



Characterization, Anti-Bacterial, Anti-Cancer and Anti- Inflammatory Activity of the Green Synthesized Silver Nano Particles from Grewia Oxyphilla Fruit Extract

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ABSTRACT:

The synthesis of silver nanoparticles and their potential applications play a vital role in several fields in the advanced world. There are many technologies applied for the synthesis of silver nanoparticles. Some of them are electrochemical method, sonochemical method, sol-gel method, thermal decomposition method, mechanical milling method, and laser ablation method. Apart from the above methods, the green synthesis method is trending now because its applications are performing a significant role in this contemporary world. The availability of the key starting material is more and more easy, low-cost, high-importance, and less toxic to the environment. These nanoparticles are further characterized by using the following instruments: UV-visible absorption spectrophotometer, XRD, FT-IR, FESEM, and EDAX. It performed the good result against the antibacterial activity of gram-negative (*E. coli*) bacteria and gram-positive (*S. aureus*) bacteria and anti-cancer activity against MCF-7 breast cancer cells. The antioxidant activity is carried out with DPPH as the standard, and the performance of the silver nanoparticles was good in antioxidant activity. The anti-inflammatory activity of the synthesized silver nanoparticles was tested against BSA (bovine serum albumin). In this test, the denaturation of the protein by the effect of the synthesized silver nanoparticle was almost similar to the effect of acetylsalicylic acid. In this test acetylsalicylic acid was used as a standard.

1. Introduction

Nanotechnology is one of the most emerging areas of modern science, and green synthesis offers a sustainable alternative to physical and chemical routes [1–3]. Plants containing flavonoids, terpenoids, polyphenols, and tannins act as natural reducing and stabilizing agents in nanoparticle synthesis [2,5]. The synthesized AgNPs exhibit strong optical, structural, and biological properties, including antimicrobial and anticancer activities [3–5]. Hence, it is valuable when compared with other methods of synthesis of nanoparticles. The green synthesis method is more valuable because the plant sources containing the natural phytochemicals like flavonoids, terpinoids, polyphenols, and tannins are involved as the reducing and capping agents in the formation of silver nanoparticles. In this reduction, the Ag⁺ ion is reduced to Ag⁰ when treated with 0.01M silver nitrate solution. The colour of the solution was in dark brown and it was allowed to tested by UV-Visible absorption spectroscopy, the absorption peak was

appeared at 430 nm region, from this test the range for silver Nanoparticles are (400-500) nm further XRD for particle size, FTIR for the identification of the functional groups, SEM instruments revealed that the morphology of the agglomerated Nanoparticles from the colloidal phase and EDAX spectrum were used for the investigation of other elements present in the same sample. Then another important characterization method was applied for the identification of the size, shapes, and interplanar distance in the lattice site at its atomic level. Such nanoparticles were clearly determined by the HRTEM (High Resolution Transmission Electron Microscope) instrument. The biological applications of the synthesized silver nanoparticles were absolutely proven as excellent when treated against the pathogenic bacteria. Gram-negative and gram-positive bacteria, and the AgNPs perform impressively against the MCF-7 cancer cells and it performed well in antioxidant activity and anti-inflammatory activity when treated in the denaturation of the protein (BSA), so there are so many



applications for *Grewia Oxyphylla* fruit extract when preparing the silver nanoparticles with silver nitrate. It is environmentally safe and eco-friendly. By using such a method of synthesis of silver nanoparticles from *Grewia oxyphylla*'s fruit extract, it is much better than the chemical route. *Grewia oxyphylla* (**Fig. 1**) is an Australian species of flowering plant in the mallow family. The common name for this plant is "plum blush.

It is a shrub-type plant that grows in hill areas, in the bed of lakes and streams and in farmlands as well as in tropical rain forests, and it will grow almost 3 - 4 meters high and the diameter of the stem is recorded as about 5 cm; expanded rays are visible in the bark. It has dark green leaves. The blades of the leaves are almost 6.0 to 14 x 2.1 to 7.7 cm, and their petioles are about 0.6 to 1.2 cm long, with small branches and small flowers.

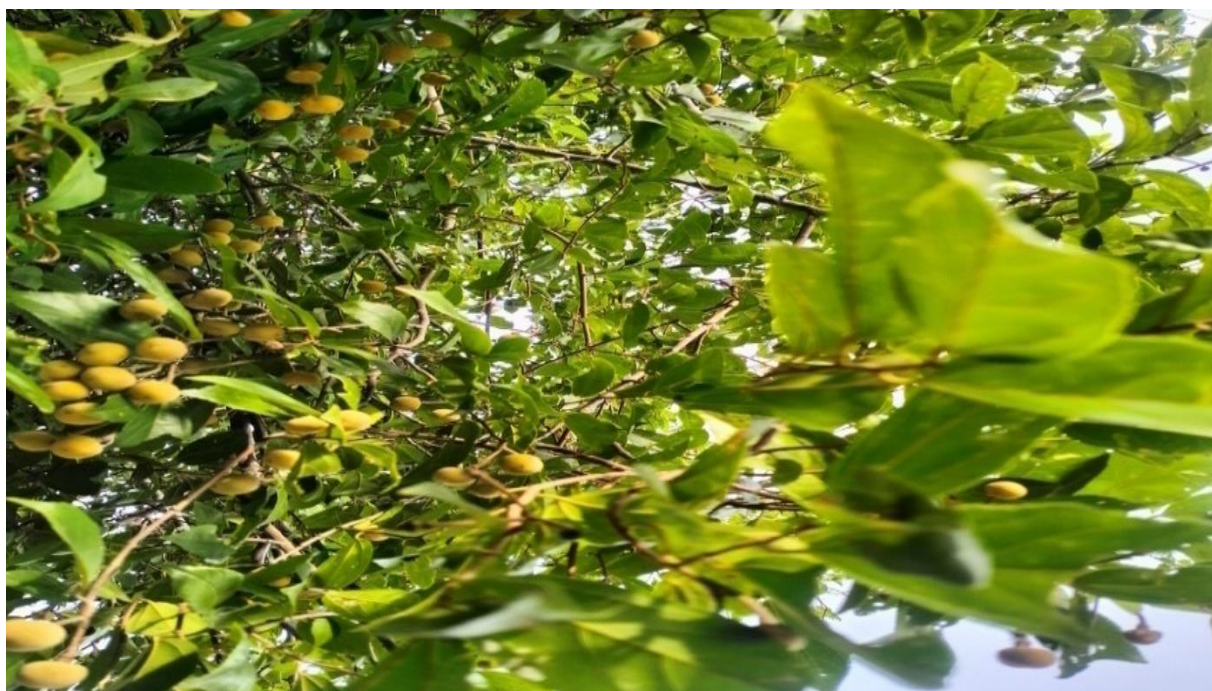


Fig.1. Plant and its fruit image

The petals of the flowers are about 6-7 mm long, and the fruits are about 10-18 x 6-12 mm in size. The seeds are very small, and its fruit extract was jelly-like while chopping with water. It loses the leaves in the autumn season. It is used as food for goats, sheep, squirrels, sparrows, and other animals and birds. In rural areas this plant is used for the treatment of bone inflammation in bone joints.

2. Materials and Methods

AgNO₃ was purchased from Merck Chemicals (99.8% assay), and the fruit has been collected from the Vilangadu forest. Double distilled water, a mortar with pestle, a funnel, a Whatman 40 filter sheet, a mechanical stirrer and magnetic pellet, a thermometer, as well as an oil bath.

2.1. Preparation of Nanoparticles:

The collected *Grewia oxyphylla* fruit was rinsed with tap water and drained well, and it was allowed to dry in a shaded area at 32°C till the tap water traces were completely removed from the fruits. After the drying, the seeds were removed from the fruits, and then 30 grams of fruit pulps were collected and weighed by using the digital weighing balance. It was transferred into the 300 ml beaker after the transfer of the plum [6]. Then 100 ml of double distilled water was added into the same beaker. The pulp has been chopped well until the plum was completely decomposed into the water. After the completion of decomposition, again 200 ml of deionized water has been poured into the beaker until the 300 ml mark. Then it was heated for 20 to 30 minutes to reach 50°C, and it was allowed to cool till it reached room temperature, and it was filtered using Wattman 40 filter



paper, and then the filtrate was collected into the clean and dry equipment. The schematic procedure is given in Fig. 2.

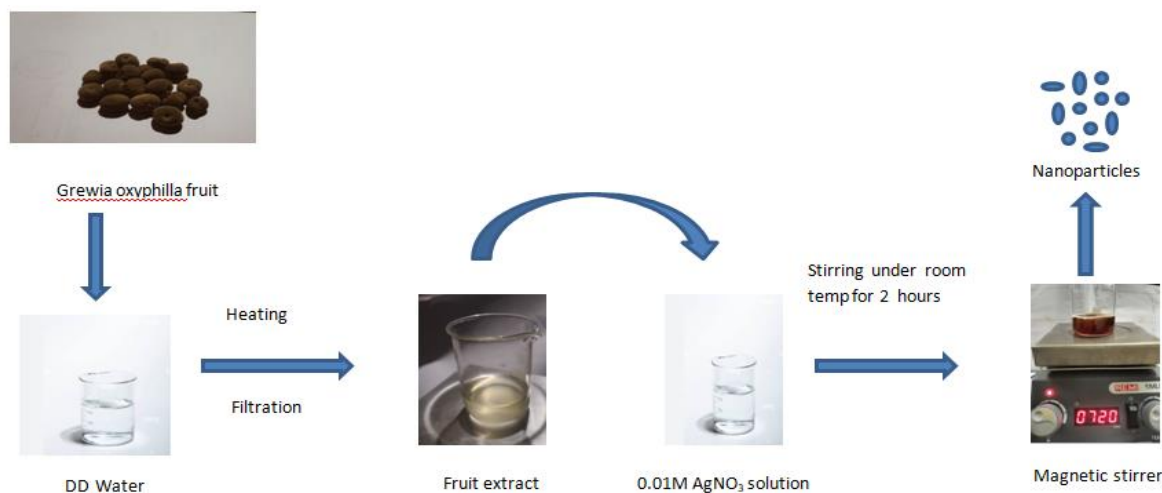


Fig.2. Synthesis of silver Nanoparticles from fruit extract with metal precursor (AgNO_3) 0.01M concentration

From the extract, only 40 ml of the extract was taken by using the measuring cylinder, and it was transferred into the beaker, and then 20.0 ml of 0.01 M concentration of AgNO_3 solution was taken into the burette, and the AgNO_3 solution was added into the fruit extract continuously in a dropwise manner with constant stirring by using the mechanical stirrer. In this agitation, the colorless jelly solution slightly changes into phenolic red; after the continuous stirring, the color changes to blood red and gradually changes to dark brown. The brown solution was considered as silver nanoparticles. The synthesized silver nanoparticles were further characterized by several analytical instruments.

2.2. Characterization:

The above nanoparticles were characterized by using the following instruments: UV-visible absorption spectrophotometer (UH5300) HITACHI. Then the functional groups were identified by using FT-IR (Fourier Transform Infrared Spectrophotometer) JASCO 1033, the XRD studies, and data were obtained from the Institute of Sathya Bhama Institute of Technology, and then the FE-SEM data were obtained from ZEISS SIGMA 300 in Sathya Bhama Institute of Science and Technology and HRTEM instrument (Talos F200S G2 HRSTEM made by Thermo-Fisher Scientific) from the Sathya Bhama Institute of Engineering and Technology.

3. Results and Discussion

3.1. UV-Visible Absorption Spectroscopy

The ultraviolet and visible absorption spectroscopic technique is one of the essential methods initially used to identify the presence of Ag NPs. In general, the range for the presence of silver nanoparticles in UV is about 400-500 nm.

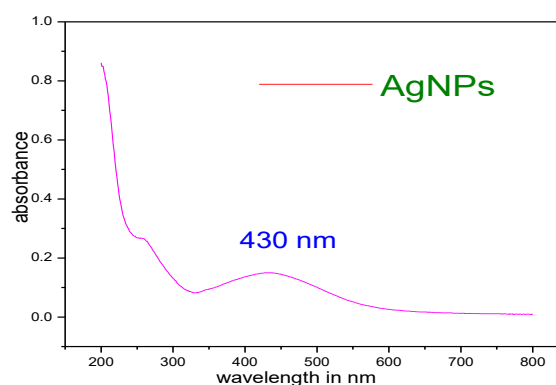


Fig. 3. UV-Vis spectrum for synthesized AgNPs from G.O fruit extract

In the synthesis of Ag NPs by using the Grewia Oxyphilla fruit extract results at 430 nm (Fig. 3), showing the presence of Ag NPs in UV-Visible absorption spectra, the result is visible through the color change, and the absorption peaks were important to explain the presence of silver nanoparticles by using the



UV-Visible absorption spectra. It occurs due to the effect of surface plasmon resonance.

3.2. XRD Analysis

It is an important technique to evaluate the crystalline nature of the synthesized silver nanoparticles from the fruit extract of the *Grewia oxiphilla* plant. The XRD pattern as shown in **Fig. 4**, displayed the five distinctive sharp diffraction peaks [7] of the 2θ values of 38.23, 44.27, 64.47, 77.30, and 81.46, corresponding to the values of (111), (200), (220), (311), and (222). The sharp peaks show the (FCC) structure in silver nanoparticles, in which the plane at (111) is highly intense; other than the above concept, the particle is confirmed as crystalline in nature.

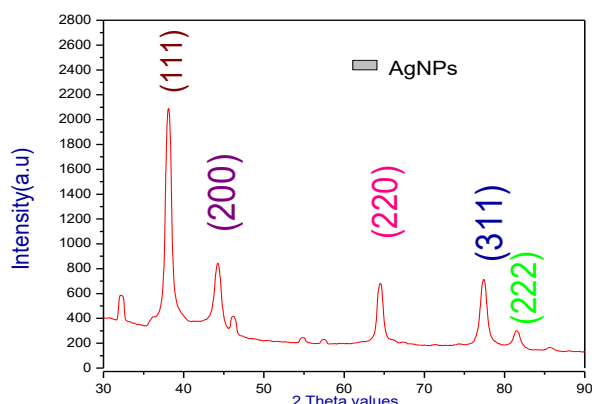


Fig. 4. XRD graph for synthesized AgNPs from *Grewia Oxyphilla* fruit extract

3.3. FT-IR Spectra

The FT-IR spectroscopic technique is an important one for the identification of the functional groups present in the fruit extract. In general, the natural sources also contain the phytochemicals like carotenoids, flavonoids, terpenoids, tannins, polyphenols, phenolic compounds, etc. In **Fig. 5 (a)**, the peak at 611 cm^{-1} represented the presence of C-H bending vibration [8]. The peak at 1634 cm^{-1} in the FT-IR spectra shown in **Fig. 5 (b)** of the extract of the *Grewia Oxyphilla* fruit represented the stretching frequency of C=C and C=O, and the peak at 2104 cm^{-1} represented the presence of amine group in the fruit extract. In this study, the broad band appears at 3330 cm^{-1} , representing the presence of phenolic compound, and then the presence of phenolic compound has been confirmed.

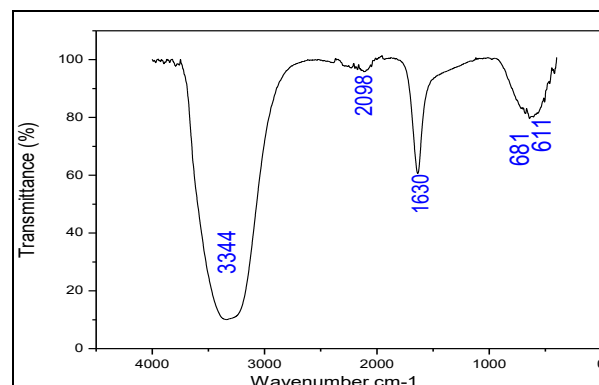


Fig. 5 (a). FTIR spectrum for G.O fruit extract

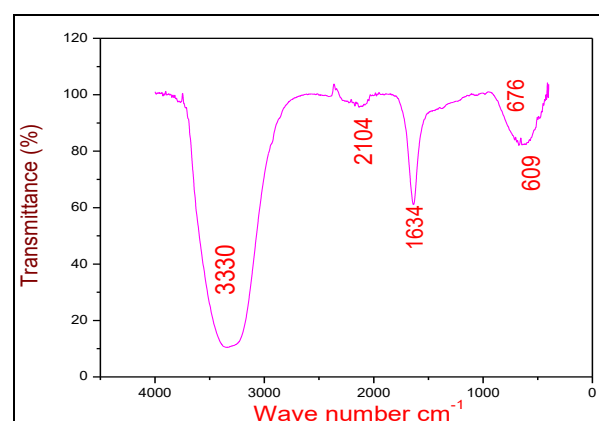


Fig. 5 (b). FTIR spectrum for AgNPs from *G.O* fruit extract

The phenolic compound is acting as the reducing and capping agent in the synthesis of silver nanoparticles, in which the Ag^+ is converted into Ag^0 nanoparticles by the effect of phenolic compound from the metal precursor.

3.4. FESEM Analysis

The field emission scanning electron microscopic technique [9] is an effective one to show the image of the Ag NPs. In which, time and temperature are considered as important parameters, it helps to determine the shape of the synthesized Ag NPs. It is temperature independent; the synthesized silver nanoparticles (**Fig. 6**) are spherically symmetrical. This instrument is an efficient and advanced one in this trending world that identifies the morphology of the synthesized silver nanoparticles, and it clearly displayed the agglomerated nanoparticles from the bulk colloidal phase.

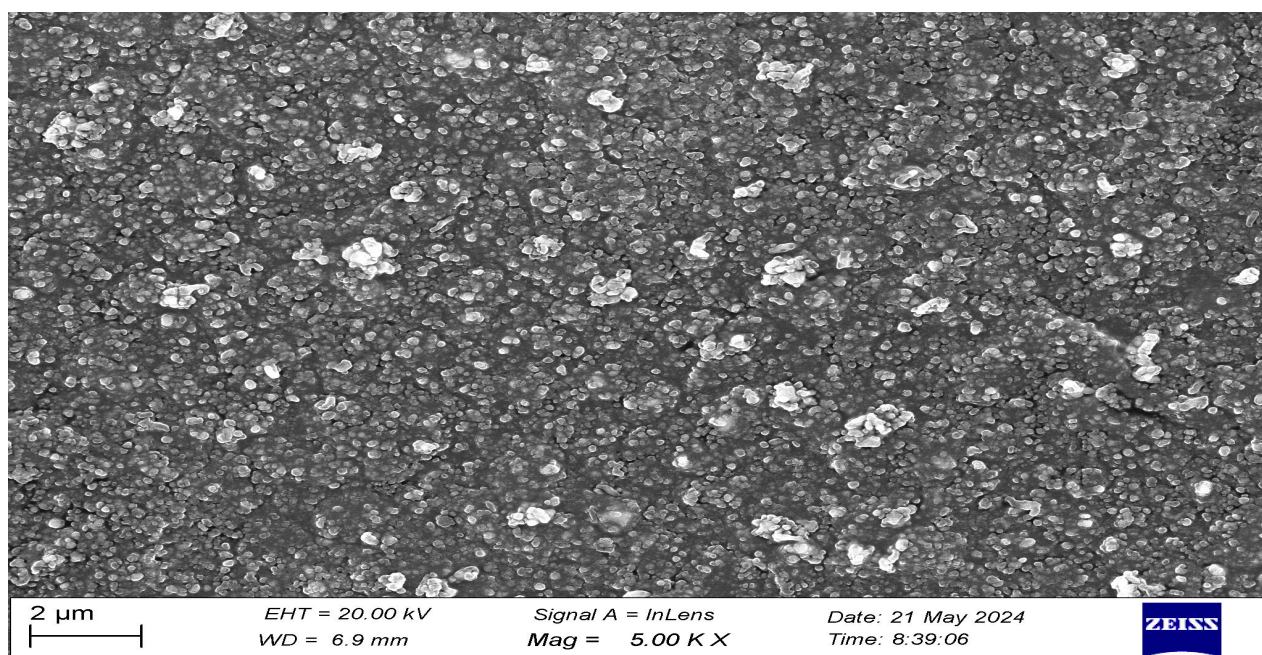


Fig. 6. FESEM- Image For Synthesized Silver Nanoparticles From G.O fruit extract

5.5. EDAX Spectrum:

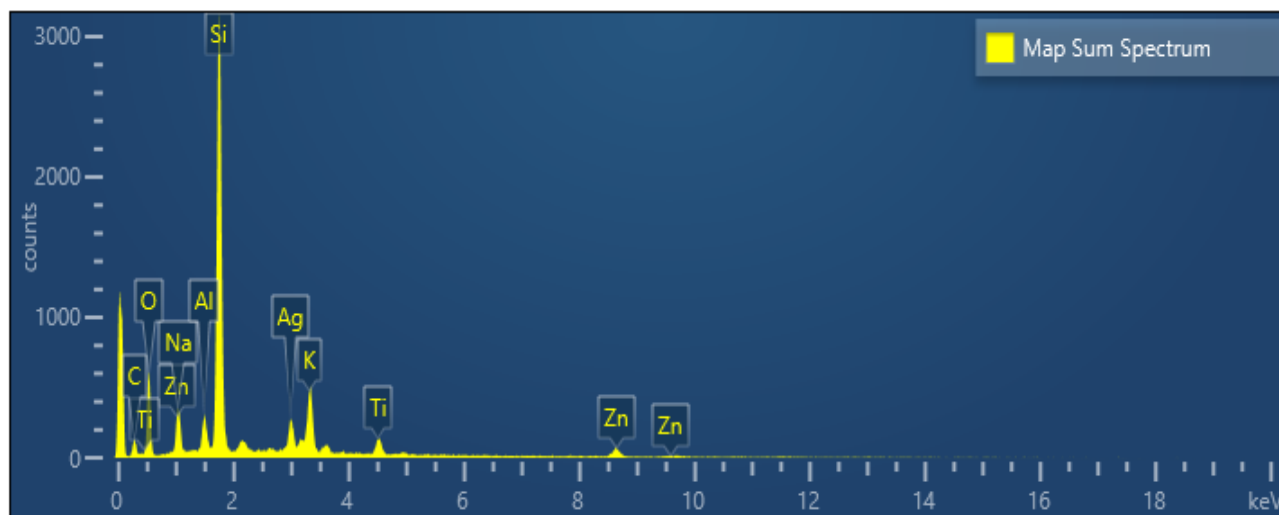


Fig.7- EDAX spectrum

This spectrum is used to identify the elements present in the synthesized silver nanoparticles from *Grewia oxiphilla* fruit extract. From Fig. 7, spectrum shows various signals for different metals like Ti, Si, Zn, Na, and Al were present in the fruit extract, among which this spectrum gives the strong signal for silver nanoparticles at 3.2 keV.

3.6. HRTEM (High Resolution Transmission Electron Microscopy)

High-resolution transmission electron microscopy [10] is an advanced method for the characterization of synthesized silver nanoparticles; this method was mostly applied for the identification of the size and shapes of the nanoparticles. This method is used to detect the size of the particle at its atom level, and the size of an atoms is



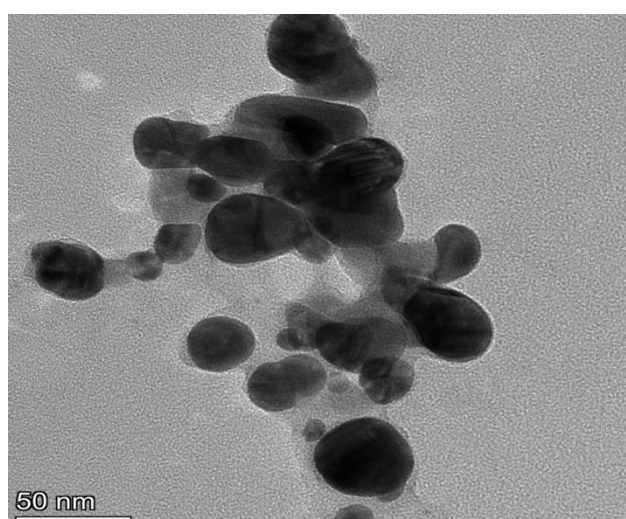
in the range of 10^{-12} m (or) (picometer); hence, the HRTEM is applied to perform exactly against the nanoparticles. Apart from that, it has given the image of arrangements of atoms in the lattice plane as well as crucial insight into the structure of the atoms, and its properties were explained with corresponding images of the silver nanoparticles. The shapes of the particles were spherical among the colloidal phase. So such methods of characterization were comfortable to show the image of nanoparticles; similarly, the size and shapes of the

synthesized silver nanoparticles in the colloidal phase were stable for a long time, which means the synthesized silver nanoparticles were in spherical shape.

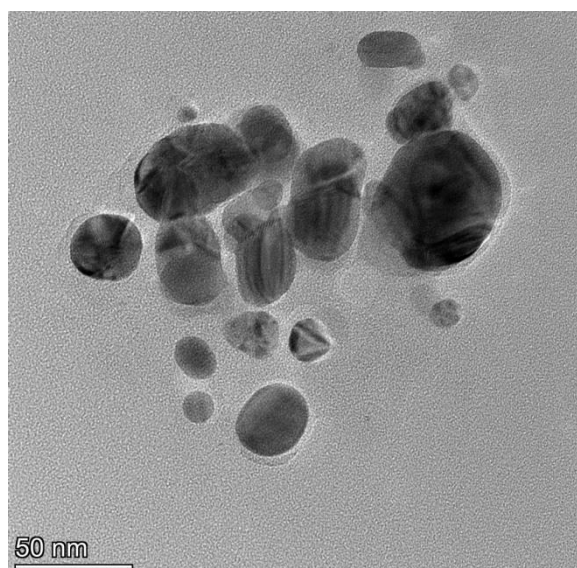
The important concepts are involved in the HRTEM instruments, like that in the generation of high-resolution image obtained due to the interference of electron waves passing through the sample only, which will create an image.



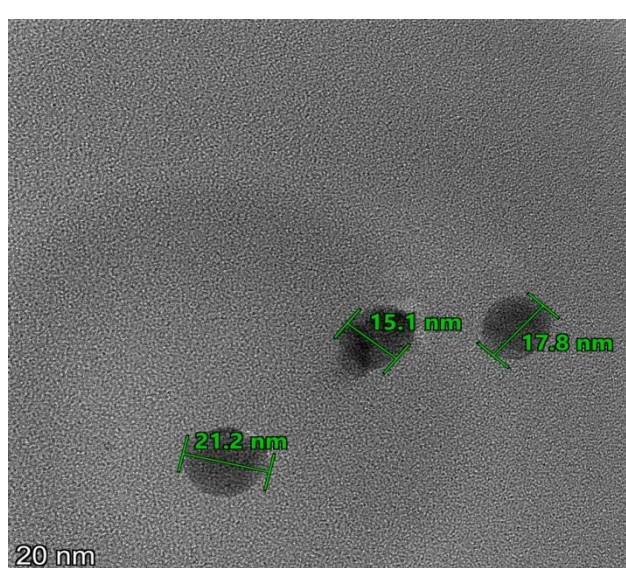
Slide (A) shows the 100 nm size of the particles



Slide (B) shows the 50 nm size of the particles



Slide (C) shows the 50 nm size of the particles



Slide (D) shows the 20 nm size of the particles

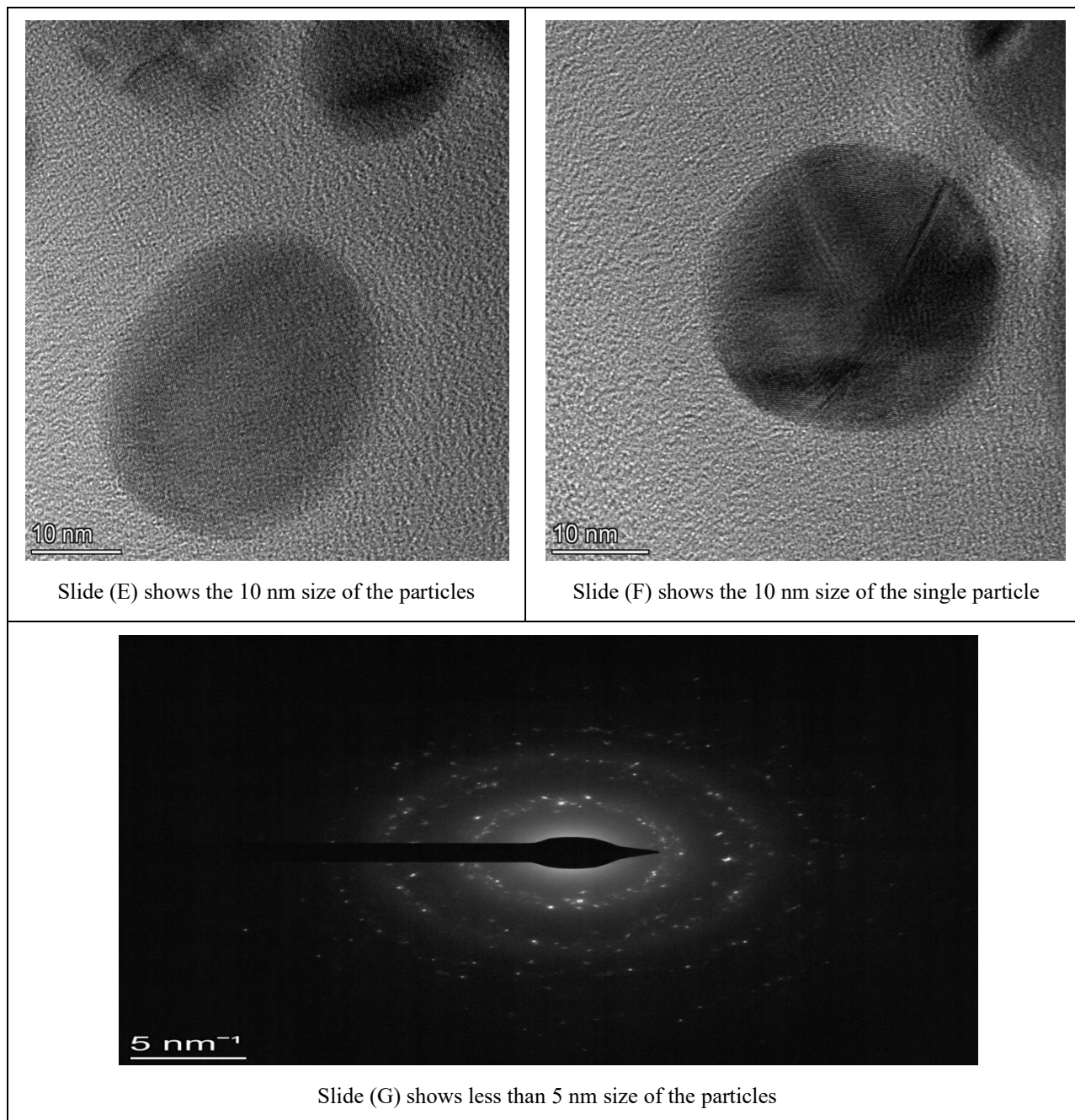


Fig.8. HRTEM images

The **Fig. 8** shows that the size of the particles are displayed. The smallest size of the nanoparticles was around 15.1 nm (Slide-D), and the largest size of the nanoparticles was around 21.2 nm; then the average size of the silver Nanoparticles in the colloidal mass were around 17.8 nm from the above data from the HRTEM instrument involved in the generation of the high-resolution images [11]. In depth, the slide (E, F) shows

the closed image of the nanoparticles, like 10 nm; from such images, the particles are almost determined as spheres.

3.7. Antibacterial Activity

Table-1 shows that the synthesized silver nanoparticles performed good antibacterial activity against gram-negative (*E. coli*) bacteria and gram-positive (*S. aureus*)



bacteria in which the agar well diffusion test method is applied to determine the performance of the silver nanoparticles against the above bacteria. The 50 μl of the synthesized silver nanoparticles is placed in the microbiological plate hole, which inhibits the growth of the (*E. coli*) bacteria around 15 mm in the plate;

similarly, for 100 μl , the inhibition zone is around 18 mm. The control sample-C is Gentamicin, which performed around 20 mm for 50 μL of the controlled sample that is known similarly for (*S. aureus*) bacteria. The corresponding plates are shown in **Fig.9**.

Table. 1. Antibacterial activity of synthesized silver Nanoparticles from G.O fruit extract testing report

Sl. No	PARAMETERS	TEST METHOD	UNIT	RESULT		
				a	b	c
1	Escherichia coli	Agar well diffusion method-antimicrobial activity	mm	15	18	20
2	Staphylococcus aureus	Agar well diffusion method-antimicrobial activity	mm	5	7	18

Note: a—50 μl , b—100 μl silver nanoparticles, and c—50 μl controlled sample gentamicin.



Slide (a) for Gram negative bacteria (*e.coli*) slide



(b) for Gram positive bacteria (*s.aureus*)

Fig.9. Plates for antibacterial activity

The concentration of 50 μl of the silver nanoparticles inhibits around 5 mm, and 100 μl of the sample inhibits around 7 mm. For gentamicin, it is around 18 mm. From this report, the synthesized silver nanoparticles [12] from *Grewia oxyphilla* fruit extract have the ultimate effect against gram-negative and gram-positive bacteria.

3.8. Anti-Cancer Activity

The synthesized silver nanoparticles were applied for the internal therapy of MCF-7 carcinogenic cells, in which the cell line have obtained from the Pune National Centre for Cell Science (NCCS). The cells were placed in Dulbecco's Modified Eagle Medium (DMEM) as well as 10% fasting blood sugar, penicillin (100 U/ml), and streptomycin (100 $\mu\text{g}/\text{ml}$) in a humidified environment

with 5% CO_2 at 37°C. Cells (1×10^5) per well were placed in 24-well plates and allowed to incubate at 37°C with 5% of carbon dioxide. After the cell reached the conflux, the samples were added gradually in different concentrations, and the brooding process continued for 24 hrs. After the brooding, the samples were taken out from the well and cleaned with phosphate-buffered saline (pH 7.4) or DMEM without serum. 100 μl per well (5 mg/ml) of 0.5% 3-(4, 5-dimethyl-2-thiazolyl)-2,5-diphenyl-tetrazolium bromide (MTT) was added in the well, and brooding was continued for 240 min. After brooding, 1.1 ml of dimethyl sulfoxide ($(\text{CH}_3)_2\text{S}=\text{O}$) was added in all the wells. In vitro assay for anti-cancer activity: (MTT assay) in all the wells.



Table 2. Anti-cancer activity of the sample against the MCF-7 cell line Testing report for anticancer activity of synthesized silver nanoparticles.

Sl. No	Conc. ($\mu\text{g/ml}$)	Dilution	Absorbance	Cell viability (%)
1	1000	neat	0.178	18.03
2	500	1:1	0.251	25.43
3	250	1:2	0.339	34.34
4	125	1:4	0.413	41.84
5	62.5	1:8	0.497	50.35
6	31.2	1:16	0.578	58.56
7	15.6	1:32	0.648	65.65
8	7.8	1:64	0.721	73.04
9	Cell control	-	0.987	100

The appearance of the absorption peak obtained at 570 nm was measured [13] by using an ultraviolet spectrophotometer in dimethyl sulphoxide medium; in this DMSO was used as the blank. Measurements were performed, and the concentration required for the 50% inhibition (IC_{50}) and the values was determined by the graphical method.

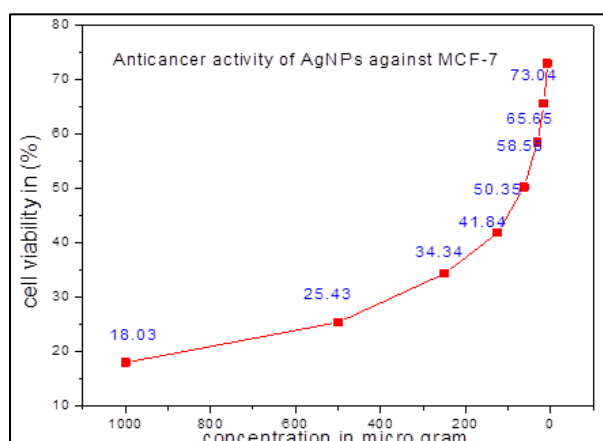


Fig.10. Anticancer activity of present AgNPs against MCF-7

The % cell feasibility was calculated as shown in **Table 2**, by using the subsequent factors. From the graphical values shown in **Fig.10**, we can explain the performance of the synthesized silver nanoparticles like, for the lower concentration, the cell viability is higher; for the higher concentration, the cell viability is low; for 62.5 $\mu\text{g/ml}$ concentrated, the cell viability is 50.35. From the data,

the anticancer activity of the synthesized silver nanoparticles is considered as good.

3. 9. Anti-Inflammatory Activity

The above activity of the certain known concentration of the green synthesized silver nanoparticles is mainly involved in protein denaturation, among which the denaturation of the protein with synthesized silver nanoparticles is compared with the standard drug acetylsalicylic acid. There are differences between these two when treated [11] in the protein denaturation process. From the above test, **Table 3** revealed the anti-inflammatory activity of the standard drug is almost similar to the newly synthesized silver nanoparticles that are given as follows: 78.83%, followed by Ag NPs at 78.45%. The anti-inflammatory activity of the green synthesized Ag NPs from *Grewia Oxyphilla* fruit extract was determined. The acetylsalicylic acid was used as the standard drug for this test. About 2 ml of the synthesized silver nanoparticles was added to 500 μl of BSA (1%). The mixture was allowed to incubate at 37 $^{\circ}\text{C}$. Then the whole mixture was heated to 51 $^{\circ}\text{C}$ for 20 min., which was introduced to cool for 33 $^{\circ}\text{C}$, and the blank sample's absorbance was checked at 660 nm. In which the acetylsalicylic acid was used for positive control and water was used as negative control.

**Table 3.** Testing report for Ag NPs against Anti-inflammatory activity

Sl. No	Conc.($\mu\text{g/ml}$)	Optical density in absorbance	% inhibition
1	20	0.235	35.08
2	40	0.201	44.47
3	60	0.160	55.80
4	80	0.116	67.95
5	100	0.078	78.45

4. Conclusion

The green synthesized Ag NPs from the *Grewia oxyphylla* fruit extract is very important, and it is environmentally safe and eco-friendly, as well as very cheap to consume from the natural source to the research field, so the researchers are mostly taking an interest in selecting this route nowadays. The physical observation is the pale yellow color solution is converted into dark brown, indicating the presence of silver nanoparticles. The above UV-visible absorption spectrum gives the peak at 430 nm. Like that, the way the Ag^+ is converted into Ag^0 is occurring due to the effect of the phytochemicals present in the fruit extract, in which the phytochemical is the phenolic compound, which is confirmed from the FT-IR spectral analysis. The XRD analysis has given more than four sharp peaks, which confirmed the particle nature is in an FCC-type crystal. The SEM analysis confirmed the synthesized silver nanoparticles are agglomerated to each other. The much better and highly validated technique is HRTEM (High Resolution Transmission Electron Microscopic technique) showed the electron image of the synthesized silver nanoparticles in the colloidal phase, and it was explained about the dispersion of the particles in the solution as well as the particles interplanar distance, and the insight structure in the lattice is also explained from HRTEM. From the spectral analysis, the particle is spherically symmetrical. The synthesized silver nanoparticles from *Grewia oxyphylla* fruit extract are much better at killing pathogenic bacteria like the gram-negative bacteria (*E. coli*) and gram-positive bacteria (*S. aureus*), and these nanoparticles are also good against the anticancer activity of the MCF7 cell line. The anti-inflammatory activity of the synthesized silver nanoparticles was much better in the denaturation of the

protein than that of the standard, acetylsalicylic acid, which was used as the standard.

Author Contributions:

M. Karthikeyan: writing original draft, visualization, validation, investigation, formal analysis, data curation; **R. Sureshkumar:** Writing, review & editing, Investigation; **K. Dinakaran:** Writing review & editing, resources, All authors have read and approved the manuscript.

Data Availability: The data obtained and analyzed during the current study are available from the corresponding author on reasonable request.

Declaration of Interest:

There are no actual or potential conflicts of interest, including any financial, personal, or other relationships with other people or organizations.

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