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Detection of the effect of silver nanoparticles prepared by using extract leaves *Prosperis juliflora* on pathogenic fungus *Trichophyton tonsurans* isolated from human scalp

Hussein A. R. Abbood *

Department of Biology, College of Education for Pure Sciences, Kirkuk University, Kirkuk- Iraq.

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Abstract

The silver nanoparticles were prepared using *Prosopis juliflora* leaf extract and extracted from trees in Kirkuk city and showed that the silver nitrate solution in 1 mm turned its color brown which will be preliminary evidence of the formation of nanoparticles. The UV and visible absorption spectrum of a solution of silver nanoparticles was tested as a second step in confirming the formation of the particles, and it was found that they were present at the wavelength (440) nm, where it turned out that the peaks of X-ray diffraction were at (111), (220), (311) at angles (31.77, 48.11, 54.21) respectively. *Trichophyton tonsurans* were isolated from the scalp of a person with ringworm from Kirkuk General Hospital. The fungus was diagnosed on the basis of the phenotypic and microscopic characteristics and hair penetration. it was found that the solution of silver nanoparticle had a clear inhibitory effect on the fungus *Trichophyton tonsurans*. The highest and lowest inhibitor concentration reached 90% and the highest inhibitor concentration was 100 % Also, the ratios of the silver nanoparticles effects on the growth and production of fungi spores were determined.

Keywords: Silver nanoparticals; *Trichophyton tonsurans*; *Prosperis juliflora* leaf

1. Introduction

Silver nanoparticles are very small, ranging in size from 1-100 nm. These very small materials are used by studying their chemical and physical properties, and they are also concerned with full control of the composition of matter by controlling the number of atoms of the body of matter, whenever the number of atoms changes, the properties of matter change to a large extent. Researchers are looking for the most suitable ways to prepare nanoparticles, and among these methods is the best, fastest and safest way to use plant leaf extracts. and other parts of it in the preparation of silver nanoparticles (1)(2).

Many scientists have used plant materials in the preparation of silver nanoparticles, including neem (*Azadirachta indica*) (3), *aloe vera* (4) or *Prosopis juliflora*. Agricultural and botanical scientists and forestry specialists know it as an evergreen tree whose leaves are feathery with thorns and increases in height. About 15 meters, and its roots go deep into the ground 35 meters. It resists drought, and the highest temperature it resists reaches a temperature of 46 m. It grows on various types of soil.

Dermatophytes can invade keratinized tissues such as hair, skin , nails of humans and animals and cause the disease known as Dermatophytosis. This group includes three genera: Epidermophyton, Microsporum and Trichophyton.

* Corresponding author: Hussein A. R. Abbood

These fungi cannot infect the internal tissues of the body, and the infection is limited to the superficial keratinized layers (5), but in rare cases the infection can extend to the deep tissues, especially when the body is infected with suppurations and granulomas or the body is infected with acquired immunodeficiency syndrome (6).

Trichophyton tonsurans is the main cause of tinea capitis in America and Europe, and its symptoms include hair breakage and fungal secretions, as the fungus invades the hair shaft and the fungal filaments fragment, forming articular spores inside the endothrix (7).

2. Materials and Method

2.1. Isolation and identification of fungi

Samples were obtained from the scalp of a person infected with ringworm from the consulting clinic of Kirkuk General Hospital. Clinical samples were taken after sterilizing the affected area with 70% ethyl alcohol.

Small scales were taken from the edge of the skin ulcers using a sterile surgical blade, and infected hair samples were collected by sterile forceps (5). It was placed on a glass slide and drops of potassium hydroxide were added to it at a concentration of 10 %. After a while, it was placed on a glass slide, then we pass it over it with a quietly burning lamp flame, in order to dissolve the keratinized materials.

All glass slides prepared under a microscope were diagnosed using 40X power to see scattered fungal hypha and other fungal structures (8). The skin and hair scales of the affected individual were grown on the medium of dextrose agar Sabouraud containing the antibiotic chloramphenicol, and the dishes were incubated at a temperature of 28 ° C for 12 days (9). The identification of the fungus depends on the appearance of the colony. These tests include the exterior, color and smooth shape of the colony For microscopic testing, a drop of lactophenol dye was applied to a clean glass slide. A sterilization needle was used to collect a piece of fungus from the edge of the colony, the glass slide was placed on top of the mycelium ,lightly pressed and the specimen was brushed ,and the shape and branching of the mycelium and the shape the spores on the mycelium were observed under a40x microscope (5). It also relied on the hair penetration test, as the fungus showed its penetration into the hair shaft and puts chains of spores inside it, and this infection is called Endothrix (10).

2.2. Preparation of the plant extract

The plant extract of *Prosopis juliflora* leaves was prepared in the laboratory of Al-Qalam University College. Where the leaves of the plant were collected from the trees of the garden Kirkuk city. collect the plant leaves, wash them with distilled water, then dry them for 24 h in the incubator at 45 °C , then mix 5 g of dry leaves, add 500 ml of distilled water, transfer the mixture to a conical flask and centrifuge it at a rate of 3000 rpm, then filter it and prepare the resulting extract at concentration 100 % (11).

2.3. Preparation of silver nanoparticles

1mM $AgNO_3$ silver nitrate solution was prepared and placed in the incubator under completely dark conditions (12). 15 ml of silver nitrate solution was gradually added to the *Prosopis juliflora* leaf extract and homogenized at 5 ± 35 °C for 1 hour with a concentration of 1M. After color change, the AgNPs particles were examined using an ultraviolet-visible spectrophotometer (JASCO) in the laboratories of the Medical Laboratories Department at Al-Qalam University College in Kirkuk, and X-Ray spectrometer (shimadzu) in a laboratory in the Department of Physics - College of Science. Baghdad University.

2.4. Fungi sensitivity test *T. tonsurans* towards silver nanoparticles

2.4.1. Determination of minimum inhibitory and minimum lethal concentrations of nanoparticles

For the purpose of measuring the value of the minimum inhibitory concentration (MIC) and maximum concentration of inhibitory fungicide (MFC) of the nanoparticles solution against dermatophytes, the silver nanoparticles solution produced by the leaves of the *Prosopis juliflora* plant was prepared at the following concentrations:

- The first concentration: contains 1mM of AgNPs solution and represents 100 % concentration.
- Second concentration: 9 ml of the initial concentration was added and dilute 90 % concentration with 1ml of distilled water.

- Third concentration: 8 ml of the initial concentration was added and dilute 80 % concentration with 2 ml of distilled water.
- Fourth concentration: 7 ml of the initial concentration was added and dilute 70 % concentration with 3 ml of distilled water.
- Fifth concentration: 6 ml of the initial concentration was added and dilute 60 % concentration with 4 ml of distilled water.
- Sixth concentration: 5 ml of the initial concentration was added and dilute 50 % concentration with 5 ml of distilled water.
- Seventh concentration: 4 ml of the initial concentration was added and dilute 40 % concentration with 6 ml of distilled water.
- Eighth concentration: 3 ml of the initial concentration was added and dilute 30 % concentration with 7 ml of distilled water..
- Ninth concentration: 2 ml of the initial concentration was added and dilute 20 % concentration with 8 ml of distilled water.
- Tenth concentration: 1 ml of the initial concentration was included and dilute 10 % concentration with 9 ml of distilled water.

As counting the lowest concentration of the extract in which fungal growth did not appear, it is the minimum inhibitory concentration.

After preparing multiple concentrations of the nanoparticles solution, placing each concentration in 100 ml of culture medium (SDA) and pouring it into sterilized Petri dishes, to obtain the MIC and MFC of the pathogenic fungus against the silver nanoparticles by cultivating young discs of the pathogenic fungus in the center of the dishes containing these concentrations. . All dishes were incubated at a temperature of 28 °C for 7 days, and growth was observed or not, and growth was compared with control dishes that were devoid of any addition (13).

2.4.2. Effect of a silver nanoparticle solution on the growth of T. tonsurans

The inhibition ability of nanoparticles on the growth of pathogenic fungi was calculated. The same previous concentrations were used for these nanoparticles mixed with medium (SDA). The incubation period was measured with two perpendicular diameters from the back of the fungal colony, and the inhibition ratio was measured, compared to control dishes that were devoid of any additive (14).

2.4.3. Effect of a silver nanoparticle solution on sporulation of the fungus T. tonsurans

Conidia pathogenic fungi was calculated using a sliding hematology and the same concentrations in practice . So, 1 g of the culture medium of the pathogenic fungus treated with nanoparticles was taken and placed in 50 test tube, mix with 10 ml of sterile distilled water, then add two drops of liquid (tween 80) to reduce the surface tension of conidia and to reduce their aggregation or precipitation , and after shaking, leave the solution for 10 minutes, then take 1 ml of the above solution and mix 9 ml of sterile distilled water to obtain a dilution 10^{1-} After that, we transferred one drop of the suspension to a counting slide using a sterile pipette, and the numbers Conidia of the fungus for five large diagonal squares on a slide count and compare that with a control group to extract the percentage of inhibition of conidia numbers (15).

2.5. Statistical analysis

The results were analyzed statistically using a completely randomized design, and Arithmetic means of treatments were compared ta significance level of 0.05 using Duncan's multiple range test.

3. Results and discussion

3.1. Isolation and identification of fungi

The pathogenic fungus was isolated from the scalp of a young man with tinea capitis, and the hair was broken at the openings of the follicles, where a black substance consisting of hair residue and fungal secretions, as this fungus invades the hair shaft and mycelium fragment to form articular spores. phenotypical characteristics of the colony a texture similar to deer skin to the woolly shape, and the center of the colony is slightly raised, while the color of the colony varies from lemon to red-brown (Figure 2) this description is consistent with many researchers (16) .

Microscopic test showed mycelium divided by septa with the presence of large numbers microcinidia spores of different shapes and sizes such as pear-shaped, club-shaped and balloon-shaped, carried along the mycelium or on short conical stands, While Macroconidia are few, with smooth or thin walls, Chlamydia are found in small cultures(Figure 1). This description is identical to each of (17) (18) .

The hair penetration test showed that the infection of the hair is endothrix, and spores are formed in the form of chains inside the infected hair, which leads to its breakage near the scalp (19) , and these results are not consistent with (20), which indicated that the fungus *Trichophyton tonsurans* It does not cause penetration of infected hair outside the living body (*In vitro*).

Figure (1) Fungal mycelium and conidia of pathogenic fungi

Figure (2) colony of *Trichophyton tonsurans*

3.2. Visual observation

During the mixing process of a colorless ($AgNo_3$) silver nitrate solution and a greenish leaf extract, the results showed that The color of the solution changes to light brown during the reaction process, and this color change is an indicator of the formation of silver nanoparticles. The non-silver color is thought to be caused by the properties of surface plasmon resonance, which occurs in some metals such as silver ,changing the diameter of the particles to the nanometer order.Thus, aspectrometer is used at visible light wave lengths to prove the formation of silver nanoparticles (21) (22) .

3.3. Absorption UV-light spectroscopy

The optical characterization of the UV and visible absorption spectra of silver nanoparticle solutions formed in *Prosperis juliflora* extracts revealed that the intensity of absorbed light at the wavelength corresponding to the highest peak of the absorbance curve of the *Prosperis juliflora* solution was in the 440 nm range, one of the main techniq ues to detect nanostructures ,which is consistent with the following results (23).

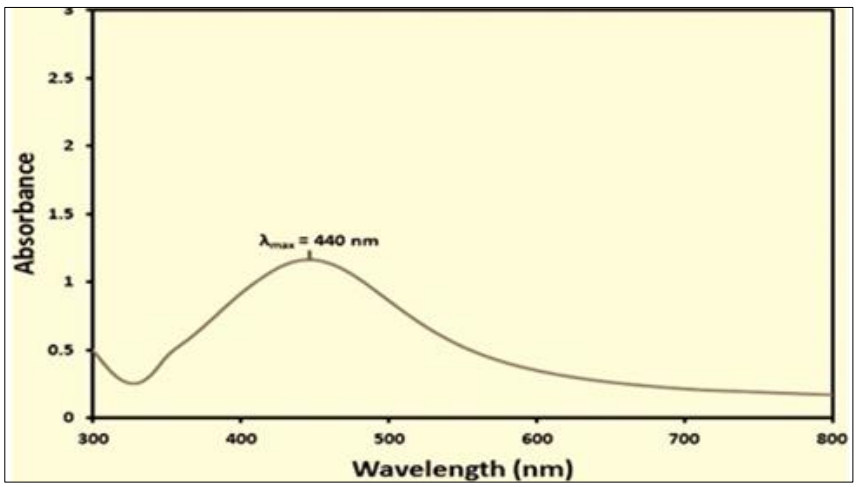

Figure 3 UV-visible absorption spectra of silver nanoparticle solutions

3.4. X-Ray Diffraction

Figure (4) shows the X-ray diffraction spectrum of silver nanoparticles (AgNPs) prepared by *Prosperis juliflora* extract, and we note from the figure the diffraction peaks (111), (220), and (311) at angles (31.77, 48.11 and 54.21), respectively. These angles are close to those reported with the JCPDS card and agree with (24) (25).

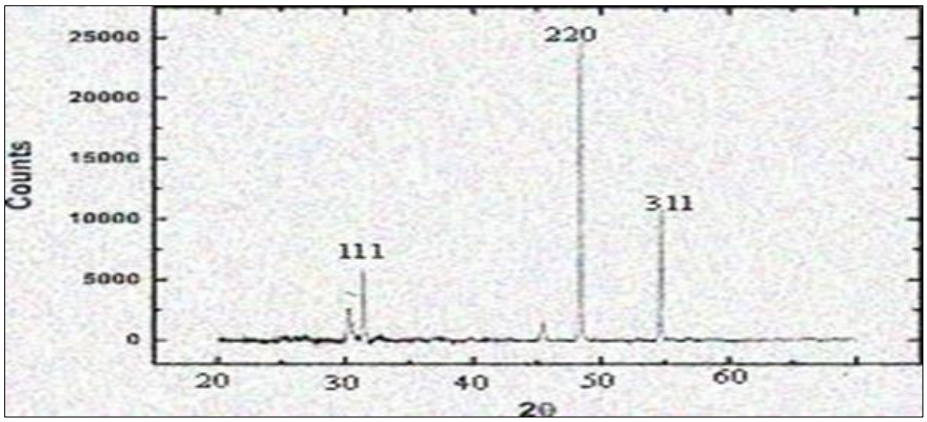

Figure 4 X-ray diffraction (XRD) of silver nanoparticles (AgNPs)

3.5. Testing the effect of silver nanoparticles prepared from *Prosperis juliflora* extract against pathogenic fungi *Trichophyton tonsurans*

3.5.1. Determination of minimum inhibitory and minimum lethal concentrations of nanoparticles

Minimum inhibitory concentration (MIC) is defined as the minimal concentration that inhibits 80 % of fungal growth, and MFC is defined as the minimal concentration that completely inhibits (100 %) fungal growth or growth does not appear even after transferring the fungus to a medium completely free of any addition (26). In this testing , 10 concentrations of silver nanoparticles solution were used to obtain MIC and MFC towards the pathogenic fungus at a temperature of 28 °C and SDA medium, and after 7 days of incubation, as the minimal inhibitory concentration was 90% and the maximum inhibitory concentration was 100 %, as the results showed that there were no significant differences.

3.5.2. Effect of silver nanoparticles on the growth of the fungal colony Trichophyton tonsurans

The results presented in Table (1) indicate that the silver nanoparticles have effect on the growth of the pathological fungus colony, as the fungus growth inhibition rate was 100 % at a concentration of 100 % for the nanoparticles solution and at a concentration of 90 % it reached 88.33 % for growth and at a concentration of 80 % it reached 80.56 % and at a concentration of 70 % it reached 75.98 % and at a concentration of 60 % it reached 63.53 % and at a concentration of 50 % it reached 53.56 % and at a concentration of 40 % it reached 42.98 % and at a concentration of 30% it reached 30.22 % and at a concentration of 20 % it reached 15.46 % and at a concentration of 10% it reached 8% . Silver nanoparticles cause changes in the external structure of fungal mycelium, cell wall castrate, as well as changes in fungal spores and germination depending on nanoparticle concentrations (27) . Silver nanoparticles have been observed in previous studies to disrupt transport systems , including ion flow(28). Disruption of ion flow leads to rapid accumulation of silver ions, leading to silver ions interacting with molecules to disrupt cellular processes such as metabolism and respiration. Silver ions are also known to interact with oxygen to producer active oxygen species, which can damage cells ,damaging proteins, lipids, and nucleic acids (29) (30).

3.5.3. Effect of silver nanoparticles on spore production of Trichophyton tonsurans

The results presented in Table (1) indicate that the silver nanoparticles have effect on sporulation of pathogenic fungi, as the percentage of inhibition of sporulation of the fungus reached 100 % of the nanoparticles solution, and at a concentration of 90 % it reached 90.27 % spore / ml. At a concentration of 80 % it reached 82.09 % spore/ml and at a concentration of 70 % it reached 76.98 % spore/ml and at a concentration of 60 % it reached 65.99 % spore/ml and at a concentration of 50 % it reached 52.09 % spore/ml and at a concentration of 40 % it reached 44.48 % and at a concentration of 30 % It reached 32.09 % spore/ml, and at a concentration of 20 % it reached 17.48 % spore/ml, and at a concentration of 10 % it reached 5.40 % spore/ml.

Table 1 Effect of silver nanoparticles on the percentage inhibition of growth and sporulation of fungi *Trichophyton tonsurans*

Inhibiting the growth of spores %	Inhibition of %fungal growth	nanoparticle %concentrations
5.40 j	8 j	10
17.48 i	15.46 i	20
32.09 h	30.22 h	30
44.48 g	42.98 g	40
52.09 f	53.56 f	50
65.99 e	63.53 e	60
76.98 d	75.98 d	70
82.09 c	80.56 c	80
90.27 b	88.33 b	90
100 a	100 a	100

Numbers followed by different letters mean that there are significant differences in the transactions at a significant level of 0.05.

4. Conclusion

Through the search results, we conclude the following

- *Prosperis juliflora* leaves showed the ability to produce AgNPs nanoparticles.
- The results of the study showed a color change of the plant extract after mixing it with silver nitrate $AgNO_3$ from colorless to light brown, evidence of the formation of silver nanoparticles AgNPs.
- Efficiency of nano silver oxide in affecting the growth of colonies and production of spores of *Trichophyton tonsurans*.

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