Biosynthesis of silver nanoparticles using (*Carissa macrocarpa*) leaves extract and study its antibacterial activity

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الملخص:

حققت جسيمات الفضة النانونية مكانه خاصهومهمه من بين جميع الجسيمات النانونية المحضَرة وذلك بسبب تطبيقاتها المهمة كاستخدامها كمضادات للبكتريا والمكروبات. ان تحضير جسيمات الفضة النانوية يشمل استخدام طرق كيماويه غير امنه او طرق فيزيائية مكلفه. اما طريقه التحضير البيولوجية فتعتبر افضل من الطرق السابقة ولها العديد من المزايا؟ أهمها ان استخدام النباتات ومستخلصاتها تعتبر من اكثر الطرق الأمنه. فالنباتات ليست لها اهميه جمالية فحسب وانما لها فوائد عظيمة لاحتوائها على مواد مهمه في المجالات الطبية. في هذه الدراسة المقدمة جرى تحضير جسيمات الفضة النانوينة بطريقه التحضير البيولوجي التي تعتبر من الطرق السهلة وغير المكلفة والفعالة . تم الاختزال البيولوجي باستخدام مستخلص اوراق نبات الكاريزيا في المحلول المائي لأيون الفضة التي تم اختزالها الى جسيمات الفضة النانونية . ان استقرار جسيمات الفضة وثباتها تم متابعته باستخدام العديد من التقنيات وهي: مطياف الاشعة المرئية وفوق بنفسجية و طيف الاشعة تحت الحمراء وحيود الاشعة السينية و مجهر القوه الذرية والمجهر الالكتروني الماسح والمجهر الالكتروني النافذ، وقد وجد ان جسيمات الفضةالنانونية المحضرة ذات شكل كروي وبأحجام تتراوح بين (٣٨–٧٤ nm). أن الفعالية البيولوجية لجسيمات الفضةالنانونية المحضرة تم اختبارها ضد البكتريا المسببة للأمراض الموجبة لصبغه غرام (*Staphylococcus aureus*) والسالبة لصبغة غرام (*Shigella*) وتم تأكيد فعاليتها الطبية.

Abstract:

Among all the nanoparticles synthesized; silver nanoparticles have attained special place in the area of nanotechnology because of their antimicrobial and medical applications. In general; their syntheses involves the use of unsafe chemicals or costly physical methods. However, the biological processes are making their ways in between and proving their advantages over them. The use of plants and their extracts is one of the most valuable methods which are gaining concerns due to their imperative biological benefits. Plants are not only beautiful but majestic, they are rich sources of various medicinally important substances. In the presented work "one pot synthesis of silver nanoparticles" were described. A simple, cost effective bio-reduction on the principle of "green synthesis" of silver nanoparticles using the *Carrisiamacrocarpa* leaves extract.The aqueous silver ions were reduced to silver nanoparticles when exposed to leaves extract. The bio-reduction and stabilization of so formed silver nanoparticles was monitored by UV-Vis spectrophotometry. The synthesized silver nanoparticles were also characterized by various other techniques viz. FTIR spectroscopy, XRD diffraction, SEM, and TEM. Revealed that the synthesized silver nanoparticles were spherical in shape with a size of (38-74 nm). Biological evaluations of silver nanoparticles were also done against gram positive (*Staphylococcus aureus*) and gram negative bacteria (*Shigella*) for their future applications in biomedicines especially for the treatment of wounds

Keywords:*Carissa macrocarpa*leaves extract, capping agent, silver nanoparticles, biosynthesis

1. Introduction

Nowadaynanotechnology owes to the tremendous improvement in human life and it has a multidisciplinary research area. Among the various fields of nanotechnology, green nanotechnology provides more effective nanoparticles synthesis with expected products and economical manner [1]. Nanomaterials are new, emerging and creating progress due to their interesting electrical, optical, magnetic and chemical properties than bulk materials . Noble metal nanomaterialshave great attention in various fields due to their unique properties. Noble metals such as Ru, Pd, Ag, Pt and Au [2] are exhibiting a particularly wide range of material behavior along the atomic to bulk transition[3]. Synthesis of nanoparticles using microorganisms or plants can potentially eliminate this problem by making the nanoparticles more bio-compatible . Using plant extract for the synthesis of nanoparticles have advantageous over other environmentally benign biological processes by eliminating the elaborate process of maintaining cell cultures. Jose-Yacaman and co-workers first reported the formation of gold and silver nanoparticles by living plants [4,5]. A number of biomolecules in the extract successfully act as reducing agents in the green synthesis of AgNPs. For example, black tea leaf extract has been used for the biosynthesis of AgNPs with sizes averaging 20 nm[6]. The extract of Mangiferaindica leaf also produces AgNPs with sizes of about 20nm [7]. Extracts from fruits such as the red fruits of the pepper (*Capsicum annuum*) have also been shown to produce AgNPs in the range of 3–10 nm [8]. The aqueous extract of *Hoveniadulcis* fruit produces AgNPs with sizes of 45nm [9]. Biosynthesis of silver nanoparticles has already been reported as clean, cost effective and non- toxic to environmental routes. Green synthesis offers improvement over synthetic, chemical or micro-organisms methods due to its cost effective ,environmentally friendly and can easily be scaled up for large scale synthesis. The methods used for the synthesis of silver nanoparticles,usetoxic chemicals for the reduction process of substances such as citrates, $NaBH_4$, or ascorbates [10]. The present study was aimed to a rapid synthesis of silver nanoparticles using aqueous leaves extract of *Carissa macrocarpa*and evaluates its antibacterial activity against water borne pathogens such as *Escherichia coli*, *Staphylococcus*, and *Shigella*.

2. Experimental

2.1 Materials

Silver nitrate $AgNO_3$ was obtained from sigma-Aldrich chemicals and used as received. Deionized water was used throughout the reactions. All glass wares were washed with dilute nitric acid HNO3 and distilled water, then dried in hot air oven. 2.0 of *Carissa macrocarpa* leaf broth was boiled for 15 min, filtrated and completed to 100 ml to get the extract. The filtrate used as reducing agent was kept in the dark at 10 ºC to be used within one week .A stock solution of $AgNO_3$ 2×10^{-2}M was prepared by dissolving 0.34 g/100 ml de-ionized water

2.2 Synthesis of silver nanoparticles

10ml of plant extract of *Carissa macrocarpa* was added to the aqueous solution of 1mM Silver Nitrate .Then the sample was incubated in dark for 24 h. The absorbance is measured by using UV-Visible spectrophotometry. The reduction of Ag^+ to Ag^0 nanoparticles indicated by the change in color of the solution from yellow to brownish yellow to deep brown. This process affected by many parameter such as plant extract concentration, $AgNO_3$ concentration, temperature, pH value, and contact time. The sample was then dried to obtain the synthesized silver nanoparticles for characterization

2.3Instruments for characterization

The UV–vis spectra were recorded at room temperature using a Shimadzo UV-1800 spectrophotometer. Transmission electron microscopy (TEM) studies were performed using a Carl Zeiss EM 900. For the TEM measurements, a drop of solution containing the particles was deposited on a copper grid covered with amorphous carbon. Fourier transform infrared (FTIR) spectra were recorded at room temperature on a ShimadzoFTIR 84005 spectrometer,for the plant extract containg silver nanoparticles, (0.01) g dried at 60 ºC for 4 h usingKBr. X-ray diffraction (XRD) pattern was obtained using a Shimadzu XRD-6000 diffractometer with Cu K, α (λ= 1.54056 A°) to confirm the biosynthesis of AgNPs. An aliquot of this filtrate containing silver nanoparticles was used for SEM, using SEM S-4160.

2.4 Anti-bacterial activity

Anti-bacterial activity of AgNPs was determined by using well diffusion method for *Staphyloccocus* and *Shigella* . The culture was inoculated by spread plate method. Nutrient broth was used to sub culture bacteria and were incubated at 37°C for 24 h. Mueller-Hinton Agar plates incubated with pathogenic bacteria were taken. Sterile paper disk of 5mm diameter saturated with plant extract as control and silver nanoparticles were placed in each plate. The plates were then incubated for 24 h at 37°C. The inhibition zones was measured and tabulated.

3. Results and discussion

3.1 Effect of concentrations of plant extract

The UV-visible absorption spectra of the synthesized silver nanoparticles were recorded at its λ max against distilled water as a reference. (Fig. 1) shows the UV–visible spectra of silver nanoparticle formed using constant (AgNO3) concentration (10^{-3}M) with different concentration of *Carissa macrocarpa* extract at room temperature after 24 h. The color of the solutions changed from pale yellow to yellowish brown to deep brown depending on the extract concentration indicating silver nanoparticle formation as the color change observed is due to excitation of surface Plasmon vibration in the silver nanoparticles. It can be seen that the surface plasmon resonance (SPR) of AgNPs is (290 to 460) nm depending on concentration of plant extract, The absorption peak gets more sharpness and blue shift was observed from (424 to 460) nm. This blue shift indicates a reduction in the mean diameter of the silver nanoparticles, spherical and homogeneous distribution [15].

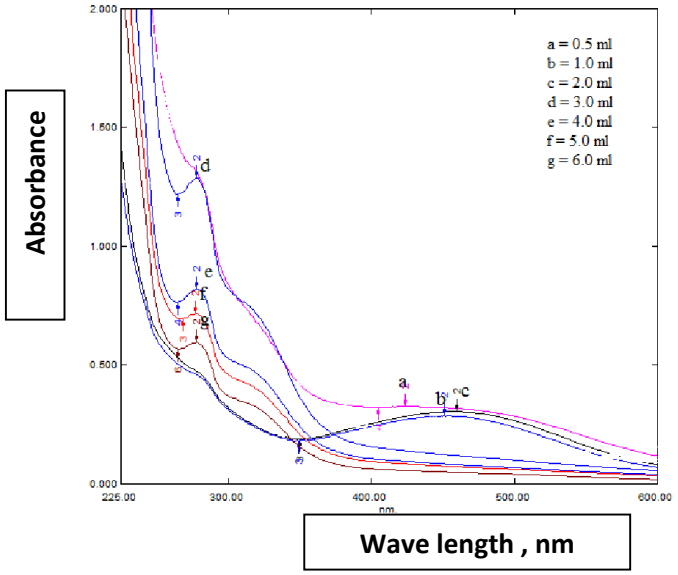


Figure:1 UV-vis spectra of silver nanoparticles at different concentration of *Carissa* extract

3.2 Effect of silver nitrate concentration

The UV-visible spectra recording after 24 h (Fig.2) shows the effect of silver ion concentration onAgNPs prepared by using constant carissa extract concentration (2 ml) with different silver ion concentration (0.5 to 6ml). The observed peaks shows that the wavelength rangewere (450-460 nm). As resultincreasing the concentration of Ag ion lead to the formation of larger AgNPs [16].

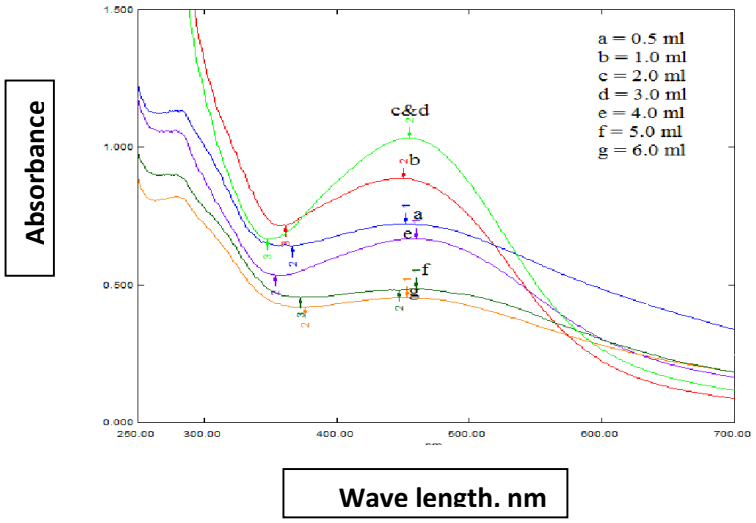


Figure:2 UV-vis spectra of silver nanoparticles at different concentration of silver ion

3.3 Effect of PH

The pH solution affect the size and shape of AgNPs, a major influenced of the reaction pH is its ability to change the electrical charge of biomolecules which might affect their capping and stabilizing abilities and thereafter the growth of nanoparticles(Fig. 3) shows this effect at different range of pH (1.48, 2.10, 5.21, 7,and 9.33). The pH is adjusted using H_3PO_4 (0.1 N) and NaOH (0.1 N) at room temperature . The absorbance increase with increasing pH from (1.48 – 7) and then decrease. The maximum absorbance and blue shift were seen in (451 nm) in sample (d) pH=7 [17].

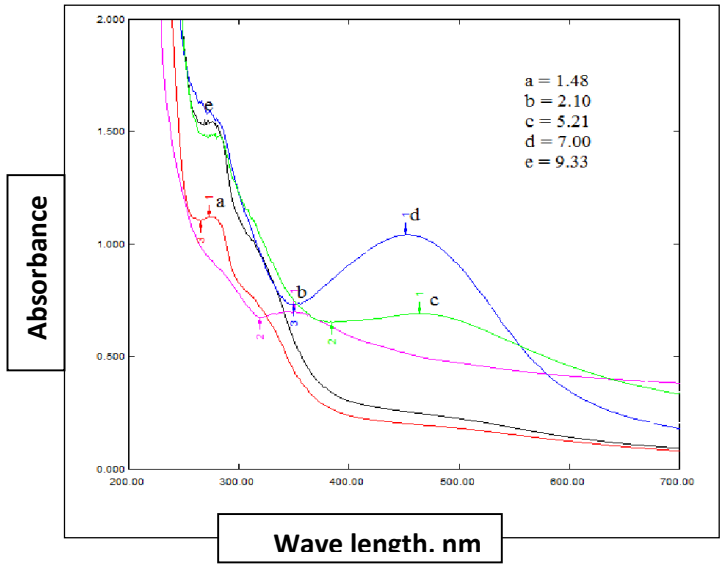


Figure: 3UV-vis spectra of silver nanoparticles at differentpH

3.4 Effect of contact time

The effect of contact time of AgNPs formation by using *Carissa* extract was recorded by UV-visible spectroscopy. Fig.4shows the UV-vis spectra in wavelength range (232- 455 nm). Absorption band increase as contact time increased. A sharp peak and blue shift observed at the time of (2 h) and above to (96 h).The blue shift and (SPR) signified the formation of spherical shape of AgNPs [18].

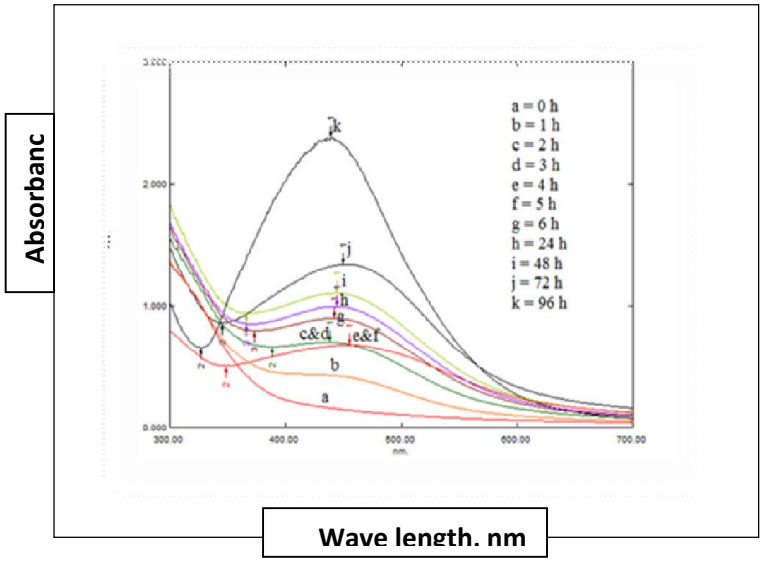


Figure:4 UV-vis spectra of silver nanoparticles at differentcontact time

3.5 Effect of temperature

The effect of temperature has an important physical parameter on the prepared AgNPs . Fig. 5 shows the UV-vis spectra of AgNPs formation by using Carissa extract at different temperature (30, 40, 50, 60, and 70ºC). The absorbance band observed at wavelength (444 - 452 nm), the intensity of absorption increase with increasing temperature. The higher rateof reduction of Ag ions was occurred at high temperature due to the formation of homogenous nucleation ofAgNps[19]

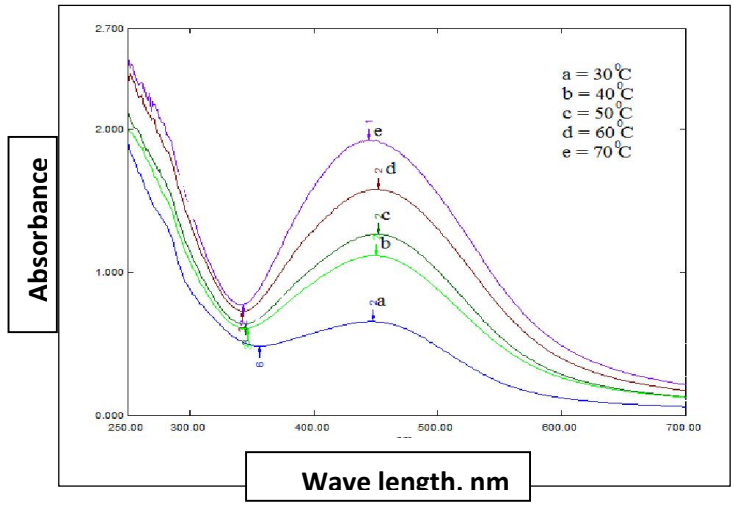


Figure:5 UV-vis spectra of silver nanoparticles at different Temp

3.6 Fourier transform infrared spectroscopy (FTIR)

The FTIR spectrumidentify the different functional groups presented in plant extract which perform a position responsible for reduction AgNO3 as capping and efficient stabilization of silver nanoparticles. Fig. 6shows atypical Infrared spectra ofthe synthesized using this extract (A) andCarissa leaf extract AgNps (B). When we compare these two spectrum we observed that the Peak which appear at 3446 cm^{-1} and 3375 cm^{-1} which correspond to amine groups were shifted to 3406 cm^{-1}, 3361cm^{-1}. A peak which correspond to a carbonyl group at 1610 cm^{-1} has a little shift to 1606 cm^{-1} with a change in its intensity. A peak at 1051 cm^{-1} and 1089 cm^{-1} shift to 1068 cm^{-1} that correspond to ether or alcohol or ester, implying the binding of silver ion with hydroxyl, carboxylate groups and amide of the extract [20],[21].

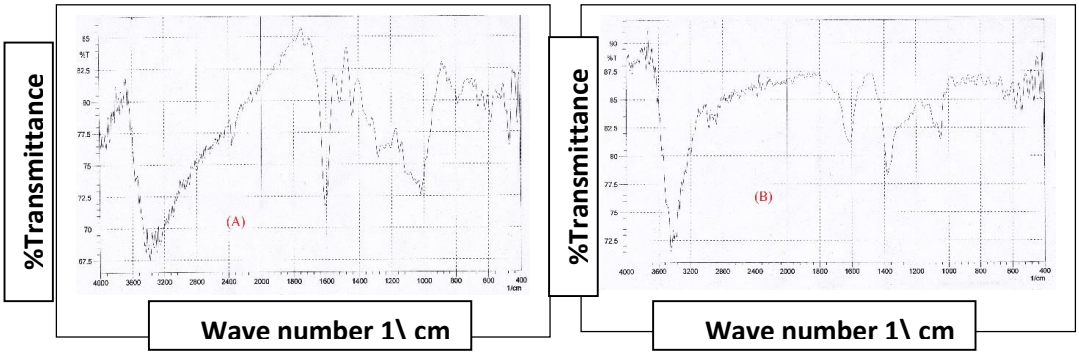


Figure: 6 FTIR spectra of capped silver nanoparticles

3.7 X-Ray diffraction (XRD)

The XRD pattern shows a significant amount of boarding line which are characteristic of nanoparticles . The crystallite size can be calculated according to Debye- Scherrer formula [22].

$D=k\lambda/(\beta\cos\theta)$

D= Average crystallite size (Diameter of the crystal)

B= Line broadening in radians (Full width at half maximum)

θ= Bragg angle.

λ= X-ray wave length.

The XRD pattern of the prepared AgNps is shown in Fig.6. The three diffraction peaks at (38.12°, 44.25°, and 64.45°) related to (111, 200, and 311) planes of the cubic Ag structure on powder diffraction standards (JCPDS 04-0783) can be seen, indicating that the AgNps are made of pure crystalline Ag[23]. The average grain size of AgNps was determined by application Scherrer formula of AgNPs with approximately (21.1 nm) in diameter.

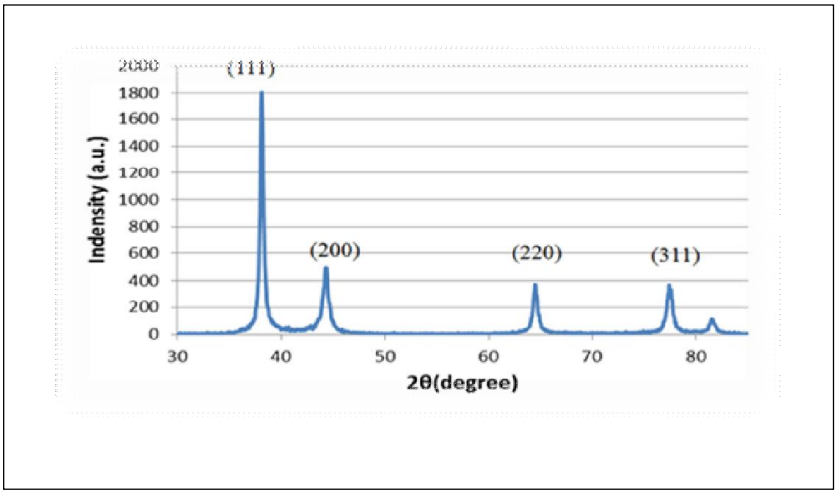
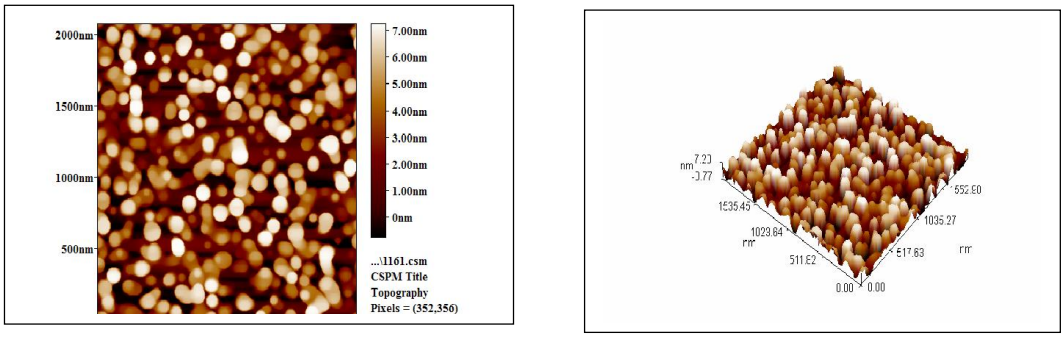


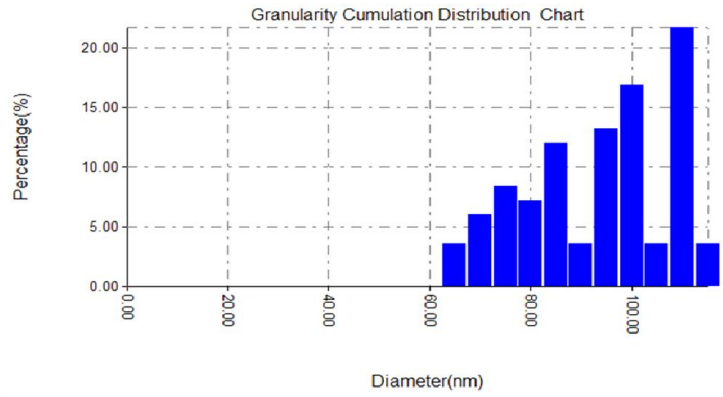
Figure:7 X-ray diffraction pattern of silver nanoparticles Prepared with *Carissa* extract

3.7 Atomic Forces Microscope (AFM)

Atomic force microscope (AFM) uses to know the surface morphology and to determine topography. The (AFM) gives a three dimensional image of the surface of a nanoparticles at an atomic level. The average particle diameter is calculated in nanoscale size as in fig.7shows the three-dimensional image of AgNps prepared using *Carissa* plant extract [24].



(a)



(b)

Figure: 7 AFM (A) image of silver nanoparticles prepared with *Carissa* extract,(B) percentage of diameters of silver nanoparticles

3.8 Scanning Electron Microscope (SEM)

The size, shape and distribution of green synthesized silver nanoparticles were Characterized by (SEM).(Fig. 8) shows particles are spherical with average size between(74-38 nm) and also individual nanoparticles were aggregated shows large nanoparticles. This aggregation took place due to the presence of the extract is a biological cell components on the surface of nanoparticles and acts as capping agent [25].

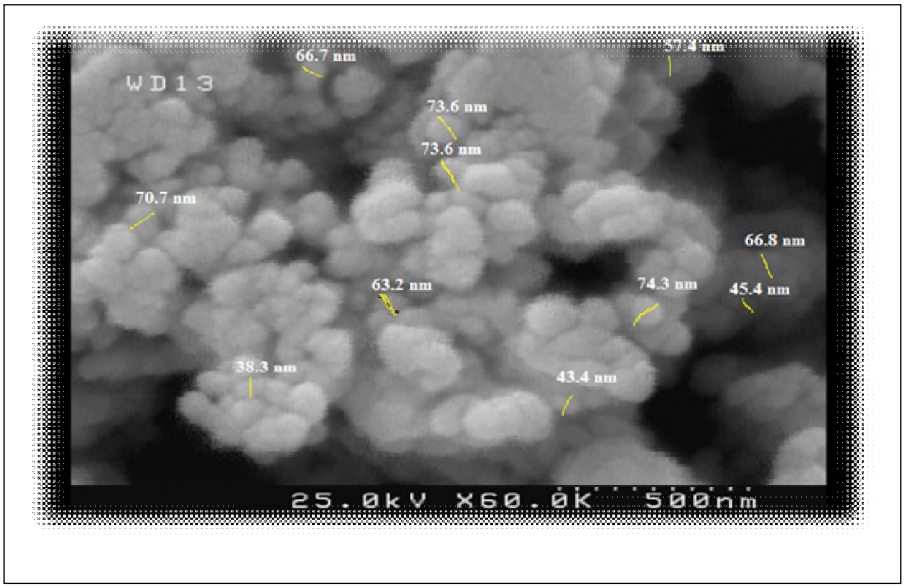


Figure: 8 SEM image of silver nanoparticles prepared with*Carissa* extract

3.9 Transmission Electron Microscope (TEM)

The silver nanoparticles synthesized by using *Carissa* leaves extract when scanned using TEM from which we conclude that the average mean size of silver nanoparticles was in between 17-46 nm and seems to be spherical in morphology as shown in (fig. 9). Thus the transmission electron microscopy gave a detailed descriptive image of the silver nanoparticles synthesized with their structural details and their size [26].

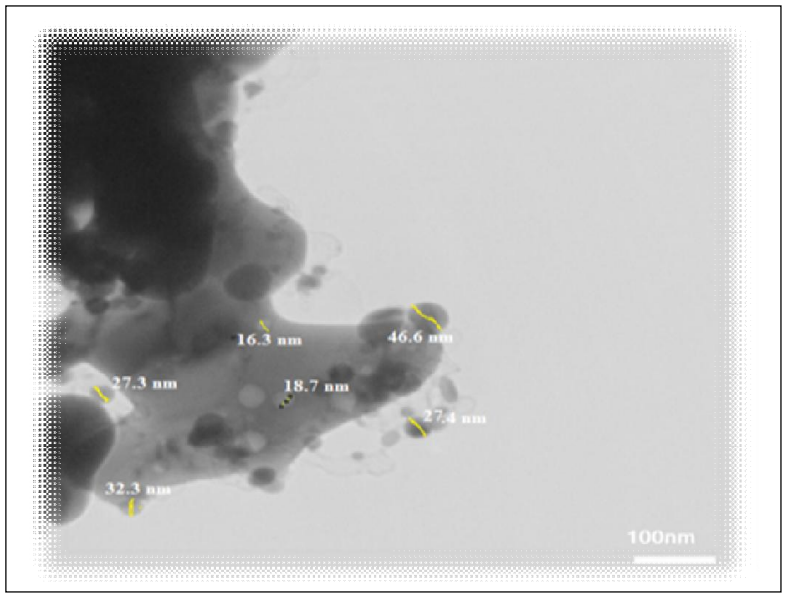
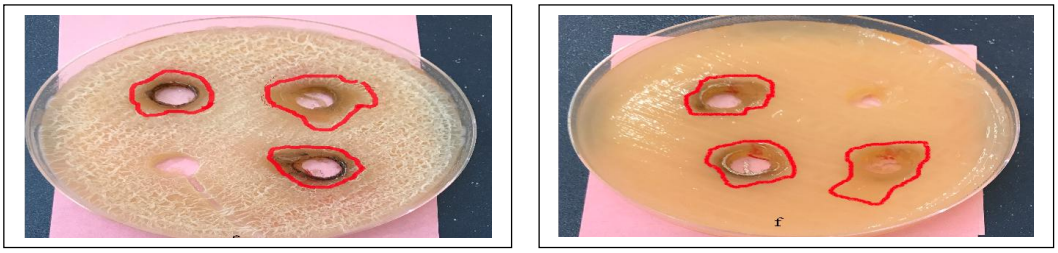


Figure: 9 TEM image of silver nanoparticles prepared*Carissa* extract

3.8 Antimicrobial assay

Antimicrobial activity of synthesized silver nanoparticles against Gram negative (*Shigella*) and Gram positive (*Staphyloccusaureus*) bacteria wasrevealed and zone of inhibition was measured (fig. 8 and table 1). Silver nanoparticles were use with its plant extract as experimental in the methods. The results indicatedthat silvernanoparticles showed effective antibacterial activity both in Gram negative and Gram positive bacteria in different concentration . Several studies have confirmed that the effect of silver nanoparticles AgNPs on bacteria is through the effect of silver nanoparticles AgNPs on the cell walls of bacteria where interaction with proteins contacting sulfur, leads to damage to the respiratory function of the bacteria, leading to their distraction [28], [29], and [30].



a b

Figure 8: Antibacterial activity (a) *Shigella* and (b) *S.aureus*

Table 1: Zone of inhibition (mm)

Name of the organism	AgNPs 2 ml	AgNPs 3 ml	AgNPs 4 ml	Carrisa Leafes extract
Shigella	9 mm	10 mm	12.5 mm	0 mm
Staphyloccocusaureus	9 mm	10.5 mm	10.5 mm	0 mm

4.Conclusions

In this study, silver nanoparticles were synthesized using *Carissa macrocarpa*leaves extract as reducing agent and capping agent, showed antibacterial activity against pathogens Gram positive and Gram negative. Average size of silver nanoparticles AgNps was adjusted by changing the extract concentration, pH, and original article of the reactions, this were done by taking the best conditions. Quantitative pH 7 is the best pH for this synthesis due to increased activity of Carissa extract constituents.Theprepared of silver nanoparticles have been characterized by different techniques UV-visible spectrophotometer, FTIR, AFM, SEM, TEM. The biosynthesis method developed in this study for producing silver nanoparticles has distinct advantage over chemical methods such as high biosafety, eco-friendliness, and nontoxicity to the environment.

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