyzed by GC–MS |
|---------|----------------------------------|
| NO:     | Type of Component | Area (%) | Time (min) |
| 1       | Chavicol            | 11.9     | 27.13      |
| 2       | Isopropyl           | 2        | 26.52      |
| 3       | ACETONITRILE        | 11.8     | 26.41      |
| 4       | Coronene            | 13.5     | 26.33      |
| 5       | Pentenamide         | 2.22     | 26.23      |
| 6       | Phosphate           | 1.27     | 26.07      |
| 7       | trans-Caryophyllene | 12.2     | 25.55      |
| 8       | Benzoic acid        | 1.10     | 25.42      |
| 9       | Homobrend           | 4.49     | 25.24      |
| 10      | Benzaldehyde        | 1.10     | 25.11      |
| 11      | Tricyclo            | 3.26     | 25.02      |
| 12      | EPOXYSPIRO          | 5.36     | 24.73      |
| 13      | Longiverbenone      | 3.34     | 24.57      |
| 14      | Phenanthrene carboxylic acid | 3.18  | 24.38      |
| 15      | Seneciphylline      | 1.91     | 23.21      |
| 16      | Cyclohexene         | 4.06     | 22.36      |
| 17      | TETRAHYDROQUINOLINE | 5.03     | 22.1       |
| 18      | Benzopyran          | 0.55     | 9.35       |
| 19      | 1H-Benzimidazole    | 0.79     | 8.14       |
| 20      | 2,3-Dimethylbenzofuran | 0.63  | 8.08       |
| 21      | 7-Methyl-1-indanone | 0.48     | 7.89       |
| 22      | Benzimidazole       | 0.56     | 7.8        |
| 23      | Triazolo            | 0.09     | 3.81       |
| 24      | Quinoxaline         | 0.37     | 3.55       |
| 25      | 5-ethyl-5-fluorobarbituric acid | 0.61 | 2.92      |
0.5 M NaOH (2 ml), and 0.1 N folin (0.1 ml) phenol reagent; absorbance of the solutions was read at 550 nm after 10 min.

**Statistical analysis**

Standard deviation (SD) was used for antibacterial and protein leakage assays. In this study, for protein leakage assay, the differences between treated and control groups’ data were assessed with Student’s t test. \( p < 0.05 \) was significant.

**Results**

**Phytochemical analysis**

GC–MS analysis for ginger extract showed 101 compounds (Table 1). These results revealed that the major compounds were Coronene (13.5%), trans-Caryophyllene (12.2%), Chavicol (11.9%), ACETONITRILE (11.8%), and EPOXYSPIRO (5.36%).

**Characterization of biosynthesized nanoparticles**

The visual examination or color change test was used to indicate the green and chemic synthesis of Au NPs (Fig. 1) and Ag NPs (Fig. 2), which confirmed that the reduction of metal ions to metal NPs results in a color change in the solution. After that, the UV–Vis spectrum was used to determine the stability and bio-reduction of metal NPs in the solution. In the present study, UV–Vis analysis revealed the maximum absorption peaks of green synthesized Au NPs and Ag NPs were at 523 and 432.5 nm, respectively (Fig. 2). Also, the amount of Au and Ag NPs was measured by atomic absorption spectroscopy (AAS), and results show that those amounts are 1.531 and 2.025 mg/L, respectively.

DLS method indicated that the average particle size for green synthesized Au and Ag NPs are 314 and 225 nm, respectively (Fig. 3). Also, the average particle size for chemical synthesized Au and Ag NPs are 42 and 27 nm, respectively. Zeta potential values give information about the stability of the NPs for green synthesized Au and Ag NPs values were −7.11 and 4.83 mv, respectively, which might Au NPs are given more suitable stability as the zeta potential of NPs with values > +25 mV or < −25 mV usually have a high degree of stability [15].

TEM techniques were used to study the morphology and sizes of Au NPs and Ag NPs (Fig. 4). TEM images of NPs showed the particles distributed individually in different shapes, such as hexagons and spheres, with sizes ranging from 15 to 25 nm for green synthesized Au NPs and less than 15 nm for green synthesized Ag NPs. Also, a TEM study has shown that sizes range for Ag NPs from 15 to 25 and 20 to 70 nm for chemical synthesized Au NPs.

FTIR spectrum (Fig. 5) of ginger root extract shows the band at 3441 cm\(^{-1}\), which is assigned to the O–H stretching of phenolic compounds, water, and fatty acids. The band 2933 cm\(^{-1}\) is set to C–H stretching of methylene group in esters, fatty acids, and aliphatic hydrocarbons, 1738 cm\(^{-1}\) is assigned to C = O stretching of aldehyde, esters, fatty acid, and ketones, 1620 cm\(^{-1}\) is set to (H–O–H) bending of water, 1517 cm\(^{-1}\) is due to C = C stretching of aromatic elements, 1462 cm\(^{-1}\) is assigned to C–O–H in-plane bending of with the use of trisodium citrate. c Green synthesis of Ag NPs using Ginger root extract. d Chemical synthesis of Ag NPs using trisodium citrate
fatty acids and other compounds, 1269 cm⁻¹ is set to C–O stretching of ester and fatty acid, and 1044 cm⁻¹ is due to C–O stretching of alcohols, phenols.

By comparing the infrared spectra of plant extract and green synthesized NPs (Fig. 5), it is observed that the intensity of the peaks at 3450 cm⁻¹ for Ag, 3435 in Au, 2928 cm⁻¹ for Ag, and 2928 cm⁻¹ in Au NPs, 1735 cm⁻¹ in Ag and 1738 cm⁻¹ in Au NPs, 1460 cm⁻¹ in Ag and 1384 cm⁻¹ in Au NPs, 1108 cm⁻¹ in Au, and 1107 cm⁻¹ in Ag in compare with plant extract spectrum has decreased/increased and then shifted to higher/lower wavenumbers. The band at 3450 cm⁻¹ is assigned to O–H stretching of water, 2928 cm⁻¹ is assigned to methyl symmetrical C–H bending of esters and band at 1107 cm⁻¹ is set to C–O stretching of carbohydrates, ester. The peak of 2928 cm⁻¹, related to the C-H stretch of aliphatic fatty acids, esters, and hydrocarbons in the plant root extract, became less intense and shifted to 2923 cm⁻¹. The band at 1620 cm⁻¹ assigned to H–O–H bending of water became less severe and moved to 1628 cm⁻¹. The band at 1517 cm⁻¹ assigned to C = O stretching of esters, aldehydes, ketones, and fatty acids disappeared. Furthermore, the peak at 1383 cm⁻¹, which is set to methyl symmetrical C–H bending of esters, became sharp.
Antibacterial activity

To prove the antibacterial activity of the extract, this study first evaluated the antibacterial activity of ginger extract root (Table 2). Better than chemically synthesized once, the green synthesized Au and Ag NPs have shown acceptable bacterial growth inhibitory and led to a mean zone of inhabitation of *S. aureus* and *E. coli* (Table 3). The **IC**<sub>50</sub> and **IC**<sub>90</sub> values for Au NPs and Ag NPs were 7.5 and 7.3 µg/ml and 15 and 15.2 µg/ml for both bacterial strains, respectively (Figs. 6, 7).

Protein leakage

The total protein leakages were quantified upon treatment with green synthesized AuNPs and Ag NPs. The result showed that protein leakage for treated bacterial cells with green and chemical synthesized NPs was higher when compared to the untreated groups. Still, the amount for Ag NPs was more elevated than for Au NPs (Table 4). This indicates that NPs disrupted the bacteria cell membrane and enhanced the pin leakage.
Earlier studies showed that the gr extract contains n. hexane, ethyl acetate, and soxhlet, which those compounds have an antibacterial effect and also inhibit the growth of the bacterial biofilm [16]. In the present study, chemical composition of ginger root extract is made up of gingerol, shogaols, zingerone, paradol, and starch. The rhizome, consisting of 6-gingerol and 6-shogaol, is the principal source of gingerol, and shogaol, as previously reported, was found in high levels in ginger extract [17, 18]. The essential compounds responsible for reducing Au and Ag ions to NPs are water-soluble ingredients in ginger root extract. Ginger holds chemical compounds like oxalic acid, ascorbic acid, phenylpropanoids, and zingerone. The Au NPs and Ag NPs can be reduced by the ascorbic acid and oxalic acid present in the ginger root extract. The possible stages of the formation of NPs from ginger extract during the chemical reaction include nucleation, condensation, surface reduction, and stabilization, as previously described [19, 20]. Results indicated that during NPs synthesis, the biodegradable components of root extract could act as reducing and capping agents, thus promoting the formation of NPs while inhibiting their aggregation via increasing their stability [21]. This finding also presents the potential of plant root extract as biological “nano-factories” providing non-toxic reducing-capping agents and offering a clean, highly tunable, and environmentally benign method for producing desired NPs [22]. Although the idea of utilizing living plants is revolutionary; nevertheless, the difficulty of purification of the intracellularly formed NPs directed studies to use the extracts of plants for extracellular syntheses of NPs [23]. The development of nanotechnology has given humankind new opportunities and a broader view of what it can do.
by modifying matter at the nanoscale [24]. Using various nanomaterials has consequently changed both the industrial and biomedical fields. Due to their high surface-to-volume ratio, strong reactivity, size distribution, morphology, surface charge, surface chemistry, capping agents, metallic, and metal-oxide nanostructures have demonstrated tremendous potential [25, 26].

The UV–Vis analysis peaks indicate that green NPs were synthesized and consistent with the results of previous studies that have shown the range of 400–450 nm for Ag NPs and the range of 500–550 nm in the case of Au NPs [27]. According to Zeta potential data, the surface charge of Ag NPs is more favorable than Au NPs; this might enhance the better binding to the outer membrane of Gram-negative bacteria [28]. Also, Zeta potential for chemical synthesized Au, and Ag nanoparticles values are 0 and -10.1mv, respectively. Elia et al. synthesized the Au nanoparticle from P. granatum and characterized them using DLS spectroscopy, in which the particle size range was 34–312 nm [29]. Inconsistency with our study, Sujitha et al. [30] reported that the lower concentration of the plant extract leads to Au NPs with a lower Zeta potential value. These findings reveal that biological extracts from plants’ roots provide the method for producing NPs with a broad range of sizes [4], and since they are originally natural, NP’ covering the NP’s surface improves their biocompatibility for in vivo applications [22].

Addressing the TEM results, as previously studied, the plant extract ratio, type of components, and the initial metal salt in the reaction medium affected the Au NPs’ size and shape [31]. Similarly, a study showed the synthesis of NPs using a marigold flower, where TEM analysis showed spherical and hexagonal shape particles in the range of 10 to 90 nm [32]. Thus, the TEM and DLS studies gave similar results for the size range of the NPs.

The FTIR analysis indicated the presence of phenolic groups, which are suggested to be responsible for reducing
silver ions [33]. The presence of other FTIR-associated peaks confirmed that the NPs were covered by ginger root extract with functional groups such as carboxylic acid, ketone, aldehyde, and other functional groups. The presence of these functional groups is due to the bio-stability of the NPs. It confirms that NPs synthesized from ginger root extracts are stabilized by phytoconstituents through functional groups [34, 35].

Prakash Patil groups synthesized Ag NPs using the flower extract of Madhuca Longifolia as a reduction agent and synergic effect. Synthesized NPs show potential antibacterial activity against Gram-negative and Gram-positive bacteria. Results indicate that the Madhuca Longifolia flower is a good source for NPs synthesis. According to obtained data, synthesized Ag NPs are applicable as an antibacterial agent in therapeutics. This can be explained by the antibacterial activity being due to the change in membrane permeability [36]. After entering the cytoplasm, Ag NPs induce reactive oxygen species (ROS) production by binding the phosphate group of effector molecules, disturbing the protein synthesis and thus, causing bacterial growth inhibition or killing [37]. Due to the high prevalence, antibiotic resistance, and pathogenicity of S. aureus and E. coli, we studied them. These two pathogens are the causative agents for several infections,

Table 2  Antibacterial activity of ginger root extract

| Concentration (µg/ml) | 500 | 250 | 125 | 62.5 | 31.25 | 15.62 | 7.81 | 3.90 |
|-----------------------|-----|-----|-----|------|-------|-------|------|------|
| S. aureus             | −   | −   | −   | −    | +     | +     | +    | +    |
| E. coli               | −   | −   | +   | +    | +     | +     | +    | +    |
Table 3 Antibacterial activity of green and chemical synthesized Ag/Au NPs

|                | 27.72 | 13.86 | 6.93  | 3.46  | 1.73  | 0.86  | 0.43  | 0.21  |
|----------------|-------|-------|-------|-------|-------|-------|-------|-------|
| Ag (µg/ml)     |       |       |       |       |       |       |       |       |
| Concentration  |       |       |       |       |       |       |       |       |
| S. aureus      | −     | −     | −     | +     | +     | +     | +     | +     |
| E. coli        | −     | +     | +     | +     | +     | +     | +     | +     |
| Green-Ag       |       |       |       |       |       |       |       |       |
| S. aureus      | −     | −     | −     | −     | −     | −     | +     | +     |
| E. coli        | −     | −     | −     | −     | −     | −     | −     | +     |
| Au (µg/ml)     | 17.95 | 8.97  | 4.48  | 2.24  | 1.12  | 0.56  | 0.28  | 0.14  |
| Concentration  |       |       |       |       |       |       |       |       |
| S. aureus      | −     | +     | +     | +     | +     | +     | +     | +     |
| E. coli        | +     | +     | +     | +     | +     | +     | +     | +     |
| Green-Au       |       |       |       |       |       |       |       |       |
| S. aureus      | −     | +     | +     | +     | +     | +     | +     | +     |
| E. coli        | +     | +     | +     | +     | +     | +     | +     | +     |

Fig. 6 Antibacterial activity of chemical and biosynthesized silver NPs

(a) Green Ag – E. coli
(b) Ag – E. coli
(c) Green Ag – S. aureus
(d) Ag – S. aureus
such as endocarditis, urinary tract infection, osteomyelitis, and septicemia [38, 39].

Metal NPs, especially once having a relatively large size/surface ratio or smaller than 20 nm, act as destroyers of the cell membrane by binding to cells, causing structural alterations and eventually the loss of the semi-permeability of the membrane [9]. Studies have shown that bacterial cell membrane disruption by Psidium guajava leaf extracts agrees with this study’s result [40]. The synthesized NPs exhibited pronounced antibacterial activities on *S. aureus* and *E. coli*. Similar to these findings, Janaki et al. showed that zinc oxide nanoparticle (ZnO NPs), synthesized using ginger extracted root, has an efficient antimicrobial activity [41]. In the other study, Kumor and co-workers utilized ginger extract green synthesized gold NPs and evaluated their blood compatibility. The result showed that biosynthesized NPs are suitable vectors for medical applications [17]. These findings explain the possible antibacterial actions of green synthesized NPs: (i) may be due to DNA damage, (ii) protein synthesis inhibition and denaturation, and (iii) formation of free radicals causing cell wall damage [42] (Fig. 8).

Table 4 Quantification of protein leakage level in NPs treated bacterial species

| Bacteria    | Control (%) | G-Ag NPs treated cell (%) | G-Au NPs treated cell (%) |
|-------------|-------------|----------------------------|---------------------------|
| *S. aureus* | 7.18 ± 2.5  | 10.23 ± 1.77               | 10.07 ± 1.71              |
| *E. Coli*   | 11.01 ± 0.69| 15.41 ± 0.37               | 13.19 ± 0.23              |

* a p < 0.05, Experiment performed in triplicates and statistical analysis using a student t test.
Conclusion

The present study reports the chemical and green synthesis of Au and AgNPs using citric acid and aqueous root extract of ginger, respectively. Biosynthetic NPs were spherical and hexagonal, with an average size of ≤ 100 nm. The biosynthesized Au and Ag NPs showed an excellent antibacterial effect against *S. aureus* and *E. coli* in comparing chemical synthesized NPs. Furthermore, the possible antibacterial mechanisms were studied by protein leakage. This study proves that biosynthesized Au and Ag NPs could be used as an alternative therapy to control and eliminate the infection caused by *E. coli* and *S. aureus*.

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Authors’ contributions  MY: a significant contributor to doing and writing the manuscript. MA, HD-M, and MA: collaborated on the thesis that resulted in the paper. AA and MM: designed and supervised the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials  The corresponding authors’ data supporting this study’s findings are available upon reasonable request.

Declarations

Conflict of interest  The authors declare that they have no conflicts of interest in the publication.

Ethics approval  Not applicable.

Consent to participate  All authors consent to participate.

Consent for publication  All authors consent for publication.

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Fig. 8 A possible mode of antibacterial action of green synthesized NPs
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