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Green synthesis of silver nanoparticles using *Lawsonia inermis* leaves extract and it[s] Antibacterial activity

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Abstract

Nanotechnology is defined as a highly developed technology due to the wide range of applications in various fields of medical science, technology and other fields of research. Green synthesis methods are environmentally friendly, readily available, safe, and nontoxic. Nanoparticles are particles with lengths ranging from 1 to 100 nm in two or three dimensions. Silver nanoparticles are important because their unique properties eg, size and shape Depending on the optical, electrical and magnetic properties. In this study, methanolic extract of *Lawsonia inermis* has been used for synthesis of silver nanoparticles. The change in color from brown to yellowish brown indicates the formation of nanoparticles. To improve the biological composition of natural nanoparticles, many variables were investigated, and these were concentration of the extract, pH and the reaction temperature. The characterization process was performed using techniques of UV-visible spectroscopy, Energy Dispersive X-Ray (EDX) and scanning Electron Microscopy (SEM). The results illustrated that silver nanoparticles had a size of (150-600) nm and a spherical shape, UV-Vis spectroscopy showed SPR sharp peak between (390- 451) nm and EDX attested that Ag, W, Cl and C elements found by weight percent of 57%, 35%, 5.8% and 2.2 % respectively. The antibacterial activity was examined against four types of strain bacteria, two Gram positive bacteria (S. aureus and B. subtilis) and two Gram negative bacteria (E.coli and P. aeruginosa) by using the agar disk diffusion method. The results showed that silver nanoparticles had a

strong effect against Gram positive bacteria and it is more than Gram negative bacteria.
Keywords: Silver nanoparticles, Lawsonia inermis, SEM, EDX, Antibacterial activity.

1. Introduction

Nanotechnology is a technique capable of achieving high degree of precision in the functions, sizes and forms of materials and components by controlling the reaction of molecules and directing the atoms involved in the reaction with specific guidance from the production the most accurate and refined materials of traditional manufacturing as well as a decrease in energy consumption. The word ' Nano originated from a Greek word that means too small or the little thing infinitely [1]. According to the ASTM standard definition, Nanoparticles are particles with lengths ranging from 1 to 100 nanometers in two or three dimensions [2]. In this scale, the chemical and biological properties of nanomaterials vary in basic ways that are characteristic of both atoms/individual molecules and corresponding materials. Nanoparticle shows new or fully optimized properties, such as size, distribution and morphology of molecules [3]. Noble metal nanoparticles are well known to their important applications in the fields of electronic, magnetic, optoelectronics, and information storage [4]. Silver nanoparticles, as a significant member of the noble metal nanoparticles. Silver nanoparticles are important because of their unique properties which can be integrated into antimicrobial applications, biological sensor materials, composite fibers and electronic components. Several physical and chemical methods have been used to synthesize and stabilize silver nanoparticles [5]. Several protocols are designed to synthesize different types of nanoparticles that meet the requirements of various nanoparticle-mediated applications. The synthesis protocols are broadly divided into two categories top-down path and bottom-up path [6]. Nanoparticles are created in the bottom-up technique through the accumulation method from the bottom, while Nanoparticles are created by breaking down the bulk material in the top-down path method [7].

Biosynthesis of Nanoparticles is a type of bottom-up production based on oxidation-reduction of interaction. Scholars used microorganisms and plant extracts to synthesize Nanoparticles and this called the "green synthesis"[8]. In this research focuses our attention to the green synthesis using extract plants "phytosynthesis of nanoparticles". Green synthesis, also known as phytosynthesis, of noble metal nanoparticles (NPs) is one of the emerging fields in nano science and nanotechnology [9]. Particle size and nanoparticles strongly affect the physical, chemical, electrical and optical properties of nanomaterials. At present, silver (Ag) and gold (Au) NPs are used in a wide range of biomedical, drug delivery, catalytic, agriculture antioxidant, anticancer antibacterial, anti-fungal, anti-biofilm, entomological and parasitological applications [10]. The three main steps in the green synthesis of nanomaterial's that use plants include plant varieties and the type solvents used to extract the effective compound of the plant, environmental agent [11]. The main mechanism considered for this process is the reduction by phytochemicals in plant. The phytochemicals involved are terpenoids, flavones, ketones, aldehydes, amides, and carboxylic acids. Flavones, organic acids, and quinone's are responsible for the immediate reduction of Ag ions. It has been found that the phytochemicals are directly involved in the reduction of the ions then releasing hydrogen and thus formation of silver nanoparticles [12]. To improve the biological composition of natural objects, many variables such as concentration of the extract, pH and temperature strongly influence the chemical composition, particle size, volume distribution, particle morphology, Characteristics of product applications. The characterization is a necessary stage to understand the structure and applications of nanoparticles. The characterization process is achieved using techniques of UV-visible spectroscopy, Energy Dispersive X-Ray (EDX) and scanning Electron Microscopy (SEM). UV-VIS test was considered to understand the electronic transitions of synthesized AgNPs. The EDX technology was wilised to illustrate the elemental composition of the synthesized AgNPs of the methanolic extract of Lawsonia inermis. While the size, morphology and surface charge of AgNPs was analyzed using scanning electron microscopy (SEM).

The study aimed to synthesize silver nanoparticles using methanolic extract of Lawsonia inermis. Moreover, to Characterize the prepared AgNPs using SEM, UV-Vis spectroscopy and EDX. Furthermore, studying and evaluating the activity of Lawsonia inermis methanolic extract and silver nanoparticles.

2. Experimental work

2.1 The Collection of *Lawsonia inermis* leaves

Lawsonia inermis leaves were collected from North West of the city of Nasiriyah in southern of Iraq in September, 2016. The verification of the plant was performed at the department of Biology- College of Science - Dhi-Qar University. The Collected leaves were washed and dried in the shade at room temperature for 10 days, then crushed to obtain powder.

2.2 Preparation of *Lawsonia inermis* Extract

Dried leaves (50 g) were extracted with (350 ml) of methanol and shacked for 48 hr using a shaking water bath. Then, the extract was filtered three times by gauze cloth and titron cloth. The solvent was evaporated using a rotary evaporator (45 °C). The extract was preserved at −4 °C to be used in the experiments.

2.3 Synthesis of Silver Nanoparticles

Aqueous solution of silver nitrate was prepared by adding 1 mM of AgNO3 to 250 ml of distilled water at room temperature and stored in an amber colored bottle to evade auto oxidation of silver ions. Silver nitrate (2mL ; 1 mM) was added drop wise into Extract (0.6 mL) while stirring and heated (45 °C) in a water bath at pH 9. The resulting solution became yellowish brown after 30 min of heating, indicating the formation of silver nanoparticles [13], as showing in figure1. The colloidal suspension thus obtained was centrifuged at 4000 rpm for 30 min and the pellet obtained after discarding the supernatant was re dispersed in deionized water. The centrifugation process was repeated 2 to 3 times for the removal of any adsorbed substances on the surface of silver nanoparticles. The synthesized nanoparticles were lyophilized and recovered in powdered form.

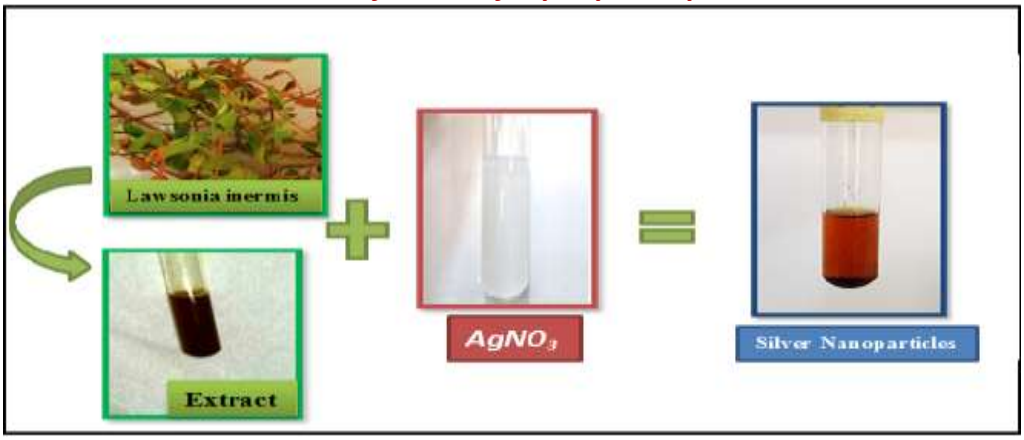

Figure1: The Formation of silver nanoparticles

The biological composition of natural nanoparticles was improved by varying many variables these were, the concentration of the extract, pH and the temperature.

2.3.1 Optimization factors

2.3.1.1 The Effect of pH

PH of the reaction mixture was maintained at 4, 7 and 9, respectively, by using 0.1 N Hcl and 0.1 N NaOH. The absorbance of the resulting solutions was measured spectrophotometrically.

2.3.1.2 The Effect of temperature

To study the effect of temperature on the synthesis of AgNPs, a typical sample was synthesized at 25 °C, 35 °C and 45 °C. Electronic absorption spectra of the aqueous colloidal suspensions were recorded at each temperature range.

2.4 Characterization of silver Nanoparticles

2.4.1 UV-Visible spectrophotometer:

The optical property of AgNPs and The reduction of pure Ag + ion were monitored by measuring the UV-Visible spectrum of the reaction mixture after diluting of the samples with 4ml of deionized water after regular period of time. UV-Vis spectral analysis was performed by using UV-Vis spectrophotometer UV-1700 (Shimadzu, Tokyo, Japan) that was operated in the scanning range of 250-750 nm.

2.4.2. Scanning-Electron-Microscopy-(SEM):

Scanning electron microscope procedures in physics department, faculty of science, AL-nahrain University. The morphology and size of silver nanoparticles were identified using Scanning Electron Microscope (SEM) (Model INSPECT S50). According to the microscope specifications, resolution up to 7.0 nm at 3 KV (in high vacuum mode) ensured, while magnification reported to be in the range of 13-1000000X; though, these data is dependent on the characteristic of each sample.it can be different depending on the samples with heavier elements lead to obtained analysis that is much more accurate. The sample were prepared by dropping a very small amount of the sample on glass plates and then allowed to dry at room temperature. Then the SEM slides were prepared by taking dried of the solution then covered with thin layer of gold to make the samples conductive.

2.4.3 Energy Dispersive X-ray spectroscopy (EDX):

EDX analysis been working in physics department, faculty of science, AL-nahrain University. The elemental composition of silver nanoparticles was investigated by using energy dispersive X-ray spectroscopy (EDX). For the EDX analysis the suspension of nanoparticles was dried into powder and about 1mg fine powder was used for the analysis. The EDX analysis Software was sourced from Oxford instruments Analytical Ltd. All measurements were performed at an accelerated voltage of 10 KV.

2.5 Antibacterial Activity

The AgNPs that were synthesized by alcoholic extract was tested for antimicrobial activity by using agar well diffusion method. Muller Hinton Agar plates were used and swabbed with pathogenic organisms from fresh cultures (105-106 CFU/mL) using a sterile cotton swab. With the help of sterile subereous drilling subsequently four adequately spaced wells (holes) of 6 mm diameter each were made per plate at the culture agar surface Pure cultures of microorganisms were sub cultured on Mueller-Hinton agar. A sterile cotton swab was then used to spread the resulting suspension on the nutrient agar and allowed to dry for 10 min. In each hole, 0.2 mL of each extract and control were put under aseptic conditions, and kept at room temperature for one hour to allow the

biosynthesized extracts to diffuse into agar medium and incubated accordingly. The plates were then incubated at 37 °C for 24 h. At the end of the incubation period, the zones of inhibition were measured to the nearest millimeter [15]. The inhibition zone is the area surrounding the hole with no growth of inoculated microorganisms.

3. Results and Discussion

3.1 Characterization of silver nanoparticles

The measurements and characterization such as UV-Visible spectroscopy, Scanning Electron Microscope (SEM) and Energy Dispersive X-ray spectroscopy (EDX) and measurements of the silver nanoparticles produced by the green reduction method using the L. inermis extract are presented with the effects of the extract quantity, pH variations, and reaction temperatures.

3.1.1 Scanning Electron Microscope (SEM)

The morphology and size of silver nanoparticles were revealed by scanning electron microscopic (SEM) analysis and shown in fig3. AgNPs are distinctly seen from the SEM images and Ag+ ions have been completely consumed. The obtained size of the AgNPs in the synthesis is in the range 200–600 nm in sample. The various sizes of Ag particles might be due to the agglomeration of Ag in the preparation for SEM analysis. SEM image provides the details about the surface topography, composition and other properties such as electrical conductivity of silver nanoparticles. The results also showed that the particles had a spherical shape. The silver spherical nanoparticles spherical are suitable for applications in medicine and pharmaceutical preparations. The size of the prepared nanoparticles was more than the size of nanoparticle i.e.; between 1-100 nm. This was because the proteins were bound to the surface of the nanoparticles [17].

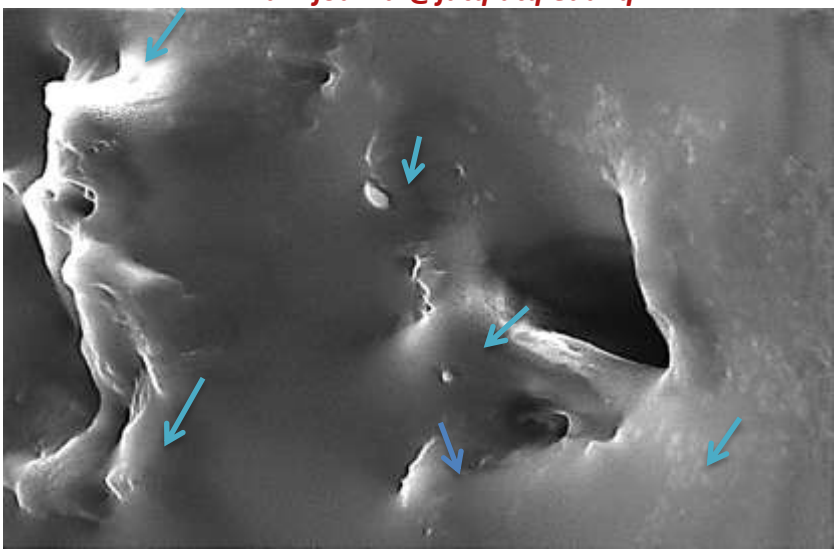

Figure 2: Images of SEM of AgNPs. → Indicate to Silver nanoparticles.

3.1.2 Energy Dispersive X-ray Spectroscope (EDX)

Figure 8 shows the compositional analysis (spectrum EDX) of synthesized nanoparticles. Elemental composition of AgNPs was synthesized using Henna leaves extract was determined by using EDX analysis. It was attested that silver Ag, tungsten W, chlorine Cl and carbon C elements exhibited weight percentages of 57%, 35%, 5.8% and 2.2% respectively. Carbon appears due to plant constituent. The energy dispersive X-ray analysis (EDX) reveals strong signal in the silver region and confirms the formation of silver nanoparticles. Metallic silver nanocrystals generally show typical optical absorption peak approximately at 3 keV due to surface plasmon resonance [18]. Weak elemental signals of W, C and Cl were also detected, reported in the spectrum are due to the phytochemical elements present in the *Lawsonia inermis* leaves

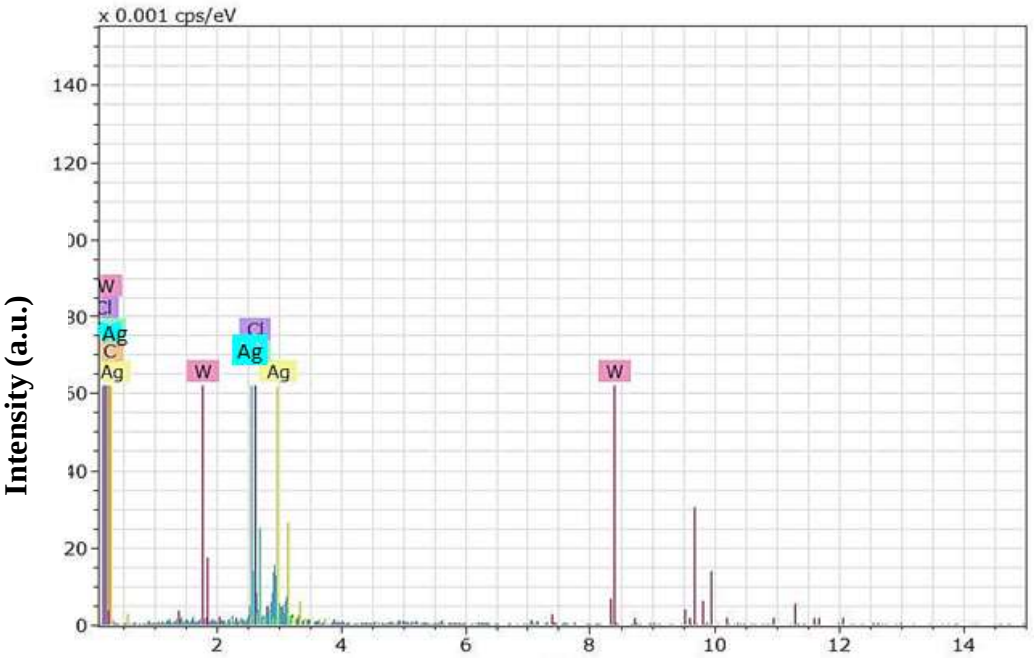

Figure 3: EDX s **Energy (keV)** **particles.**

3.1.3 UV-Vis Spectrophotometer Analysis

The formation and optimization of Ag NPs was monitored using UV–Vis spectroscopy by measuring the absorbance in the range of 200–700 nm, by varying the temperature, pH and amount of *L. inermis* root extract. UV–Vis absorption measurements in the range 200–700 nm can provide deep insight into the optical properties of the formed nanosized silver particles. The change in color indicates the formation of Ag NPs which was further confirmed by the appearance of the SPR band between 400 to 500 nm [19]. The UV-VIS Spectral analysis of the green synthesized nanoparticles was observed a sharp peak around 386 – 450 nm indicates the formation of silver nanoparticles, which was identified as "surface Plasmon resonance band" and this band is ascribed to excitation of valence electrons. The position of absorption band also mainly depends upon dielectric constant of the medium and surface-adsorbed species. The shape of the band was symmetrical, suggesting uniform scattering of spherical shape nanoparticles [20]. The relative percentage of scatter from the measured spectrum depends on the size, shape, composition and aggregation of sample. From literatures it was observed that the silver

nanoparticles of L. inermis extract show SPR peak at around 370- 420 nm. In the present study, it was found the SPR peak at 390-451 nm. There are factors affecting the intensity absorption band and thus affect the synthesis of nanoparticles. This effect is explained as below:

3.1.3.1 The effect of temperature

The affect Temperature is one of the factors influencing the synthesis of silver nanoparticles. This was confirmed by studying the UV-Vis spectra shown from fig3 at three different temperatures 25°C, 35°C and 45°C..

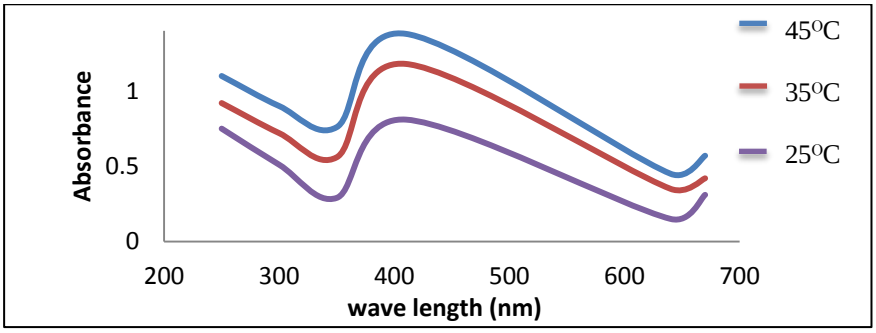

Figure 4: The effect of temperature on wavelength and absorbance for AgNPs of in UV spectra of AgNPs.

Figure 5 shows a spectral comparison of the effect of temperature 25°C, 35°C and 45°C on the synthesis of silver nanoparticles. Notice from the Figure that when the temperature increased from 25° C to 45° C SPR peak became clearer, the absorption band was obtained from 410 to 435 nm which suggested the formation of silver nanoparticles, it was noted that the rate of reduction of silver ions increased with increasing temperature. Similar results were received by Pastoriza Santos and Les-Merzan [21].

This sharpness at the peak absorption depends on the size of the synthesized nanoparticles, as with the high temperature the particle size may be smaller, leading to sharpness of the plasmon resonance of AgNPs [22]. Maximum absorption was observed at 445, 430 and 410 nm at 25, 35 and 45 °C, respectively which correspond to the wavelength of the surface plasmon resonance (SPR). This means that at a higher temperature, reactants are consumed rapidly, leading to the formation of small nanoparticles [23]. The temperature also increases the kinetic energy of the AgNPs in the solution, by the collision frequency between

the particles and this leads to the higher rate of agglomeration, reduce of the size due to the reduction in aggregation of the growing nanoparticles [24]. Our results are in agreement with recent previous reports [25].

3.1.3.2 The effect of pH

Changes in pH lead to changes in charge of natural phytochemicals, affecting their binding ability and reducing metal ions during the synthesis of nanoparticles. This in turn may affect the morphology and yield of nanoparticles. The effect of pH was studied in three different conditions including acidic, neutral and basic forms. The fig4 shows the effect of changes in pH on UV-Vis spectra of silver nanoparticles synthesized in pH = 9.0, 7.0 and 4.0, respectively.

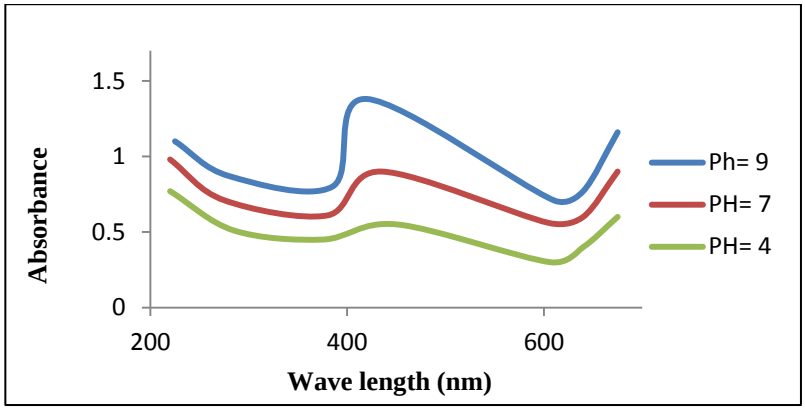

Figure5: The effect of pH on wavelength and absorbance for AgNPs of in UV spectra of (2ml of AgNO3: 0.6ml of extract).

The results show that the formation of nanoparticles is completed at neutral and Basic conditions, where it is observed that in acidic conditions we cannot observe any absorption band for nanoparticles and therefore do not form nanoparticles, so There was no reaction to the pH value of 4, but the monodispersive silver nanoparticles were obtained in the pH equal to 9. The peak surface plasmon resonance (SPR) in the wavelength range of 390-440 nm corresponds to the AgNPs that absorbs radiation intensively at a wavelength of 410 nm due to the transmission of electrons. In acidic pH values, the accumulation of nanoparticles is dominated more of reduction process. This effect may be related to the fact that a large

number of functional groups that bind and intend a metal ion become available in pH 7 and 9 compared to pH 4. At pH 4, the most accessible metal ions are in a smaller number of nuclei Events, leading to a metal conglomerate. In basic pH, the extracts may contain functional groups with more negative charge, which are able to be effectively binding and reduce silver ions, thus forming more nanoparticles [26].

3.2 Antibacterial Activity

The antibacterial activity for AgNPs with four microorganisms were detected against Gram-negative bacteria (P. aeruginosa, E.coli) and Gram- positive bacteria (S. aureus, B. subtilis) using agar disk diffusion method was detected and compared with the antibacterial activity of antibiotic (ciprofloxacin) because it is a strong antibiotic.

The disc diffusion method, a most commonly used technique to access the antimicrobial activity, has been employed by many researchers to confirm antibacterial action of the AgNPs solution. The results of AgNPs showed higher ability to suppress the microbial growth than methanolic extract. The maximum inhibition of AgNPs was recorded against S. aureus (28) mm, (25) mm for E.coli , (22) for P. aeruginosa and (21) of B. subtilis for AgNPs , and in comparison with antibiotic (ciprofloxacin) showing synergistic effect, Ciprofloxacin destroys microorganisms by Interaction with DNA which prevents formation the bacterial chromosome and will kill bacteria in all active and inactive growth stages.

The Inhibition zones of ciprofloxacin antibiotic discs were 0, 0, 28, 30 mm for P. aeruginosa, E.coli, B. subtilis and S. aureus respectively. Generally, the methanolic extract of *L. inermis leaves* and biologically synthesized AgNPs showed good antibacterial capability against Gram-positive than Gram-negative bacteria. S. aureus was the most sensitive microorganism to silver nanoparticles with inhibition zone (28) mm. The clear inhibitory zone appeared around against S. aureus and B. subtilis after incubation for 24 h, more of Gram-negative bacteria E.coli and P. aeruginosa. The difference in the effect of the antibacterial is due to the difference in the structure of the cell wall, where the cell wall of the gram positive bacteria contains a single layer, while the cell wall of Gram-positive bacteria composed of a rigid thicker multiple layer of peptidoglycan, as it prevented the nanoparticles from entering into cell

wall. The difference in the structure of the cell wall is due to its cell wall containing compounds such as lipopolysaccharides, lipoprotein and protein lipid for gram-negative bacteria while Gram-positive bacteria wall, have a lipid content that makes them more permeable and therefore more effectively to gram-negative bacteria [28].

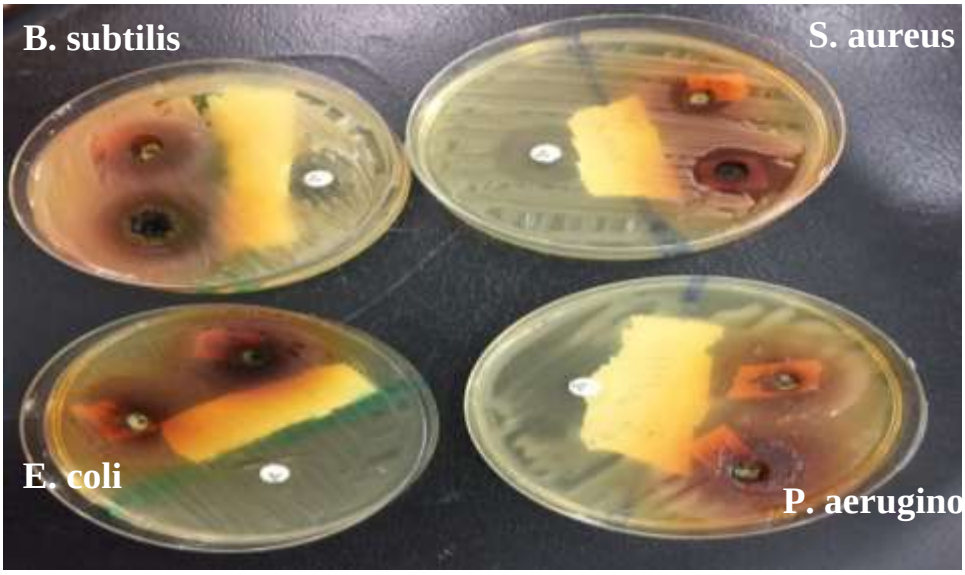

Figure 6. Antibacterial activity of AgNPs and L. inermis extract against bacteria Gram-negative (E. coli, P. aeruginosa), and Gram-positive (S. aureus, B. subtilis) and were compared with Ciprofloxacin.

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التخليق الحيوي (الأخضر) لدقائق الفضة النانوية باستخدام المستخلص الكحولي لنبات الحناء, والتحقق من فعاليتها البيولوجية

الخلاصة

يعرف النانو تكنولوجي بأنه تقنية متطورة وحديثة نظرا لمجموعة واسعة من التطبيقات في مختلف مجالات العلوم الطبية والتكنولوجيا وغيرها من مجالات البحث. التخليق الحيوي (الاخضر) هو طريقة خالية من التلوث(صديقة للبيئة) ، متاحة بسهولة، آمنة، وغير سامة. الجسيمات النانوية هي جزيئات ذات أطوال تتراوح من 1 إلى 100 نانومتر في بعدين أو ثلاثة. تمتاز الجسيمات النانوية الفضية بأهميتها الكبيرة بسبب خصائصها الفريدة مثل الحجم والشكل. في هذه الدراسة، تم استخدام المستخلص الميثانولي لأوراق الحناء لتخليق الجسيمات النانوية الفضية حيث تم مزج 2 مل من نترات الفضة بتركيز 1 ملي مولاري مع 0.6 مل من المستخلص الكحولي حيث لوحظ تغير اللون الى اللون البني المصفر دلالة على تكوين دقائق الفضة النانوية. تم تحسين التركيب البيولوجي للجزيئات النانوية المخلقة طبيعيا ، من خلال دراسة العديد من المتغيرات، مثل تركيز المستخلص ودرجة الحموضة ودرجة حرارة التفاعل. نفذت عملية التوصيف باستخدام تقنيات التحليل الطيفي للأشعة فوق البنفسجية المرئية، الأشعة السينية المشتتة للطاقة (إدكس) والمسح المجهري الإلكتروني (سيم). بينت نتائج المجهر الالكتروني الماسح أن الجسيمات النانوية الفضية كانت بحجم (150-600) نانومتر وذات شكل كروي، وأظهر التحليل الطيفي للأشعة فوق البنفسجية أن حزمة سبر حادة بين(451- 390) نانومتر, كما اثبت طيف تشتت الاشعة السينية(إدكس) أن عناصر الفضة, التنجستن, الكلور والكربون وجدت بالنسب الوزنية الاتية 57٪، 35٪، 5.8٪ و 2.2٪ على التوالي . كما درست الفعالية المضادة للبكتيريا ضد أربعة أنواع من الاحياء المجهرية البكتريا الموجبة الجرام (العنقودية الذهبية, العصوية

الشمعية) والبكتيريا السالبة الجرام (الاشريكية القولونية, الزائفة الزنجارية) باستخدام طريقة الانتشار بالحفر. وأظهرت النتائج أيضا أن الجسيمات النانوية الفضية لها تأثير قوي ضد البكتيريا الموجبة لصبغة غرام أكثر من البكتيريا السالبة لصبغة غرام.

الكلمـــات المفتاحيـــة : دقـــائق الفضـــة النانويـــة , التخليـــق الأخضـــر, , المجهـــر الالكترونـــي الماســـح , طاقـــة تشـــتت الاشـــعة الســـينية و الفعاليـــة المضـــادة للبكتيريـــا .