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Green Synthesis of Silver Nanoparticles by using *Annona Muricata* Extracts and Determine the Inhibitory Effects against Some Pathogenic Bacteria

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Abstract: Silver nanoparticles (AgNPs) were manufactured using the green technique, with silver nitrate (AgNO3) as a precursor and an alcoholic extract of Annona muricata (Graviola) as a reducing agent. The color change from light yellow to dark brown indicated the creation of AgNPs. The average size and shape of the nanoparticles were determined using Atomic Force Microscopy (AFM), which was 60 nm. Scanning Electron Microscopy (SEM) revealed that AgNPs have a spherical and smooth surface area. The wavelength range was examined using ultraviolet-visible spectroscopy (UV-Vis) to monitor the creation of nanoparticles, which revealed a sharp peak at 425 nm. The average crystallite size of AgNPs was determined to be 50 nm using Debye Scherrer's formula and X-ray Diffraction (XRD).

Fourier-transform (FT) infrared spectroscopy (FT-IR) spectra have been used for Silver nanoparticles (AgNPs) to identify the practical groups found in the synthesis method by Annona muricata (Graviola) .

The present study showed that the bacterial isolates which were multi drug resistance to classical antibiotics; distributed on Gram positive (Staphylococcus aureus and Staphylococcus epidermidis) and Gram negative (Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli, and Proteus mirabilis). The antibacterial activity of biosynthesized Ag Nps by ethanolic extract of Annona muricata was showed the maximum diameter inhibitions zone at concentration (100) mg/ml against S. aureus, S. epidermidis, P. aeruginosa, K. pneumoniae, E. coli and P.mirabilis reaching (34, 33, 33 ,31,30,28) mm respectively, while at concentration (12.5) mg/ml lowermost ranges of inhibition zone were recorded.

Keywords: Annona muricata, Silver nanoparticles, Green synthesis, Antibacterial activity.

Introduction

Annona muricata one of the medicinal plants have used in folk medicine to treat several diseases for medicinal and immune modulatory usage including antispasmodics antiparasitic, anti-diarrhea, analgesic, anti-allergic and sedative (George *et al*., 2012). It is also known to be one of the charming fruits that play a significant role in cancer care and in preventing cancer cells from proliferating faster and more efficiently than chemotherapy, resulting in many adverse effects besides being very costly (Barbalho *et al*., 2012). Medicinal plants have active compounds that are the primary source of medicinal use. Plants were used in medicine since ancient times as a main source of medicinal and nutritious materials for action and used in the pharmaceutical substances for the treatment of many diseases (Shakya *et al*., 2016).

Annona muricata commonly called soursop is an evergreen low-branching, slender and bushy tree. For many epochs now, the *A. muricata* (Graviola) showed strong antibacterial activity against several gram-negative and gram-positive bacteria with interesting results (Nwinyi *et al*.,2008). Viera *et al*., (2010) painted and confirmed that the *A. muricata* (Graviola) extracts were active against *Staphylococcus aureus*, whereas no such activity was observed in the aqueous extract of the fruit skin.

Nanotechnology offers a way of altering the core features of various materials, including metal nanoparticles **(**Boisseau *et al*., 2011). Nanoparticles (NPS) are minor sized atoms having a size range of 1-100 nm, which show an important role in current daily lives, furthermore, it has obvious importance in the fields of biotechnology such as food, medicinal and pharmaceutical industry (Kaur *et al*., 2012). Among numerous noble metal nanoparticles, silver nanoparticles (Ag-NPs) have achieved a special focus (Ahmad *et al.,* 2016). Silver nanoparticles (Ag-NPs) are of precise interest because of their anticancer, antimicrobial, and cytotoxic activities. The current research about the successful synthesis of Silver nanoparticles (Ag-NPs) from ethanolic-extracts of *Graviola*, nevertheless, there had been few stated method or journals on the use of ethanolic extracts of *Graviola* to prepare nanoparticles from flesh of this fruit. The aim of this study was therefore to improve a method for the synthesis of Ag-NPs from ethanolic-extracts of *Graviola* as well as to describe the green synthesized Ag-NPs and determine the antibacterial activity against pathogenic bacteria.

2. Material and Methods

2.1. Sample Preparation of *Annona muricata* Extracts

The Graviola fruits were wash away with distilled water (dH_2O) and then peeled. The pulp was located in oven to arid at 50 °C for 6 days, then the dried pulp was crushed into a dust using an electrical grater to get a fine dust, which was reserved in a sterile and closed glass vial at 4°C until additional inquiries (Gavamukulya *et al*., 2014).

2.2 Preparation of the 1 mM AgNO3 Solution: A pure silver nitrate ($AgNO_3$) at 99.7% was used for the grounding of the AgNO3 solution. 0.1698 g of AgNO3 were considered on an ultrasensitive gaging balance and transported to 1000 ml flask. Then dH_2O was added to the flask with continuous shaking until the 1000 ml mark was reached. The solution was then left to totally liquefy the AgNO3.

2.3. Biosynthesis of Ag Nanoparticles from *Annona Muricata*

It was prepared according to Shah *et al*., (2015) and Shaniba *et al*., (2017) as follow:

Fifty gram of dried fruits powder were soaked in a flask containing 250 ml of absolute ethanol for 3 days. After this period, the light brown color begins to appear, ethanolic extracts of Graviola fruits were then filtered by filter paper Whatman No. 1 (Gavamukulya *et al* .,2014).

50 ml of the fruits extract added to flask contain 450ml of 1mM of AgNO3 solutions and mixed thoroughly. The mixture was then placed in a dark storage vial to avoid photochemical activation of silver nitrate at room temperature for 72 h with continuous observing. The color of the mixture changes after 3 h from light brown to yellowish-brown. After 3 days the mixture color totally changed to dark brown and located in the heater, heated up to 300 °C and were reserved at this temperature for three hours (Goudarzi *et al*., 2016). The mixture changed to light brown is visual evidence of the creation of Ag-NPs or decrease of silver ions into Ag-NPs.

2.4. Characterizations of Ag Nanoparticles

2.4.1 UV/VIS measurements to confirm formation of Ag-NPs.

The ultraviolet - visible spectroscopy (UV/VIS) technique was used to check the synthesis of Ag-NPs from Graviola (ethanolic extract) in the range of 300 - 650 nm (Ahmed *et al.,* 2016).

2.4.2 The functional groups investigation using FTIR

FTIR studies were performed to determine the potential biomolecules in the Graviola (ethanolic extract) that are responsible for silver ion reduction, as well as the capping agents responsible for the

stability of the bio-reduced Ag-NPs. The KBr pellets of samples were made by grinding 10 mg of samples with 250 mg of KBr (FTIR grade). The 13 mm KBr pellets were formed in a conventional apparatus under a pressure of 75 kN cm-2 for three minutes. The spectrum resolution was tuned to 4 cm-1, and the wavelength range was 400–4000 cm-1 (Madivoli *et al*., 2018).

2.4.3 SEM and AFM Measurements

The structure, shape, and particle size of materials were examined using a scanning electron microscope (SEM). Atomic force microscopy (AFM) was used to determine the size, surfaces geography, and particulate volume of Ag nanoparticle.

2.4.4 Crystalline size determination using XRD

XRD examination was employed to regulate the middling crystalline size of the Ag-NPs designed. The XRD (Bruker-Germany) with Cu-Kα radiation (λ=1,54060 Å) and employed at 40.1 kV/40 mA in the variety of 10°–80° with a 2°-per-minute scanning degree was used.

2.5. Antibacterial Action of the Ag -NPs

The well diffusion method was used to identify Ag-NPs have antimicrobial activity against pathogenic bacteria (MDR- positive and negative Gram). Muller Hinton agar was used to culture the isolates and then 5 wells (5mm) were mad .The 4 diverse concentrations (12.5, 25, 50,100 mg /ml) were set from the standard solution of Ag-NPs and finally the5th was considered as control by adding 100µl of water, then incubated all plates for 18-24 hs at 37°C (Hasan *et al.,* 2009 ; Obedat *et al*., 2012).

3. Results and Discussion

3.1 Biosynthesis of Ag-NPs from ethanolic extract of *Annona muricata*

Silver nanoparticles were prepared according to Shah *et al*. (2015) and Shaniba *et al*. (2017). Ethanolic extract of Graviola fruit have been used to synthesize Ag NPs. The presence of the active compounds inside Graviola acts as reducing agents to reduce silver ions to Ag NPS. Changing in color indicates for nanoparticles synthesis, color transformed from light yellow to dark brown figure (1) was an indication of the creation of Ag NPs (Gavamukulya *et al*., 2019). One of the most important uses of biosynthesis of silver nanoparticles is that it is low-cost, naturally safe, riskless, easy to operate and low in toxicity (Heer *et al*.,2017).

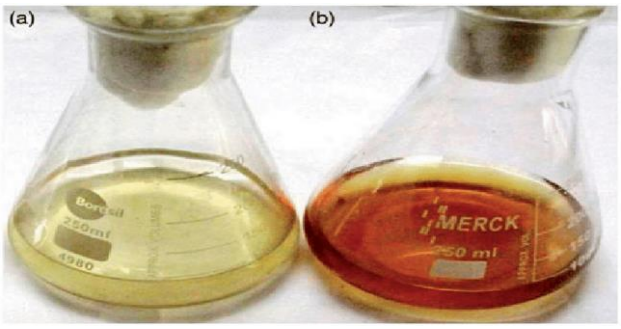


Figure (1) The **biosynthesis of Ag-NPs from Graviola color change from light yellow(A) to dark brown (B)**

3.2. Characterization of Ag-NPs

3.2.1. Atomic Force Microscopy (AFM)

Atomic force microscopy (AFM) was used to confirm the external shape of the biosynthesized Ag-NPs by using *Annona muricata* (Graviola) ethanolic extract; the two dimensions and three dimensions was determined image with AFM. The results showed there were differences in the silver nanoparticles' phenotypic properties as in the figure (2) which indicated the size of Ag-NPs formed by Graviola was 60 nm.

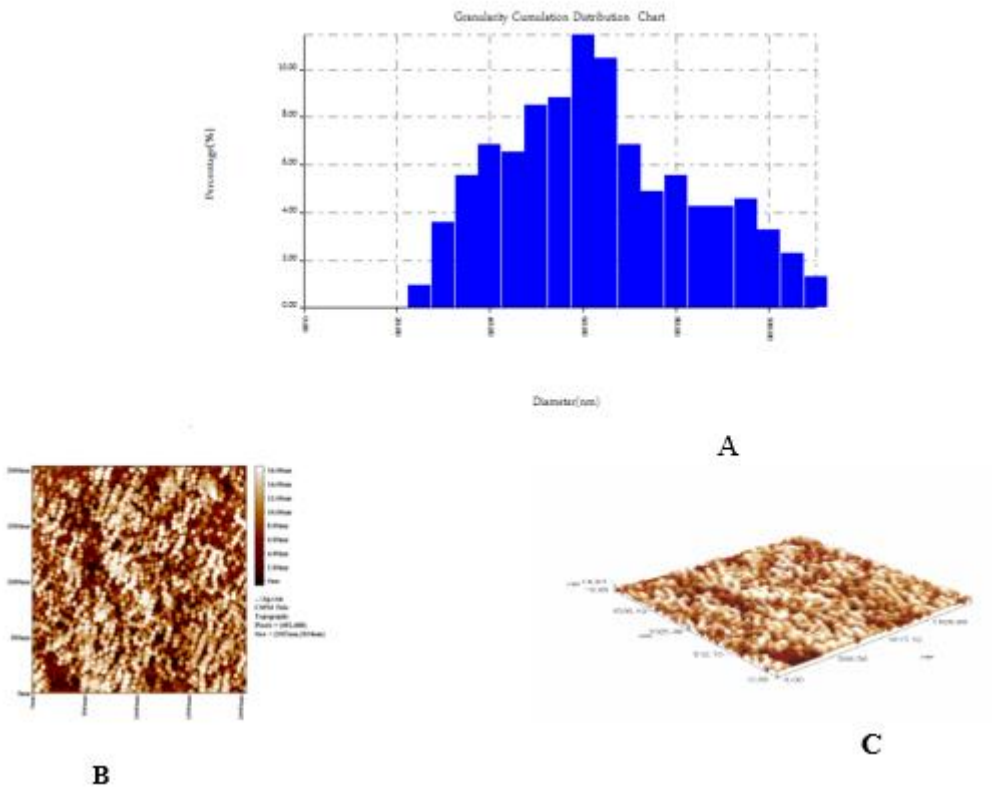


Figure (2): **A-** The range sizes of biosynthesized Ag-NPs.

B- Topo-graphy of 2-dimensional Ag-NPs.

C- Topo-graphy of 3-dimensional Ag-NPs.

3.2.2. Scanning Electron Microscopy (SEM) analysis

Additional method used to determine the shape, size, and distribution of green-synthesized silver nanoparticles was the Scanning Electron Microscope (SEM), figure (3), shows the particles were spherical with 32–68 nm of smooth surface area.

The present study successes to achieve good outcomes in establishing a fine range of silver nanoparticles sizes which in agreement with Gavamukulya *et al*., (2019) and Santhosh *et al*.,(2015) they produced Ag nanoparticles sized 30-70 nm.

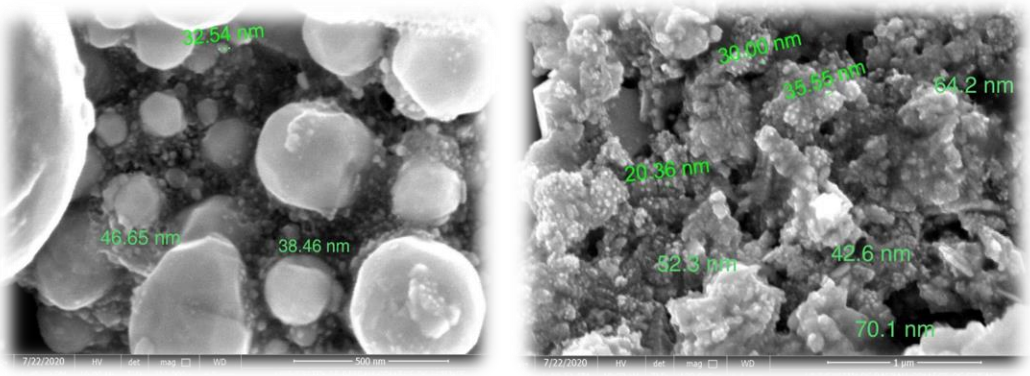


Figure (3). SEM image of Ag -NPs

3.2.3. Ultraviolet-visible spectrum

The absorption characters' spectra have essential properties of the Ag-NPs and the UV-Visible fields had proved to be very useful for Ag-NPs investigation and were a good method for characterizing Ag-NPs creation and production.

Ultraviolet-visible spectroscopy (UV / VIS) in the range between 300 and 600 nm further provoked the production of Ag-NPs from the ethanolic extract of Graviola fruits and the absorbance top was reported at 425 nm as shown in the figure (4) this outcome was similar to Gavamukulya *et al*. (2019) noting the Ag absorption peak was (427 nm). The highly maximum absorption arising about 425 nm checks the creation of the Ag-NPs constituent since the maximum absorption for the bulk Ag-NPs occurs at about 350-550 nm (Bose *et al*.,2016).

The decrease of the silver ion to Ag-NPs during contact to the plant extracts could be observed via color change and thus UV/VIS spectroscopy (Ahmed *et al*., 2016; Shankar *et al*., 2004; Song and Beom 2009). In the present study, the formation of Ag-NPs was confirmed by the change in color of the mixture from dark green to dark brown, indicating the successful green synthesis process. Silver nanoparticles exhibit a yellowish/dark brown color in solution because of the excitation of surface plasmon vibrations in Ag-NPs.

The produced Ag-NPs' UV/VIS maximum absorption spectra were observed at 429 nm, which is within the range of previous investigations on the synthesis of Ag-NPs from extracts of plants. Among other studies, synthesis of Ag-NPs with UV/VIS absorption maxima at 410 nm (Otari *et al*., 2017), 420 nm (Santhosh *et al*., 2015), 430 nm (Song and Beom, 2009), and 435 nm (Kumar *et al*., 2017). The current results also confirm for the first time that *Annona muricata* flesh extracts can be used in the green synthesis of Ag-NPs using ethanol as an inexpensive and environmentally friendly method.

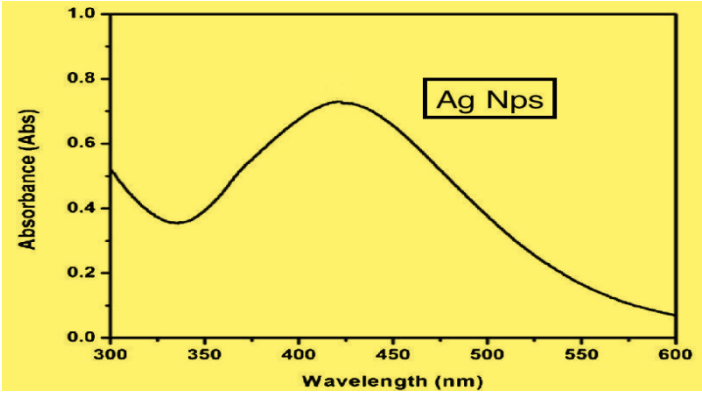


Figure (4) UV-Vis spectrum of produced AgNPs

3.2.4.X-ray Diffraction (XRD)

The bio-synthesized Ag nanoparticles were analyzed by using X-ray diffraction devices to knowing the crystallinity and average particle size. Figure (5) shows the protuberant peaks conforming to the diffraction levels observed at 2θ levels 38.1 ° (111), 44 ° (200), 64.2 ° (220) and 77.2 ° (311) are in agreement with the Jhoint Committee on Powder Diffraction Station(JCPDS) card No.4-783. The average oparticle size (D) of synthesized nanoparticles was estimated using the formulation Ddebye Scherrer (Bokuniaeva *et al*., 2019)

$D = 0.9\ \lambda / \beta \cos \theta$

where λ is the wavelength sof X-ray sources (CuKα lines – 0.1541 nm), β is the fulls width ast half maximums (FWHM) in radianss and θ is Brag's diffraction angle. The calculated value of D was found to be 50 nm that was less than what he found (Gavamukulya *et al*., 2019) which reached 60 nm.

The 2θ peak found at 38.31°, 38.41°, and 77.50° in the XRD diffraction patterns correlates to the (111), (111), and (311) reflection planes, which, respectively, depict the face-centered spherical structure of silver (Kumar *et al*., 2017; Kumar *et al*., 2015). Additional peaks near 28.07°, 32.47°, 46.56°, 55.50°, and 57.50° are caused by the bio-organic phase that is present on the particle surface. According to Kumar *et al*. (2017) and Umadevi *et al*. (2012), the broadening of peaks in solid XRD patterns generally indicates smaller particle size and represents the impact of the experimental circumstances on the nucleation and growth of the crystal nuclei.

The significant reflection at 32.47°, in contrast to the other seven peaks, would indicate the nanocrystals' growth trajectory. It was estimated that the AgNPs produced during the bioreduction process had an average size of 87.36 nm. The AgNPs were about spherical in shape, had a smooth surface, and were widely dispersed throughout, as seen in Fig. 9(A) and (B). These findings are consistent with the SPR band shape identified from the UV-visible spectrum, which has a maximum absorption at 429 nm.

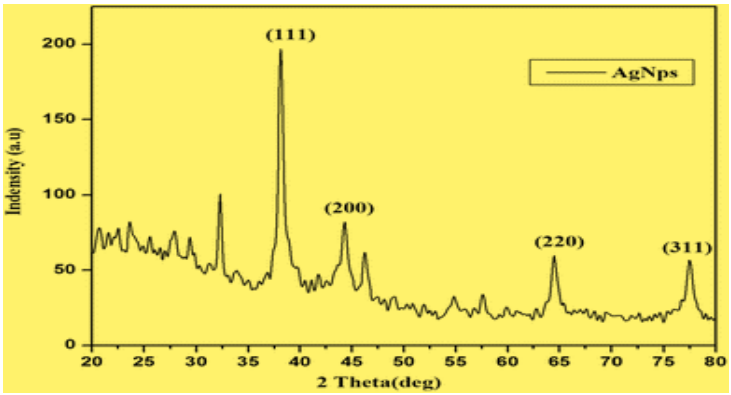


Figure (5) XRD spectra of Ag Nps

3.2.5. Fourier Transform Infrared Spectroscopy (FTIR)

FTIR was conducted to evaluate the possible functional classes of biomolecules involved in silver ion decrease and steadying of biosynthesized AgNPs created by *Annona muricata* ethanolic extract. The test sample's band intensities were analyzed as shown in the figure (6) below.

The prominent peak at 3788 cm-1 is related to the N-H stretching amine vibration while the observed peak at 3381 cm-1 is due to the – OH stretching vibration of flavonoid, polyphenol, and alcohol functional groups of carboxyl. The peaks of the alkanes and alkynes at 2921 and 610 cm-1 are unique to methyl groups or – CH.

The prominent peak at 1640 cm-1 is due to the alkene stretching vibration C = C while the observed peak at 1075 cm-1 is attributable to the Aliphatic amine stretching vibration C-N. Alkenes are related to the peaks at 610 cm-1. This outcome was very nearby Gavamukulya *et al*., (2019). These comprised the functional crowds responsible for Ag-NP formation; alkanes and alkyls, alcohol groups, carboxylic acids, amides, alkenes, acids, and alkyl halides.

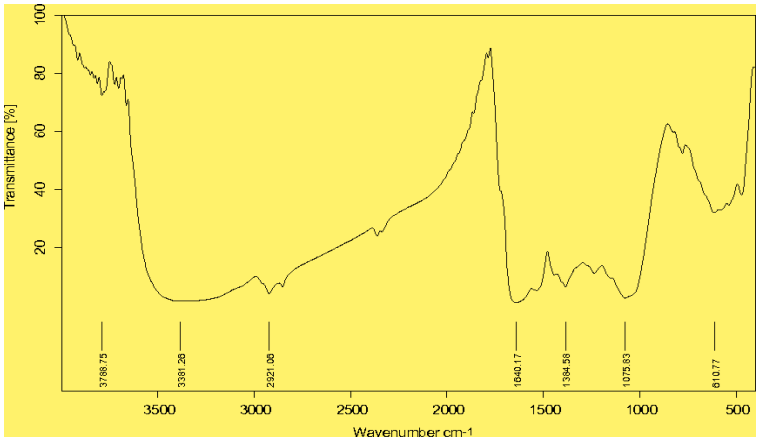


Figure (6) FTIR spectra of functional groups of the Ag-NPs synthesized from Soursop fruit extract.

3.3. Antibacterial activity of Ag-NPs against pathogenic bacteria

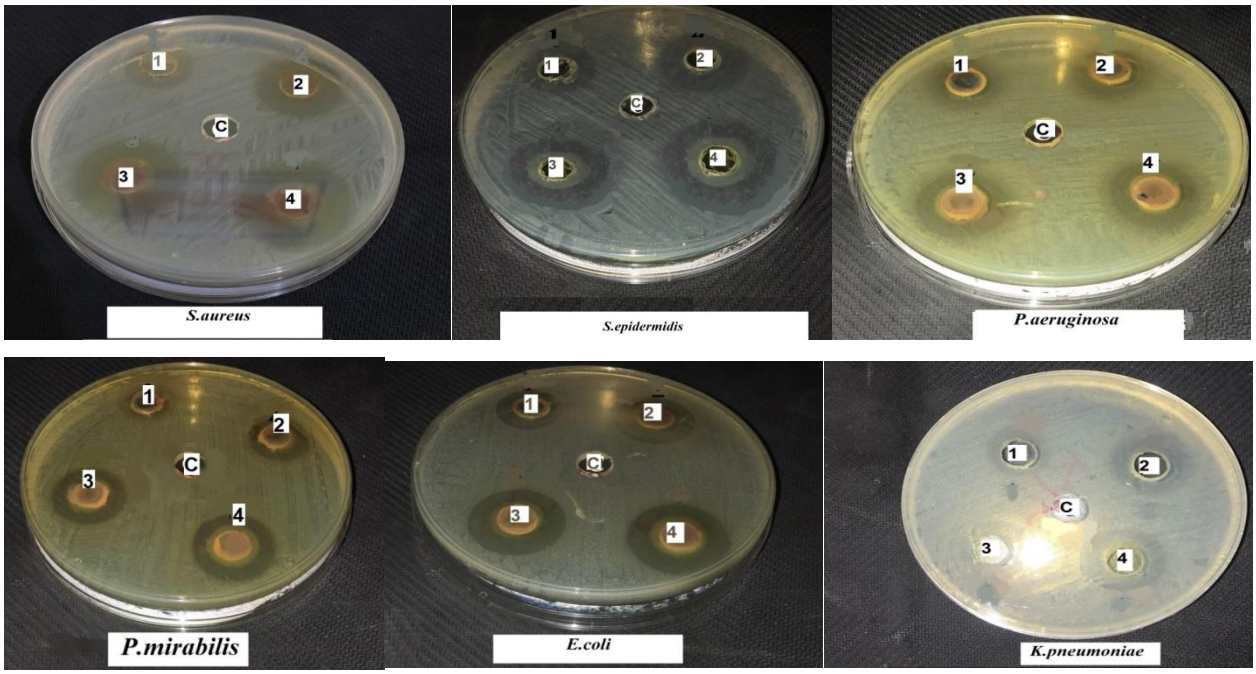
The antibacterial action of biosynthesized Ag-Nps by ethanolic extract of *Annona muricata* was evaluated against Gram positive and negative bacteria (Multidrug resistance) isolated from burns and wounds specimens shown in table (1) and figure (7).

The Ag NPs indicated the highest width of inhibition zone at concentration (100) mg/ml of *S.aureus, S.epidermidis, P.aeruginoas, K .pneumoniae, E.coli* and *P.mirabilis* reaching (34, 33, 33 ,31,30,28) mm respectively, while the Ag NPs noted at concentration (12.5) mg/ml lowest areas of inhibition zone against the same isolates reaching (18,17,16,16,15,13) mm respectively. This results were close to Akintelu *et al*. (2019).

Table (1) The Effect of Ag NPs on Bacterial Growth

(zones of inhibition)

Type of isolate	12.5 mg/ml	25 mg/ml	50 mg/ml	100 mg/ml
Staphylococcus aureus	**18mm**	**24mm**	**29mm**	34mm
Staphylococcus epidermidis	**17mm**	**22mm**	**28mm**	33mm
Pseudomonas aeruginosa	**16mm**	**21mm**	**28mm**	33mm
Klebsiella pneumonia	**16mm**	**22mm**	**26mm**	31mm
Escherichiae coli	**15mm**	**19mm**	**24mm**	30mm
Proteus Mirabilis	**13mm**	**17mm**	**23mm**	28mm



Fiqure (7) Show the inhibition zone of AgNPs on Bacteria isolates (C1=12.5,C2=25,C3=50,C4=100) mg/ml and C = Negative Control (ddH_2O)

Ag-NPs antimicrobial activity against bacterial pathogens was attained by enhancing the levels of reactive oxygen, including that of the formation of free radicals (Kim *et al*., 2011), which allows them to penetrate the walls of the bacterial cell by the small size of the particles.

Silver nanoparticles have the ability to release Ag+ions, which bind to the thiol group in bacterial proteins, impacting the role of DNA and damaging bacteria. Silver nanoparticles may penetrate the bacterial cell wall and act on inactivating some bacterial cell enzymes, creating hydrogen peroxide H2O2 (Patra and Beak 2017).

Although silver nanoparticles used with a size of 60 nm showed a strong (positive) effect in inhibiting gram positive and negative bacteria, silver nanoparticles possess antimicrobial properties as these particles bind to the cell wall and pierce the bacterial cell wall negatively to the gram stain, thereby increasing cell absorptivity and contributing to uncontrolled cell permeability. In addition, the biological membrane matrix in bacteria contains extracellular DNA (eDNA) and there is an association

in between positively charged of Ag-NPs and the negatively charged of eDNA) and this plays an important role in the removal of microbes (Hendiani *et al*., 2015).

Conclusions

Several techniques such as AFM, SEM, XRD, UV-vis, and FTIR were utilized to study Ag-NPs. The biosynthesis of Ag-NPs nanoparticles from plant extracts was a simple, ecologically friendly, and cost-effective process.

Both Gram-positive and Gram-negative bacteria are susceptible to the antibacterial action of Ag-NPs produced by the alcoholic extract of *Annona muricata*. Ag-NPs had more efficacy against gram-positive bacteria than against gram-negative bacteria.

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