DETECTION OF THE ANTIMICROBIAL ACTIVITY OF SILVER NANOPARTICULES BIOSYNTHESIZED BY *STREPTOCOCCUS PYOGENES* BACTERIA

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ABSTRACT

This study was aimed to biosynthesized silver nanoparticles by *Streptococcus pyogenes* bacteria and its antimicrobial activity against (*S.aureus* ,*P. aeruginosa, E.coli,* and *C. albicans* yeast) at different concentrations (20, 40, 60 ,80 and 100) µg/ml by agar well diffusion assay. Fifty sample was collected from Wounds and burns, from Baghdad Teaching City Medicine Laboratories. Samples identified by culture, VITEK 2 Compact system ID-YST kit. The sensitivity of bacterial isolates to antibiotics ware tested and the microbes were more sensitive, resistant and moderate range to antibiotics. Several techniques where used to characterize AgNPs: X-ray Diffraction (XRD), UV–Visible Spectroscopy(UV) and Scanning electron microscope (SEM).The results show that biosynthesized silver nanoparticles are more effective than bacterial supernatant on human pathogenic microbes.

Key words: Biological activity; pathogenic microbes; nanoparticles.
***Part of M.Sc thesis of the 1st auther.**

الكشف عن الفعالية الضد ميكروبية لدقائق الفضة النانوية المصنعة حيويا بواسطة بكتريا *Streptococcus pyogenes*

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المستخلص

تهدف الدراسة إلى التخليق الحيوي لجسيمات الفضة النانوية باستخدام بكتريا المكورات العقدية *Streptococcus pyogenes* وتحديد نشاطها المضاد لبعض الميكروبات المرضية للانسان وهي (*P.aeruginosa S.aureus E.coli*,و *C. albicans*), بتراكيز مختلفة (20, 40, 60, 80 و100 مكغم / مل). تم جمع 50 عزلة من الجروح والحروق من المختبرات التعليمية لمدينة الطب و تم تشخيصها بجهاز الفايتك Vitek. تم اختبار حساسية العزلات البكتيرية للمضادات الحيوية، واظهرت بعض العزلات حساسية عالية واخرى كانت مقاومة لمضادات اخرى . تم توصيف دقائق الفضة النانوية المخلقة حيوياً بإستعمال جهاز spectrophotometer UV–Visible (UV) و X–Ray Diffraction (XRD) و Scanning electron microscope (SEM). تم دراسة التأثير التثبيطي للراشح ولدقائق الفضة النانوية المصنعة حيويا على عزلات الاحياء المجهرية واظهرت النتائج أن دقائق الفضة النانوية تمتلك تأثيرا تثبيطيا على نمو الاحياء المجهرية اعلى من تأثير الراشح البكتيري.

كلمات مفتاحية: الفعالية البايولوجية، الميكروبات المرضية، الدقائق النانوية

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INTRODUCTION

Silver nanoparticles are characterized by unique properties that are not available in other metals and are characterized by antimicrobial properties as well as physical and optical properties that are not available in silver when they are larger in size. The properties of nanoparticles are used in many applications and are important for human life, Medical industry, food industry, food conservation (6).Silver Nanoparticles are a killing agent for a wide range of G+ve bacteria, including, *Staphylococcus and Streptococcus* ,and G-ve bacteria, including *Acinetobacter, E.coli ,Pseudomonas* spp., *Salmonella , Vibrio, Bacillus, Clostridium, Enterococcus* ,and *Listeria* (20). It is a killing agent of antibiotic resistant strains, which represent methicillin resistant strains and Vancomycin resistant strains. In addition to Antibacterial properties , Silver Nanoparticles have Antifungal, Anti-inflammatory properties (19) To overcome problem utilizing toxic chemicals and high energy required physical procedures, the nanoparticles have been combined by natural materials which have been used for the union of different metal and oxide nanoparticles. Thus, the biogenic methodology, the use of regular living beings or materials specifically, has offered a dependable, basic, nontoxic and eco-accommodating technique (7). In addition to different types of plants ,many microorganisms, such as algae, fungi and bacteria have been used in nanoparticles synthesis (1). Biosynthesis of metal nanoparticles can occur either intracellularly or extracellularly (9) (10). In this study, *Strept. pyogenes* bacteria were selected to the biosynthesis of silver nanoparticles.

MATERIALS AND METHODS

Collection of microbial isolates

50 microbial isolates belong to *Strept. pyogenes, E. coli, C. albicans S.aureus* and *P. aeruginosa* were collected from Baghdad teaching city medicine laboratories The isolates were diagnosed by gram stain and biochemical tests .These identified cultures were transferred to nutrient agar slants for preservation and then store in the refrigerator at 4°C (14).

Antibiotic susceptibility test: By using disk diffusion assay , by transferred part of colony of microbe grown in BHIB by loop to test tube that contained 5 ml of normal saline and The bacterial suspension was compared to the standard McFarland , then 0.1 ml of the microbe suspension has been spread on the surface of Mueller-Hinton agar, and left to dry. Antibiotic disks were placed on the agar by sterile forceps. After incubation period , The zone of inhibition diameter around the disc is estimated and compared to the CLSI reference table to determine if the organism is susceptible, intermediate or resistant against the antibiotic agents tested (16).

Biosynthesis of silver nanoparticles by *Streptococcus pyogenes*

The growing *S. pyogenes* strains were freshly injected on Muller_Hinton broth and incubated at 37°C for 24hours. The culture was centrifuged at 14,000 rpm for 10 minutes, supernatant added separately for reaction container containing silver nitratea concentration of 10^{-3}(1% v/v). The interaction between this supernatant and Ag^{+} ions was carried out in bright status for 24 hours (8).

Characterization of biosynthesis silver nanoparticles

Characterization of Silver nanoparticle were analyzed by (UV–Vis spectrophotometer and X-Ray diffraction) . Color change of the reaction mixtures was checked by estimating UV–visible range of the reaction mixture .SEM used to determination of the morphology and size of nanoparticles and X-ray diffractometer by casting (CuKα) over the model to be measured at different angles from 20 ° to 60 ° and measured the results. The Debye-Scherer formula was applied to obtain the size of the synthesized nanoparticles (4).

$D = K \lambda / \beta \cos.$

D is the particle size; *K* is a dimensionless shape factor; λ refer to X-ray wavelength; β is the line broadening at half the maximum intensity (FWHM); θ is the Bragg angle (in degrees).

Antimicrobial activity assay

To determine the antimicrobial activity of the biosynthesized AgNPs by using *S. pyogenes* against other microbial strains that cultured on media and after the plates were permitted to dry, wells ware formed . A 100µL volume of

biosynthesis AgNPs was applied in the wells , incubated at 37ºC for 24hrs. the diameter of inhibition zone was estimated around each well , 3 replicate trials were conducted against each microbes (2) .

RESULTS AND DISCUSSION

Collection of microbial isolates and identification

microbial isolates belong to *S. pyogenes*, isolated from wounds and burns and diagnosed with VITEK ,API strips, gram stain and subjected to biochemical tests for further confirmation (17)**.**

Antibiotic susceptibility test

The results in (Table 1) showed that *S. pyogenes* bacteria were the most resistant to antibiotics. The number of antagonists was 6 antibiotics of a total of 10 antibiotics, followed by *P.aeruginosa, E. coli* and *S.aureus*. The number of antagonists was 4 antibiotics of a total of 10 antagonists, and *C. albicans*, were resistant to 3 of the 10 antibiotic agents. The results showed that there is a difference in the sensitivity or resistance of microorganisms to antibiotics, due to the common use of some antibiotics for a long time, led to the evolution of strains resistant to them, and the manufacture of new antibiotics working on sensitive sites in bacteria has made the resistance of bacteria have something so that bacteria are sensitive to these antibiotics (5).

Table 1. Effect of different antibiotics against some human pathogens

Microbes	Inhibition zone diameter in (mm) Antibiotics symbol									
	C	TMP	AZM	DO	AK	PY	CIP	TOB	E	AM
P. aeruginosa	12	6	20	18	32	6	33	15	6	6
E.coli	10	10	6	15	30	6	25	12	6	6
S. aureus	10	11	6	13	31	6	25	12	6	6
C. albicans	6	22	18	32	25	6	33	15	12	6
S. pyogenes	20	6	6	30	33	6	6	14	6	6

Note: In the absence of bacterial growth inhibition zones, the disc diameter is (6 mm) .
C= Chloramphenicol , TMP= Trimethoprim, AZM= Azithromycin, DO=Doxycycline, AK= Amikacin ,PY= Carbenicillin , CIP= Ciprofloxacin, TOB= Tobramycin , E= Erythromycin , AM=Ampicillin

Biosynthesis of AgNPs

The results in (Fig.1) shown of the biosynthesis nanoparticles by using supernatant of *S. pyogenes* with silver nitrate solution on Muller-Hinton broth at a concentration of 1 mM at pH 7 and at room temperature for 24 hrs. The change in the mixture color to brown was refer to a positive synthesis (13).

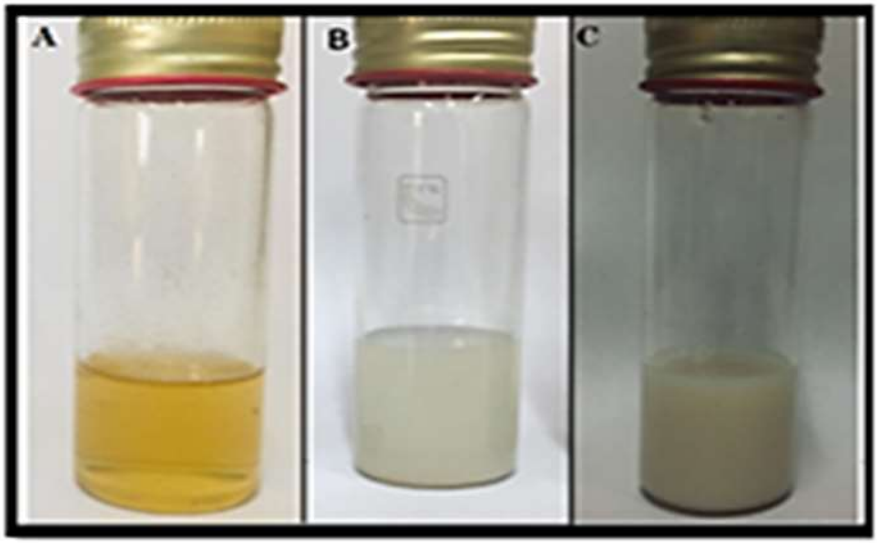

Figure 1. Stages of AgNPs biosynthesis

A= supernatant of *S. pyogenes* B = when adding silver nitrate to the supernatant of *S. pyogenes* C =after 24 hours in room temp. synthesis AgNPs

Characterization of AgNPs

The characterization of nanoparticles show in (Fig.2). By using the UV-Visible spectrophotometer showed the absorption at λmax at 410 nm wavelength, The phenotypic properties of nanoparticles formed by (SEM),

Silver nanoparticles appear spherical in shape and Through the X-Ray Diffraction process, the formation of nano-silver at the angle 3θ was shown in degrees (29.39.32.21 and 31.77). By applying Scherrer equation, the calculated nanoparticles were 30.3 nanometers (13).

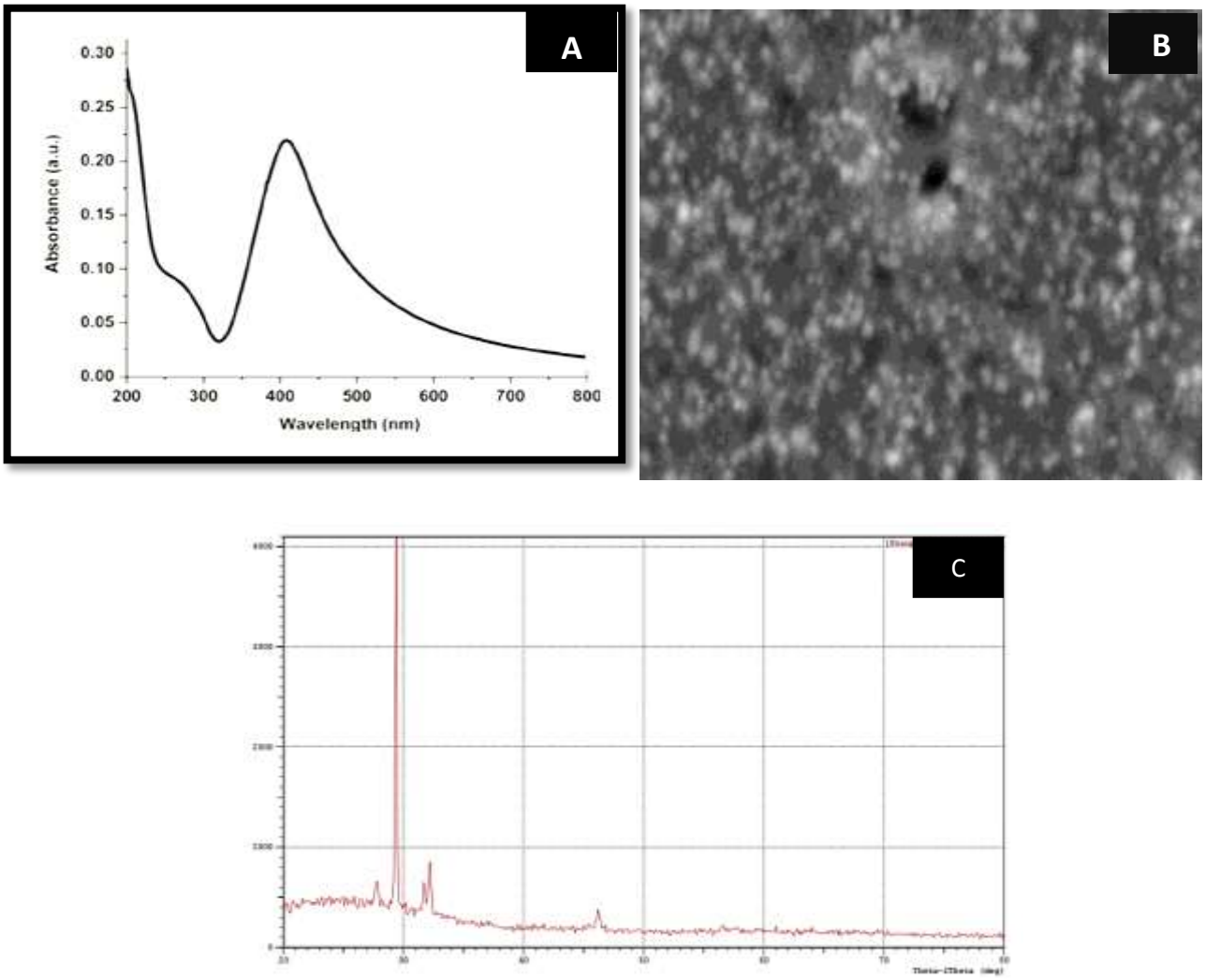

Figure2. Characterization of silver nanoparticles using A= UV-Visible Spectrophotometer B= SEM image of AgNPs C= X-ray diffraction of biosynthesis nanoparticles

Antimicrobial activity of biosynthesis AgNPs

Biosynthensis AgNPs displayed antibacterial properties against bacterial pathogens with close connection of the nanoparticles themselves with the microbial cells. The results showed that the biosynthesized AgNPs by bacteria has a strong inhibitory effect on the growth of pathogenic microbes compared to bacterial supernatant. The supernatant of *S. pyogenes* showed less effective inhibitory effect on *P.aeroginosa* bacteria in an inhibition zone reached to 13 mm at concentration of 100% only (Fig. 3A) . The effect of biosynthesized silver nanoparticles was the highest inhibition on the growth of *P.aeroginosa* with an inhibition zone reached to of 18 mm at concentration of 100%,16mm at concentration 80% ,13mm at concentration 60% (Fig. 3B).

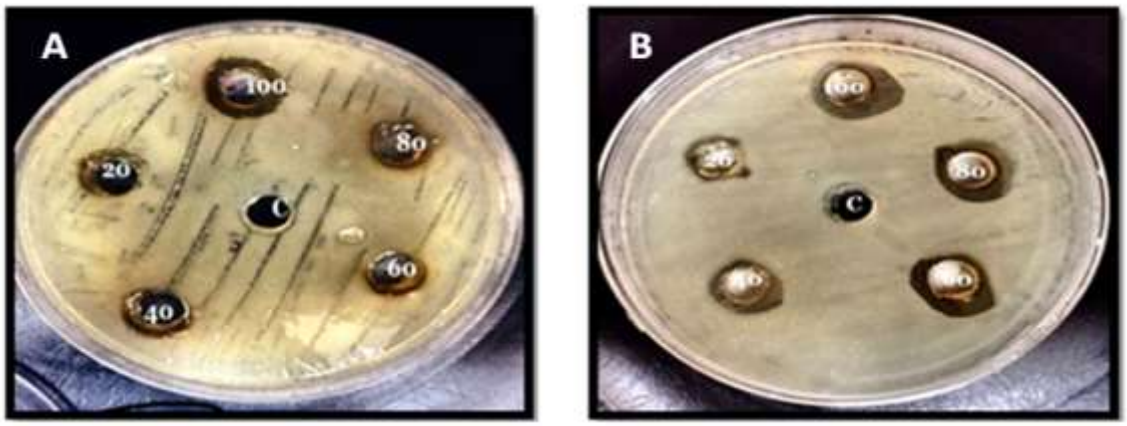

Figure 3.A. The effect of *S. pyogenes* supernatant on *P.aeruginosa*, B. The effect of biosynthesized sliver nanoparticles on *P.aeruginosa*

As for *S.aureus* bacteria where bacterial supernatant showed less inhibitory effect with a 13 mm at 100% conc., and 11mm at 80% conc. (Fig.4A) , but there's no effect for the other conc. The highest inhibition zone recorded to the biosynthesized silver nanoparticles at 26 mm for 100% conc., and 16mm at 80% conc., 14mm at 60% conc., 8mm at 40% conc., and 12mm at 20% conc. (Fig. 4B)

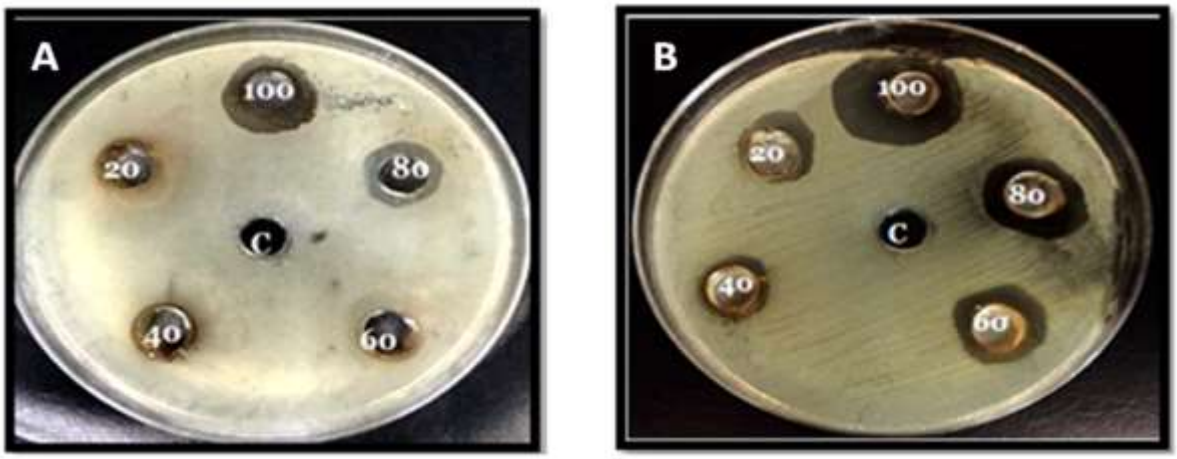

Figure 4. A. The effect of *S. pyogenes* supernatant on *S.aureus* , B. The effect of biosynthesized sliver nanoparticles on *S.aureus*.

Followed by *E. coli*, where bacterial supernatant was less effective inhibition with a 12 mm inhibitory at 100% conc. , and 13mm at 60% conc.(Fig.5A) The highest effect of the biosynthesized silver nanoparticles reached to 21 mm at 100% conc. , 20mm at 60% conc.(Fig. 5B).

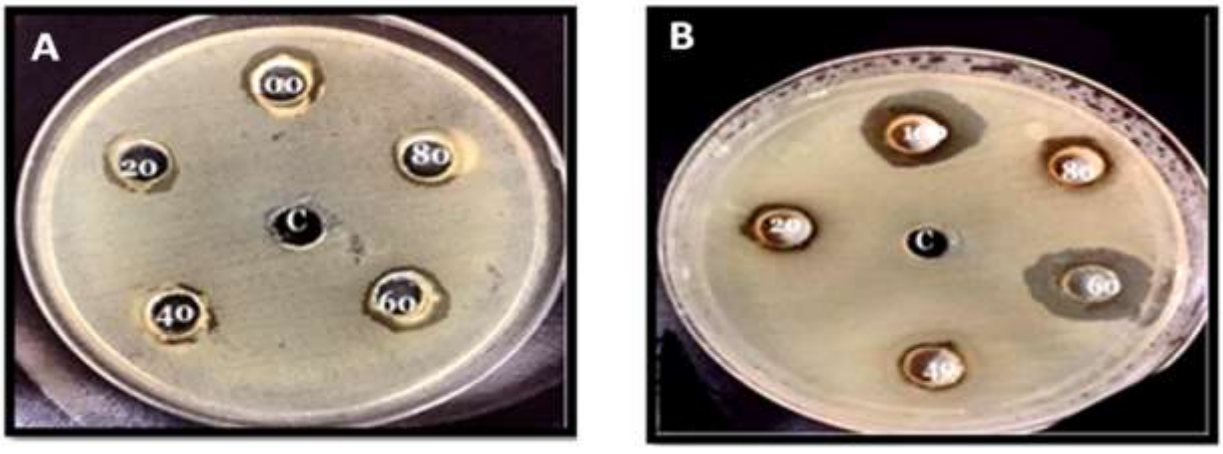

Figure 5.A.The effect of *S. pyogenes* supernatant on *E. coli* , B. The effect of biosynthesized sliver nanoparticles on *E. coli*

Finally, the yeast *C. albicans* where the effect of bacterial supernatant was less effective inhibition with a 12 mm inhibitory at100% conc. and 12 mm at 80% conc.(Fig.6A), The highest effect of the biosynthesized silver nanoparticles at 100% reached to 23 mm ,13 mm at 60% conc. ,17mm at 60% conc. ,and 14mm at 40% conc. (Fig6B).

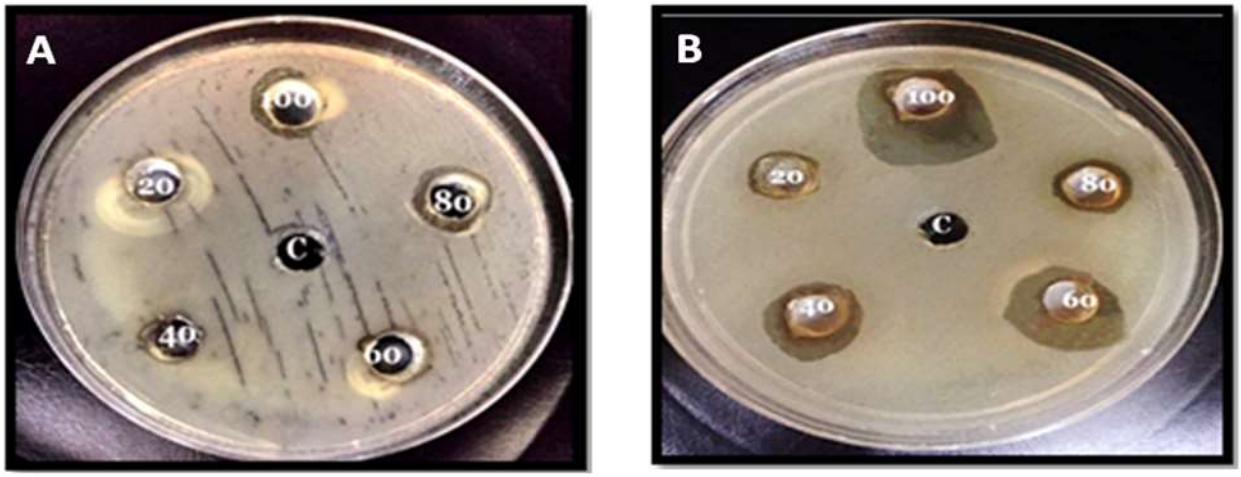

Figure 6 A. The effect of *S. pyogenes* supernatant on *C. albicans*, B. The effect of biosynthesized sliver nanoparticles on *C. albicans*

Table2. Effect of bacterial supernatant on the growth of microbes

Microbes	Control	20%	40%	60%	80%	100%
P. aeruginosa	**- ± 0.00**	**- ± 0.00**	**- ± 0.00**	**- ± 0.00**	**11.66±1.52**	**14±1.00**
S.aureus	**- ± 0.00**	**- ± 0.00**	**- ± 0.00**	**- ± 0.00**	**11±1.00**	**14±1.00**
E. coli	**- ± 0.00**	**- ± 0.00**	**- ± 0.00**	**- ± 0.00**	**11±1.00**	**12±1.00**
C. albicans	**- ± 0.00**	**- ± 0.00**	**- ± 0.00**	**- ± 0.00**	**11.66±0.57**	**13±1.00**

Table3. Effect of biosynthesized silver nanoparticles on the growth of microbes

Microbes	Control	20%	40%	60%	80%	100%
P. aeruginosa	**- ± 0.00**	**- ± 0.00**	**16±4.16**	**17±3.60**	**12±2.00**	**20±2.00**
S.aureus	**- ± 0.00**	**- ± 0.00**	**11.33±1.52**	**15±1.00**	**17±1.00**	**21±1.15**
E. coli	**- ± 0.00**	**- ± 0.00**	**- ± 0.00**	**17.33±3.05**	**11.66±1.52**	**21±1.00**
C. albicans	**- ± 0.00**	**15±1.00**	**15±1.00**	**18.66±1.52**	**15.66±3.78**	**24±1.00**

***(-)= no inhibition occurs**

The results show that biosynthesis of Silver nanoparticles are more effective than bacterial supernatant. The supernatant of *S. pyogenes* bacteria have Inhibitory effect on the growth of microbes related to the nature of the substances produced by the bacteria but this effect is weak (3) , But The biosynthesized silver nanoparticles by supernatant of *S. pyogenes* bacteria have a strong inhibitory effect because silver nanoparticles have a very effective against bacterial and fungal infections. These properties are due to silver nanoparticles having a large surface area of the volume ratio which increases their association with bacterial cell well causing changes in the membrane and thus cell death (18) That can also release silver ions that interfere with thiol groups in biomass enzymes, which inhibit them. Silver ions also inhibit respiratory enzymes and during the inhibition process reactive oxygen species are generated. (ROS) that attack the cell itself and thus die. (11) Silver nanoparticles have the ability to interfere with the sulfur and phosphorus bases of DNA and thus lead to the breakdown of DNA and cell death due to a disturbance in the DNA replication of bacteria and microbes (12). This agreement with that the biosynthesis of Silver nanoparticles by *S.pyogenes* has great effectiveness against broad spectrum of pathogenic microorganisms (15). The present investigation support the use of biosynthesized AgNPs by *S.pyogenes* elicited a strong antimicrobial activity. Thus, the biological approach could be an economical alternative to conventional chemical and physical assays of AgNP synthesis and would be suitable for the development of a biological process for commercial large-scale creation. AgNPs have wide application in different fields, such as antibacterial. Therefore, the improvement of their synthesis for nanoparticle production is the main objective of nanotechnology (21).

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