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# Mycosynthesis of Silver Nanoparticles using *Terminia* sp. Desert Truffle, Pezizaceae, and their Antibacterial Activity

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**Abstract**

Desert truffle *Tirmania* sp. is appropriate as a greener reducing agent to synthesize silver nanoparticles at room temperature in a dark place by utilizing the aqueous extract of ascocarps of *Tirmania* sp. and $10^{-3}AgNO_3$ solution. Characterization of those nanoparticles was accomplished by changing color, UV-visible spectrum, FT-IR, AFM and SEM analyses. The changing in color is considered a first indicator for the formation of AgNPs which changed from pale yellow to dark brown after seventy-two hours. UV-visible spectrum showed the formation of AgNPs at peak 415 nm. AFM and SEM images demonstrated nanoparticles with spherical or irregular-shaped, agglomerated and highly heterogeneous. The higher zone of inhibition was 14 mm against *Pseudomonas aeruginosa* at a concentration of 5 mg/well while, the lower zone of inhibition was 9.5 mm toward *Staphylococcus aureus* at the level of 5 mg/well. These *Tirmania*-AgNPs had a promising antibacterial activity toward gram-negative and gram-positive bacteria, mainly the infectious bacteria of the eyes, *Pseudomonas aeruginosa*.

**Keywords:** Ascocarp, Tuber, Antibacterial effects, Green nanotechnology, Fungi.

## 1. Introduction

Desert truffles are hypogeous ascomycetes which develop underground such as *Terfezia* sp. and *Tirmania* sp. which grow in mycorrhizal association with *Helianthemum* sp., Cistaceae family (Bradai *et al.*, 2014). Desert truffles grow naturally after rainfall in arid and semi-arid zones of some deserts in Middle Eastern and Arabian countries like Iraq, Saudi Arabia, Jordan, Syria, Turkey, Iran, Kuwait, United Arab Emirates, Qatar, Bahrain, Morocco, Algeria, Tunisia and Libya (Owaid, 2018). The desert of Iraq in Anbar province is rich in desert truffles especially *Terfezia* sp. and *Tirmania* sp. (Owaid, 2016).

These truffles have a sufficient nutritional value because of their compositions of proteins, crude fibers, polysaccharides, fat and low energy (Dogan and Aydin, 2013). *Tirmania* sp. is rich in fatty acids (Bokhary *et al*., 1989). Thus it is considered one of the natural health products (Dogan *et al.*, 2013). Truffles have therapeutic benefits including anticholinesterase, antioxidant (Tel-Cayan *et al*., 2018), anticancer, anti-cholesterol, anti-cardiovascular activities. They can be useful in improving the immune system, in the prevention of sleep and prostate disorders, hormonal imbalances in females and in increasing the absorption of calcium from milk (Gajos *et al*., 2014).

Several studies have discussed the antimicrobial effects of desert truffles especially against microbes (Owaid, 2018), such as *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Janakat *et al*., 2004; Janakat *et al*., 2005). But only few researchers have investigated the biosynthesis of desert truffle-nanoparticles and their bioactivity recently. Muhsin and Hachim (2016) managed to synthesize AgNPs from *Tirmania nivea*, while Khadri *et al.* (2017) produced silver nanoparticles from *Terfezia claveryi* which showed excellent cytotoxicity toward the breast cancer cell line. Some researchers have focused on using macro-fungi as natural products to mycosynthesize metallic nanoparticles in the last decade. The increase in using edible mushrooms in the field of nanoscience can be attributed to the ability to produce huge amounts of fungal biomass (Owaid and Ibraheem, 2017).

This study is one of its kind conducted to biosynthesize silver nanoparticles from Iraqi truffles without heating, and to characterize the biosynthesized AgNPs using the changing color, UV-Visible spectrum, FT-IR, AFM and SEM analyses also to investigate the bioactivity of AgNPs against some pathogenic bacteria *in vitro*.

## 2. Materials and Methods

### *2.1. Desert Truffles Samples*

Ascocarps (fruiting bodies) of Iraqi desert truffle *Terminia* sp. were obtained from Hit market, Iraq. The ascocarps (Figure 1) were cleaned, sliced, dried in the direct sun at 30-35 °C until stability of weight, and were

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ground to obtain powder. The truffle powder was kept at room temperature in a dry place until use.

**Figure 1** Slices of Ascocarps of Iraqi desert truffle *Terminia* sp.

*2.2. Bacterial Isolates*

Four human pathogenic bacterial isolates were obtained from Al-Ramadi Hospital Lab in Iraq to investigate the bioactivity (antibacterial activity) *in vitro* of the synthesized silver nanoparticles using agar-well diffusion testing. They are *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella* spp., and *Staphylococcus aureus*.

*2.3. Extraction of Desert Truffle Ascocarps*

Ten grams of powder of the dried *Terminia* sp. desert truffle with 200 ml distilled water were extracted by stirring on magnetic starrier for sixty minutes to get the crude extract. The crude aqueous extract was filtered using filter paper Whatman No. 1, centrifuged at 4000 cycle/min for fifteen minutes and the aqueous extract was kept at 2 °C for future studies, while the residue was omitted.

*2.4. Biosynthesis and Characterization of Silver Nanoparticles Using Terminia sp.*

Ten milliliters of *Terminia* sp. desert truffle extract (concentrations 5, 10, 15 mg/ml) and 5 ml of $10^{-3}$ M $AgNO_3$ were mixed in glass test tubes separately and incubated at 30 °C for twenty-seven hours in a dark place. Changing in color, UV-Visible spectrum, FT-IR, SEM, and AFM analyses were performed to characterize the synthesized silver nanoparticles from desert truffles.

*2.5. Antibacterial Activity of the Truffle-AgNPs*

The mycosynthesized Ag nanoparticles using *Terminia* sp. desert truffle were tested the antibacterial action by agar-well diffusion testing against the Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella* spp.) and Gram-positive bacteria (*Staphylococcus aureus*). The tested pathogenic bacteria were maintained in nutrient broth for 24 hr and kept undisturbed until use. Muller-Hinton agar plates were prepared for this test. Then 100 µL of 24 hr activated bacterial cultures of individual organisms were spread over Muller-Hinton plate using a cotton swab. Wells of seven millimeters (diameter) were made on each dish by sterile cork borer. Then, 50 µL per well of the biosynthesized Ag nanoparticles solution was poured, and 25 µg/well of Gentamycin was used as a control in this work. The plates were left in a cool place for 30 min to spread the biomaterials then incubated at 37 °C for 24 hr. Zone of inhibition of each bacterium was recorded.

*2.6. Statistical Aspect*

The data of zones of inhibition of the antibacterial activity test were analyzed in one way analysis of variance by table of ANOVA (SAS program version 9, SAS Institute Inc., USA) Significance of differences were calculated by DMRT (Duncan's Multiple Range Test). Probability value < 5% ($p$<0.05) is considered to be statistically significant.

## 3. Results and Discussion

*3.1. Characterization of Truffle-AgNPs*

The visual observation of color changes from pale yellow to dark brown as shown in Figure 2 after seventy-two hours of incubation in a dark place at 30 °C showed the formation of desert truffle silver nanoparticles (truffle-AgNPs). The synthesis of AgNPs was confirmed by UV-Visible spectra which agree with the results of Owaid *et al*. (2015). Figure 3 showed higher UV-visible peak of 415 nm which confirmed the formation of truffle-AgNPs in this test.

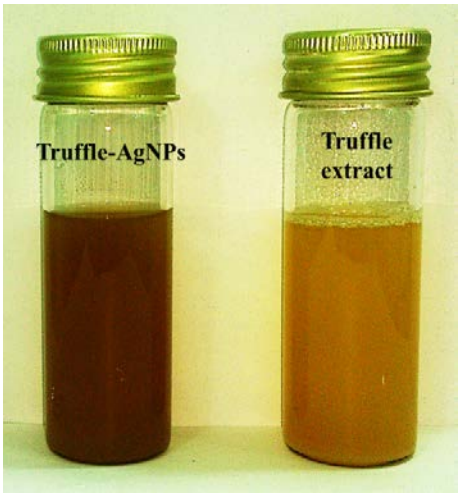


**Figure 2.** Changing the color of truffle extract with $AgNO_3$ solution after 72 hr

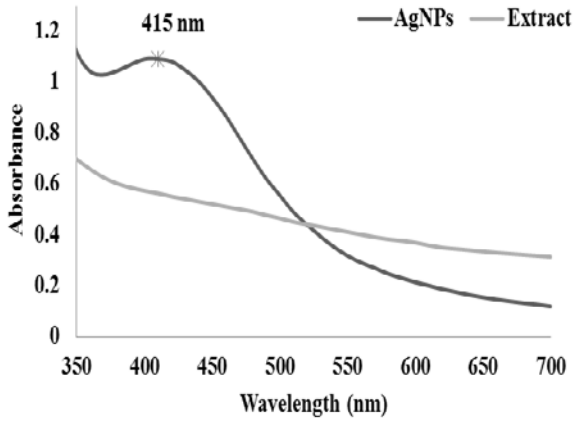


**Figure 3,** UV-Visible spectrum of the desert truffle-AgNPs solution

The surface topography was investigated using Atomic Force Electron microscopy (AFM) image (Figure 4) which shows the histogram of the percentage of AgNPs as a

function of the grain size. The AFM images illustrate the surface morphology and roughness. Figure 4 shows that the synthesis leads to the formation of spherical, irregular and hummock-like structures in the silver layers. The formation may be connected with an enhanced diffusion of silver particles during their aggregation into more massive structures. Furthermore, SEM image showed an aggregate of dispersed particles of AgNPs as in Figure 5.

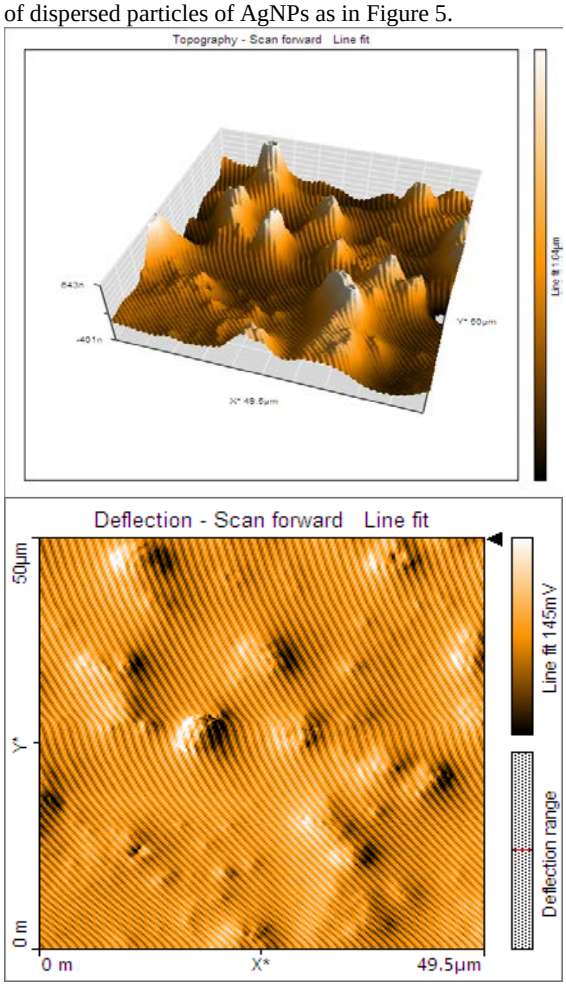


**Figure 4.** AFM of desert truffle-AgNPs

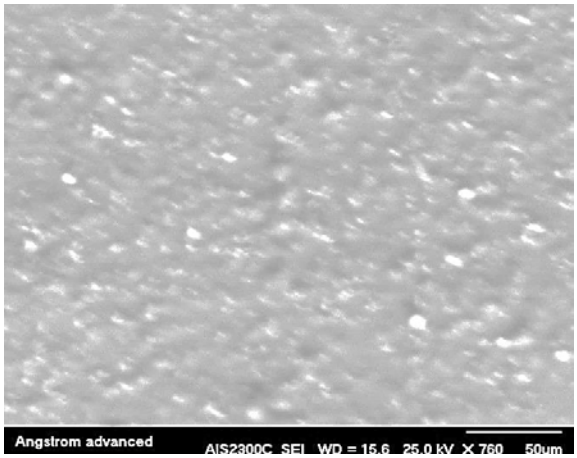


**Figure 5.** SEM of desert truffle-AgNPs

The FT-IR spectrum of silver nanoparticles was used to identify the potential bio-reductants of the aqueous extract of *Terminia* sp. involved in the reduction of silver nitrate ($10^{-3}$ M). Absorbance peaks from 3461.99 $cm^{-1}$ to 445.53 $cm^{-1}$ represent the role of different functional groups in the bio-reduction of $AgNO_3$ (Figure 6).

The absorbance peaks at 3461.99, 3431.13, 3411.84, 3388.70 and 3290.33 $cm^{-1}$ correspond to a stretch group hydroxyl (O-H). Peaks between 2931.60 and 1969 $cm^{-1}$ correspond to a C=O vibration at the α- and β-unsaturated aldehydes, while the peak 1636 $cm^{-1}$ indicates C=O stretch. Peaks of 1094 $cm^{-1}$ and 611 $cm^{-1}$ are strong indications of heterocyclic compounds such as alkaloids (Meghwal and Goswami, 2012).

In the current study, peaks of 1094 $cm^{-1}$ and 1261 $cm^{-1}$ disappeared after the nanoparticles synthesis indicating the involvement of amine group (C-N) and alcohol group (C-O) respectively in the bio-reduction process. The wide range 2400-2600 $cm^{-1}$ indicates the presence of a carboxylic group (-COOH). The carboxylic group indicates the presence of free amino acids and proteins as well as the presence of fatty acids, but may be small in comparison to the amino acids and proteins. The presence of the peak at 3411 $cm^{-1}$ and the existence of lactam group (-NH-CO-) at the peak of 1631 $cm^{-1}$ are considered clear evidences of the existence of proteins. The lactam group is called amid group which links the different amino acids to make up the protein. The wideband range (2900-3600 $cm^{-1}$) indicates a hydroxyl group (OH), which means the presence of Tyrosine, Serine, and Threonine, or at least one of them. The peaks of 923 $cm^{-1}$, 1031 $cm^{-1}$ and 1078 $cm^{-1}$ report the existence of CS CN and CO groups which also indicates the presence of proteins and amino acids. The two packs at 2517 and 2768 $cm^{-1}$ are clear evidence on the existence of -SH group, and this is another evidence of the presence of Cysteine and is likely to be free or existing at the end of the protein.

In addition, the researchers believe there are lipoproteins and lipopolysaccharides but in small amounts. Peak 1373 $cm^{-1}$ indicates the presence of methyl group ($CH_3$) and is present in the composition of some free amino acids or proteins. The three consecutive peaks at 1147, 1201 and 1244 $cm^{-1}$ are clear and definitive evidences on the presence of the methyl group in the composition of lipids because of the saturated and unsaturated fatty acids starting with its chemical composition by an analog group. The peak at 2931 $cm^{-1}$ indicates the presence of the group (CH) of amino acids, proteins or lipids. Peaks from 636 to 707 $cm^{-1}$ are indicative of the out-of-level curve of the group -N-H. The peaks of 445-559 $cm^{-1}$ indicate that single bonds are connecting an organic compound with individual elements (maybe silver) in the biosynthesized silver nanoparticles, but the type of the element could not be determined here by FT-IR alone (Silverstein *et al*., 2005).

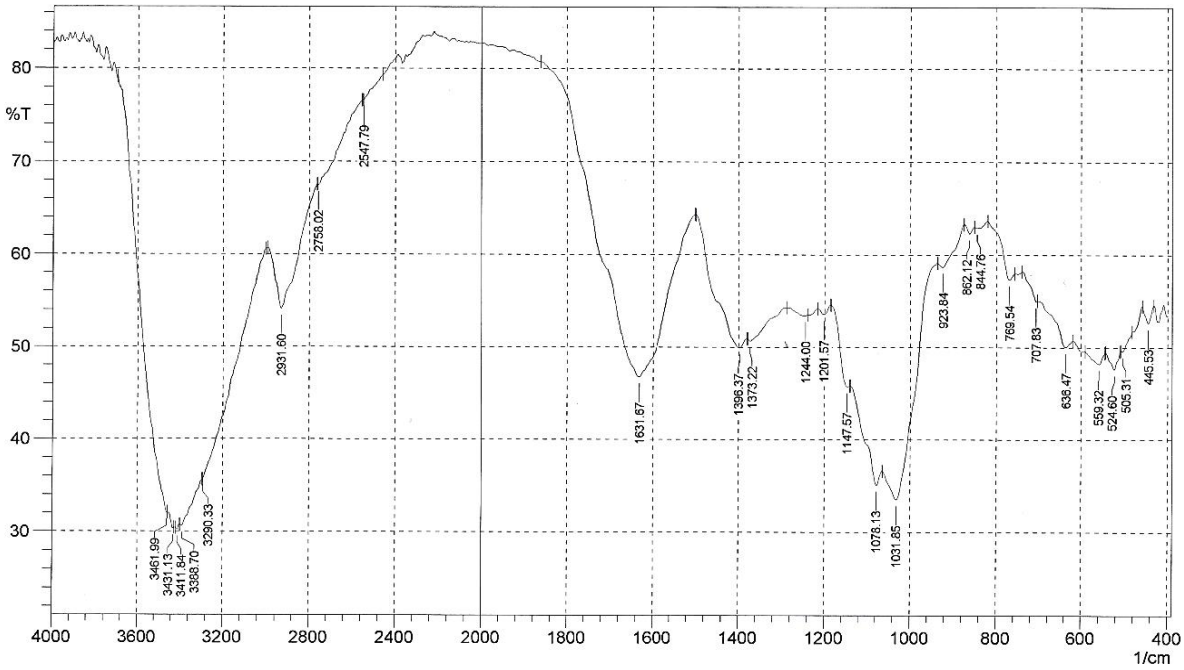


**Figure 6.** FT-IR spectrum of truffle-AgNPs

### *3.2. Antibacterial Activity of Truffle-AgNPs*

The concentration 2.5 mg/well did not exhibit any inhibitory efficacy as shown in Figure 7. The level 5 mg/well had the best zone of inhibition 10.5 mm against *Pseudomonas aeruginosa* (Gram-negative bacterium) followed by *Staphylococcus aureus* (Gram-positive bacterium) of 9 mm. However, the higher concentration 10 mg/well showed an inhibitory effect against all the studied pathogenic bacteria. The higher inhibition was 14 mm against *Pseudomonas aeruginosa* compared with 31 mm by the control (50 µg/well of gentamycin), followed by *Staphylococcus aureus* (12 mm compared with 25 mm by the control). As shown in Figure 8, the zone of inhibition of *Escherichia coli* and *Klebsiella* spp. recorded 11 and 9.5 mm in the case using 10 mg/well of truffle-AgNPs compared with the control (27 and 24 mm) respectively. The effectiveness of the synthesized AgNPs (as antimicrobial agents) is related to their ratio of high surface area to volume. This property is enabling specific interactions with the bacterial cell membrane (Morones *et al.,* 2005). The truffle-silver nanoparticles play an inhibitory role against the pathogenic bacterium which opens the door towards using this biomaterial (desert truffle) as reducing and stabilizing agents in producing green nano-drugs against human pathogenic bacteria, in particular *P. aeruginosa*.

As the AgNPs come in contact with the bacteria, they adhere to the cell wall and cell membrane. Some of the silver adheres to the sulfur-containing proteins on the membrane (Klasen, 2000). The silver-sulfur interaction at the membrane causes many structural and morphological changes in the cell wall such as the formation of pores and pits. Through these pores, cellular components are released into the extracellular fluid, just due to the osmotic difference. While another portion passes to the inside of bacteria and interacts with DNA and RNA thus AgNPs will inhibit the cell's replication proteins that lead to the death of bacterial cells (Feng *et al*., 2000). This interaction has been linked to the suppression of enzymes, and inhibited the expression of proteins that relate to the ability of cells to produce ATP (Yamanaka *et al.*, 2005).

As the AgNPs interact with the microbes, they adhere to the wall and membrane of cells. A portion of the silver atoms adheres to the sulfur-containing proteins on the cell membrane (Klasen, 2000). The silver-sulfur interaction at the membrane causes numerous basic and morphological changes in the cell wall, for example, the formation and development of pores and pits. Through these pores, some cellular components are discharged into the extracellular fluid, just due to the osmotic difference. While another portion passes to the inside of the bacteria and interacts with DNA and RNA thus AgNPs will inhibit the cell's replication proteins that lead to the death of bacterial cells (Feng *et al.*, 2000). This interaction has been linked to the suppression of enzymes and inhibited the expression of proteins that relate to the ability of cells to produce ATP (Yamanaka *et al*., 2005).

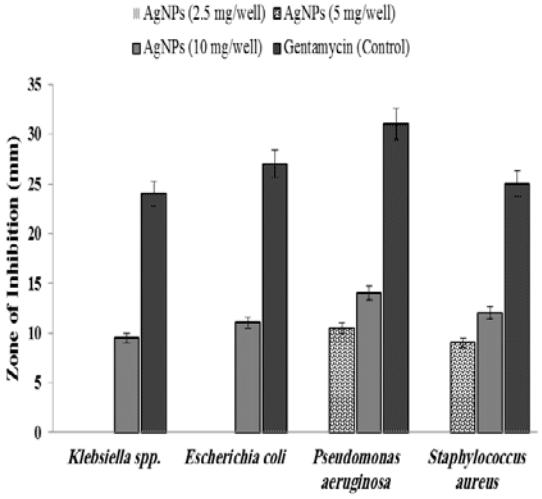


**Figure 7.** Antimicrobial activities of desert truffle-AgNPs

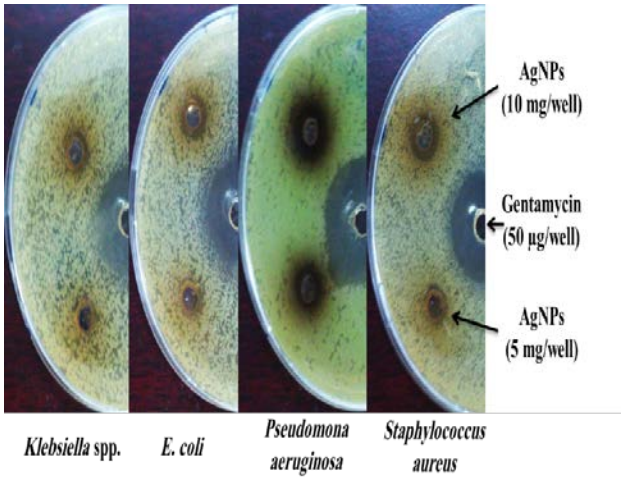


**Figure 8.** Zone of inhibition of desert truffle-AgNPs by agar-well diffusion testing

## 4. Conclusion

Desert truffle *Tirmania* sp. is suitable as a greener-reducing agent to mycosynthesize silver nanoparticles at the room temperature in a dark place using the aqueous extract of its ascocarps and $10^{-3}AgNO_3$ solution. The changing in color from pale yellow to dark brown after seventy-two hours is considered a first indicator of the formation of AgNPs. The characterization of those nanoparticles was also achieved by UV-Visible spectrum, FT-IR, AFM and SEM analyses. The UV-visible spectrum showed the formation of AgNPs at peak 415 nm. The AFM and SEM images exhibited silver nanoparticles with spherical or irregular-shaped, agglomerated and highly heterogeneous. The higher inhibitory effect was 14 mm against *Pseudomonas aeruginosa* at the concentration of 5 mg/well. These *Tirmania*-AgNPs have exhibited zones of inhibition of gram-negative and gram-positive bacteria especially the infectious bacteria of the eyes *Pseudomonas aeruginosa*.

## References

Bokhary HA, Suleiman AAA and Basalah MO. 1989. The Fatty acid components of the desert truffle "AI Kamah" of Saudi Arabia. *J Food Protection*, **52 (9)**: 668-669.

Bradai L, Bissati S and Chenchuni H. 2014. Desert truffles of the North Algerian Sahara: Diversity and bioecology. *Emir J Food Agric*., **26 (5)**: 425-435.

Dogan HH and Aydin S. 2013. Determination of antimicrobial effect, antioxidant activity and phenolic contents of desert truffle in Turkey. *Afr J Tradit Complement Altern Med*., **10 (4)**: 52-58.

Dogan HH, Duman R, Ozkalp B and Aydin S. 2013. Antimicrobial activities of some mushrooms in Turkey. *Pharm Biol*., **51 (6)**: 707-711.

Feng QL, Wu J, Chen GQ, Cui FZ, Kim TN and Kim JO. 2000. A mechanistic study of the antibacterial effect of silver ions on *Escherichia coli* and *Staphylococcus aureus*. *J Biomed Mater Res*., **52 (4)**: 662-668.

Gajos M, Ryszka F and Geistlinger J**.** 2014**.** The therapeutic potential of truffle fungi: a patent survey. *Acta Mycol*., **49 (2)**: 305–318.

Janakat S, Al-Fakhiri S and Sallal AK. 2004. A promising peptide antibiotic from *Terfezia claveryi* aqueous extract against *Staphylococcus aureus* in vitro. *Phytother Res*., **18 (10)**: 810-813.

Janakat SM, Al-Fakhiri SM and Sallal AKJ. 2005. Evaluation of antibacterial activity of aqueous and methanolic extracts of the truffle *Terfezia claveryi* against *Pseudomonas aeruginosa*. *Saudi Med J*., **26 (6)**: 952–955.

Khadri H, Aldebasi YH and Riazunnisa K. 2017. Truffle mediated (*Terfezia claveryi*) synthesis of silver nanoparticles and its potential cytotoxicity in human breast cancer cells (MCF-7). *African J Biotechnol*., **16 (22)**: 1278-1284.

Klasen HJ. 2000. A historical review of the use of silver in the treatment of burns. *Burns*, **26 (2)**: 117-130.

Meghwal M and Goswami TK. 2012. Chemical Composition, Nutritional, Medicinal and Functional Properties of Black Pepper: A Review. *Sci Rep*. **1**: 1-5.

Morones JR, Elechiguerra JL, Camacho A, Holt K, Kouri JB, Ramirez JT and Yacaman MJ. 2005. The bactericidal effect of silver nanoparticles. *Nanotechnol.*, **16**: 2346-2353.

Muhsin TM and Hachim AK. 2016. Characterization and Antibacterial Efficacy of Mycosynthesized Silver Nanoparticles from the Desert Truffle *Tirmania nivea*. *Qatar Foundation Annual Research Conference Proceedings.* HBPP1149 http://dx.doi.org/10.5339/qfarc.2016.HBPP1149.

Owaid MN, Raman J, Lakshmanan H, Al-Saeedi SSS, Sabaratnam V and Al-Assaffii IAA. 2015. Mycosynthesis of silver nanoparticles from *Pleurotus cornucopiae* var. *citrinopileatus* and its inhibitory effects against *Candida* sp. *Materials Letters*, **153**: 186-190.

Owaid MN. 2016. Biodiversity and bioecology of Iraqi desert truffles (Pezizaceae) during season 2014. *J Aridland Agriculture*, **2**: 22-25.

Owaid, MN and Ibraheem, IJ. 2017. Mycosynthesis of nanoparticles using edible and medicinal mushrooms. *Eup J Nanomedicine*, **9 (1)**: 5-23.

Owaid, MN. 2018. Bioecology and uses of desert truffles (Pezizales) in Middle Eastern. *Walailak J Sci Technol*., **15 (3)**: 179-188.

Silverstein R, Webster F and Kiemle D. 2005. **Spectrometric Identification of Organic Compounds**. 7th edition, John Wiley and Sons, Inc., p. 72-126.

Tel-Cayan G, Deveci E, Cayan F and Duru ME. 2018. Comparative assessment of phytochemical composition, antioxidant and anticholinesterase activities of two Basidiomycota truffle fungi from Turkey. *Marmara Pharm J*., **22 (1)**: 59-65.

Yamanaka M, Hara K and Kudo J. 2005. Bactericidal actions of a silver ion solution on *Escherichia coli*, studied by energy-filtering transmission electron microscopy and proteomic analysis. *Appl Environ Microbiol*., **71 (11)**: 7589-7593.