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Antimicrobial Activity of Silver and Gold Nanoparticles Biosynthesized Using Ginger Extract.

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ABSTRACT

Biofabricated metal nanoparticles is under exploration due to wide biomedical applications and research interest in nanotechnology. Biosynthesis of silver and gold nanoparticles was achieved by reduction of silver nitrate ($AgNO_3$) and chloroauric acid ($HAuCl_4$), respectively, using ginger rhizome (*Zingiber officinale*) extract. The biosynthesized silver (AgNPs) and gold (AuNPs) nanoparticles were characterized by using UV–Vis spectroscopy and TEM. The UV–vis spectrum showed maximum absorption peak at 450 and 547 nm corresponding to the plasmon absorbance of AgNPs and AuNPs, respectively. The AgNPs were generally found to be spherical in shape with diameter of 5-50 nm, whereas AuNPs were found to be in the range of 5-35 nm. The antibacterial potential of the biosynthesized nanoparticles was measured by the Kirby-Bauer method against 5 Gram-negative and 5 Gram-positive bacteria. AuNPs did not show any inhibition potential. However, AgNPs showed antimicrobial activity against, except *Bacillus cereus* TUB8, which observed complete resistance to AgNPs and 1 µg oxacillin. AgNPs displayed synergistic improving effect when it was combined with oxacillin (1 µg) or tetracycline (30 µg) in ready to use disks. In conclusion, Ginger rhizome water extract could be considered as a potential bioreductant for the synthesis of AgNPs and AuNPs. AgNPs showed high antimicrobial potential against Gram-positive and Gram-negative bacteria. For the antibiotic resistant bacteria, the combination of antibiotics with AgNPs could improve its susceptibility to these antibiotics.

Keywords: Silver and gold nanoparticles, Nanobiotechnology, Plant extract, Antimicrobial, antibiotics

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INTRODUCTION

The fields of application for metal nanoparticles are wide ranging. They play a major role in materials technology. The great expectations we place on today's modern nanoparticles containing materials is based on the hope that the different material properties such as conductivity, weight, stability, flexibility, heat resistance etc. can be specified independently from one another. Several techniques of chemical and physical methods such as sol-gel process, micelle, chemical precipitation, hydrothermal method, pyrolysis, chemical vapor deposition, have been established for synthesizing of silver and gold nanoparticles [1]. The bio-protocol is the most important and ecofriendly production method. Therefore, there is a growing need to develop eco-friendly processes for nanoparticles synthesis without using toxic chemicals. Biosynthetic methods employing either microorganisms or plant extracts have raised as a simple and viable alternative to chemical synthetic procedures and physical methods. The use of plant materials for the synthesis of nanoparticles could be more advantageous, because it does not require elaborate processes such as intracellular synthesis and multiple purification steps or the maintenance of microbial cell cultures [2]. Biosynthetic processes for nanoparticles would be more useful if nanoparticles were produced extracellularly using plants or their extracts and in a controlled manner according to their size, dispersity and shape. Plant use can also be suitably scaled up for large-scale synthesis of nanoparticles [3]. Noble metals, especially Au and Ag, have been extensible tested for the biosynthetic process assisted by plants, in order to obtain metallic nanoparticles with control over shape and size. Various plants have been used for efficient and rapid extracellular synthesis of silver and gold nanoparticles such as Alfalfa [4], and *Aloe vera* [5]. Huang, Li [6] demonstrated that, sundried *Cinnanonum camphora* leaf extract, which are novel, greener, and cost-effective, can be used as capping as well as reducing agents in the synthesis of AuNPs and AgNPs. Fruit extract of *Emblica officianalis* have been explored for synthesis of gold and silver nanoparticles. Their Phase Transfer and Transmetallation in an organic solution have been studied [7]. Furthermore, Synthesis of plant mediated silver nanoparticles using *Carica papaya* fruit extract and evaluation of their anti microbial activities have been reported [8]. Gold nanotriangles biologically synthesized using leaf extract of *Parthenium hysterophorus* and their application in vapor sensing have been studied [7]. Parthenium leaf extract of *Diopyros kaki*, a novel approach towards weed utilization for synthesis of silver nanoparticles [9]. The potential plants as biological materials for the synthesis of nanoparticles is yet to be fully explored. The uncontrolled use of antibiotics led to increase the drug-resistant bacteria worldwide; therefore, finding alternative antibacterial agents is required. Silver nanoparticles has been suggested as antimicrobial agents in different fields to control drug-resistant bacteria [10, 11]. The present study was designed to biosynthesis and characterizes of silver (AgNPs) and gold (AuNPs) nanoparticles using aqueous ginger rhizome extract. Antimicrobial potential of biosynthesized AgNPs and AuNPs was also studied.

MATERIALS AND METHODS

Preparation of plant extracts

Ginger (*Zingiber officinale*) rhizomes were purchased from local market, Taif, KSA. Rhizomes were washed with distilled water to remove dirt and soil. Ginger rhizomes were cut into small pieces, and dried in a vacuum oven for 3 h. A portion of 25 g was crushed in an electric blender with adding 200 mL of Milli-Q water during crushing. The extract was stirred 1 h, and filtered using a Whatman No 1 filter paper. The extract was stored at 4°C until further use.

Synthesis of silver and gold nanoparticles

For the synthesis of silver nanoparticles, 90 ml of 1mM AgNO3 (in Milli-Q water) was taken in a sterile reaction bottle and 10 ml of aqueous plant extract was added to it. The solution was mixed well and kept in a rotator shaker for overnight at 37 oC. As a result, a brown color solution was formed, indicating the formation of silver nanoparticles. It showed that aqueous silver ions could be reduced by aqueous extract of plants part to generate extremely stable silver nanoparticles in water [9]. Similarly for the bioreduction of Au (III) into the Au (0), 10 ml of plant extract was added to 90 ml of 10^{-3} M $HAuCl_4$ solution. After the addition of leaf extracts, both the solutions were kept in the incubator at 37°C, it showed a color change from yellow to bright ruby red which signed to gold nanoparticles formation

Characterization of silver and gold nanoparticles

Visual observations and UV–Vis spectra analysis

The biologically synthesized of silver and gold nanoparticles using ginger extract were characterized by UV-Vis spectroscopy (Perkin Elmer, Lambda 25) instrument scanning in the range of 200-900 nm, at a resolution of 1 nm [12, 13]. All Samples were prepared by centrifuging an aliquot of plant extract filtrate (1.5 mL) at 10000 rpm for 10 min and diluted 10-fold for all experiments involving measurement of UV–vis spectra. $AgNo_3$ or $HAuCl_4$ solution without addition of plant extract was used as a control throughout the experiment.

Transmission electron microscopy

Samples of silver and gold nanoparticles for transmission electron microscopy (TEM) analysis were prepared on carbon-coated copper TEM grids. Studies of size, morphology and composition of the nanoparticles were performed by means of transmission electron microscopy (TEM) operated at 120 kV accelerating voltage (JTEM-1230, Japan, JEOL) with selected area electron diffraction (SAED). Finally, the obtained images were processed using the software ImageJ. ImageJ developed at the National Institutes of Health (NIH), USA is a Java-based public domain image processing and analysis program [14].

Antimicrobial potential of AgNPs and AuNPs

Ten bacterial strains; 5 Gram-negative and 5 Gram-positive bacteria were used for studying the antimicrobial potential of the biogenic AgNPs and AuNPs. These strains have been previously isolated from slime layer of microbial biofilms in drinking water network in Taif City, KSA [15]. Antimicrobial potential was assayed by Kirby Bauer disc diffusion as described by Concepcion, Verzosa [10]. Easy-to-use Thermo Scientific™ Oxoid™ Blank Antimicrobial Susceptibility Disks (6 mm) were saturated with AgNPs or AuNPs (10 µg/mL) and then evaporated until dryness. Disks were placed equidistantly on the MHA plate, which was swabbed with the test organism. Plates were incubated at 35 °C for 24 h. Antimicrobial potential was subsequently assessed by measuring the zone of inhibition (in millimeters) around each disk. Inhibitory action was categorized according to the zone of inhibition (ZI) as described by NCCLS [16]. These categories were: Strong inhibitory action (++++), ZI >/=22mm; Complete inhibitory action (+++), ZI =18-21mm; Partial inhibitory action (++), ZI =14-17mm, Slight inhibitory action (+), ZI </=13mm or No inhibitory action (-), ZI=0. All experiments were repeated in triplicates and data were averages.

Synergistic effect between AgNPs and antibiotics

The synergetic effect of AgNPs and five common antibiotics was investigated. These antibiotics were Gentamicin (CN;10 µg), Norfloxacin (NOR; 10 µg), Oxacillin (OX; 1 µg), Tetracycline (TE; 30 µg), and Vancomycin (V; 30 µg). As a control antibiotics were tested in the absence of AgNPs. For studying the synergetic effect of AgNPs antibiotic disks were saturated with 20 µL AgNPs (10 µg/mL). Disk of each set were placed on MHA plate inoculated with the tested bacterium. The zone of inhibition was measured after incubation at 35 °C for 24 h.

RESULT AND DISCUSSION

Characterization of silver and gold nanoparticles

In this work, biosynthesis of AgNPs by plant extract was described. Visual observation of the plant extract filtrate incubation with silver nitrate at room temperature showed a color change from colorless to yellowish brown whereas no color change could be demonstrated in AgNO3 alone (Fig. 1). It is well known that silver nanoparticles exhibit yellowish brown color in aqueous solution due to excitation of surface Plasmon vibrations in silver nanoparticles [17]. UV-vis spectrum of the biosynthesis AgNPs is shown in Fig. 1. Surface plasmon peak observed at 450 nm. It may be due to the excitation of Surface Plasmon Resonance (SPR) effect and reduction of $AgNO_3$ [18]. On the other hand, visual observation of the plant extract incubated with chloroauric acid at room temperature in dark showed a color change from yellow to bright ruby red. This color change indicated the gold nanoparticles formation due to the excitation of surface plasmon vibrations, which is the characteristic for gold nanoparticles and provides a convenient spectroscopic signature of their

formation [19]. However, no color change could be demonstrated in plant extract without chloroauric acid (Fig 1). UV–Vis absorption spectroscopy was also used to measure the biosynthesis of gold nanoparticles, a well-defined absorption peak at ca. 547 nm appears in Fig. 1 that corresponds to the wavelength of the SPR of gold nanoparticles [19].

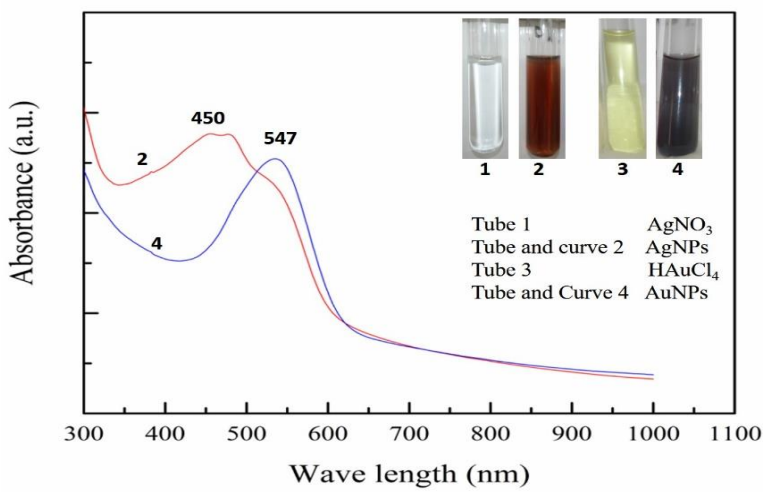

Figure 1: The visualization of color change due to formation of AgNPs ad and AuNPs and their absorption spectra.

TEM analysis

For transmission electron microscopy (TEM) study, a drop of the AgNPs or AuNPs solution was deposited onto a TEM grid which was coated with carbon support film. After drying, this grid was imaged using TEM. Figure 2 showed a representative TEM image, with a size distribution on its right side. The TEM image of AgNPs and their size distribution showed that the particles were spherical, and monodispersed with average diameter of 5 – 50 nm. On the other hand, TEM analysis of AuNPs presented in Figure 3 showed spherical shape and reasonably mondispersed AuNPs with size range of 5 – 35 nm . The presence of spherical, mondispersed and small particles in TEM image is in accordance with the UV–Vis spectral study [12, 19].

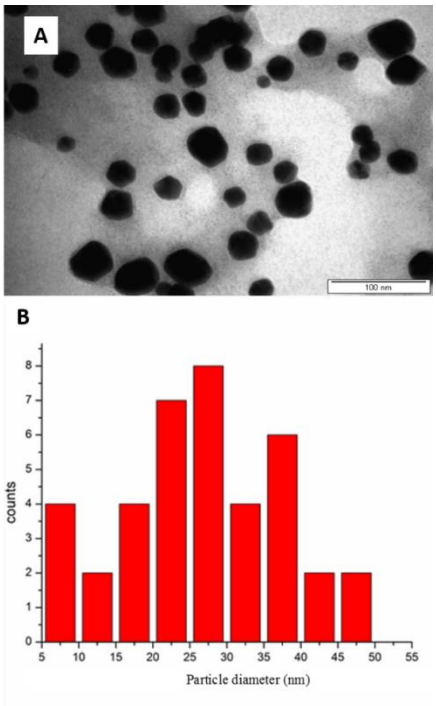

Figure 2: TEM image (a) and particle size distribution (b) of the silver nanoparticles produced by the reaction of 1 mM aqueous $AgNO_3$ solution with ginger rhizome extract at pH 7.

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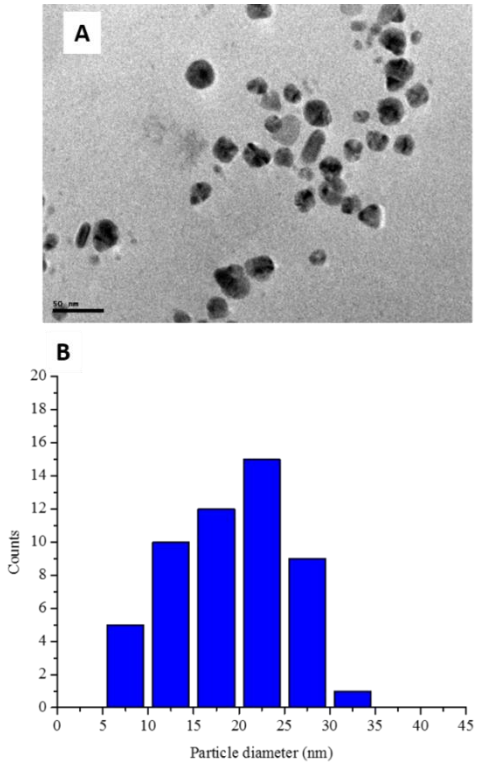

Figure 3: TEM image (a) and particle size distribution (b) of the gold nanoparticles produced by the reaction of 1 mM aqueous $HAuCl_4$ solution with ginger rhizome extract at pH 7.

Antimicrobial potential of AgNPs and AuNPs

Table 1: Antimicrobial potential of the biosynthesized AgNPs and AuNPs

Strains	Antimicrobial potential of	
	AgNPs	AuNPs
Micrococcus flavus TUB1	+	-
Micrococcus luteus TUB3	+	-
Aerococcus viridans TUB6	+	-
Bacillus cereus TUB8	-	-
Geobacillus stearothermophilus TUB9	+	-
Escherichia coli TUB17	+++	-
Aeromonas punctata TUB18	+	-
Aeromonas hydrophila TUB19	+++	-
Aeromonas hydrophila TUB20	+++	-
Aeromonas hydrophila TUB21	+++	-

Inhibitory action was categorized according to the zone of inhibition (ZI) as described by NCCLS [16]. Strong inhibitory action (++++), ZI >/=22mm; Complete inhibitory action (+++), ZI =18-21mm; Partial inhibitory action (++), ZI =14-17mm, Slight inhibitory action (+), ZI </=13mm or No inhibitory action (-), ZI=0.

Generally, AuNPs did not showed any antimicrobial potential against all strains (Table 1). On the other hand, AgNPs (10 µg/mL) displayed antimicrobial potential against all tested Gram-negative. Complete inhibitory action (+++) was recorded against *Escherichia coli*, and three different strains of *Aeromonas hydrophila* (Table 1). Previous studied has demonstrated the antimicrobial activity of AgNPs against *Escherichia coli* [11, 20] and *Aeromonas hydrophila* [21, 22]. However, slight inhibitory action (+) was obtained when *Aeromonas punctate* has been tested. This result indicated that, *A. punctate* as an environmental isolate, it may be adapted with the presence of high concentration of heavy metals. In this respect, Sudheer Khan, Bharath Kumar [23] stated that *A. punctate* isolated from sewage has showed tolerance to 200 µg/ml AgNPs. Slight inhibitory action (+) of AgNPs was observed against the tested Gram-positive strains, except *Bacillus cereus* TUB8 (Table 1). This result indicated that, treatment with AgNPs was more effective against Gram-negative bacteria compared with Gram-positive bacteria. This finding was in harmony with those mentioned

by [24, 25]. They stated that inhibition of Gram-positive bacteria is generally more difficult to obtain with AgNPs alone, this may be attributed to the nature of Gram-positive bacterial cell wall. [24]. Interestedly, *Bacillus cereus* TUB8 displayed complete resistance to AgNPs. Therefore, this strain was selected for examination of the synergistic effect between AgNPs and five different antibiotics.

Synergetic effect between AgNPs and antibiotics

Synergistic effect between AgNPs and five different antibiotics was studied against the AgNPs-resistant *B. cereus* strain. This strain showed slight and complete resistance to 30 µg of tetracycline and 1 µg oxacillin, respectively (Fig 4 A), while it was susceptible to treatment with gentamicin (CN;10 µg), norfloxacin (NOR; 10 µg) and vancomycin (V; 30 µg). No visible change in the antibiotic efficiency was obtained when AgNPs has been added to gentamicin, norfloxacin, and vancomycin disks. However, considerable improvement in the tetracycline and oxacillin actions was obtained when AgNPs were incorporated into the antibiotic disks (Fig 4 B). This interesting finding was in harmony with those demonstrated by Fayaz, Balaji [11]. They stated that, the antibacterial activities of some antibiotics including ampicillin, kanamycin, erythromycin, and chloramphenicol have been enhanced in the presence of AgNPs against Gram-positive and Gram-negative bacteria. They added also that, the highest enhancing effect has been observed for ampicillin, one of the β-lactam antibiotic group to which oxacillin (used in the present study) is also considered as a member of this group. Generally, the obtained result demonstrated that the combination of antibiotics with AgNPs have better antimicrobial potential.

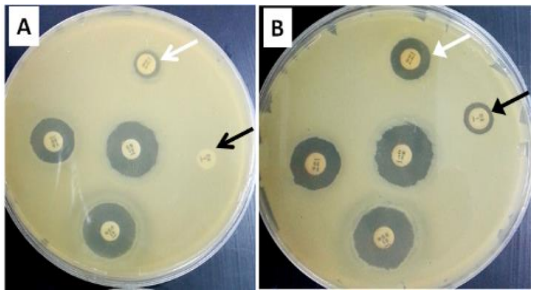

Figure 4: Synergetic effect between AgNPs and five antibiotics against *B. cereus* TUB8 (A), antibiotic disks were saturated with 20 µL AgNPs (10 µg/mL). Antibiotic susceptibility of *B. cereus* TUB8 in the absence of AgNPs was measured as a control (B). Improving effect of AgNPs on the action of oxacillin (black arrows) and tetracycline (white arrows) against *B. cereus* TUB8 was demonstrated.

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