Current Applied Science and Technology Vol. 22 No. 5 (September-October 2022)

Research article

Biosynthesis of Silver Nanoparticles from Truffles and Mushrooms and Their Applications as Nanodrugs

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Received: 17 August 2021, Revised: 4 December 2021, Accepted: 12 January 2022

DOI: 10.55003/cast.2022.05.22.009

Keywords

green chemistry;
antibacterial activity;
Tirmania;
mushroom;
AgNPs

Abstract

This study aims to seek a new bio-agent as a reducer to synthesize silver nanoparticles (AgNPs). Dried fruiting bodies of truffles were used to mycosynthesize AgNPs, which were described by optical vision, UV-visible spectroscopy, SEM, AFM, Zetasizer, Granularity Cumulation Distribution, and FTIR analyses. The brown color is a sign of mycosynthesizing AgNPs. The UV-visible spectrum exhibited an absorption peak of 450 nm. Images of SEM showed that most of the AgNPs have an average of sizes reached 72 nm with irregular and spherical shapes. The concentration 10 mg/well exhibited 9.5 mm, and 20 mm toward *Klebsiella*, and *P. aeruginosa*, respectively. The synergism effect between gentamicin antibiotic and desert truffle-synthesized AgNPs is remarkable to increase the antibacterial activity. The zone of inhibition recorded 18 mm, 18.3 mm, and 20 mm by the conjugation between gentamicin and 5 mg/well of AgNPs compared with 21 mm, 20.6 mm and 23 mm by the conjugation between gentamicin and 10 mg/well against *S. aureus*, *Klebsiella*, and *P. aeruginosa*, respectively.

1. Introduction

Tirmania, one of the hypogeous ascomycetes (desert truffle) genera, develops underground and grows with *Helianthemum* (Cistaceae) plant in a mycorrhizal association [1]. After rainfall, *Tirmania* grows naturally in arid and semi-arid districts in Iraq, Iran, Jordan, Saudi Arabia, Kuwait, Turkey, Syria, Qatar, Bahrain, United Arab Emirates, Algeria, Morocco, Libya and Tunisia [2]. Moreover, the desert of Anbar province (Iraq) is rich with hypogeous ascomycetes such as *Tirmania* and *Terfezia* [3].

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Iraqi desert truffle species have an important nutritional value because of the presence of proteins, polysaccharides, fat, crude fibers and low calories [4]. Species of *Tirmania* contains a remarkable amount of different fatty acids [5]; therefore, this desert truffle is considered an important healthy natural products [6]. The desert truffle has been used as it has therapeutic properties such as anticancer [7], antioxidant [8], antibacterial [9], anti-cholesterol, and anti-cardiovascular activities. Desert truffles have been used as improvers for the human immune system, against disorders of prostate and sleep, as balancers of the body hormones in females, and as encouragers of Ca absorption from milk [10].

Few researchers have studied the anti-microbial effects of the desert truffle, particularly against human pathogenic microbes [2] like *Staphylococcus aureus* [11] and *Pseudomonas aeruginosa* [12]. The desert truffle also has hepato-protective efficacy [13]. Furthermore, *Terfezia claveryi* ascocarp extract was used to synthesize AgNPs and examine potential cytotoxicity against MCF-7 cancer cells (human breast line cells) [7]. But little work has been done on the desert truffle mediated-mycosynthesis of NPs [14]. While an extract of *Tirmania nivea* was used to mycosynthesize AgNPs [15], *Terfezia claveryi* was used to mycosynthesize AgNPs with excellent cytotoxicity against breast cancer cell line MCF-7 [7]. Over the last decade, some scientific researchers have focused on the use of edible mushrooms in the biosynthesis of ecofriendly metallic NPs (nanoparticles). The increased usage of macrofungi in the nano-science field can be related to the capability of producing huge amounts of myco-biomass [16].

In this work, we aimed to mycosynthesize AgNPs (silver nanoparticles) from Iraqi desert truffles, and to describe the mycosynthesized silver nanoparticles through color alteration, UV-vis spectroscopy, AFM, SEM, and FT-IR analyses. Furthermore, the antibacterial activity of AgNPs against some pathogenic bacteria was studied and compared to AgNPs conjugated with gentamicin *in vitro*.

2. Materials and Methods

2.1 Microbial samples

The pathogenic bacteria: *Escherichia coli, Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella* spp. were obtained from laboratories of Ramadi Hospital, Iraq, and used to test the synergistic effects of gentamicin conjugated with truffle-synthesized AgNPs.

2.2 Truffle sample

Tirmania sp. (desert truffle) fruiting bodies grown in Anbar desert were purchased from a local market, in Hit, Iraq. After cleaning, slicing, and drying, the fruiting bodies were ground to obtain desert truffle powder, which was kept at 2°C for further studies.

2.3 Extraction of truffle fruiting bodies

To extract the desert truffle, 5 g *Tirmania* sp. powder was extracted with 100 ml D.W and stirred using a magnetic starrier hot plate at room temperature for 1 h [17]. The aqueous extract was filtered with a Whatman No. 1 filter paper and centrifuged at 4000 rpm for 15 min. The extract was then stored at 2°C to until use.

2.4 Mycosynthesis and characterization of *Tirmania* assisted-silver nanoparticles

To synthesize AgNPs, 50 ml of the aqueous extract of *Tirmania* sp. was mixed with 100 ml of 1 mM $AgNO_3$ in a 250ml-flask in the dark and at room temperature for 1 day. Change of color, UV-visible spectroscopy, FTIR (Fourier-transform infrared spectroscopy), Zetasizer, Particle Cumulation analysis, SEM (Scanning Electron Microscopy), and AFM (Atomic Force Microscopy) were used to analyze and characterize the mycosynthesized AgNPs from *Tirmania* sp. truffle. The accumulation of nanoparticles was checked with the electron microscop.

2.5 Synergistic effect of *Tirmania*–synthesized AgNPs conjugated with gentamicin

The synergistic effect of the truffle-synthesized AgNPs was checked and compared with gentamicin and with truffle-synthesized AgNPs. Two concentrations of the truffle-synthesized AgNPs (5 and 10 mg/well) were conjugated with 25 µg gentamicin antibiotic, then tested against a Gram-positive bacterium (*Staphylococcus aureus*) and three Gram-negative bacteria (*Klebsiella* spp., *Escherichia coli,* and *Pseudomonas aeruginosa*) using the agar-well diffusion method and the zone of inhibition was calculated and compared with gentamicin (alone as a positive control) using Muller-Hinton agar dishes (90 mm), as described by Owaid [18]. Also, the two concentrations of AgNPs were used as negative controls (alone) in this work. After incubation at 37°C for 24 h, the zones of inhibition were recorded. This method was done to examine the antibacterial efficacy of AgNPs synthesized from truffle and the synergistic effect with the antibiotic.

2.6 Statistical analysis

The zones of inhibition data of the synergistic effect with gentamicin were collected and analyzed using ANOVA table in one way (SAS program 9.0, SAS Institute Inc., USA). The significance of the difference (p<0.05) was calculated by Duncan's Multiple Range Test (DMRT).

3. Results and Discussion

Figure 1 showed that the lambda max was 410 nm with an absorbance of 1.091. The optical vision changed from very bright yellow to brown after the reaction between the truffle extract and $AgNO_3$ (1mM), which was considered as a sign that AgNPs had formed [19]. Changes in visual optical confirmed the synthesis of AgNPs from truffle extract [20, 21], as seen in Figure 1. The UV-vis spectrum exhibited a maximum peak at 410 nm, which agreed with the results of Muhsin and Hachim [16, 17].The values of AgNPs bands in the UV-vis spectrum are related to the Localized Surface Plasmon Resonance property which is affected by size, shape and assembly of particles [22]. This optical phenomenon is generated when light interacts with conductive NPs smaller than the incident wavelength [23]. Plasmonic features are important in the determination of the shape and size of NPs, which may be spherical in this study [22]. Furthermore, in a recent study, colloidal *Tirmania nivea*-AgNPs exhibited a lambda max at 420 nm [15], which agreed with the results of the current work.

The FTIR spectrum of the truffle extract was achieved in the region from 4000.00 to 400.00 cm^{-1} as shown in Figure 2A. The peak at 600.29 cm^{-1} (intensity of 44.938) is considered a strong sign of the existence of some heterocycles like alkaloids [24]. Besides, the peaks at 988.85 cm^{-1} (intensity of 29.330), and 1024.11 cm^{-1} (intensity of 29.228) relate to the CS and CO groups, which indicate the existence of amino acids and proteins. The peaks at 1147.03 cm^{-1} (intensity of 60.593)

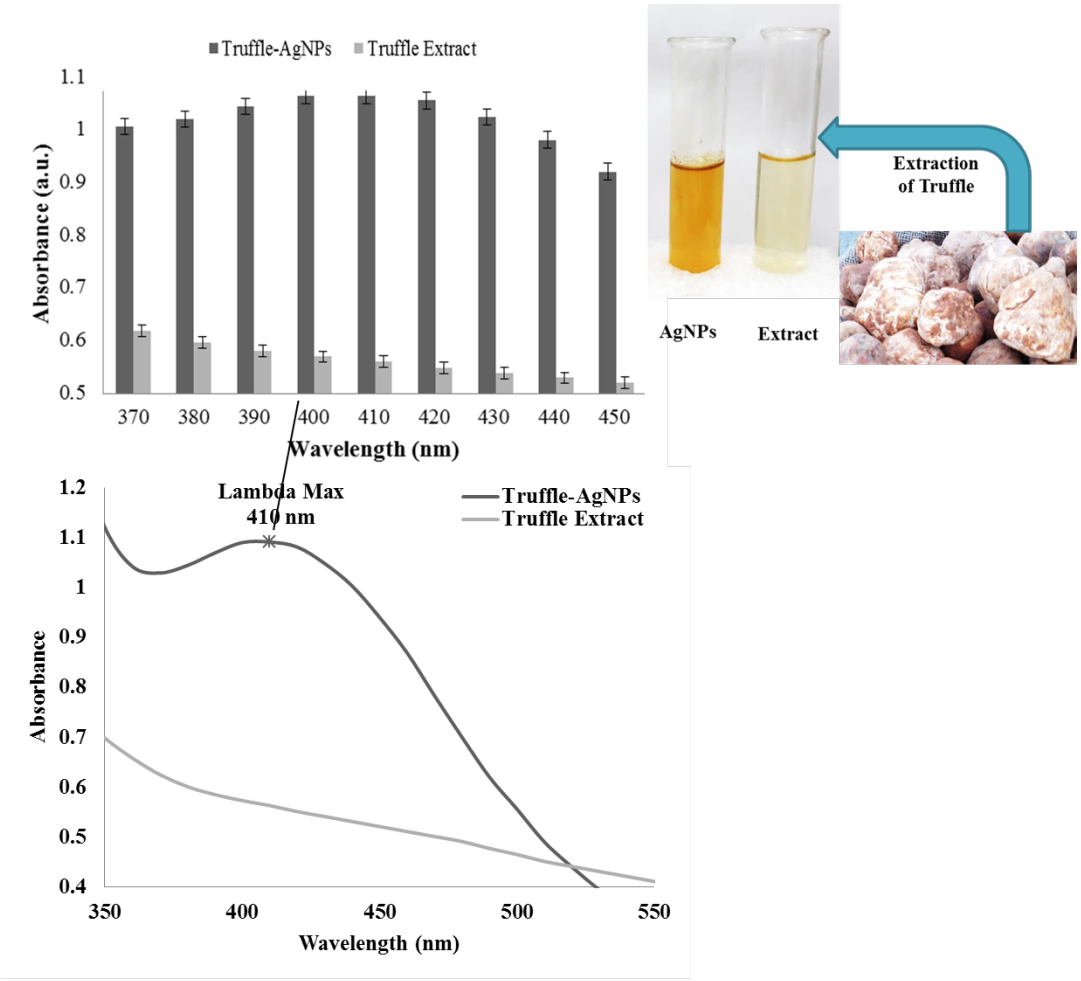

Figure 1. UV-visible spectrum and optical vision of the truffle-synthesized AgNPs

and 1370.46 cm^{-1} (intensity of 69.474) show the presence of the methyl (CH_3) group in lipids and proteins, respectively. The peak at 1633.84 cm^{-1} (intensity of 72.157) is related to carboxylic (C=O) as well as amino acids (N-H), and is a clear sign of proteins. The peak at 2926.24 cm^{-1} (intensity of 77.051) is associated with the vibration groups of -CH_2 and -CH_3 in carboxylic acids. Finally, the peak at 3271.78 cm^{-1} (intensity of 63.192) shows the stretching of O-H and -NH groups (hydroxyl and amine) for proteins.

The matches of all AgNPs bands with aqueous truffle extract is shown in Figure 2B. The differences in identical peaks between the extract and the as-prepared AgNPs are distinguished. Nevertheless, the presence of the broad stretching vibration at 3388.70 cm^{-1} due to the hydroxyl group (O-H) stability on AgNPs confirms the role of -NH from protein in the reduction of silver ions.

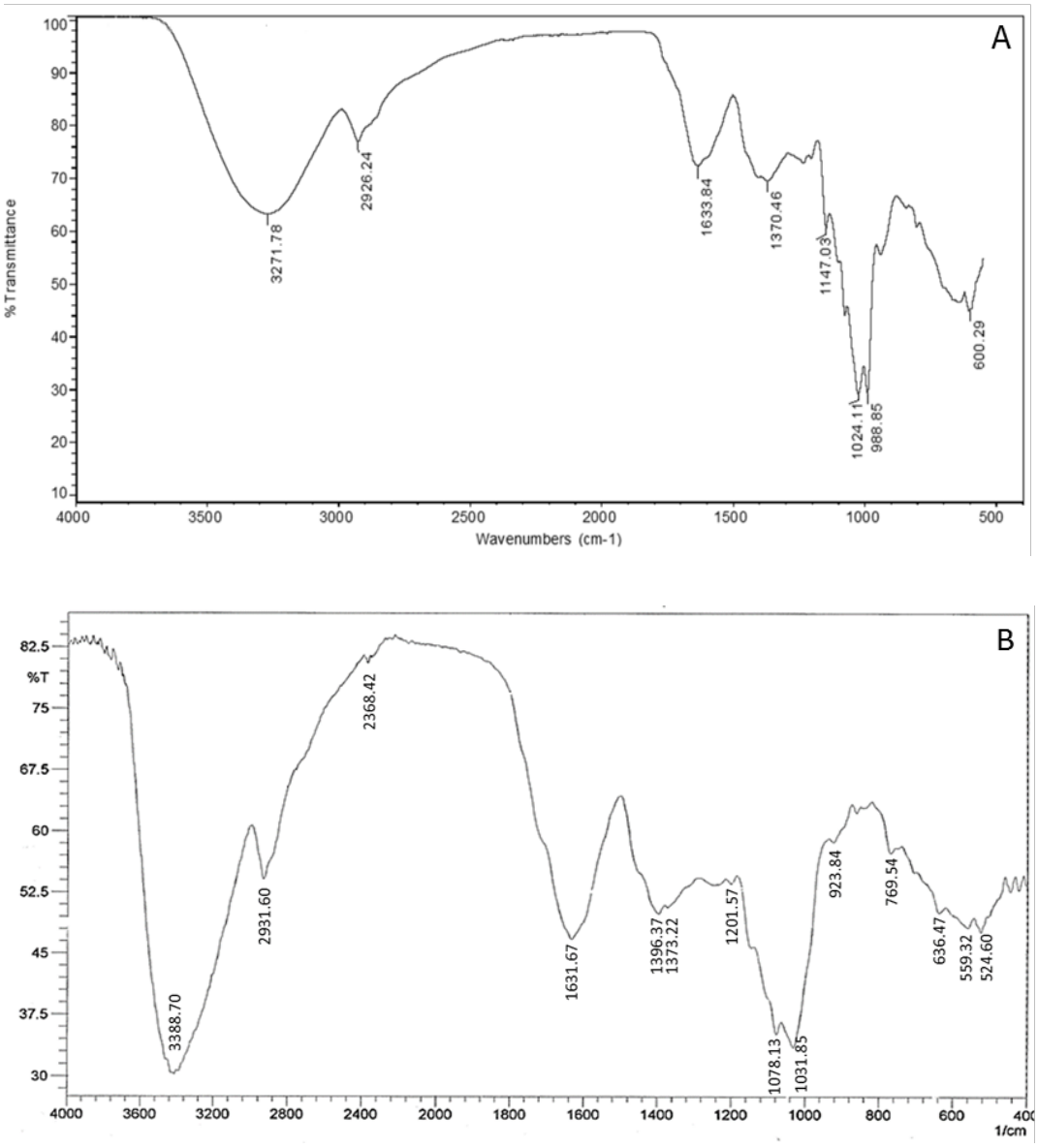

Figure 2. FTIR of truffle extract (A) and truffle-synthesized AgNPs (B)

The topography images of the Atomic Force Microscope (2042 nm×2037 nm) showed surface roughness analysis. Figure 3 shows the 2D and 3D images performed with the AFM, which showed some physical parameters, including amplitude, hybrid, functional and spatial parameters. Amplitude parameters were Roughness Average, Surface Skewness, Root Mean Square, Surface Kurtosis, Ten Point Height, and Peak-Peak of 21.1 nm, -0.152, 24.7 nm, 1.94, 48.6 nm, and 94.2 nm, respectively. While the hybrid parameters shown were Surface Area Ratio, Root Mean Square Slope, and Mean Summit Curvature of 66.7, 1.42 nm^{-1}, and -1 nm^{-1}, respectively.

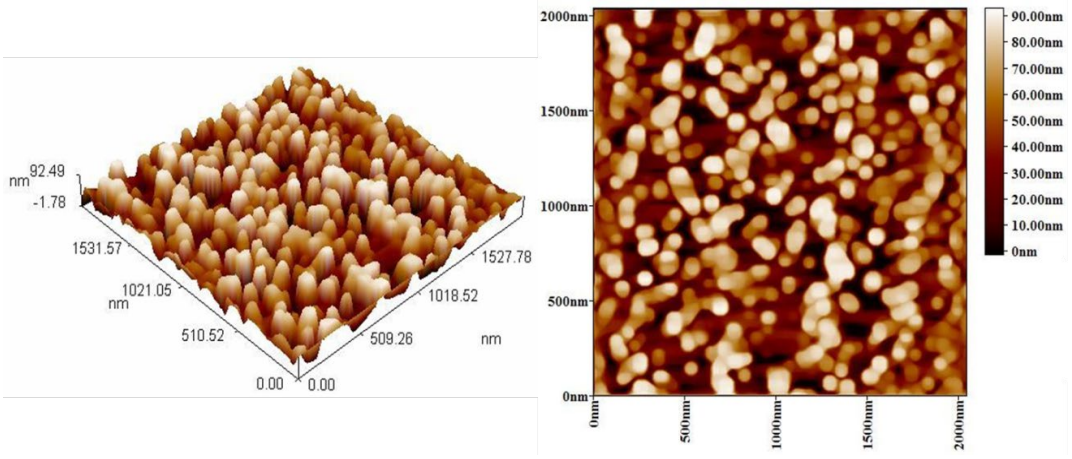

Figure 3. AFM 2D and 3D images of the truffle-synthesized AgNPs

Functional parameters exhibited were Core Fluid Retention Index, Surface Bearing Index, Reduced Summit Height, Valley Fluid Retention Index, Reduced Valley Depth, and Core Roughness Depth of 1.42, 3.19, 1.56 nm, 0.0905, 10.4 nm, and 78.9 nm, respectively. Finally, spatial parameters exhibited Density of Summits and Fractal Dimension of 0 um^{-2} and 2.58, respectively.

Besides, Figure 4 (SEM pattern and Zetasizer) exhibited the shape of the truffle-Ag nanoparticles as irregular particles with a size average of 92.63 nm. DLS showed a range of AgNPs sizes from 65 nm to 120 nm at different percentages. The diameter 65 nm, 70 nm, and 80 nm exhibited volumes of 1.39%, 4.17%, and 5.56%, respectively. The sizes of 100 nm, 105 nm, 110 nm, 120 nm showed volumes reaching 8.33% for each, while 90 nm and 115 nm exhibited volume reaching 10.42% for each. The diameters of 75 nm and 95 nm showed the volumes of 9.03 % and 9.72%, respectively. The highest volume was 15.97%, with the diameter of 85 nm. Irregular, spherical, and hummock-like forms of *Tirmania*-AgNPs was reported by Muhsin and Hachim [17]. Moreover, aggregated or dispersed spherical *Tirmania nivea*-AgNPs mycosynthesized from truffles with sizes ranging from 3 nm to 41 nm were reported [15].

The cumulation of silver nanoparticles mycosynthsized from desert truffle varied according to the diameter parameter in nanometer scale, as shown in Figure 5, which shows Granularity Cumulation Distribution. The cumulation of NPs together did not occur with small sizes of NPs in this study and that agreed with many studies [23], compared to the big sizes. The least cumulation was 1.39%, with AgNPs 65 nm followed by 5.56% with the size of 70 nm (as single NPs); it was important for stability of the colloidal AgNPs. The particles with sizes 75, 80, and 85 nm showed cumulation percentages of 14.58%, 20.14% and 36.11%, respectively. The particles ranging from 90-100 nm exhibited cumulations of 46.53-56.25%. While 105, 110 and 115 nm particles showed cumulation percentages reaching 72.92%, 81.255, and 91.67%.

Finally, the biggest cumulation percentage was 100%, with AgNPs of 120 nm. However, large silver nanoparticles accumulate, and small AgNPs do not stay accumulating in the colloid [25]. These results lead to precipitate cumulative AgNPs and enhanced colloid AgNPs properties [26].

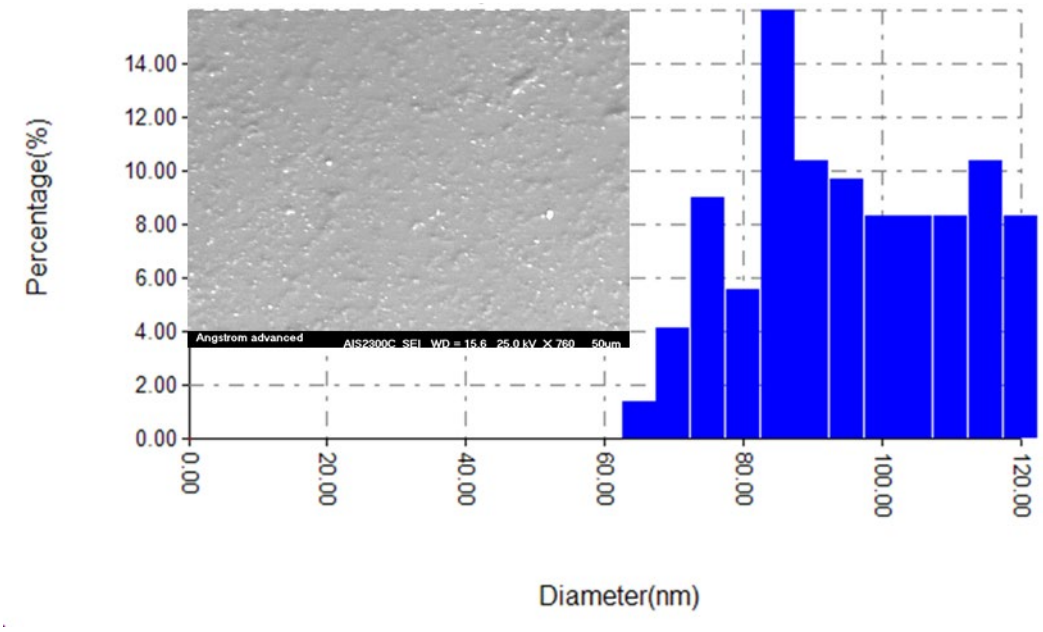

Figure 4. DLS (Dynamic Light Scattering: including SEM and Zetasizer) of the mycosynthesized AgNPs

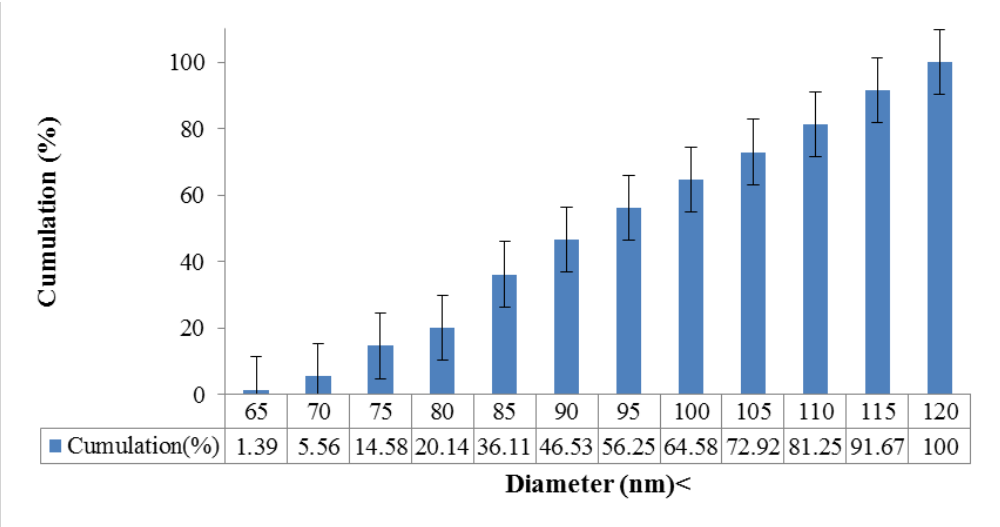

Figure 5. Granularity Cumulation Distribution chart

Bioactivity of the mycosynthesized silver nanoparticles AgNPs from *Tirmania* sp. revealed suitable antibacterial activity and remarkable synergistic effect with gentamicin (Figure 6) against three human pathogenic microbes, including *Klebsiella* spp., *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The concentration of 5 mg/well *Tirmania* extract showed a zone of inhibition reaching 10.5 mm against *Pseudomonas aeruginosa,* but other bacteria did not show any

inhibitory effect in this concentration. While, a 10 mg/well concentration of *Tirmania* extract exhibited inhibition zones of 9.5 mm, 11 mm, 18 mm, and 20 mm towards *Klebsiella* spp., *E. coli*, *S. aureus* and *P. aeruginosa*, respectively. Moreover, a synergistic effect of *Tirmania*-AgNPs with gentamicin was done between 5 mg and 10 mg AgNPs mixed with just 25 µg gentamicin per well separately. The concentration of 10 mg AgNPs with 25 µg gentamicin per well was more effective than 5 mg AgNPs with 25 µg gentamicin per well, as shown in Figure 6. The zones of inhibition recorded 18 mm, 18.3 mm, and 20 mm by the conjugation between gentamicin and 5 mg/well of biosynthesized AgNPs compared with 21 mm, 20.6 mm and 23 by the conjugation between gentamicin and 10 mg/well against *S. aureus*, *Klebsiella* spp., and *P. aeruginosa*, respectively. Besides, in the case of *Escherichia coli*, the same effect (about 12-13 mm) was seen in the case of the conjugation between gentamicin and the 10 and 5 mg AgNPs. However, 50 µg/well of gentamicin exhibited antibacterial activity that reached 24 mm, 25 mm, 27 mm and 31 mm against *Klebsiella* spp., *S. aureus*, *E. coli* and *P. aeruginosa,* respectively, as the control.

Generally, an extract of *Terfezia claveryi* has antibacterial action against *Streptococcus pneumoniae* [9]. Also, the AgNPs synthesized from fungi have antibacterial efficacy [27]. Moreover, the AgNPs of *Tirmania nivea* are active against *Staphylococcus aureus*, *Proteus mirabilis*, *Escherichia coli*, *Salmonella typhi*, and *Pseudomonas aeruginosa* [15]. Thus, using AgNPs against microbes enhances the antibacterial activity against pathogens [28]. The current results of the synergistic effects agree with some studies which applied various antibiotics with fungal-NPs to enhance bioactivity against pathogenic bacteria [29, 30] by affecting the genomic DNA sequence [31]. Also, a remarkable increase of bacterial growth inhibition zones (25.5-35.5 mm diameter) was achieved by the combination of gentamicin with fungus-NPs to increase the zone of inhibition of the growth of bacteria [20]. The truffle-silver nanoparticles play an inhibitory role, working after the antibiotic (gentamicin), which itself weakens the resistance of bacteria and eases the work of the AgNPs. The use of the AgNPs thus could lead to the use of antibiotics at lower concentrations in treatment, which may reduce the mutations of microbes by antibiotics.

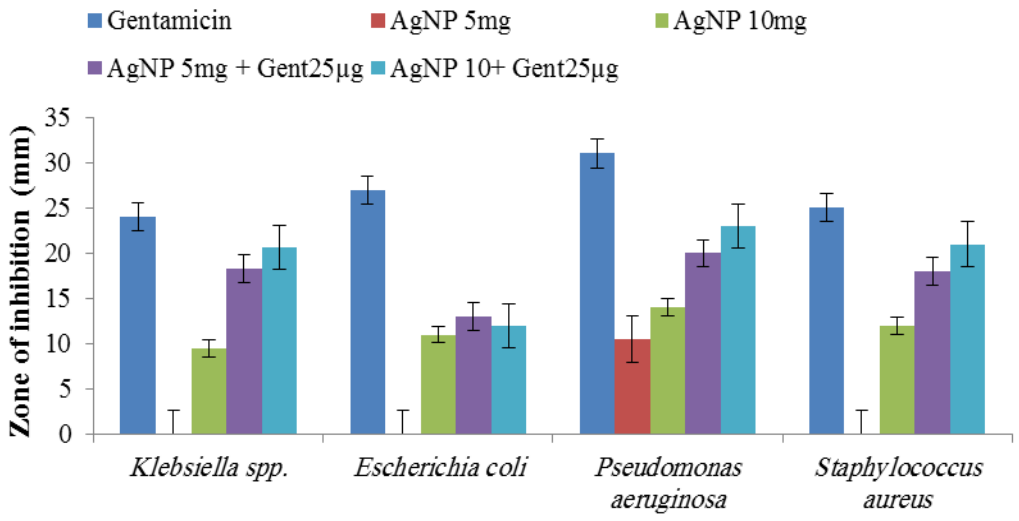

Figure 6. Synergistic effect (zone of inhibition, mm) of truffle-synthesized AgNPs conjugated with gentamicin

4. Conclusions

The truffle-silver nanoparticles play an inhibitory role, working after the antibiotic (gentamicin), and weaken the resistance of bacteria and ease the work of the AgNPs. Bioactivity of the mycosynthesized silver nanoparticles AgNPs from *Tirmania* sp. revealed suitable antibacterial activity and remarkable synergistic effect with gentamicin against some human pathogenic microbes. The 10 mg/well concentration exhibited 9.5 mm, and 20 mm towards *Klebsiella* spp. and *P. aeruginosa*, respectively. The zones of inhibition recorded 18 mm, 18.3 mm, and 20 mm by the conjugation between gentamicin and 5 mg/well of biosynthesized AgNPs compared with 21 mm, 20.6 mm and 23 by the conjugation between gentamicin and 10 mg/well against *S. aureus*, *Klebsiella* spp. and *P. aeruginosa*, respectively. This method can lead to the use of antibiotics in lower concentrations in the treatment, an effect which in turn slow down or reduce the mutations of microbes due to antibiotic resistance.

5. Acknowledgements

I thank the Iraqi Ministry of Education for their support of this project No. 31406/6/13, registered on 08/08/2019 and I also thank the Iraqi Ministry of Higher Education and Scientific Research, order No. 23649, registered on 17/09/2019.

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