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Preparation and Characterization of Silver Nanoparticles using *Gardenia* Leaf Extract and Study its Antimicrobial Activity

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Abstract: ***The present work investigates the synthesis of silver nanoparticles (Ag NPs) by biological method using Gardenia leaf extract and silver nitrate as precursor. Silver nanoparticles were successfully synthesized from Gardenia extract by green synthesis method. The detailed characterization of the Ag NPs was carried out using UV-visible spectroscopy, Scanning Electron Microscopy (SEM), X Ray Diffraction (XRD), FTIR, and Transmission Electron Microscopy. Finally, the antibacterial activity of silver nanoparticles were determined by spread plate method, and found that silver nanoparticles have significant antibacterial activity against Staphylococcus aureus and E. coli.***

Keywords: Silver nanoparticles, *Gardenia* leaf extract, Biosynthesis

1. Introduction

Nanoparticles are considered as important structural masses ofnanotechnology.Theuniqueandmostimportantproperty of the nanoparticles is that they unveil superior activity. There are remarkable applications of metal nanoparticles in the areas of diagnostic biological probes, catalysis, display devices, and optoelectronics [1].The silver metal has a great toxicity against a wide range of microorganisms, particularly; silver nanoparticle which has promising antimicrobial properties. Silver nanoparticles are found to be effective as antiinflammatory, anti-angiogenesis, antiviral, anti-platelet activity and against cancer cells which makes them vital [2-7].A number of approaches are available for the synthesis of silver nanoparticles such as electrochemical method, thermal decomposition, laser ablation, microwave irradiation and sonochemical synthesis [8-12]. However, there is still a need for economic, commercially viable as well environmentally clean route to synthesize silver nanoparticles. Silver nanoparticles were synthesis by biosynthesis method, reducing the silver ions present in the solution of silver nitrate.Although there are many routes available for the syntheses of silver nanoparticles including chemical, physical, electrochemical, irradiative, photochemical and biological techniques [13,14]. Drawbacks associated with physico-chemical methods of silver nanoparticles synthesis such as use of toxic chemicals, high temperature, pressure and production of hazardous by-products etc. therefore; it become necessary to search for safer alternative methods of silver nanoparticles syntheses. Bio-inspired synthesis using microorganisms, and plant extracts for silver nanoparticles have been suggested as valuable alternative to chemical methods as it avoids use of toxic chemicals and use of high and temperature. The plant extracts have come up nano factory for synthesizing metal nanoparticles of gold and silver. Its use for the synthesis of nanoparticles is potentially advantageous over microorganisms due to the ease of scale up, less biohazard, eco-friendly and elaborate process of maintaining cell cultures [15]. It is considered to be the best platform for synthesis of nanoparticles being free from toxic chemicals as well as providing natural capping agents for stabilization of silver nanoparticles. Moreover, use of plant extracts has drawn special attention becauseit reduces the cost of micro-organisms isolation and culture media enhancing the cost competitive feasibility over nanoparticles synthesis by microorganisms. A lot of literature is available on green synthesis of silver nanoparticle till date. Gold and silver nanostructures were produced using C. sinensis extracts, as a reducing and stabilizing agent, in aqueous solution at ambient conditions [16]. In the present study, we established that an aqueous extract of *Gardenia* were used in reduction of silver ion and formation of stable silver nanoparticles and tested the effect of antimicrobial activities.

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. 2.0g of *Gardenia* 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 *Gardenia* was added to the aqueous solution of 1mM Silver Nitrate .Then the sample was incubated in dark for 24 h. After 24 h, it measured at its maximum absorbance 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, AgNO3 concentration, temperature, pH value, and contact time. The

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sample was then dried to obtain the synthesized silver nanoparticle- for characterization.

2.3 Instruments for characterization

The UV–visible 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 Shimadzo FTIR 84005 spectrometer, for the plant extract containg silver nanoparticles, (0.01) g dried at 60 ºC for 4 h using KBr. 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. Morphology and contact surface ofsilver nanoparticles were performed using AFM Model AA300 Angstrom advanced. An aliquot of this filtrate containing silver nanoparticles was used for SEM, using SEM S-4160.

2.4Anti-bacterial activity

Anti-bacterial activity of silver nanoparticles was determined by using well diffusion method for Staphyloccocus aureus and E. coli. 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

3.1Effect of concentrations of plant extract

The UV-visible absorption spectra of the synthesized silver nanoparticles were recorded at its λ max. (Fig. 1) shows the UV–visible spectra of silver nanoparticle formed using constant (AgNO3) concentration (10^{-3}M) with different concentration of Gardenia 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 (447 to 451) nm depending on concentration of plant extract, The absorption peak gets more sharpness and blue shift was observed in (451) nm. This blue shift indicates a reduction in the mean diameter of the silver nanoparticles, spherical and homogeneous distribution [17]. This concludes the best concentration of *Gardenia* extract is (3 ml)

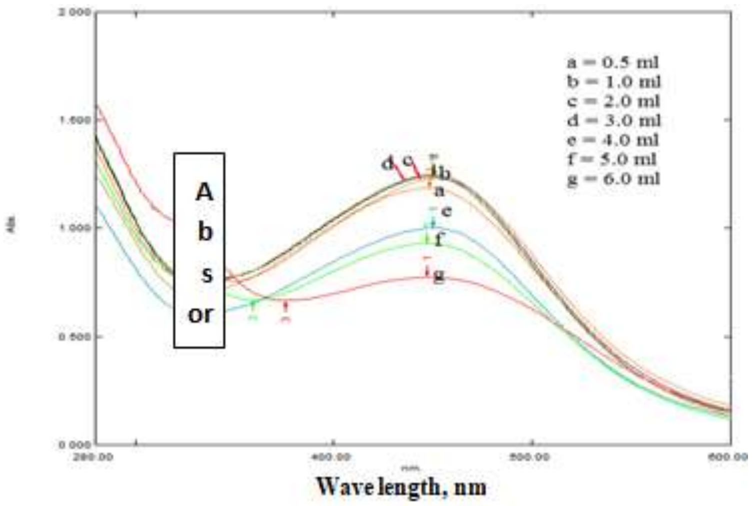

Figure 1: UV- visible spectra of AgNPs synthesized using different concentration (0.5- 6 ml) Gardenia extract

3.2 Effect of silver nitrate concentration

The UV-visible spectra recording after 24 h (Fig.2) shows the effect of silver ion concentration on AgNPs prepared by using constant *Gardenia* leaves extract concentration (3 ml) with different silver ion concentration (0.5 to 6 ml).For all the silver ion Ag^{+} concentrations, the samples changed in color after addition of the plant extract, indicating that a reduction reaction took place. The color of mixture was a slightly yellowish liquid; as red and brown (Fig 3). The observed peaks shows that the wavelength range were (442-447 nm). As result increasing the concentration of Ag ion lead to the formation of larger AgNPs [18].

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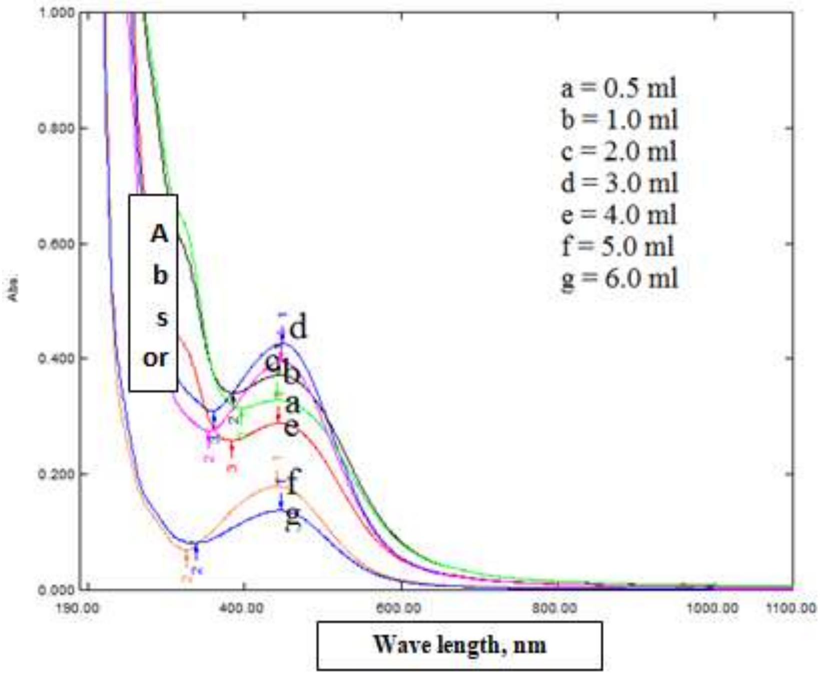

Figure 2: UV-visible spectra of silver nanoparticles at different concentration of silver ion

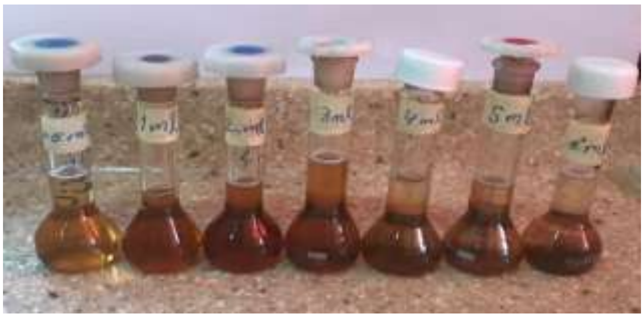

Figure 3: AgNPs synthesized using 4ml Gardenia extract with different concentration of Ag+ ion

This concludes the best concentration of silver ion Ag^+ to prepare AgNPs is (4 ml)

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.4) shows this effect at different range of pH (1.48, 2.10, 5.21, 7,and 9.33). The pH is adjusted using H3PO4 (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 (444 nm) in sample (d) pH=7 [19].

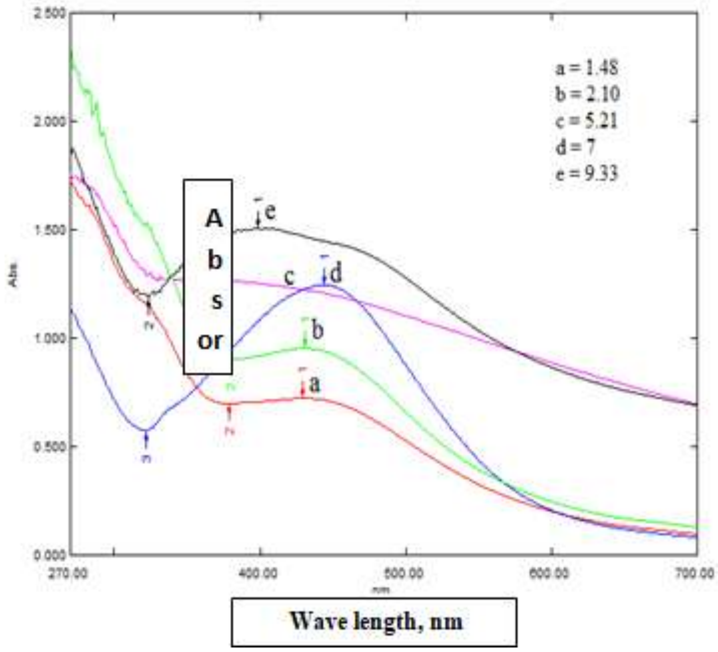

Figure 4: UV-visible spectra of silver nanoparticles at different pH

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3.4 Effect of temperature

The effect of temperature has an important physical parameter on the prepared AgNPs . (Fig. 5) shows the UV-visible spectra of AgNPs formation by using *Gardenia* extract at different temperature (30, 40, 50, 60, and 70° C). The absorbance band observed at wavelength (439 - 440 nm), the intensity of absorption increase with increasing temperature. The higher rate of reduction of Ag ions was occurred at high temperature due to the formation of homogenous nucleation of AgNps [20].

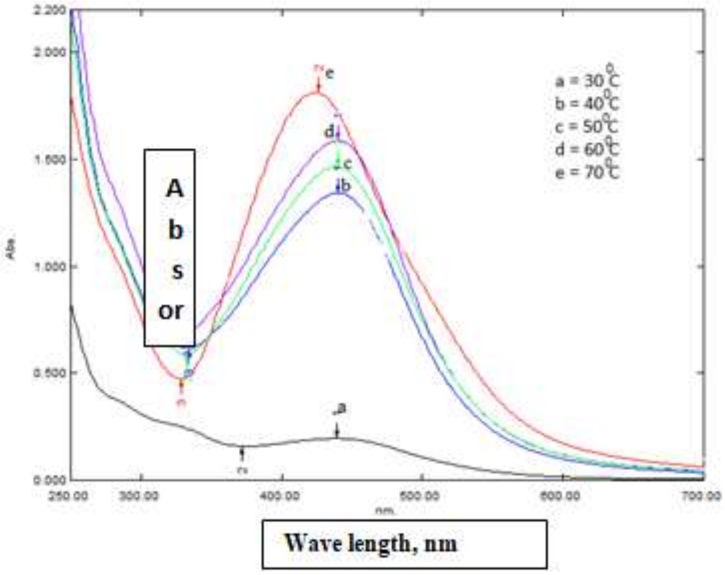

Figure 5: UV-visible spectra of silver nanoparticles at different Temperature

3.5 Effect of contact time

The effect of contact time of AgNPs formation by using *Gardenia* extract was recorded by UV-visible spectroscopy. (Fig.6) shows the UV-visible spectra in wavelength range (231- 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 [21].

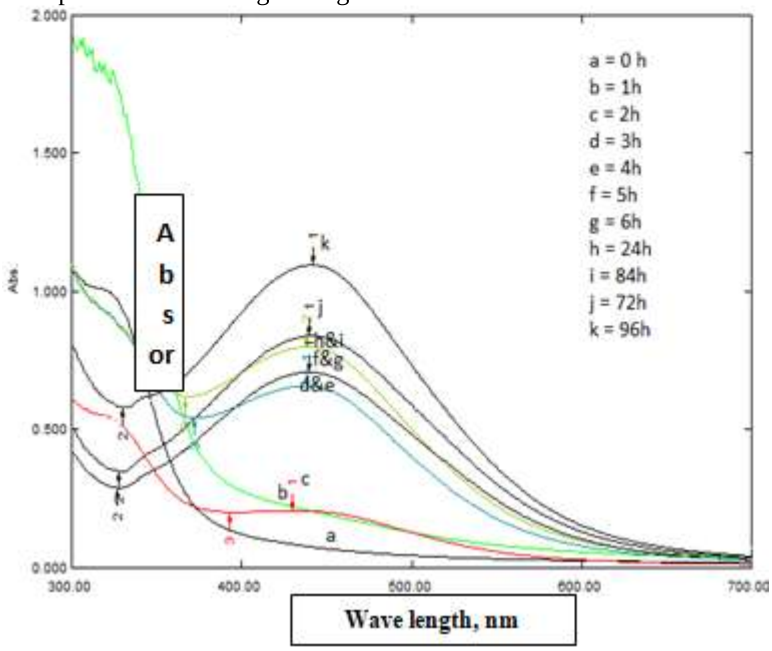

Figure 6: UV-visible spectra of silver nanoparticles at different contact time

3.6Energy gap and Tauc Plat of silver nanoparticles using Gardenia extract

The UV-visible spectra of silver nanoparticles AgNPs synthesized at optimum concentration of silver ion(3 ml) and plant extract(2 ml) used to determine the energy gap (Eg) by edge of absorption. Energy gap (Eg) were calculated in (ev) using Tauc plot curve.

Typically, a Tauc plot shows the quantity hν "The energy of the light " on the absicissa and the quantity (αhν)1/2 on the ordinate, where (α) is the absorption coefficient of the material. The resulting plat has a distinct linear regime which denotes the onset of absorption. Thus, extrapolating this linear region to the abscissa yields the energy of the optical band gap of the material.

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Tauc formula:
αhν = β*(hν - Eg) m β* is the edge width parameter representing the materials quality and is calculated from linear part of this relation, hν is the energy of the photon

$$h\nu = hc/\lambda \qquad hc = 1240\ ev$$

$$h\nu=1240/\lambda \qquad ....(1)$$

Eg = optical energy gap of the material
m= number which characterize the mechanism transition process
m = 1/2, 1/3 for direct transition
m = 1, 2, 3 for indirect transition
α(υ) is the absorption coefficient defined by the Beer-Lambert law
α= 2.303 Abs/d
d = path length = 1
α = Abs

The Absorption value were obtained from UV- visible spectrum at different wave length [22] (Fig. 7) shows the Tauc plot for silver nanoparticles AgNPs synthesized using Gardenia extract as reducing agent at the optimum concentration of silver ion and *Gardenia* extract the calculated value of energy gap is (3.4 ev).

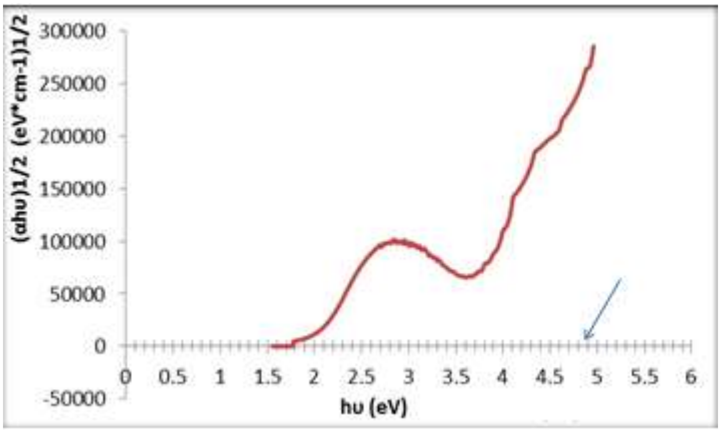

Figure 7: Tauc plot from UV-visible analysis of AgNPs synthesized using Gardenia extract

3.7 Fourier transform infrared spectroscopy (FTIR)

The FTIR spectrum identify 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. 8) shows a typical Infrared spectra of the synthesized using this extract (A) and *Gardenia* leaf extract AgNps (B). When we compare these two spectrum we observed that the beak 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 [22],[23].

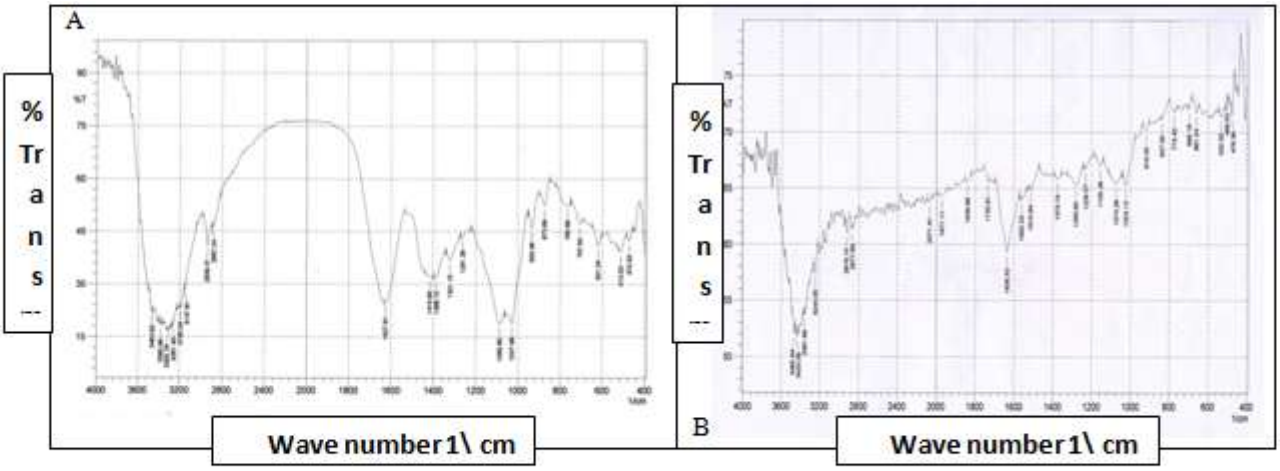

Figure 8: FTIR spectra of capped silver nanoparticles

3.8 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 [24].

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

D= Average crystallite size (Diameter of the crystal)
B= Line broadening in radians (Full width at half maximum)
Ө= Bragg angle.
λ= X-ray wave length.

The "XRD pattern" of the silver nanoparticles AgNPs is shown in (Fig.9) The three diffraction beaks at (38.30˚,

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77.50, and 44.46˚) related to (111, 200, and 311) planes of the cubic Ag structure. The average grain size of AgNPs was determined by application Scherrer formula of AgNPs with approximately (17.8 nm) in diameter [25].

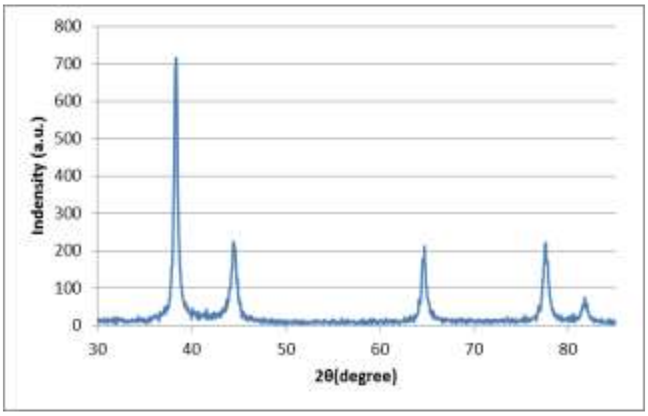

Figure 9: X-ray diffraction pattern of silver nanoparticles Prepared with *Gardenia* extract

3.9Atomic 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 calculate in nanoscale size [26].(Fig.11) shows the three-dimensional image of AgNps prepared using *Gardenia* plant extract.

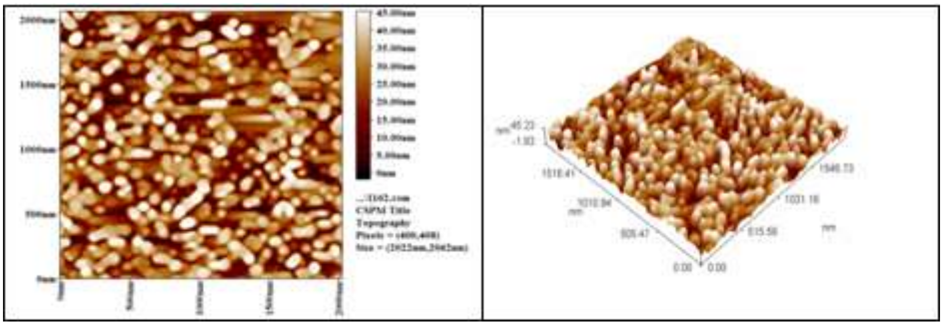

Figure 10: AFM image of silver nanoparticles prepared with *Gardenia* extract

3.10Scanning Electron Microscope (SEM)

The size, shape and distribution of green synthesized silver nanoparticles wereCharacterized by (SEM). (Fig. 11) shows particles are spherical with average size between (75-35 nm) and also individual nanoparticles were aggregated shows large nanoparticles. This aggregation took place due to the presence of the extract components on the surface of nanoparticles and acts as capping agent [27].

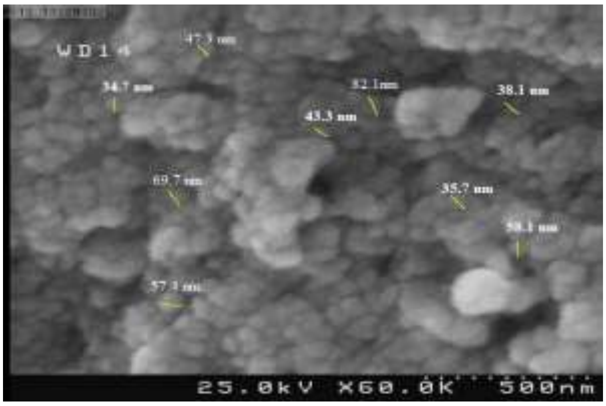

Figure 11: SEM image of silver nanoparticles prepared with Gardenia extract

3.11 Transmission Electron Microscope (TEM)

The silver nanoparticles synthesized by using Gardenia 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. 12). Thus the transmission electron microscopy gave a detailed descriptive image of the silver nanoparticles synthesized with their structural details and their size [28].

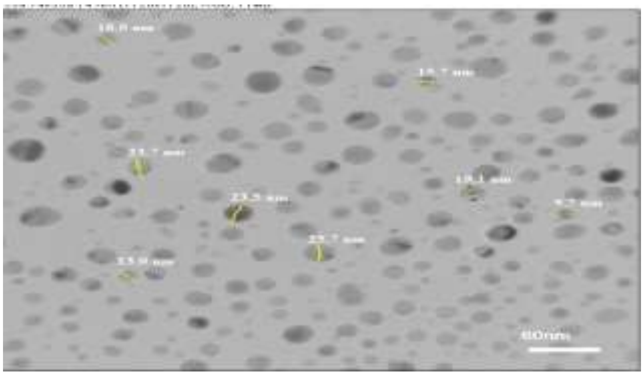

Figure 12: TEM image of silver nanoparticles prepared Gardenia extract

3.8 Antimicrobial assay

Antimicrobial activity of synthesized silver nanoparticles against Gram negative E. coli and Gram positive Staphyloccus aureus bacteria were revealed and zone of inhibition was measured (Fig.13 and table 1). AgNPs were use with plant extract as explainthe methods.The results

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indicated that silver nanoparticles showed effective antibacterial activity both in Gram negative and Gram positive bacteria in different concentration . The results indicated that silver nanoparticles 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 [29], [30].

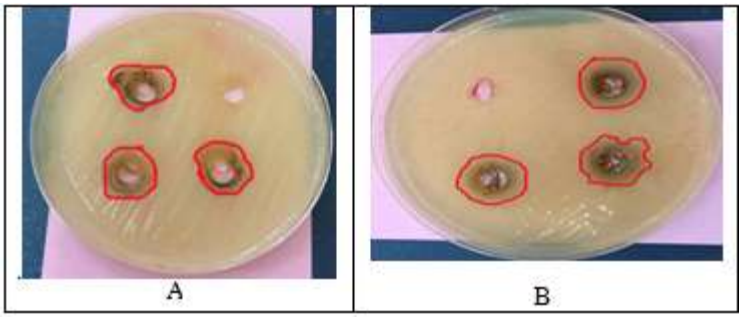

Figure 13: Antibacterial activity (a) *E.coli* and (b) *Staphylococcus. Aureus*

Table 1: Zone of inhibition (mm)

Name of organism	AgNPs 2 ml	AgNPs 3 ml	AgNPs 4 ml	*Gardenia* leaf extract
E.Coli	8 mm	10.5 mm	13 mm	0 mm
Staph. Aureus	9 mm	10 mm	11.5 mm	0 mm

4. Conclusions

In this study, silver nanoparticles were synthesized using *Gardenia* 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 . The prepared 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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