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Green Synthesis of Bio-Active Silver Nanoparticles from Insect Gall Extracts as Potent Anti-microbial Agents

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Keywords

Insect gall,
Ziziphus jujube,
Biogenic synthesis,
silver nano particles

Abstract

Synthesis of nanoparticles of metals using natural plant extracts has gained much attention these days. Here silver nano particles are produced by facile method of biogenic synthesis using extracts of insect gall which is a common infection on *Zizipusjijube* plant. The aqueous extracts of the dried galls were used for the synthesis of silver nanoparticles (AgNPs) which were then screened for their antimicrobial activity. The morphology, size and functional groups present in AgNPs was evaluated using electron microscopy and Fourier Transform Infrared Spectroscopy- FTIR analysis. Also, UV–Vis spectroscopy confirmed the formation of particles. X-ray diffraction analysis-XRD. The diffractogram revealed the characteristics of particles. The mean diameters of the nanoparticles were between 20 nm, and they had moderate antimicrobial activity against gram positive and gram-negative bacteria than gall extract alone.

Introduction

Gall formation involves an intimate association between the plant host and gall maker. Galls can be found on any part of the plant, but are most often observed as large, swollen growths on a leaf, petiole, twig, or branch. Most galls are caused by mites and insects. Galls are formed mainly by gall midges and some other flies (Diptera), gall wasps (Hymenoptera), and mites (Acarina), but are also caused by aphids (Homoptera), sawflies (Hymenoptera), and few moths (Lepidoptera) and beetles (Coleoptera). (Khatamifar et al. 2022, Felt et al. 1940, Gagne et al. 1989, Russo et al. 1979)

The word nano is derived from Greek word for “dwarf, it denotes one-billionth part of something. These day nanoparticles are utilized for various purposes, especially metallic nano particles are considered as the most encouraging because they contain wonderful antimicrobial properties due to their enormous surface zone to volume proportion, which is of special interest to scientists because of the developing microbial obstruction against metal particles, anti-infection agents and the improvement of safe strains. (Khalil *et al.*2013, Nayem *et al.* 2020, Odeniyi, M. A *et al.* 2020)

For biomedical applications; being added to wound dressing, topical creams, germ-free showers and textures, silver capacities' as a germ-free and shows a wide biocidal impact against microorganisms through the interruption of their unicellular layer in this way upsetting their enzymatic exercises. (Yoram et al. 2008, Panel Seema et al. 2018, Alomar et al. 2020, Hano et al. 2021). Union of silver nanoparticles is of much enthusiasm to the scientific network due to their wide scope of utilization. These silver nanoparticles are infect effectively utilized in the malignancy conclusion and its treatment as well. (Popescu et al. 2010a, Baruwati et al. 2009 b) As of late, Mocking and associates (Hawar et al. 2022, Siddiqi et al. 2017) showed that half of breeds of silver nanoparticles with amphiphilic hyper branched macromolecules, display powerful antimicrobial surface covering specialist properties (Aymonieretal.2002). Design and development of biological synthesis of active silver Nanoparticles as a hopeful predominance of the interaction of Biotechnology and Nano technology has received raised concentration due to growing to require expansive environmentally good technology in material synthesis. (Xin et al. 2010, Iqbal et al. 2020)

There are so many methods present for biological design and synthesis of nano materials. (Bindu and Mahalingam 2015) in recent times, fabrication of silver nanoparticles has drawn considerable attention due to their physical and chemical properties and applications in biomedicine, antiangiogenic activities against bovine retinal endothelial cells, controlling HIV infection (Luis et al. 2005), and detection of bacterial pathogens (Valodkar et al.2011), good Catalytic activity. (Xiaojun et al. 2004) and anticancer against lung carcinoma cells (Kanniah et al. 2004).

In this research, we have represented the green synthesis of silver Nanoparticles by reducing the silver ions present in the soln. of silver nitrate ($AgNO_3$) by an aqueous insect gall extract grown on Ziziphus jujube. Silver Nano particles (AgNPs) were successfully designed and generated bytreating $AgNO_3$ soln at 90˚ C for 1 hour 30 min. and then colour change of whole gall extract of Ziziphus jujube treated solution. from light brown to dark brown confirmed formation of silver Nanoparticles and the nature of synthesized Nanoparticles were characterized by UV-Vis spectroscopy, FT-IR, X-ray diffraction, SEM and HR-TEM. AgNPs show excellent antibacterial property. (Barabadi et al. 2019b)

This research is to provide a significant base for applying NPs as antimetabolites and medicated AgNPs are playing a major role in the field of Nanomedicine.

Materials and Methods

Collection of the material

The insect gall of *Ziziphus jujube* was collected from TheDangs districts area. The galls were washed, shade-dried and powdered in a grinder mixer. Silver nitrate was purchased from Sigma-Aldrich Chemical Company.

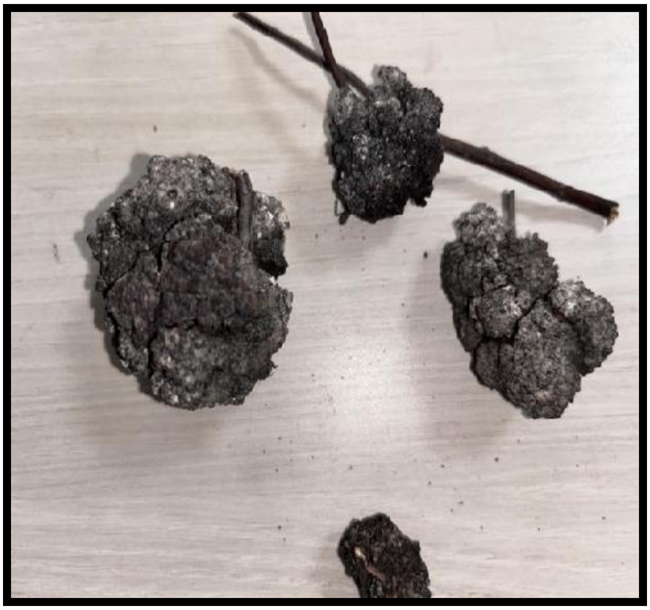


Fig.1

Fig.2

Fig.1& Fig.2.Insect gall of *Ziziphus jujube*

Method

Preparation of aqueous gall extract.

Fresh galls were collected and washed with double distilled water then shade dried and pulverized using a kitchen grinder. Powdered galls were then stored in air-tight container for future use in green synthesis of the silver Nanoparticles. 10 gm gall powder was added in Milli Q water and shaken well. The prepared mixture was heated for 3 hours at 50˚C and the hot mixture was filtered through Whatman filter paper no 1. Aqueous extract was stored under refrigeration at 4˚ C and used as stock solution for AgNPs synthesis.

Fig.3 Powder form of Galls

Fig. 4. Powdered Galls

Fig.5. Extract reaction apparatus

Fig.6. Gall extracts

Synthesis of AgNPs using of aqueous gall extracts

For the bio mediated synthesis of silver Nanoparticles, 30ml of 1mM $AgNO_3$ salt solutions lowly added to the 20 ml of the gall extracts. After completing the addition, the reaction mixture was kept on the magnetic stirrer with hot plate for 1 hr 30 min. at 90˚ C. The colour changewas observed between reaction solution after adding $AgNO_3$. The colour change indicated that the AgNPs are generated. Here colour change was from red to dark brown. The reduction of Ag^{+1} to Ag^{0} was mediated by the biomolecules present in plant which act as a reducing agent as well as capping agent. (Issaabadi et al. 2017)

Fig.7. AgNPs

Further confirmation of AgNPs was done using by using UV–Vis. spectral analysis with absorbance peak 453.4 nm. After this confirmation, the reaction mixture was subjected to centrifugation to isolate the silver Nano particles at 10000 rpm for 30 min. The separated Nano particles settled at the bottom and were collected and washed with double distilled water; they were then dried in hot air oven at 60˚ C for 2 hr. Finally, the powdered AgNPs were stored at 4˚ C for further characterization and antimicrobial activity.

Results and Discussion

The formation of AgNPs was indicated by colour change of the solution from light red to dark brown. (Fig.8.)

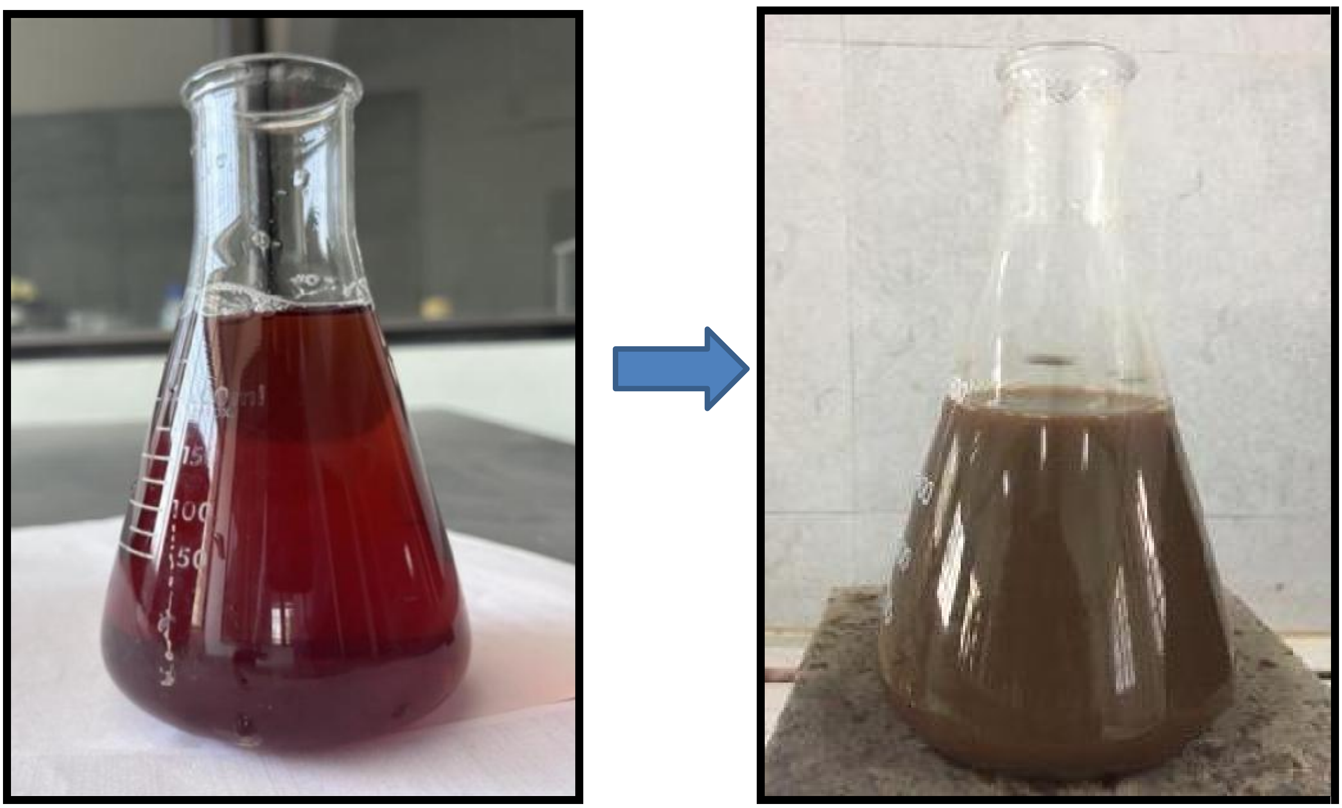


Fig.8.Difference in the colour change before and after the formation of AgNPs.

UV-Visible Spectroscopy

The absorption spectra of the synthesized silver Nanoparticles were recorded against milli Q water. Fig.9a shows the UV-Visible spectra of $AgNO_3$ soln. which absorption peak at 214 nm Fig.9b shows the UV- Visible spectra of gall extracts and instantly adding $AgNO_3$ soln. which absorption peak at 344nm. Fig.9c shows the UV-Visible spectra of silver Nano particles formation and colour change of the solution dark red to dark brown, depending on the concentration of extract and inclusion of silver nitrate solution with time the concentration of AgNPs increases and also peak value increase in accordance and takes more sharpness at 453.4nm. (Sana et al. 2015, Sana et al. 2018)

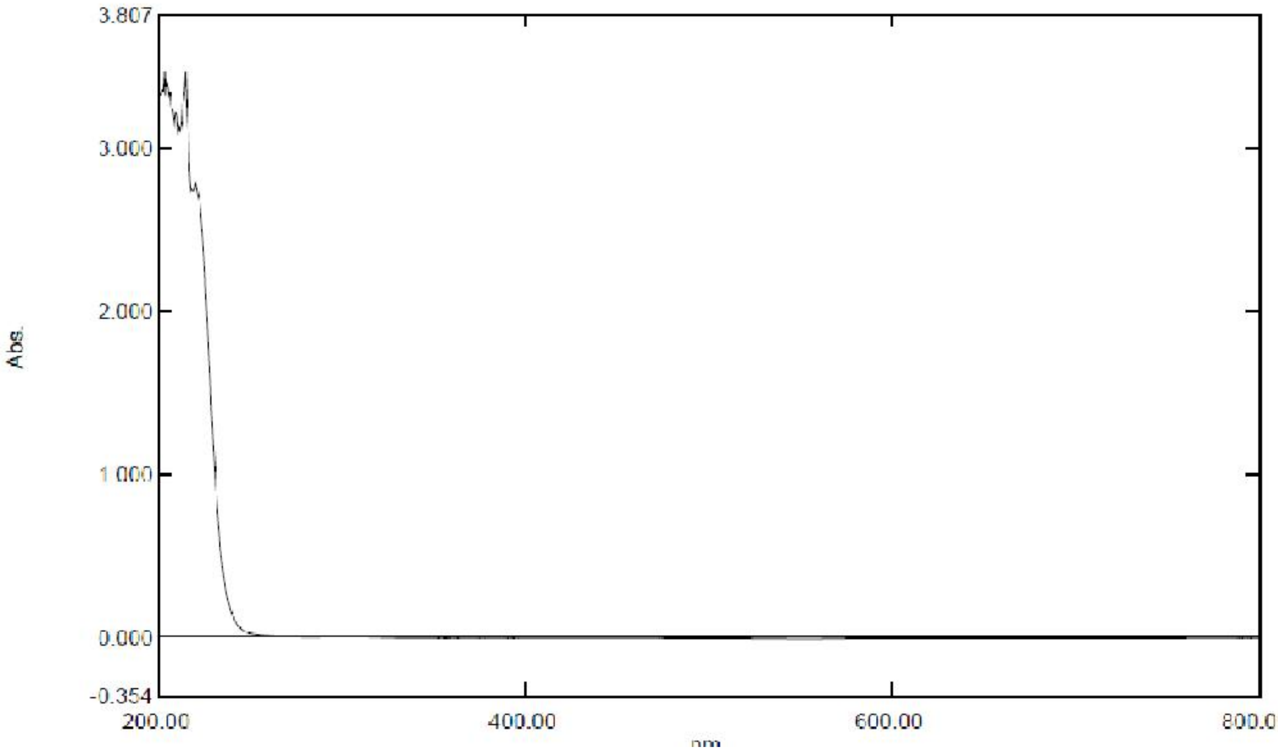


Fig.9a. UV- Visible spectra of $AgNO_3$ soln.

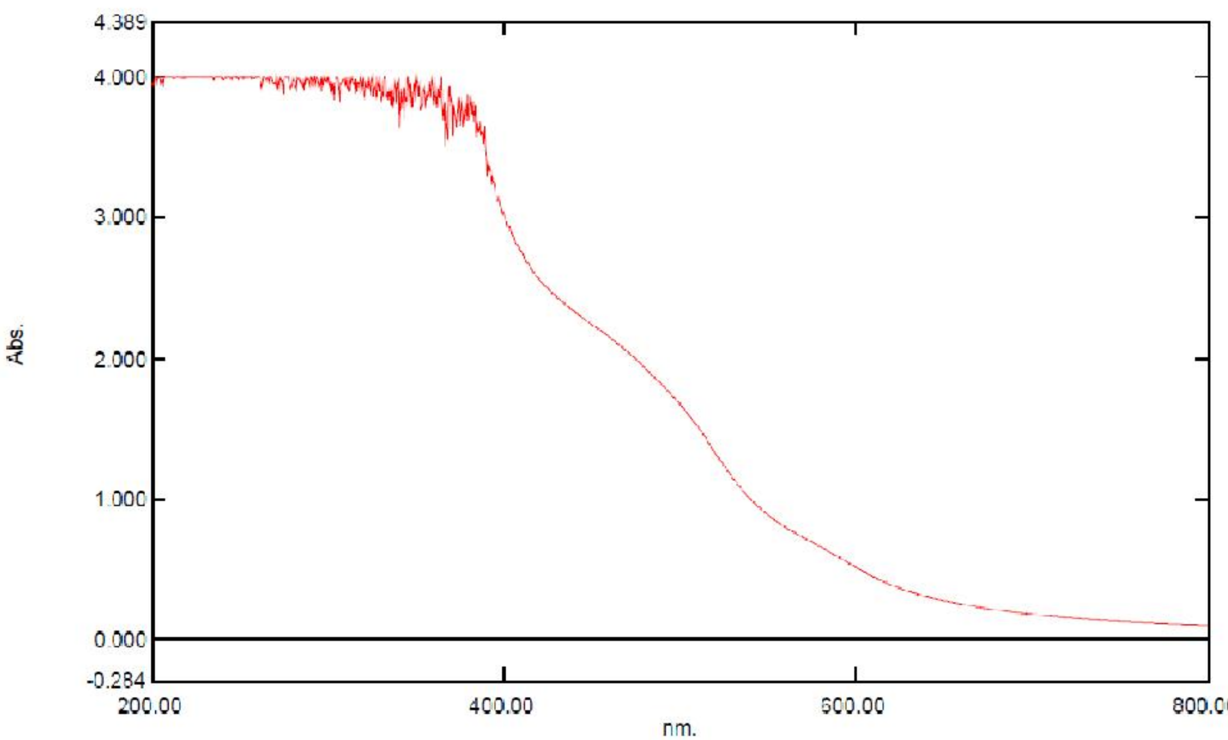


Fig.9b.UV-Visible spectra of gall extracts instantly after addition of $AgNO_3$

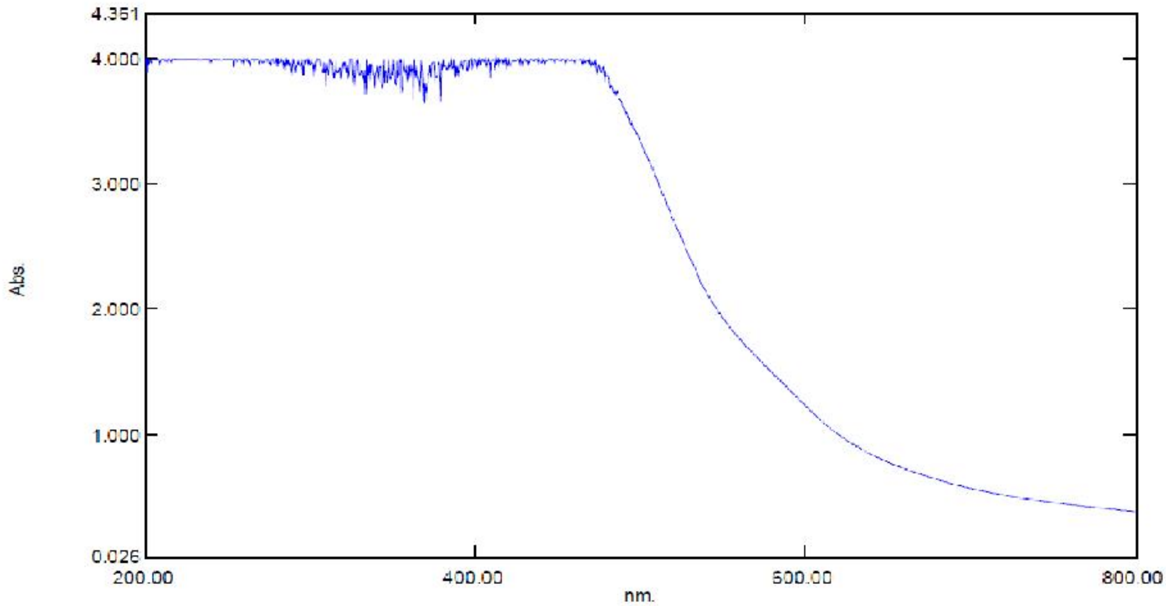


Fig.9c. UV- Visible spectra of formed AgNPs

Fourier Transform Infrared spectroscopy (FTIR)

FT-IR analysis was performed using Burker Alfa model ranging 400cm^{-1} to 4000cm^{-1}. Approximately 4 mg of dried powder of AgNPs was mixed with dried KBr to prepare pellets and subjected to FT-IR spectroscopy measurement range set from 400 cm^{-1} to 4000 cm^{-1}. Fig. 10 shows the FT-IR spectra peaks viz. 511.69 cm^{-1}, 647.04cm^{-1}, 724.56cm^{-1}, 1040.76cm^{-1}, 1069.38cm^{-1}, 1145.64cm^{-1}, 1210.96cm^{-1}, 1368.24cm^{-1}, 1448.71cm^{-1}, 1550.32cm^{-1}, 1615.63cm^{-1}, 2851.30cm^{-1}, 2920.72cm^{-1}, 3418.20cm^{-1}, 3730.28cm^{-1} and 3839.83cm^{-1}.

Here, 3730.28cm^{-1}, 3839.83cm^{-1} indicate water-OH strong stretching. 3418.20cm^{-1} indicate N-H strong stretching of Amide group the functional groups confirmed the presence of Protein (Amino Acid) which is responsible for the bio- reduction of Ag^{+} to Ag^{0} ion. 2920.72 cm^{-1} indicates –C-H aldehydic strong and weak variable bond stretching, 2851.30 cm^{-1} indicate –C-H weak stretching bond, 1615.63 cm^{-1} indicate –C=O amide weak bond stretching, 1448.71 cm^{-1} and1550.32 cm^{-1} indicated C=C alkene weak bond stretching.

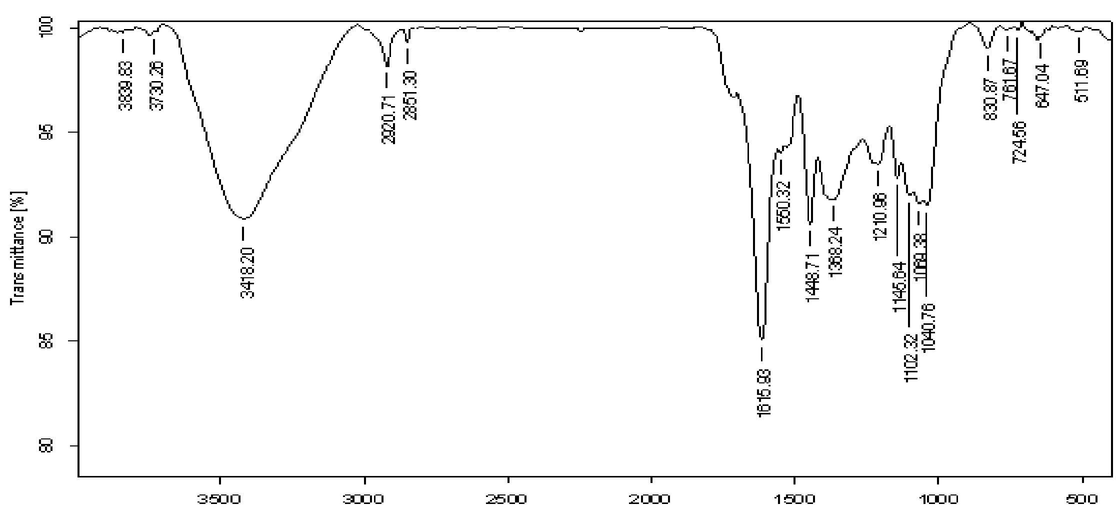


Fig.10. FTIR spectra of AgNPs

X-Ray Diffraction

X-ray Diffraction (XRD) measurement of the bio reduced silver Nanoparticles performed using Rigaku Mini flex 600 analytical X–ray diffractometer instrument at 200˚ C their obtained sharp diffraction patterns of the XRD spectra indicate that the nature of the AgNPs is pure crystalline. The fig.11 indicates3 peaks at 2θ values of 38.22, 44.26, 64.45 corresponding (hkl) values are (1,1,1) (2,0,0) (2,2,0) planes which confirm the presence of crystalline structure AgNPs. All diffraction peaks correspond to the characteristic Face Central Cubic (FCC) phase. The crystalline average particle size of the AgNPs calculated by Bragg's Law comes out to size= 0.1947 nm

Measurement profile

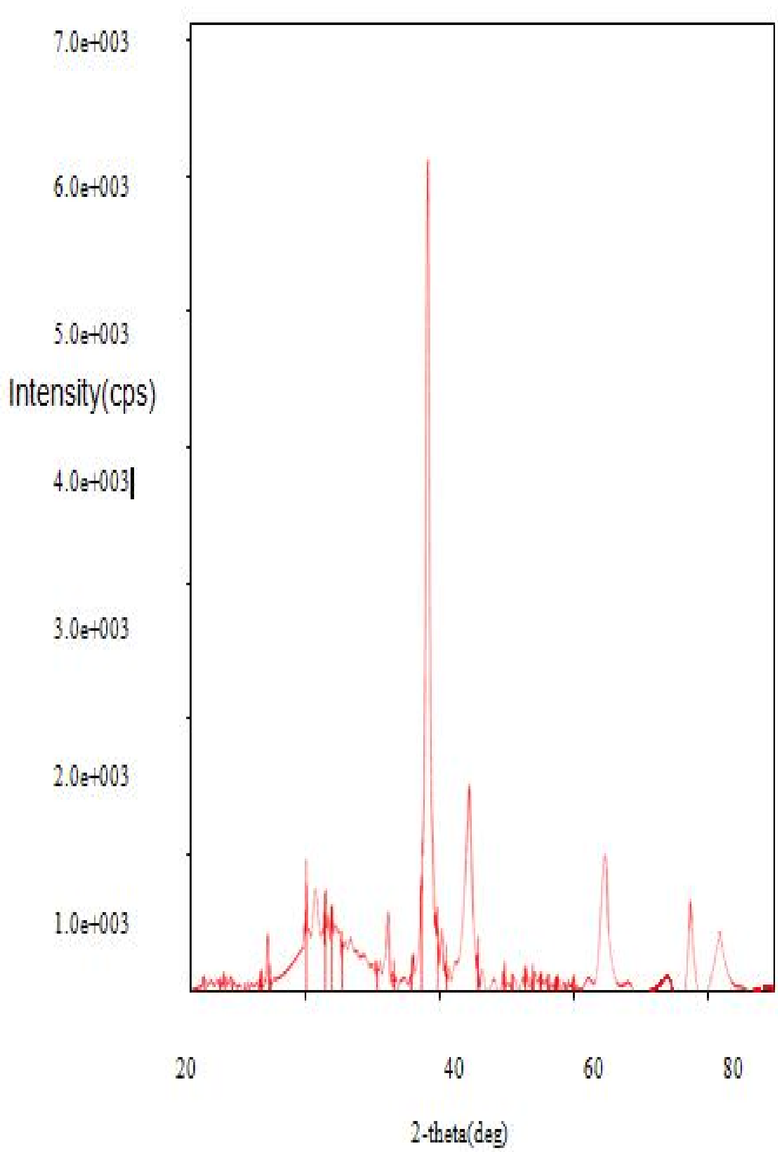


Fig.11. XRD scale of AgNPs

Scanning Electron Microscopy Analysis

Field Emission Gun - Scanning Electron Microscopic (FEG-SEM) analysis was done using JSM-7600F machine and operating on the voltage 0.1 to30kV and for operation need a very small amount of dry powder sample, and here we get types of images as shown in Fig-12.

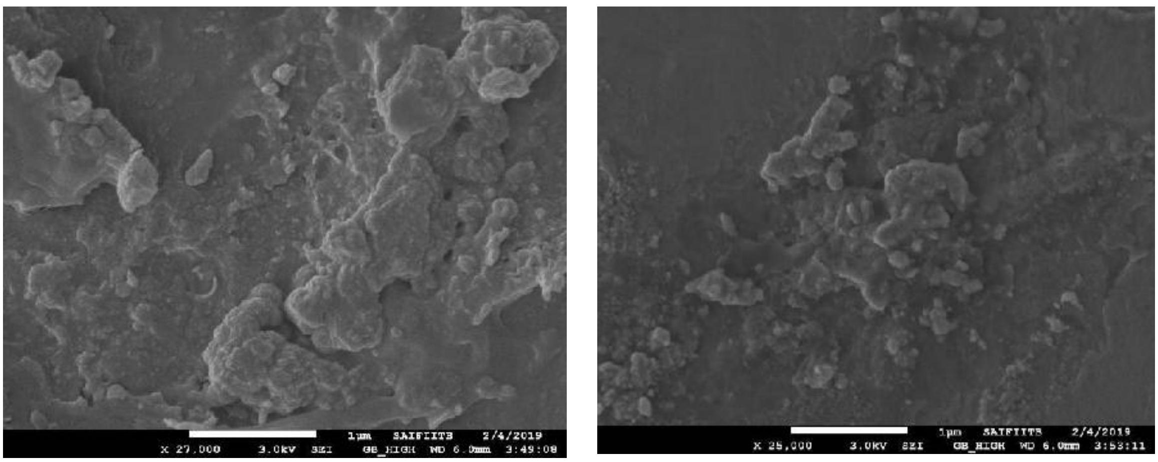


Fig. 12. FEG- SEM of AgNPs

The scanning electron microscope analysis was carried out to the shape and size of synthesized AgNPs. The obtained NPs were spherical in shape and size ranging from 0.2nm.

High Resolution Transmission Electron Microscopy (HR-TEM) Analysis

HR-TEM 200kV (Field Emission Gun–Transmission Electron Microscope 200kv) on AgNPs done by using JEOL (Model JEM 2100F) The image of AgNPs synthesized using an aqueous gall extract of Ziziphus jujube shown in fig.13 the synthesized AgNPs were almost spherical in shape with a preponderance of average diameter of 0.2 nm.

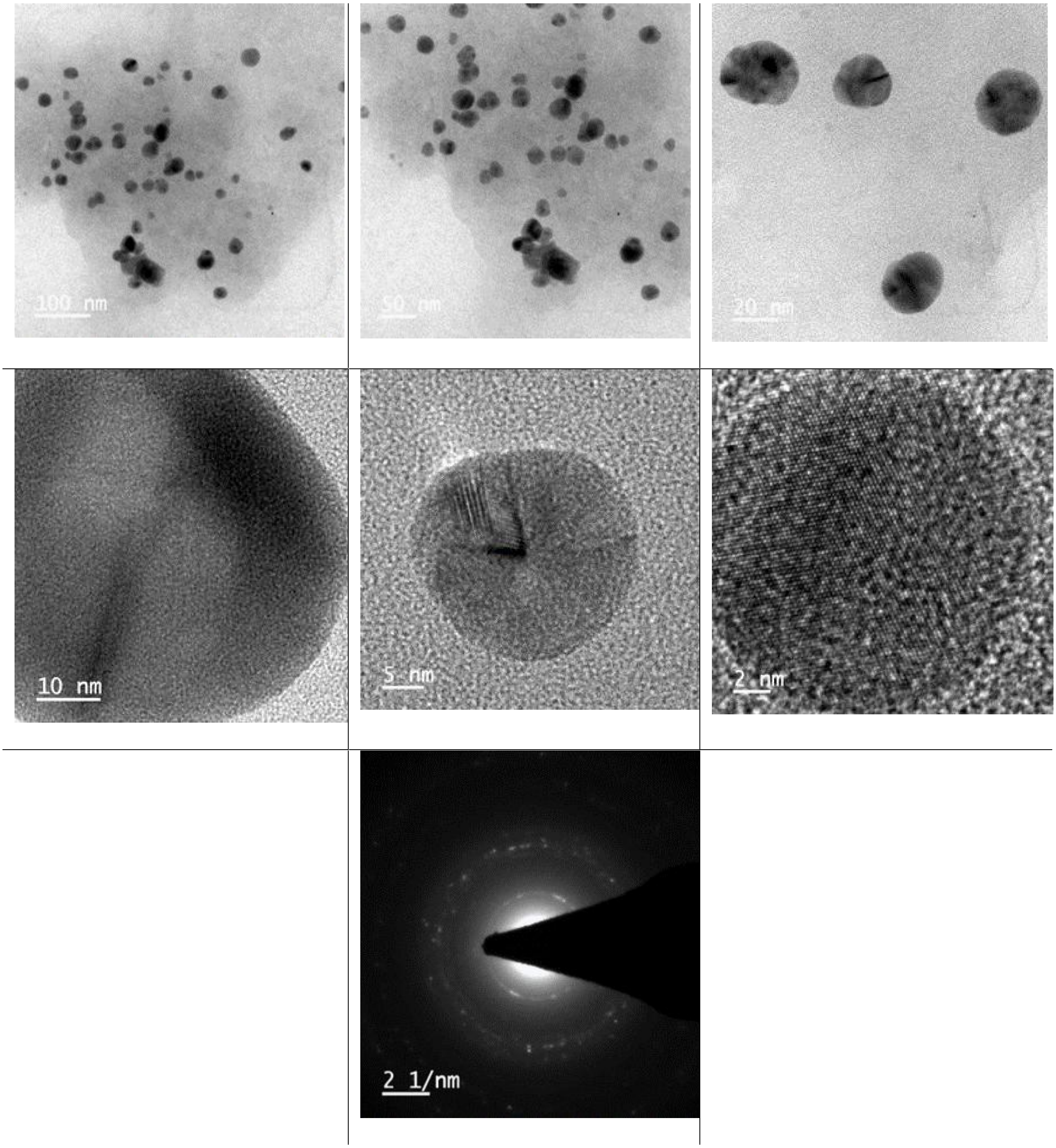


Fig.13 HR-TEM of AgNPs

Antimicrobial activity

Determination of antimicrobial activity:

Microorganisms:

The strains of bacteria used were *Staphylococcus aureus*, *Streptococcus pneumonia*, *Escherchia coli*, and *Salmonella typhi*, obtained from laboratory cultures. These are common human pathogens and hence were used to study the antibacterial activity. The nutrient broth, nutrient agar was used growing the test bacterial strains and were maintained on corresponding agar slants at 4°C.

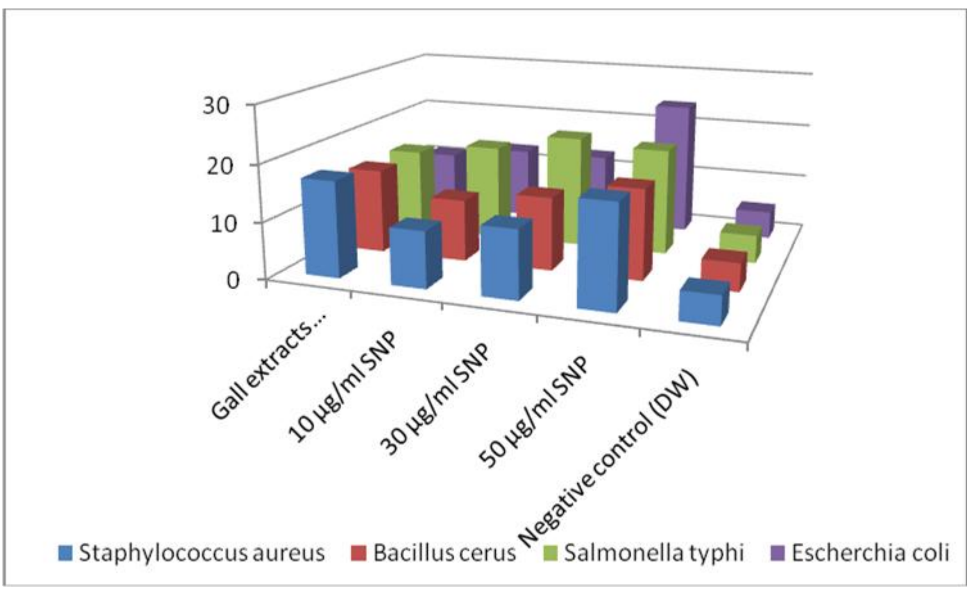
Preparation of inoculums:

The bacterial pathogens were inoculated into sterile nutrient broth and incubated at 37°C for 24 hours until the culture attained a turbidity of 0.5 McFarland units. The final inoculum was standardized to 105 CFU/ml by diluting fresh cultures with sterile distilled water. Colonies were suspended in 5 ml of sterile 0.85% saline. The resulting suspension was agitated and the turbidity was adjusted to yield 2×106 0.5 McFarland standards).E cells/ml (Antibacterial activity: - Antibacterial activity of AgNPs was determined by the agar disc diffusion method (Balouiri et al. 2016,Harathi et al. 2017)

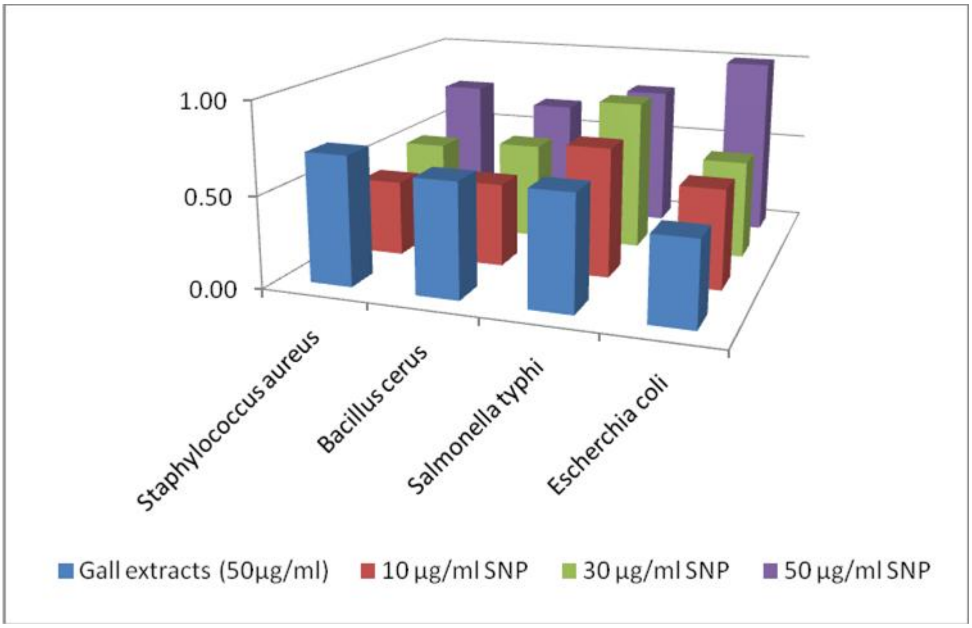
Plates of Nutrient agar were evenly streaked across the complete surface throughout the Petri plates so as to get a lawn growth of the inoculums with the help of spread plate technique with a known volume of 0.01 ml of active young culture with approx. (Qais et al. 2019)

Microbial count as 105 CFU/ml. Sterile filter paper discs (5 mm diameter) were immersed in the 50 µl of synthesized AgNPs (10, 20, 30, 40, 50 µg/ml) and allowed to dry at room temperature and it was placed over the Nutrient agar plates. Streptomycin 10 mcg/disc was used as positive control and the disc immersed in distilled water was used as negative control. (Hashemi et al. 2020)

The plates were incubated overnight at 37 ºC and the zone of inhibition around each disc was measured. Experiments were done in triplicate and mean values of zone diameter were taken.



Graph-1: Anti-Bacterial Activity of formed SNPs using gall extracts



Graph-2: - Activity Index of the SNPs at various concentrations

Note: Zone of inhibition by streptomycin as a standard drug = 24 mm (Mean Value)

$$Activity\ index\ (A.I.) = \frac{Mean\ of\ Zone\ of\ inhibition\ by\ SNPs}{Zone\ of\ inhibition\ obtained\ for\ standard\ Antibiotic\ Drug}$$

A safe easy method for eco-friendly synthesis of silver nanoparticles using aqueous gall extract of *Zizipus jujube* as a reducing agent, under the laboratory condition has been used without the use of poisonous inducers or hazardous chemical additives and/or elaborate chemical reactions. The formation of AgNPs was detected visually from a colour change and confirmed using UV-visible spectroscopy (410 nm), Fig.9c, further authenticated by SEM, Fig. 12, and also EDX, Fig.11, of AgNPs. Also using FT-IR Spectroscopy the characteristics of AgNPs was confirmed, Fig.10, FTIR studies show peaks at 1634.4, 2933.7 and 3295.4 wave numbers confirming the formation of AgNPs. Size was determined c.a. as 20 nm. this was further avowed from the TEM studies moreover the almost spherical shape of the particles was known using transmission electron microscopy, Fig.13. The silver nanoparticles exhibited more antibacterial activity than gall extracts alone, Graph-1 and 2. The effects were more pronounced on Gram-negative bacteria Salmonella typhi and Escherichia coli. The nanoparticles also showed prominent activity on Gram-positive bacteria *Staphylococcus aureus* and *Bacillus cerus*.

Conclusion

In this study design and development of biologically active Nano material as potent antimicrobial agents, was carried out using aqueous extracts from insect galls of *Ziziphus jujube*, which provides eco-friendly process. The benefit to use this method is that it is less time consuming, from simple materials obtained from nature and relatively easy way to the synthesis of Silver Nanoparticles. The Silver Nano Particles (AgNPs) were characterized by UV–Visible Spectroscopy, FTIR, XRD, SEM, TEM and their in vitro anti-microbial activity was investigated. UV – Visible Spectroscopy result confirmed formation of particles. The change in colour of the solution from dark red to dark Brown indicated reduction of Ag^{+} to Ag^{0}. FTIR analysis revealing bends of stretching and vibration confirmed various bonds present in the sample. The particle size of the Silver Nano particles is 0.1947 nm, which was confirmed by XRD and FEG - SEM analysis. Spherical in shape and size in the range 0.14 to 0.23 nm of AgNPs was confirmed by HR-TEM analysis and all are consistent.

Experimental results thus suggest that AgNPs which have been tested in the present study for their antimicrobial activity are in tune with other literatures. Thus, the present study does seem to justify the use of AgNPs for the treatment of infectious diseases our information recommends that silver nanoparticles can be effectively applied as bactericidal agent.

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