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ORIGINAL RESEARCH PAPER

Antileishmanial and antibacterial activities of biologically synthesized silver nanoparticles using Alcea rosea extract (AR@AgNPs)

Zahra Hashemi, Mina Shirzadi-Ahoodashti, Mohammad Ali Ebrahimzadeh*

Pharmaceutical Sciences Research Center, Hemoglobinopathy Institute, and School of Pharmacy, Mazandaran University of Medical Sciences, Sari, Iran.

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ABSTRACT

Recent developments in nanotechnology lead to draw scientist's interest in green synthesis nanoparticles because of their importance in all fields of sciences. This paper is an overview of Ag nanoparticles biosynthesis (AgNPs) by aerial part of Alcea rosea extract. Synthesis procedures were described, with no stabilizers or surfactants. The synthesized AR@AgNPs were characterized using scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDS), X-ray diffraction analysis (XRD), and UV-Vis analysis. The UV-Vis spectrum of AR@AgNPs shown a characteristic surface plasmon resonance (SPR) peak at 425 nm. Scanning electron microscope revealed a spherical shape with a diameter range of 10-30 nm. Energy-dispersive X-ray spectroscopy analysis demonstrated the peak in the silver region confirming the presence of elemental silver. Evaluation of the antibacterial and antileishmanial activity of biosynthesized silver nanoparticles was performed. AR@AgNPs exhibit effective antibacterial activity against seven ATCC strains of bacteria and eight strains of drug-resistant bacteria. Also, their activity against leishmaniasis was studied on both promastigotes and amastigotes.

Keywords: *Green synthesis, Ag nanoparticles, Alcea rosea, antileishmanial, antibacterial.*

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INTRODUCTION

Nanotechnology can be introduced as the knowledge of design, synthesis, characterization, and application of materials and devices with the smallest useful organization [1]. The size of nanomaterial is defined almost at the nanometer scale or one billionth of a meter. Attention to individual molecules and interacting groups of molecules correlated to the bulk macroscopic properties becomes essential at these levels, allowing control over the chemical and physical characterization [2]. So, nanoscience is nearly an area of all science branches that involved chemistry, engineering, physics, medicine, and biology affected by nanomaterial. The nanoscale materials display different important roles in preventing, diagnosing, and treating various sorts of diseases. A study has demonstrated interesting biological properties of nanoscale materials that could be applied as new pharmacological and treatments. [3]. Bacterial infections are increasing in the human life system. These infections are affected by diverse strains of bacteria resistant to conventional antibiotics, and there is a vital need for the development of novel therapeutic platforms. Infections caused by protozoa of the genus Leishmania are a major worldwide health problem,

* Corresponding Author Email: *Zadeh20@gmail.com*

causing significant morbidity and mortality in Asia, Africa, and Latin America. Cutaneous leishmaniasis (CL) is a neglected tropical disease (NTD) caused by Leishmania spp. The condition is transmitted to humans by the bite of infected female phlebotomine sandflies. Several species of Leishmania cause the disease. It is estimated that the actual number of infected cases to be 6- to 10-fold higher than that reported. Due to host susceptibility, incomplete treatment duration, route of drug administration, and Leishmania species, healing results may be varied [4]. Various types of nanomaterials such as titanium, zinc, gold, copper, magnesium, and silver have been reported, but AgNPs have proved to be most effective as it has very good antimicrobial efficacy against bacteria, parasite, viruses, and other eukaryotic micro-organisms [5-7]. Due to the broad-spectrum antimicrobial activities of AgNPs, it could be future alternatives to current antimicrobial agents. The metal nanoparticles have been prepared by different techniques like physical, chemical, irradiative, photochemical, electrochemical, and biological methods. Although most of the methods effectively synthesis pure nanoparticles, they can be dangerous to the environment and costly. Among different methods of preparing AgNPs, the biological method as green synthesis is environment-friendly, simple, cost-effective, and can be applied for large-scale synthesis. Due to the elimination elaborate procedure of maintaining cell cultures via plant extract, this method is superior to other techniques [8, 9]. Different bio-molecules in the extract of the plant play a fundamental role in reducing, capping, and stabilizing agents on the surface of nanoparticles [10]. Genus *Alcea* is a part of ornamental promising medicinal plants. It belongs to the family Malvaceae; it includes approximately 60 species, primarily of the East Mediterranean region. *Alcea rosea* (*A. rosea)*, is widely cultivated in Egypt's gardens as an ornamental plant and has a great history of folkloric medicinal uses as all parts of the plant have been used in traditional medicine. Different biological activities have been reported for *A. rosea* such as antiurolithiatic, immunomodulatory, Analgesic, Anti-inflammatory, antiulcer, anticancer, and cytotoxic activities [11].

Recently, we have reported good reducing power and antioxidant properties of *Alcea hyrcana* [12], in this report, we made use of another species, *Alcea rosea* for the green synthesis of Ag nanoparticles. Studies demonstrated that the methanolic extracts of *A. rosea* can be applied as chemotherapeutic or chemopreventive agents as the extracts remarkably suppressed the neoplastic cell transformation by inhibiting the kinase activity of the epidermal growth factor receptor (EGFR) and promoted apoptosis in some cancer cells [11,13]. In the present study silver nanoparticles were synthesized using the extract of *Alcea rosea*. The characterization of AR@AgNPs was performed by several analytical vehicles such as SEM (Scanning electron microscopy), EDS (energy-dispersive x-ray spectroscopy), XRD (x-ray diffraction analysis), UV–Vis spectroscopy. The antibacterial activity of AR@AgNPs against seven human pathogenic microorganisms at both ATCC strain and clinically isolated strains was investigated. Furthermore, the antileishmanial properties of AgNPs have been reported for the first time.

MATERIALS AND METHODS

Silver nitrate ($AgNO_3$) was purchased from Fluka Company. Mueller Hinton broth was purchased from QUALAB and Mueller Hinton agar was purchased from Pronadisa. Double-distilled deionized water was used for making solutions as well. The antibacterial experiments were carried out using various bacteria strains. *S. aureus* (ATCC No: 29213), *E. faecalis* (ATCC No: 29212), *P. aeruginosa* (ATCC No: 27853), *E. coli* (ATCC No: 25922), *K. pneumonia* (ATCC No: 700603), *A. baumannii* (ATCC No: 19606) and *P. mirabilis* (ATCC No: 25933) were originally procured from Microbial collection of Iran. Eight clinical isolates of strains including *S. aureus A*, *S. aureus B*, *E. faecalis*, *P. aeruginosa*, *E. coli*, *K. pneumonia*, *A. baumannii,* and *Proteus* were obtained from medical hospitals of Sari, Iran. The Source of these isolates included: phlegm, chip, peripheral, urine, and wound. The initial characterization of AgNPs was prepared by UV–Visible spectroscopy with scanning in the range of 300-500 nm (Series UV/Vis spectrophotometer PG Instrument Ltd., T80+, China). Scanning electron micrographs (SEM) were used for observing the morphology of nanoparticles (TESCAN BRNO-Mira3 LMU, Czech Republic) Crystalline metallic silver was studied using X-ray diffraction (XRD) analysis to determine the particle sizes and natures of the AgNPs (Philips PW 1800 with Cu Kα radiation). The purity and chemical composition of AR@ AgNPs were determined via energy dispersive

spectroscopy (EDS, Ultima IV XRD system).

EXPERIMENTAL

Preparation of extraction of Alcea rosea

A. rosea were identified and collected from Mazandaran, Iran. 50 g of Aerial parts of *A. rosea* was dried at room temperature and extracted by the maceration method. Then, the extract solution was filtered and the resultant extracts were concentrated in a vacuum by a rotary evaporator at 35 °C to achieve crude solid extract. For complete dryness, the crude extracts were freeze-dried (MPS-55 freeze-drier, Operon Co., Ltd., Gimpo, South Korea) for complete solvents removal.

Biosynthesis of silver nanoparticles

The silver nanoparticles were synthesized by reduction of $AgNO_3$ in presence of *A. rosea* extract. Therefore, 0.02 g of silver nitrate (12 ml, 5 mM) was added into 12.5 ml of extract (0.025 g) in pH 10 at 65 °C for 60 minutes. The appearance of dark brown color in the solution will indicate the formation of Ag nanoparticles. The product was determined using a UV/Vis spectrophotometer at 420–400 nm. Finally, the aqueous nanoparticles were centrifuged at 14,000 rpm for 15 min and washed three times with methanol and distilled deionized water. Silver nanoparticles in solid form were obtained by setting in the oven at 60 °C.

Antibacterial activity

The antibacterial activity of the AR@AgNPs was evaluated against seven pathogenic bacteria and eight drug-resistant clinical isolates by the micro broth dilution method according to Clinical Laboratory Standard Institute (CLSI). All the microorganisms were grown in Mueller Hinton agar (MHA) as a culture medium at 37 °C for 24 h. Each well in the microplate was filled with 100µL of nutrient broth and 100 µL of the as-synthesized AR@AgNPs (250 µg/ml) was added to the first well. Then, 100µL of different pathogens selected (which has been equal to 0.5 McFarland suspension 1:10 in sterile normal saline) were added to the wells. At the end of the incubation period, the minimum inhibitory concentration (MIC) assay was conducted to measure the minimum concentration of AR@AgNPs needed to inhibit the growth of the microorganism. Minimum bactericidal concentration (MBC) was determined by plating 10 µL samples from the tubes on a nutrient agar medium. The MBC was defined as the lowest concentration of the reagent that did not permit any visible growth on the blood agar medium [14]. Ciprofloxacin (ATCC 27853; IROST, Iranian Research Organization for Science and Technology, Tehran, Iran) and *A. rosea* extract were used as the positive and negative control, respectively.

Antileishmanial studies

Parasite culture

Iranian strain of *L. major* promastigotes (MRHO/IR/75/ER) was cultivated in RPMI-1640 medium (Gibco, Paisley, Scotland, UK) supplemented with 10% fetal bovine serum (FBS), 100 µg/mL streptomycin/penicillin (Gibco, Paisley, Scotland, UK) at 24 °C. The medium containing the parasite was sub-cultured every 3 days.

Assessment of antipromastigotes activity of Leishmania

Evaluation of the anti-promastigote activity was performed for 72 hours in 96-well plates. At first, serial dilutions of AR@AgNPs were prepared at final concentrations of 7.8- 3.9- 1.95- 0.97 in 100 µL of RPMI-1640 medium. 100 µL of RPMI-1640 medium containing 1×10^5 promastigotes was added to each well. Three untreated wells containing the parasite were considered as the negative control. It should be stated that the final volume of the DMSO in each well did not exceed 0.2%. After incubating the plates at 24 °C for 72 h, the number of promastigotes/mL was calculated using a Neubauer hemocytometer (Martand Medical Services) light microscope (400× magnification) by mixing 20 µL of 2% formaldehyde solution in phosphate-buffered saline (PBS; pH = 7.2) and 20 mL of each well content. Amphotericin B and Glucantime as positive controls were evaluated at different concentrations of 0.00098-0.0078 µg and 0.14-75 µg, respectively. The experiments were accomplished in triplicate. The death rate (DR) of the promastigotes was measured using the following formula:

$$DR\ (\%) = [(NC - DT)/NC] \times 100$$

NC is the number of promastigotes in the negative control; DT is the number of promastigotes in each treated well.

Assessment of anti-amastigote activity of Leishmania

The murine macrophage cell line J774A.1 was obtained from the Iranian National Cell

Bank (Pasteur Institute, Tehran, Iran). After cells culturing in the RPMI-1640 medium, 2×10^5 cells were added to each well of a 96-well plate in a volume of 200 µL. The plates were incubated for 5 h at 37 °C and 5% CO_2. The supernatants were removed, and 200 µL of RPMI-1640 medium containing the promastigotes at a ratio of 1:10 (cell: promastigote) was added to each well. After 24 h incubation at the same condition, the free parasites were removed by washing with RPMI-1640 medium. After that, different concentrations of AR@AgNPs as described for the treatment of promastigotes were added to each well in a volume of 200 µL of RPMI-1640 medium. The plates were incubated for 72 h at 37 °C and 5% CO_2. Three untreated wells were considered as a negative control. After 72 h, the supernatants were discarded, and 50 mL of MTT solution (5 mg/mL stock solution in PBS) (Sigma, Lyon, France) was added to each well, and the plates were incubated at 37 °C and 5% CO_2 for 4 h. After 30 minutes, the wells' optical absorbance was read using a scanning multi-well spectrophotometer (BioTek, Winooski, VT, USA) at a wavelength of 570 nm. The following formula calculated cell death rate:

$$1- (AT/AC) \times 100$$

AT is the mean absorbance of treated wells for each concentration of AR@AgNPs or compounds, AC is the mean absorbance of negative control wells.

Cytotoxicity assessment and selectivity index (SI)

Different concentrations of AR@AgNPs or compounds at high values were added to the wells containing only the cells. The death rate was calculated for each concentration as described for the amastigotes. Furthermore, the selectivity index (SI) was calculated by dividing CC_{50} by IC_{50} (amastigote) for each of AR@AgNPs. The safety of the medication was represented as SI > 10 (2). Graph Pad Prism v.6 was used to calculate IC_{50} and CC_{50} values. Statistical analysis was performed by a two-tailed *t*-test using IBM SPSS v20 software (IBM Corp., Armonk, NY) [15].

RESULTS AND DISCUSSION

Preparation and characterization

The biomacromolecules of plants have been broadly studied for the synthesis of different metal nanoparticles. Biogenic systems like plant extracts contain various bio-molecules which reduce and stabilize metals at a nano size. Many researchers have suggested that the green synthesis approach displays the appropriate choice in contrast to chemical and other physical synthesis methods. Recently, biosynthesized AgNPs have peaked interest due to their capability to reduce the impacts of toxic by-products that are being produced during the fabrication of nanoparticles. During the biological synthesis of AgNPs, natural extracts are used to reduce Ag^+ into AgNPs due to the presence of phytochemical compounds such as polysaccharides, polyphenolic compounds, vitamins, amides, alkaloids, terpenoids tannin, organic acids, and aromatic dicarboxylic acids in it [16]. Silver nanoparticles can be fabricated from many medicinal plants such as *Saccharum offcinarum* under increased pH and microwave irradiation [17]. At another research observed that different biogenic materials in *Multani mitti (Fullers earth), Tomato (Solanum lycopersicum) seeds, Rice Husk (Oryza sativa)* were able to synthesis silver nanoparticles [18]. Also, *Aloe vera, Zea mays,* and *Magnolia kobus* extracts reduce silver ions to spherical silver nanoparticles [19-21].

The reduction of aqueous silver ions with concomitant formation of AR@AgNPs was monitored by UV-Vis spectroscopy at 425 nm (Fig. 1a). Due to the stimulation of surface plasmon resonance (SPR) of AgNPs, the color of the solution changes from light brown to dark brown that indicating the formation of silver nanoparticles. (Fig. 1b). This characteristic was revealed when plasmonic metals, like silver nanoparticles, have equal numbers of fixed positive ions in position and conduction mobile electrons. Such a phenomenon is following our recent reports which we used *Feijoa sellowiana* leaf and fruit extracts [22], *Allium paradoxum* extract [9], *Ferula persica* extracts [24], *Scrophularia striata* leaf extract [25], *Convolvulus fruticosus* extract [26] for green synthesis of AgNPs.

The spectroscopic analysis confirmed the biogenic synthesis of AgNPs, which displayed an SPR with a specific sharp peak at 425 nm, which was characteristic of the spherical AgNPs. Chaudhari et al. [17] reported that the SPR range for AgNPs synthesized by *Saccharum officinarum* leaves was 440 nm that the standard SPR peak for AgNPs synthesized by the green approach was from 400 to 450 nm [27-29].

The morphological studies of the prepared samples were observed by SEM analysis which is

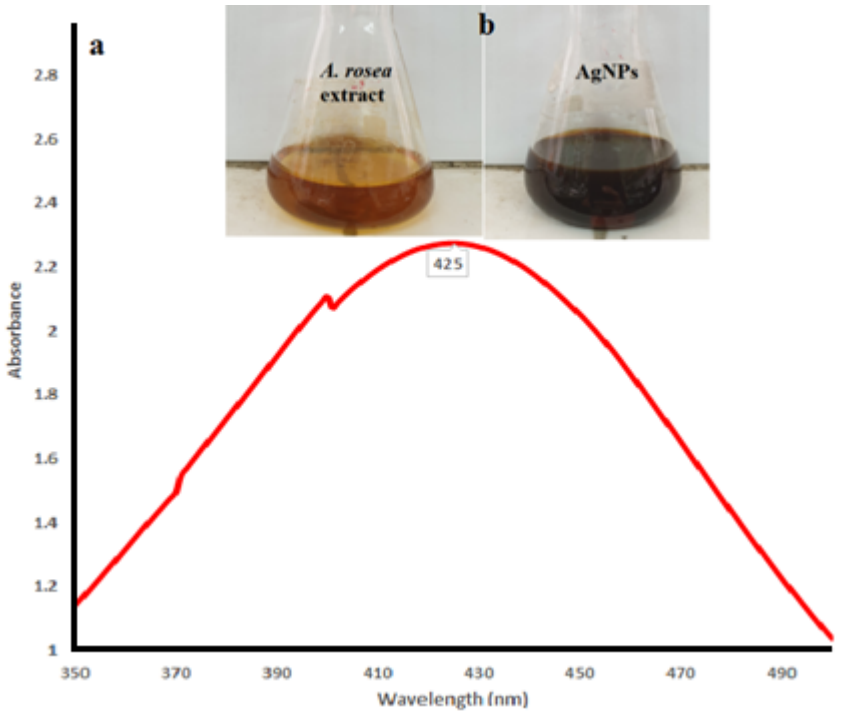

Fig. 1. A: UV-visible spectrum of silver nanoparticles b) color change related with plasmon resonance of colloidal AR@ AgNPs.

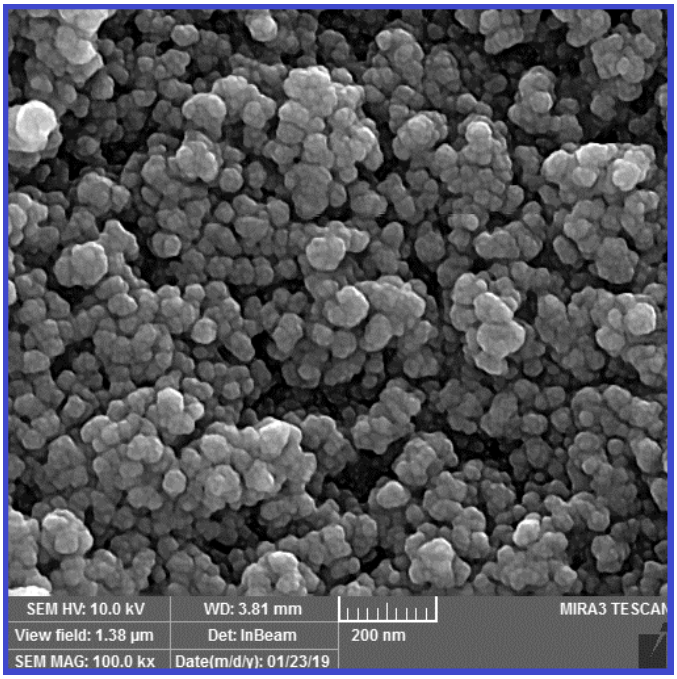

Fig. 2. Surface morphology of the synthesized silver nanoparticle from the aerial extracts of *A. rosea* by SEM.

depicted in Fig. 2a. On a scale of 200 nm, the SEM analysis displayed a relatively spherical shape of nanoparticles with a diameter range of 10-30 nm. These nanoparticles that couldn't be stabilized without extract solution were easily agglomerated and linked to each other and formed large particles. As shown in Fig. 2a, the size of synthesized AR@ AgNPs is approximately 15 nm in the presence

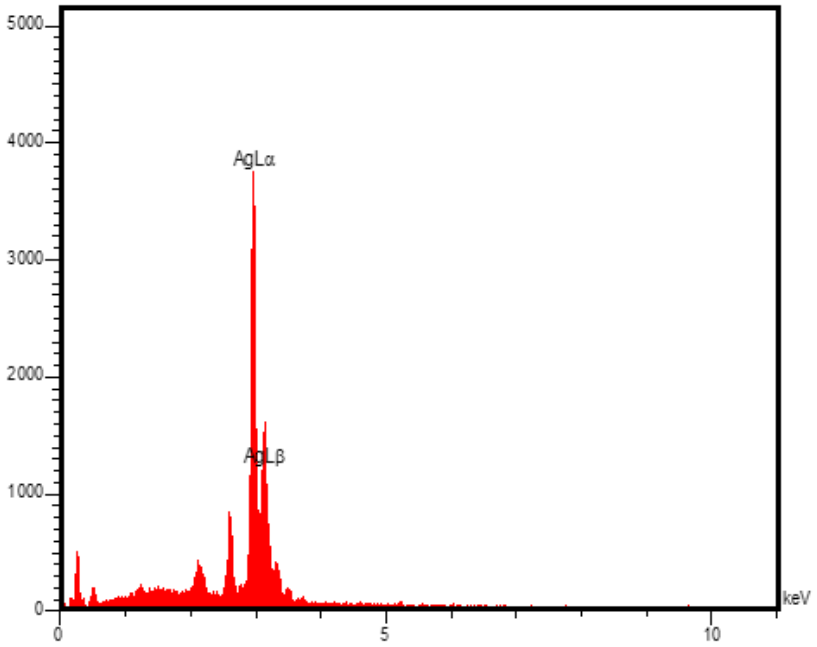

Fig. 3. EDS diagram of AR@AgNPs.

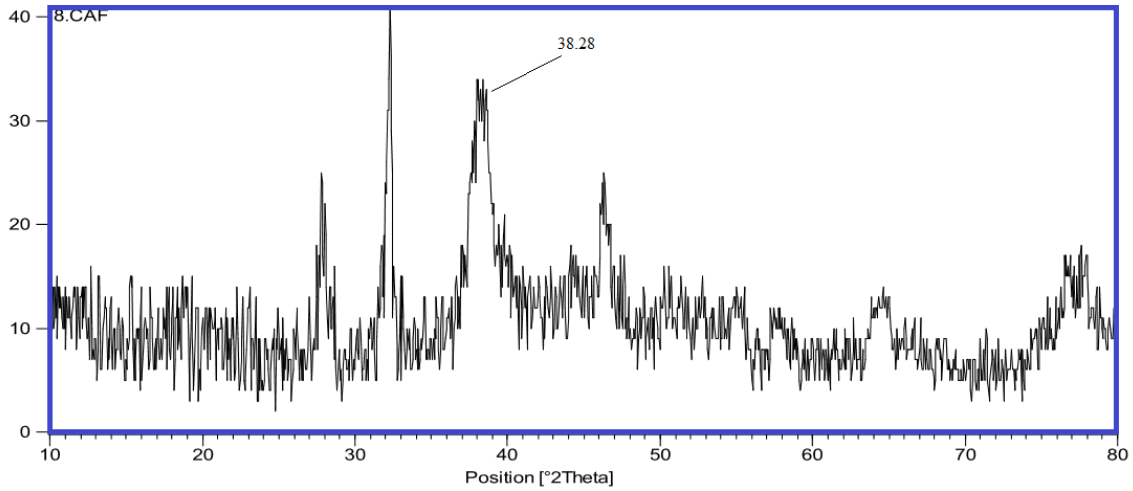

Fig. 4. XRD diagram of AR@AgNPs.

of the extract as a reducing and capping agent. In general, the calculated size for synthesized Ag nanoparticles using *Alcea rosea* extract was similar to those reported when using the other plant-mediated synthesized AgNPs. *Multani mitti (Fullers earth), Tomato (Solanum lycopersicum) seeds, Rice Husk (Oryza sativa)* that mediated AgNPs formation have been reported the values 4.6, 41.1, and 10.6 nm, respectively [18]. In the reports on green synthesis using lavender, the crystallite sizes around 20 nm as determined from scanning electron microscopy (SEM) were obtained [30].

Elemental analysis of the synthesized AR@AgNPs was performed using energy dispersive X-ray analysis. EDX profile showed in (Fig. 3a) shows a strong Ag signal from AgNPs and a carbon peak attributed either to the capping agents like biomolecules or the plant extract compounds bound to the outer layer of AgNPs and/or to the coating of the copper grids. As reported before, EDX was used to estimate the nanoparticle's elemental composition [22, 24-26].

Table 1. Antibacterial activities of AR@AgNPs against ATCC bacteria strain.

Strain	AR@AgNPs		MIC of extract	Ciprofloxacin[a]
	MIC	MBC		
S. aureus ATCC 29213	19	62.5	>4000	0.21
E. faecalis ATCC 29212	15.62	250	>4000	0.21
P. aeruginosa ATCC 27853	3.9	125	>4000	0.3
A. Baumannii ATCC 19606	7.8	125	>4000	0.25
E. coli ATCC 25922	7.8	62.5	>4000	0.1
K. pneumonia ATCC 700603	7.8	250	>4000	0.1
P. mirabilis ATCC 25933	15.62	250	>4000	0.1

>4000: not detected by the highest concentration of tested samples; [a]Positive control; MIC – minimal inhibitory concentration (µg/ml); MBC – minimal bactericidal concentration (µg/ml);ATCC- American Type Culture Collection.

XRD results revealed that AR@AgNPs could be formed at 65 °C that depicted in Fig. 3b. Four prominent characteristic diffraction peaks for Ag were observed at 2θ = 38.28, 44.76, 64.16, 77.10, which were related to Bragg's from (111), (200), (220), and (311) crystallographic planes, respectively. The peaks were assigned face-centered cubic (fcc) silver, which was in good agreement with references [31]. Two weak peaks at 2θ= 28.81 °, 32.06°, 47.5° and 58.06° were attributed to the impurity of AgCl [32, 33].

Different pure chemical techniques have been used for the synthesis of AgNPs. Generally, different reducing agents like sodium citrate, ascorbate, sodium borohydride ($NaBH_4$), elemental hydrogen, polyol process, Tollens reagent, N, N-dimethylformamide (DMF), and poly (ethylene glycol)-block copolymers have been used for the reduction of silver ions (Ag^+). These reducing agents lead to the creation of Ag^0, which is followed by agglomeration. These clusters finally lead to the fabrication of colloidal silver particles. The protective agents to stabilize dispersive NPs for nanoparticle preparation is necessary which leads to avoiding their agglomeration. The presence of surfactants containing functional groups for interfaces with the surface of the particle can stabilize particle growth. Synthesis of AgNPs from chemical ways is required at a higher concentration which enhances the toxicity of normal cells. While using the biological system for the synthesis of silver nanoparticles no stabilizer and capping agent is required [34, 35].

Biosynthetic methods of NPs provide a new possibility of conveniently synthesizing NPs using natural reducing and stabilizing agents. As possible environmentally and economically friendly alternatives to chemical approaches, biosynthesis of AgNPs using organisms has been suggested [36].

Antibacterial activity

ATCC strains

In the present investigation, the antibacterial activity of the AgNPs synthesized by *A. rosea* extracts against pathogenic microorganisms was carried out by micro broth dilution method according to the protocol as discussed in the previous part. The MIC and MBC values in this study are shown in Table 1. As shown in Table 1, *A. rosea* extracts alone did not show any antibacterial activity against the seven studied pathogenic bacterial. The bacterial growth inhibitory potential of AR@AgNPs was compared with reference antibiotics (Ciprofloxacin). The result shows that AR@AgNPs exhibited excellent antibacterial effect against *S. aureus* and *P. aeruginosa* with MIC 1.9 µg/ml and 3.9 µg/ml and 62.5 µg/ml and 125 µg/ml as MBC, respectively. The inhibition of *E. coli*, *K. pneumonia*, and *A. baumanii* was observed in the concentration of 7.8 µg/ml as the MIC and 62.5, 250, 125 as MBC, which is demonstrated in Table 1. The MIC value indicates that the green synthesized AR@AgNPs can inhibit the growth of *E. faecalis* and

Table 2. Antibiotic resistance of the bacterial isolates from chip, urine, peripheral, wound and phlegm.

MDR Bacteria	Source	MC	VC	OC	TC	GM	PC	CIF	CEZ	ERM	CM	AK
S. aureus	Chip	R	S	R	R	R	R	R	R	R	R	R
			ERM	TC	GM	LEV	TEC	VC	AM			
E. faecalis	Peripheral		R		R	S		R	R	R	R	
			IMP	MER	DOR	TOB	AMK	GM	LEVO	CIF	CAZ	AZT	PTZ	
P. aeruginosa	Wound	R	R	R	R	R	R	R	R	R	R	R	R
A. baumannii	Phlegm	R	R	R	R	R	R	R	R	R	R	R	R

		Colistine	MER	IMP
E. coli				
P. mirabilis	Urine	R	R	R
K. pneumonia	Urine	R	R	R
	Urine	R	R	R

R, resistant; I, intermediate; S, susceptible; MC, Meticillin; VC, Vancomycin; OC, Oxacillin; TC, Tetracycline; GM, Gentamicin; PC, Penicillin; CIF, Ciprofloxacin; ERM, Erythromycin; CM, Clindamycin; AK, Amikacin; LEVO, Levofloxacin; TEC, Teicoplanin; AM, Ampicillin; IMP, mipenem; MER, Meropenem; DOR, Doripenem; TOB, Tobramycin; AMK, Amikacin; CAZ, Ceftazidime; AZT, Aztreonam; PTZ, Piperacillin. MIC – minimal inhibitory concentration (μg/ml); MBC – minimal bactericidal concentration (μg/ml); [a]The presence of the genes encoding carbapenemase OXA- 23 and OXA- 24 is positive; [b] The presence of the genes encoding carbapenemase OXA- 23^+, PhosA3^+, VIM^+ and NDM^- is positive; [c] The presence of the genes encoding carbapenemase OXA- 23^+, PhosA3^-, VIM^- and NDM^- is positive; [d]The presence of the genes encoding carbapenemase OXA- 23^+, VIM^- and NDM^+ is positive.

Table 3. Antibacterial activities of AR@AgNPs against clinical isolated bacteria strain.

Strain	AR@AgNPs		MIC of extract
	MIC	MBC	
S. aureus	3.9	31.25	>4000
E. faecalis	3.9	62.5	>4000
P. aeruginosa	0.5	15.6	>4000
A. baumannii	1.9	62.5	>4000
E. coli	1.9	62.5	>4000
K. pneumonia	31.25	62.5	>4000
P. mirabilis	1.9	7.8	>4000

P. mirabili at the concentration of 15.62 μg/ml and 250 μg/ml for two strains. In general, AR@AgNPs exhibited significant effects on the inhibition of bacteria growth around the well.

Multi-drug resistance strains

For more comprehensive antibacterial effects, AR@AgNPs were tested on isolated strains from the clinic which were resistant to several antibiotics. The drug resistance of these strains is shown in Table 2. In our study, seven clinical isolated bacteria were selected to examining susceptibility against synthesized AR@AgNPs. Based on the data presented in Table 3, *S. aureus* isolated from the chip was resistant to all antibiotics listed in Table 2 but was susceptible to vancomycin. Our synthesized AR@AgNPs showed the MIC of 3.9 μg/ml and the MBC of 31.25 μg/ml, respectively. *A. baumannii* and *P. aeruginosa* which were isolated from wounds and phlegm were resistant to all antibiotics indexed in Table 2. In these two strains of bacteria, the presence of genes that express the carbapenemase OXA-24 and OXA-23 has been confirmed. Since carbapenems are the last line of treatment and the MIC for colestine sulfate was reported at 256 μg/ml and 8μg/ml for *A. baumannii* and *P. aeruginosa*, respectively, our synthesized AR@AgNPs significantly inhibited the growth of them at 1.9 μg/ml and 0.5 μg/ml as MIC, 62.5 μg/ml and 15.6 μg/ml as MBC. Isolated *K. pneumonia* and *E. coli* from urine were resistant to carbapenems. The MIC for colistin sulfate was 128 μg/ml and 32 μg/ml, but in our research, the MIC was 31.25 μg/ml and 1.9 μg/ml, correspondingly and 62.5 μg/

ml as MBC for two strains. Although *P.mirabilis* isolated from urine was resistant to imipenem and meropenem with the MIC of 2 µg/ml and 32 µg/ml, AR@AgNPs showed the MIC value of 1.9 µg/ml with the MBC value of 7.8 µg/ml. *E. faecalis* which was isolated from peripheral was not susceptible for ampicillin, vancomycin, teicoplanin, erythromycin, tetracycline, levofloxacin, kanamycin but was poorly susceptible for gentamicin, could inhibit by AR@AgNPs in the MIC value of 3.9 µg/ml and the MBC value was around 62.5 µg/ml (Table 3). Chaudhari et al. demonstrated the antibacterial activity of silver nanoparticles biosynthesized using *S. officinarum* and tested on multiple drug-resistant hospital isolates of *E. coli*, *S. aureus*, *P. mirabilis*. Their studies revealed that silver nanoparticles are potent antibacterial agents but they found the synergistic effect of silver nanoparticles along with antibiotics was more noticeable [17]. Awwed et al. reported that the silver nanoparticles synthesized using carob leaf extract have stronger activity than a standard antibiotic. They also found that these nanoparticles inhibited *E. coli* with the MIC of 0.5 µg/ml [28]. Logeswari et al. exhibited that the silver nanoparticles synthesized by *S. tricobatum*, *O. tenuiflorum* extracts have the highest antimicrobial activity against *S. aureus* and *E. coli* [6]. In a report, it was observed that the antibacterial activity of the silver nanoparticles, synthesized using gallic acid, increased with decreases the size of silver nanoparticles [37].

Due to the antibacterial properties of AgNPs could be applied most usually in the food storage, health industry, textile coatings, and various environmental uses. The real mechanisms of antimicrobial or toxicity activities by silver nanoparticles are still controversial. The Ag ions, which created a positive charge, are vital factors for antimicrobial activities. All procedures of silver or silver nanoparticles with antimicrobial properties are attributed to their sources of silver ions (Ag^+) [38]. Silver nanoparticles have emerged as antimicrobial agents against bacteria due to their high surface-to-volume ratio and their distinctive chemical and physical properties. The antibacterial activity of AgNPs is associated with four famous mechanisms: (1) adhesion of AgNPs onto the surface of cell wall and membrane; (2) AgNPs penetration inside the cell and damaging of intracellular structures and biomolecules and with phosphate and thiols in nucleic acids form a complex with Ag^+; (3) AgNPs induced cellular toxicity and oxidative stress caused by a generation of Reactive Oxygen Species (ROS), and (4) Modulation of signal transduction pathways [39].

In vitro treatment of promastigotes & amastigote using AR@AgNPs

In promastigotes treatment with AR@AgNPs, the IC_{50} values obtained 2.2 µg/ml while the IC_{50} values for Amphotericin B and Glucantime were 0.0016 µg/ml and 19.95 µg/ml, respectively. The IC_{50} values of 4.13 µg/ml were calculated for AR@AgNPs when treated with the amastigote while the IC_{50} values for Amphotericin B and Glucantime were 0.0011 µg/ml and 33.09 µg/ml, respectively. According to the CC_{50} values obtained for the AR@AgNPs, the SI values were 65.8 µg/ml while for Amphotericin B and Glucantime were reported 19.4 µg/ml and 16.33 µg/ml. Our finding revealed that new green synthesized Ag nanoparticles can inhibit the growth of two parasite cell lines compared with the reference agent, Glucantime, operated effectively. The infectivity of Leishmania promastigotes and the production of amastigotes in macrophage cells was evaluated by incubation of the parasites with the IC_{50} concentration of the nanoparticles. Ullah et al reported the IC_{50} promastigotes of *L. infantum* as 19.42 µg/ml for *T. stocksianum* leaves mediated AgNPs, 30.71 µg/ml for *T. stocksianum* stem mediated AgNPs, and 51.23 µg/ml of chemically synthesized silver nanoparticles. As a result, the IC_{50} values indicate that biosynthesized AgNPs are more effective to inhibit the growth of Leishmania promastigotes than the chemically synthesized AgNPs [40].

The outcomes display that the contamination of the parasite reduced in contact with AgNP. The proposed mechanism for the antileishmanial activity of the biogenic synthesized AgNPs is because of its nano size. Fabricating a high level of nitric oxides (NO) by AgNPs could alter the surface morphology, chemical composition, or structure of the parasitic cell. Previously, the in vitro cytotoxicity studies reports that the intracellular form of the Leishmania is destroyed by the production of nitric oxide species [40, 41, 42]. Nitric oxide (NO) is a free radical, produced in the biological system. It reacts with superoxides in a living organism to produce harmful nitrite (NO^{2-}) and nitrate (NO^{3-}) that have lethal effects on microbes and parasites. NO free radical stimulates multiple cellular procedures that led to DNA damage [40], damage to the cell membrane, alteration in

protein structure, inhibition of protein synthesis, and lipid peroxidation [43]. In another study, it was observed that the spherical shape of NPs allows them to enter cells via phagocytosis. AgNPs with oxidation and release of free Ag^+ ions led to intracellular amastigotes to death. As confirmed previously with AgNPs, after their phagocytosis by macrophages increases the release of Ag^+ ions by oxidation and favors the death of *L. amazonensis*. In general, macrophages form high levels of ROS to kill pathogens like parasites. However, the Leishmania parasite evades ROS via regulating a few signaling cascade moieties to survive inside phagolysosomes[44].

According to our study, biosynthesized AgNPs of *A. rosea* extracts maybe kill the Leishmania parasite in the same way by producing free radicals in the macrophage cells. Though, the high level of free radicals produced by AR@AgNPs leads to its therapeutic ability in the progress of antileishmanial drugs in the future.

CONCLUSION

Biogenic synthesis of silver nanoparticles with almost 15 nm and spherical shapes was carried out using aerial part of *A. rosea*. UV-Vis and XRD measurements characterized the AgNPs. Synthesis of silver nanoparticles using green resources like *A. rosea* is a better alternative to chemical synthesis since this green synthesis is eco-friendly and cost-effective. The results demonstrated that *A. rosea* extract plays a vital role in reducing and stabilizing silver ions to silver nanoparticles. The study also found that the AgNPs display antibacterial activity on Gram-positive and negative bacteria, and for more research on antimicrobial efficacies, the clinically isolated strain was used. Antileishmanial efficacy was observed (for both amastigote and promastigotes) by *A. rosea*, inhibiting the parasite's growth. To the best of our knowledge, this research claims the first evidence in descending the IC_{50} of cell lines using biocompatible NPs, against Leishmania parasites which can be compared with Glucantime as an effective drug for the treatment of cutaneous leishmaniasis.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

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