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RESEARCH ARTICLE

Evaluation antibacterial activity of Biosynthesized Silver Nanoparticles using e *Euphorbia Pseudocactus Berger extracts (Euphorbiaceae)*

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ABSTRACT

In the current research, silver nanoparticles (Ag NPs) were created using *Euphorbia Pseudocactus Berger (Euphorbiaceae)* extracts, which played the main role in the formation and stability of nanoparticles. The physic-chemical properties of biosynthesized nanoparticles were characterized by means of Ultraviolet–Visible spectroscopy (UV-Vis), X-ray diffraction (XRD), and Transmission Electron Microscopy (TEM), and Fourier-Transform Infrared spectroscopy (FT-IR) methods. UV-Vis results illustrated that maximum plasma resonance absorption of Ag NPs are about 426 nm. Size distribution and spherical morphology was determined by TEM method. The XRD was confirmed face centered cubic (FCC) structure for synthesized nanoparticles. Monte Carlo (MC) simulations and Molecular dynamics (MD) were utilized to evaluate the nanoparticles. The antibacterial properties of biosynthesized Ag NPs were studied on *E. coli* (ATCC 25922), S. *aureus* (ATCC 2592), *P. aeruginosa* (ATCC27853) and *E. faecalis* (ATCC51299) using micro dilution broth method. The minimum inhibitory concentration (MIC) results of synthesized Ag NPs on *S. aureus* and *E. faecalis* obtained 4 and 8 µg/mL and *P. aeruginosa* and *E. coli* obtained 16 and 4 µg/mL. So, synthesized nanoparticles can be utilized as an antibacterial agent in medical and industrial devices and tools.

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INTRODUCTION

Silver nanoparticles considered the attention of researchers because of their electrical, optical, thermal and biological properties [1, 2]. These properties cause that they be suitable for applications in the fields such as drug delivery, sensing, antibacterial and catalysis [3-11].

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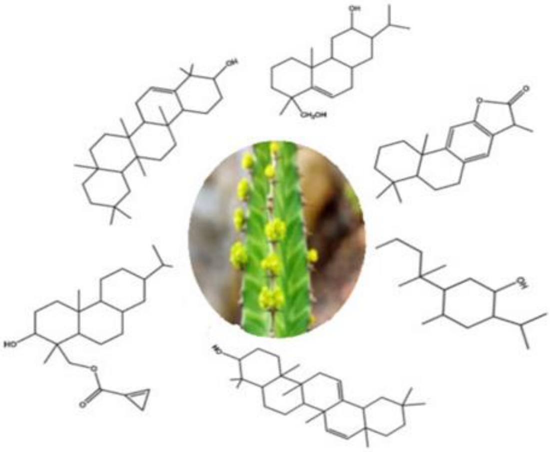

Fig. 1. shows the Euphorbia Pseudocactus Berger (Euphorbiaceaee) plant and some of the compounds and functional groups involved in the synthesis and stability of silver nanoparticles[43].

It has been reported that the excessive usage of antibiotics to eradicate the bacteria resulted in the development of resistance to various antibiotics as well as the spread of infectious diseases [12-14]. Therefore, the find of new antibacterial agents is essential for the prevention of bactericidal growth. Silver nanoparticles have significant antibacterial activity, due to having small size and the surface: volume ratios of these particles. Therefore, owing to high antibacterial properties of these nanoparticles, they could be employed for the increase of the safety in the manufacturing such as food packaging [15, 16].

These nanoparticles prepare thought several physical and chemical methods such as gamma irradiation [17], electrical irradiation[18], thermal decomposition[19], and sol-gel[20]. But these methods require to long-term experiments, a large space, use of toxic chemicals, high energy and expensive[21, 22] . So, synthesis of nanoparticles using microorganisms[23-26], enzymes[27], and plant extracts is best choose, due to advantageous such as the short-term experiments, cost-effective, easily, available, and safe[28]. Leaves, barks, stems, seeds, flowers and roots are parts of plant which can be used as reducing and stabilizing agents for the construction of nanomaterials[29-33]. The compounds like terpenoids[34], flavonoids[35], phenols [36], saponins[37], polysaccharide [38], quinones [39] present in the plants cause the reduction of metal ions.

Euphorbia Pseudocactus Berger (Euphorbiaceae)

Euphorbia pseudocactus Berger (candelabra spurge) is a multibranched, dwarf-stemmed, candelabra-shaped, succulent herb, 60–120 cm tall. The stems often have distinctive yellow V-shaped markings. It is originating in the subtropical coast of South Africa. It grows in thorny bush-lands and savannah often forming colonies.

some species of Euphorbia are useful for the treatment of boils, cuts, and wounds[40]. It is useful for cardiovascular complaints, asthma, cough,[41] and spleen disorders[42]. Certain Euphorbia species have been reported to possess cytotoxic[43-47], antimicrobial[48-52],larvicidal, insecticidal[53], anti-inflammatory, hepatoprotective, and antioxidant activities [54-56]. The diterpenoid ingredients, particularly those with tigliane, ingenane, and abietane skeletons, are believed to be the major bioactive and toxic agents [57] .So, in this paper, we study the synthesis of silver nanoparticles by using *Euphorbia Pseudocactus Berger (Euphorbiaceae)* extract and its antibacterial property.

EXPERIMENTAL

Materials and method

Fresh leaves of *Euphorbia Pseudocactus Berger (Euphorbiaceae)* were gathered from Jam, Bushehr, Iran. Silver nitrate ($AgNO_3$) was procured from Merck. The gathered herb was cleansed by using distilled water, and was dried at ambient temperature. Then they were powdered and stored for done experimental.

Extraction

Fresh *Euphorbia Pseudocactus Berger*

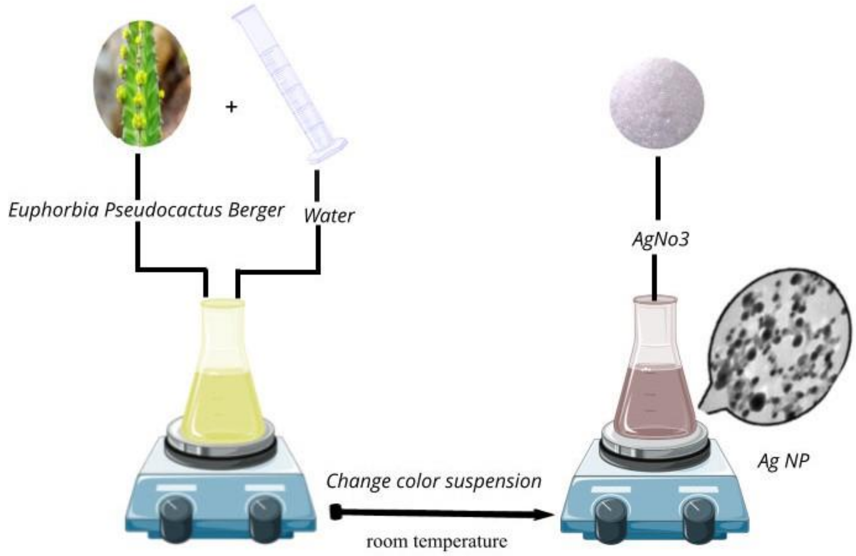

Fig. 2. shows the production of Euphorbia Pseudocactus Berger plant extract and discoloration of the plant extract with the addition of $AgNO_3$ solution.

(Euphorbiaceae) extract was preferred to reduce aqueous Ag^+ solution to Ag NPs. So, 5g powder plant was refluxed in 50 ml distilled water under stirred extremely for 2h. The resulting solution gradually cooled down at 25°C and then filtered using Whatman filter paper No. 1. Fresh extracts were employed for the preparation of Ag NPs.

Synthesis of Ag NPs

Typically, 20 ml fresh filtered extract was interacted with 5 ml aqueous 0.01 mM $AgNO_3$ under reflux by magnetic stirred. After interaction for 30 min, color of suspension was converted from light-yellow to brown which this change illustrated formation of Ag NPs. The brown Ag suspension was cooled at room temperature, and it was identified by using several techniques (Fig. 2).

Characterization of Ag NPs

UV-Vis spectroscopy (UV-Vis) was performed through Varian Cary 50 UV–vis spectrophotometer. The X-ray diffraction (XRD) of samples was done on Holland-Philips X-ray powder diffractometer using Cu Ka radiation (= 0.1542 nm). TEM of synthesized nanoparticles was performed using Transmission Electron Microscopy model of CM30 3000Kv. FT-IR spectra was recorded through Bruker VERTEX 80 v model.

Microorganisms and growth conditions

The antimicrobial activity of the prepared Ag NPs has tested through standard micro dilution technique-reveals the minimum inhibitory concentration (MIC), which MIC value is the lowest concentration of Ag NPs to inhibit bacterial growth up to 99%. To investigate of antibacterial effect of prepared Ag NPs was used several type of Gram-negative and Gram-positive bacteria. Gram-negative bacteria involved *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27852) and Gram-positive bacteria including *Staphylococcus aureus* (ATCC 25923) and *Enterococcus faecalis* (ATCC 27852). Cultivating bacteria was carried out on Mueller-Hinton agar (MHA) at 37 ˚C for 18-24 h.

Antibacterial Test

Antibacterial activity of synthesized nanoparticles was studied on standard species of bacteria including both gram-positive and gram-negative bacteria of *S. aureus*, *E. coli*, *E. faecalis*, *P. aeruginosa* by micro dilution broth method (M27-A3) documented by CLSI. In brief, for designation of antibacterial activity, serial dilutions of synthesized nanoparticles (0.5-128 μg/mL) were prepared in a 96-well micro-titer plate using Müller-Hinton broth (MHB, EMD Millipore) medium (pH=7 ± 0.1; 25°C) based on the M07-A10 protocol. Stock inoculums ready using transferring some pure colonies in 5 mL sterile DW and adjust the turbidity of the inoculums to 0.5McFarland standard at 530 nm wavelengths which are equal to

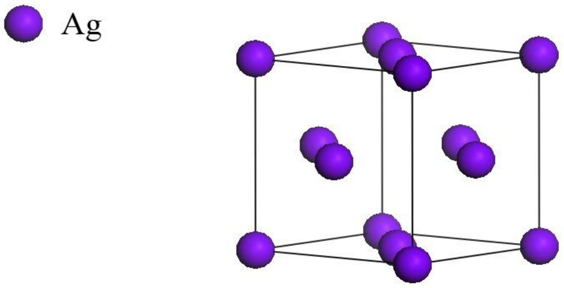

Fig. 3. Simulated crystal structure of Ag

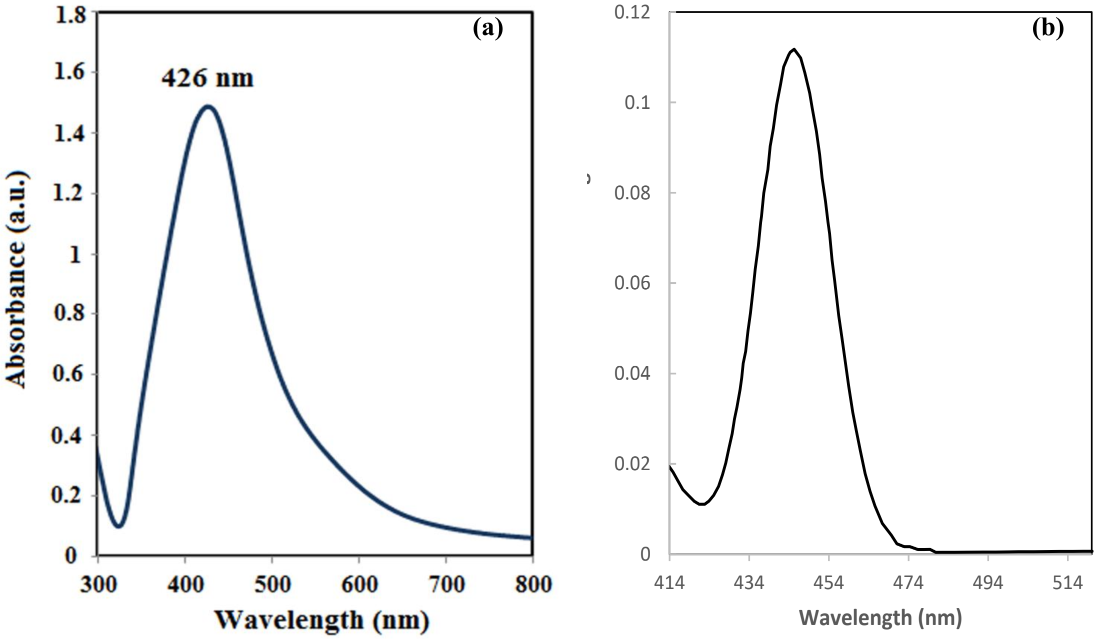

Fig. 4. UV-Vis spectrum of (a) biosynthesized Ag NPs *Euphorbia Pseudocactus Berger (Euphorbiaceae)* and (b) simulation result

$1–1.5\times10^8$ cells/mL for bacteria. Working suspension was prepared by production a 1/100 dilution with Müller-Hinton broth to add wells. The one column of 96-well microplate was filled with 200 μL of MHB media culture as positive control. The other column was filled with 200 μL of bacterial suspension as negative control. The two other columns were filled with 100 μL inoculum and 100 μL of nanoparticles dilution sequentially. The growth of bacteria treated with nanoparticles was compared with those grown in the control group. The values of MIC were defined by the lowest concentrations of the reduction in the bacterial growth in comparison with the growth in the control group. The experiments were performed in triplicate.

Simulation details

In this work, both molecular dynamics (MD) and monte carlo (MC) simulations were done by Materials Studio v17.1.0.48 to simulate the XRD and UV-vis, respectively. To geometry optimization, universal force field, the atom based van der Waals, and Ewald Electrostatic were performed. The simulated structures of Ag is face centered cubic (FCC) with lattice parameters of 4.08°A as shown in Fig. 3. UV-vis and XRD simulation can be evaluated using VAMP and diffraction module, respectively. Also, XRD pattern of Ag simulated investigate based on freely equilibrated configurations by Cu and Kα radiation (λ= 1.54 Å).

Table 1. shows some UV-Vis spectrum of biosynthesized Ag NPs that have been synthesized using various plant extracts.

Plant	The absorption peak of UV-Vis spectrum	References
Macrotyloma uniflorum	410–430 nm	[58]
macroalga Chaetomorpha linum	422 nm	[59]
Calendula officinalis	440 nm	[60]
Alternanthera sessilis (Linn.)	435 nm	[61]
Pithophora oedogonia (Mont.)	445 nm	[62]
RedApple (Malus domestica)	409 - 448 nm	[63]
Bunium persicum	400 nm	[64]

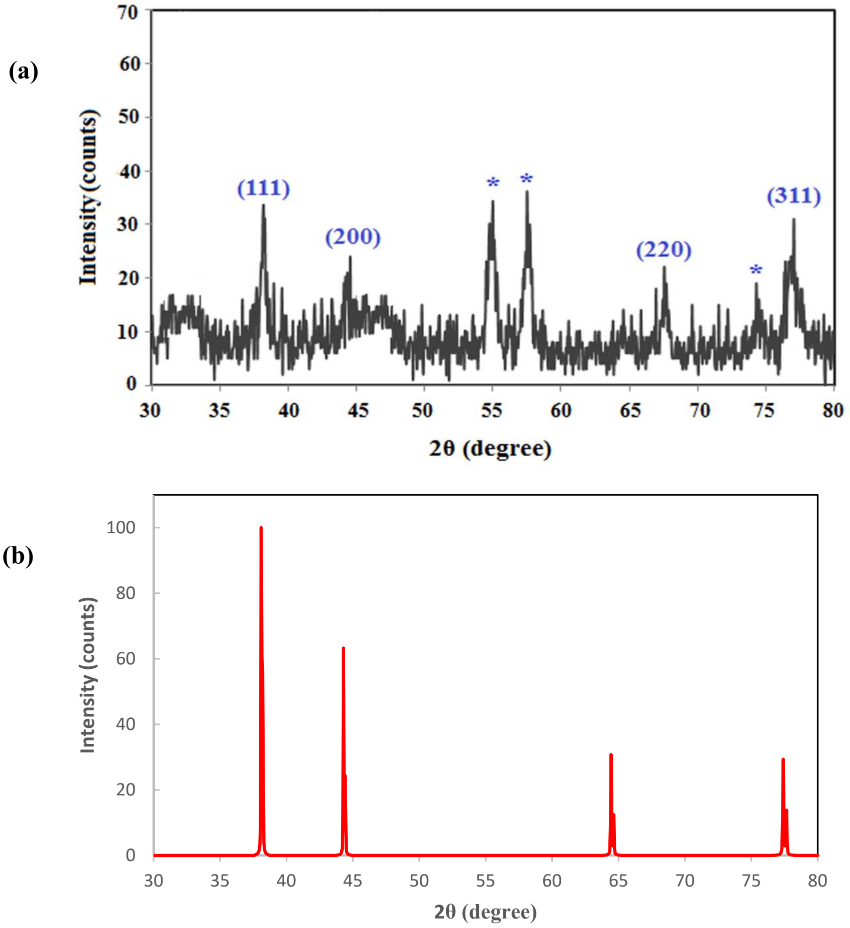

Fig. 5. (a) XRD pattern of biosynthesized Ag NPs, and (b) Simulated XRD pattern in molecular dynamics simulation for Ag NPs

RESULTS AND DISCUSSION

UV-Vis Spectroscopy a quantitative technique applied to measure the optical absorption spectra of metal nanoparticles. Absorption spectrum shows that maximum plasmon resonance absorption of simulation result and biosynthesized Ag NPs is in region of 447 and 426 nm, respectively, which confirmed formation of of *Euphorbia Pseudocactus Berger (Euphorbiaceae)* (Fig. 4).

X-ray diffraction pattern (XRD) of biosynthesized Ag NPs using *Euphorbia Pseudocactus Berger (Euphorbiaceae)* presents in Fig. 5a. All of the peaks with Miller indices of (111), (200), (220) and (311) can be indexed to the face centered cubic (FCC) structure for biosynthesized nanoparticles (JCPDS, 04–0783). The crystalline

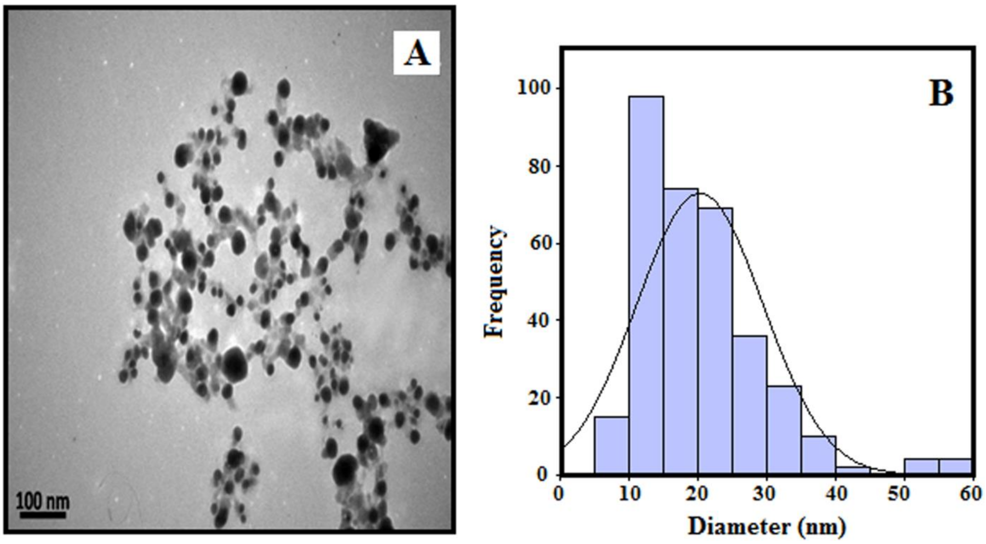

Fig. 6. (a) TEM image, (b) size distribution histogram of biosynthesized Ag NPs using *Euphorbia Pseudocactus Berger (Euphorbiaceae)*

Table 2. shows some of the different plants used to synthesize nanoparticles and their size and morphology compared to prepare Ag NPs in this study.

Plant	Type of NPS	Size (nm)	Morphology	References
Musa balbisiana (banana)	Silver	200 nm	triangles, pentagons and hexagons	[67]
Boerhaavia diffusa	Silver	25 nm	spherical	[68]
Azadirachta indica	Silver	34 nm	spherical	[69]
Emblica officinalis (EO)	Silver	15 nm	spherical	[70]
Ziziphora tenuior (Zt)	Silver	38 nm	spherical	[71]
Pulicaria glutinosa	Silver	40-60 nm	spherical	[72]
Ananas comosus	Silver	12 nm	spherical	[73]

size of Ag NPs evaluated using Scherrer's formula ($D=0.9^*\lambda/\beta\cos\theta$; where λ is X-ray wavelength, β is full width at half the maximum (FWHM) and θ is Braggs' angle) in about 42 nm [65]. Some extra peaks in XRD pattern related to bioorganic phases residue and impurities such as AgCl on surface of nanoparticles[66]. Simulated XRD pattern in molecular dynamics simulation for Ag is present in Fig. 5b. The peaks at $2\theta=38.1°$, 44.3°, 64.4°, and 77.4° are close to experimental measurement.

Transmission Electron Microscopy (TEM) is a scientific instrument that applies a beam of highly energetic electrons to evaluate the morphology, particle size, and size distribution of nanoparticles. TEM micrograph of biosynthesized Ag NPs using *Euphorbia Pseudocactus Berger (Euphorbiaceae)* extract shown in Fig. 6a. It clearly indicates that the biosynthesized Ag NPs are spherical shape and well dispersed. The histograms of particle distribution present in Fig. 6b. The biosynthesized Ag NPs have size range from 5.48-60 nm with average diameter of about 9.16 -20.31 nm.

FT-IR spectral analysis of biosynthesized Ag NPs shows in Fig. 7. The main goal of FT-IR analysis is to detect the organic functional groups present in plant extract. The spectrum shows stretching vibrations as 3500 cm^{-1} (N-H, amide), 3472 -3413 cm^{-1} (O-H, alcohol and phenol), 3231cm^{-1} (O-H, carboxylic acid), 2923-2358 cm^{-1} (C-H, methyl, methylene, and methoxy), 1640 cm^{-1} and 1618 cm^{-1} (C=O, carboxylic acid), 1383cm^{-1}(C-H, alkanes), 1111 cm^{-1} (C-OH, disaccharides), and 620 cm^{-1} (aromatic ring, carbohydrates).

Thus, FT-IR spectral showed that compounds and functional groups such as: amide, alcohol, phenol, carboxylic acid, methyl, methylene, methoxy, carboxylic acid, alkanes, disaccharides, aromatic ring and carbohydrates play significant roles in the, reduction, stabilization, and formation of these silver nanoparticles.

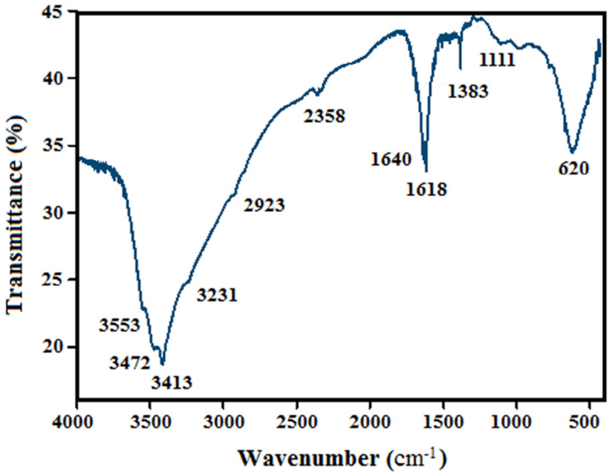

Fig. 7. FT-IR spectrum of biosynthesized Ag NPs using *Euphorbia Pseudocactus Berger (Euphorbiaceae)* extract

Table 3. shows some of the most effective compounds and functional groups in plant extracts that play an important role in the formation, reduction, stabilization, and capping of nanoparticles.

Plant	Type of NPS	effective compounds and functional groups	References
Jatropha curcas	Silver	curcain (an enzyme), curcacycline A (a cyclic octapeptide), curcacycline B (a cyclic nonapeptide)	[74]
Capsicum annuum L	Silver	Protein	[75]
Hibiscus	Gold	rosa sinensis and proteins, vitamin C, organic acids (essentially malic acid), flavinoids and anthocyanins	[76]
Cinnamomum zeylanicum	Gold	terpenoids like: eugenol, cinnamaldehyde	[77]
Murraya Koenigii	silver and gold	Carbazole alkaloids, flavonoids and polyphenols	[78]
banana peel	Silver	pectin, cellulose, hemicelluloses and protein	[79]
pongamia pinnata (L) pierre	Silver	flavones	[80]
Macrotyloma uniflorum	Silver	Polyphenol	[58]
Artocarpus heterophyllus Lam	Silver	Jacalin(lectin which is a single major protein)	[81]
Trigonella foenum-graecum	Gold	protein, vitamin C, niacin, potassium, and diosgenin	[82]
Memecylon edule	silver and gold	Saponin	[38]

Table 4. antibacterial activity of biosynthesized Ag NPs

	Staphylococcus aureus (ATCC25923)		*Enterococcus faecalis* (ATCC51299)		*Escherichia coli* (ATCC 25922)		*Pseudomonas aeruginosa* (ATCC27853)	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
EXRACT	128	ND	>128	ND	>128	ND	>128	ND
Ag NPs	16	ND	32	ND	4	ND	8	ND

ND: Not determined (>128 μg/ml)

Table 5. shows some of the different plants used in the synthesis of nanoparticles and their different sizes and their antibacterial properties compared to the preparation of silver nanoparticles in this study.

Plant	Type of NPS	Size (nm)	antibacterial effect	References
olive	silver	20-25 nm	This study showed that these nanoparticles at a concentration of 0.03-0.07 mg / ml significantly increased bacterial growth against multidrug-resistant Staphylococcus aureus (S. aureus), Pseudomonas aeruginosa (P. aeruginosa) and Escherichia coli (E coli) restrained.	[86]
Vitex Negundo L	silver	10-30 nm	The nanoparticles showed antibacterial activity in both gram-positive and gram-negative bacteria	[87]
Pedalium murex	silver	10-150 nm	Antibacterial activity increased with increasing concentration of nanoparticles	[88]
Sesbania grandiflora	silver	10-25 nm	The synthesized AgNPs showed strong antibacterial activity against multidrug-resistant bacteria (MDRs) such as Salmonella entrica and Staphylococcus aureus.	[89]

In fact, these functional groups diminish the stability of silver ions and subsequently their production yields.

Antibacterial effect of biosynthesized Ag NPs survey on gram positive and gram negative bacteria. The MIC results of biosynthesized Ag NPs on *E. faecalis* and *S. aureus* obtained 8 and 4 µg/mL and *P. aeruginosa* and *E. coli* obtained 16 and 4 µg/mL (Table 4). Synthesized nanoparticles have a significant antibacterial effect compared to the extract. The MBC test of Ag NPs showed that there was no result observed for testing all bacteria.

The antibacterial effect of biosynthesized Ag NPs is related to cell wall structure in gram-negative and gram-positive bacteria [83]. The sulfur and phosphor atoms present in cell well of bacteria. The silver tends to interact these atoms, so silver can kill bacteria by reacting with the cell wall of bacteria. Gram positive bacteria contain rigid polysaccharide in itself cell well, which makes it difficult for silver to penetrate the walls of these bacteria[84]. Hence, inhibitory activity of Ag NPs is stronger in gram-negative than gram-positive bacteria [85].

CONCLUSIONS

Silver nanoparticles were synthesized using *Euphorbia Pseudocactus Berger (Euphorbiaceae)* extract. existing Biomolecules in the plant extract act as fast bioreduction of silver ions during the formation nanoparticles. The average size of biosynthesized Ag NPs was determined 5.48-60 nm with an average diameter of about 9.16 -20.31 nm. Antibacterial results show good effect of biosynthesized nanoparticles on gram positive and gram negative bacteria. Moreover, the simulation results for XRD and UV-vis are in good agreement with experimental measurements. So, biosynthesized nanoparticles can be utilized as an antibacterial agent in medical and industrial devices and tools.

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CONFLICT OF INTEREST

The authors declare there is no any conflict of interest.

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