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ANTIBACTERIAL ACTIVITY OF SILVER NANOPARTICLES SYNTHESIZED VIA GREEN METHOD USING CARDAMOM SEED PEEL EXTRACT

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Abstract

In this novel study, rapid, simple and inexpensive methods are used to synthesis silver nanoparticles using aqueous Elachi seed peal extract. Aqueous solution of seed peal extract can acts as a capping and reducing agent. Synthesized silver nanoparticles are characterized by using various instrumentation techniques such as powder X-Ray Diffraction (XRD), Fourier Transform Infrared Spectrum (FT-IR), UV-Visible spectrum, Scanning Electron Microscope (SEM), Energy Dispersive X-Ray Analysis (EDAX), and Anti-Bacterial activity.XRD reveals the highly crystalline nature of the prepared nanoparticles and it shows FCC crystal structure. The functional groups present in the Ag-NP's were identified using FT-IR spectrum. In UV-Visible Ag absorbance peak may obtained at 440nm region. The surface morphology of the prepared silver nanoparticles was analyzed by using SEM image and their elemental analysis can be recorded using the EDAX spectrum. The antibacterial activity of the prepared nano particles was also identified using zone inhibition by disk diffusion method. Silver nanoparticles show antibacterial activity against Classmodium, Pseudomonas and Klebsilla. Results were confirmed that the prepared Ag nanoparticles as simple, low cost, non-toxic, eco-friendly, rapid and one step process.

Keywords: Nanosilver, Anti-bacterial Activity, EDAX, SEM, Powder XRD, UV-Visible Spectrophotometer, Elachi seed peal

1. Introduction

Metal nanoparticles are of various uses such as catalytic, electronics, biology and biomedical material science, physics and environmental remediation. Hence, there is a need for the development of cheap and eco-friendly nanomaterials, as nanoparticles synthesis is still on the high cost side. Consequently, the use of benign materials and biomass in the synthesis of nanoparticles is gaining a widespread acceptance. [1] Nano biotechnology is defined as a field that applies the Nano scale principle and techniques to understand and transform bio systems (living and non living) and it used biological principles and materials to create new devices and system integrated from the nanoscale. [2] In the synthesis and assembly strategies of nanoparticles and nanomaterials, precursors from liquids, solids or gas phase are used employing chemical and physical deposition approach etc. [3].

Biological method of synthesis involves the use of bacteria (Bacillus licheniformis and Bacillus subtilis), fungi (Fusarium oxysporu and Penicillium), enzymes and plant extracts [4]. Currently, sustainability initiatives that use green chemistry to improve and/or protect our global environment are focal issues in many fields of research. The development of cost efficient and ecologically being methods of synthesis of nanomaterials still remains a scientific challenge as metal nanoparticles are of use in various catalytic applications, via electronics, biology and biomedical applications, material science, physics, environmental remediation fields [5-12]. It is well known that the toxicity of nanomaterials essentially depends on the structural features such as size, shape, composition and the surface chemistry. To prolong the life span of metal nanoparticles it is vital to select stabilizing agents and pathways that are environment friendly, non toxic and easy to implement. [13]. Elachi is an odor seed available in our local market. It can be used for medicinal purpose. The current study focused on the synthesis of silver nanoparticles using the aqueous solution of seed extract at particular room temperature. This work will contribute in establishing the importance of plant sources and implanting green synthesis of nanoparticles for the future research. The preparation of silver nano particle have been already reported using several bio materials such as neem, [14], aloevera, [15] capsicum annum, [16] acacia concinna (shikakai seed), [17]. The use of plant biomass or extracts for the biosynthesis of novel metal nanoparticles (silver, gold, platinum, and palladium) would be more significant if the nanoparticles are synthesized extracellular and in a controlled manner according to their disparity of shape and size.[18]

The present investigation reports the synthesis of silver nanoparticles from elachi seed pee extract. The prepared silver nano particle is characterized using powder XRD, FT-IR, UV, SEM with EDAX. In addition to the above the bio medical activity of the prepared silver nano particle was identified using the anti microbial activity.

2. Material and methods

2.1. Materials

Silver nitrate was purchased from Sigma Aldrich chemicals. Elachi seeds were also purchased from local market. Pure form of gram positive bacteria's can collect from Dept of Biotechnology and Dept of Animal science. The antibacterial culture medium was maintained at Athmic Biotech Trivandrum.

2.2. Preparation of seed peal extract.

The collected seed peals were crushed in to small pieces then washed with double distilled water and then heat the crushed powder using stirrer at 70-80 degree Celsius for 30 minutes. Then the prepared sample was filtered using what man no.1 filter paper and the solution is stored for further purpose to synthesis the nanoparticles. Plant extracts contain much active compounds such as, polyphenols, flavonoids and sugars, which can act as reducing and stabilizing agents for the formation of CM NPs as general scheme illustrated in Fig. 5. Up to now, various types of plant extracted obtained from different parts of plant have been used to synthesize the CM NPs [19].

2.3. Synthesis of silver nanoparticles

0.1 M of silver nitrate salt is dissolved in 100 ml distilled water and stirrer the solution using magnetic stirrer. And then add 50 ml of prepared elachi seed peal extract drop by drop to the saturated silver nitrate solution. After, adding extract drop by drop color changes from greenish yellow to brownish black. This color changes confirms the presence of silver

nanoparticles. The pH of the sample is measured using pH metre and it was maintained at 8. Then the solution was centrifuged at 3000 rpm for 10 minutes. The filtered sample is placed in oven at 100 degree Celsius for removal of water content in the sample. The dried sample is powdered using mortar and pestle. Formation of silver nitrate to silver nanoparticles may take just 30 minutes at room temperature, without the use of any chemical components.

To synthesis of silver nano particles the precursor material as silver nitrate followed by 50 ml of elachi seed peal extracts. Thus by adding seed peal extract to the silver nitrate solution which finally gives the Ag nanoparticles. The use of plant systems has been considered a green route and a reliable method for the biosynthesis of nanoparticles owing to its environmental friendly nature. [18]

Green synthesis method is used in this present work. Green synthesis plays a vital role in biomedical application. Green synthesis method using plant and seed extract is much better when compared to other methods. Because plant and seed extract are low of cost and easily available when compared to proteins, DNA and other bio-molecules.

3. Characterization of silver nanoparticles.

3.1 UV- Visible spectrum analysis

The bio reductive synthesis of silver nanoparticles was monitored using a Shimadzu Elico-169 PC scanning double beam UV-Visible spectrophotometer. To confirm the reduction of silver Nano salts after visual observations, nanoparticles suspension was scanned by UV-Visible spectrophotometer in the range of 200-800 nm. Spectra were obtained from 200 µL of test volumes with a 1cm path length quartz cuvette. [20]

3.2 SEM –EDX (Scanning Electron Microscope with Energy Dispersive Spectra analysis)

Surface morphology investigation of synthesized silver nanoparticles was carried out with the help of Scanning Electron Microscope EVO18 (CARL ZEISS) and elemental analysis carried out using Energy Dispersive X-ray-Spectrometer Quantax 200 with X Flash® 6130.

3.3 XRD (X-Ray Diffraction spectrum analysis)

X-Ray Diffraction pattern was recorded by using PAN analytical X'PERT PRO diffractometer. XRD pattern are recorded from 20 to 80^{o}.

3.4 FT-IR (Fourier Transform Infrared Spectrum)

FT-IR spectrum of AgNP was recorded using Perkin Elmer RXI spectrometer in the region 400–4000 cm^{-1} with the samples in KBr pellets.

3.5 Antibacterial activity.

The antibacterial assay set up can done using disc diffusion method. The agar used was Muller Hinton agar that is rigorously tested for composition and pH the depth of the agar in the plate is a factor to be considered in the disc diffusion method. This method is well documented and standard zones of inhibition have been determined for susceptible and resistant values. There is also a zone of intermediate resistance indicating that some inhibition occurs using this antimicrobial but it may not be sufficient for inhibition to eradicate the organism from the body. The zone inhibition layer shows the anti-bacterial activity of the prepared silver nanoparticles.

4. Result and discussion

In this present novel study, the formation of silver nanoparticles by Elachi seed peal was investigated. From the investigation, the presence of silver nanoparticles was confirmed by color changes from light yellow to dark brown.

4.1 UV-Visible Spectroscopy

This method is the fast and simple technique to identify the silver Nano particles. Spectrum of the colloidal solution of silver nanoparticles was recorded from 800 – 140 nm region. The observed UV-Visible spectrum of the prepared silver nano particles is given in the Figure 1.

UV-Visible spectrum shows the absorption peak region of 440nm. It shows the excitation of surface Plasmon vibrations in silver nanoparticles. From UV spectrum peaks obtained around 283 nm – 440nm respectively. The broad absorption peaks indicates the presence of silver nanoparticles of different size. The Elachi seed peal extract might be actively involved and responsible for the reduction of Ag^{+} to Ag^{0}. UV-Visible spectrum shows silver absorption peaks in the region of 440 nm. The other peak observed in lower wavelength at 253 nm is attributed to the presence of aromatic amino acids of proteins [21]. The intensity and width of absorbance peaks depend upon the size and shape of the nanoparticles.

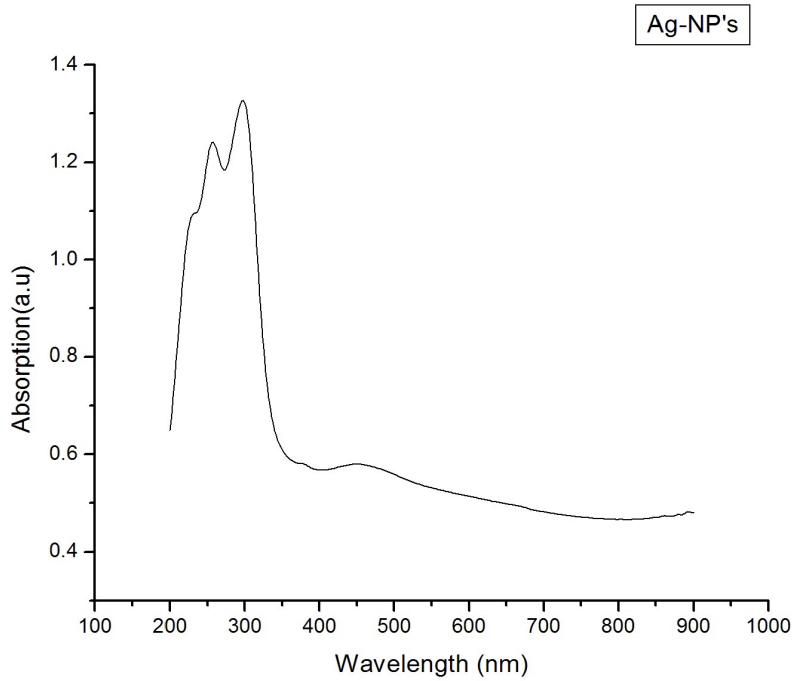

Figure 1 shows UV-Visible spectra of silver nanoparticles.

4.2 X-Ray Diffraction

Average grain size and the crystalline nature of the prepared Ag-Np's were recorded using powder XRD spectrum. The observed XRD spectrum of seed peal powder and pure silver nitrate salt is given in the figure 2 and 3 respectively. The XRD spectrum of prepared silver nano particle is shown in figure 4. The observed XRD pattern values of silver nano particle are given in Table 1. The XRD pattern (Figure 4) reveals the crystalline nature and the face centered cubic (FCC) structure of the synthesized Ag- nanoparticles.

The XRD peaks clearly indicate the prepared samples are crystalline in nature the observed peaks well matched with the JCPDS card. No: 87-0720. Prepared silver nanoparticles may have average grain size of about 27 nm respectively. Average grain size of investigated silver nanoparticles was calculated by using Debye- Scherer's formulae. Obtained XRD 2Ɵ values are 38º, 44º, 64º, and 77º .The obtained XRD patterns were coincide with the N.arvensis,

bombax cebica bark extract. [22, 23]. The observed intensity peaks (38º, 44º, 64º, and 77º) assigned with (1 1 1) (2 0 0) (2 2 0) (3 1 1) Bragg's plane. Which denote face centered cubic in structure. The comparative spectrum before and after calcinations of prepared Ag-NP's is shown in the figure 5. The observed XRD peak intensity and grain size calculation of the prepared silver nano particle are given in the table 2. It validates particle size become higher when temperature increases. The XRD pattern of seed extract, Silver nitrate and prepared silver nano particle are given in figure 2, 3 and 4 respectively.

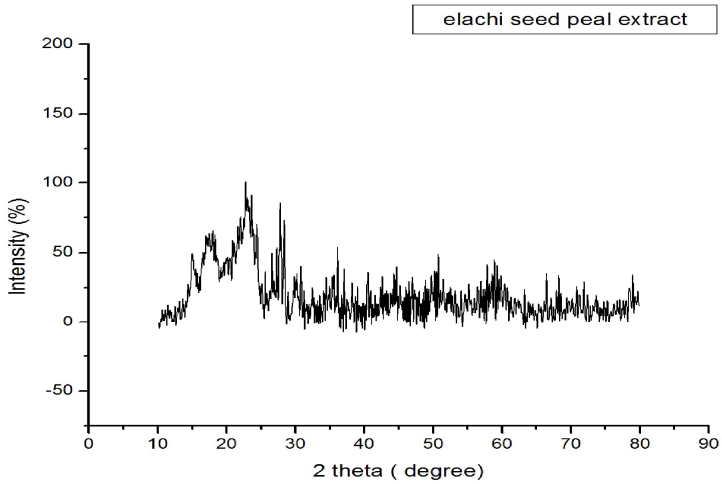

Figure 2 shows XRD pattern of elachi seed peel powder

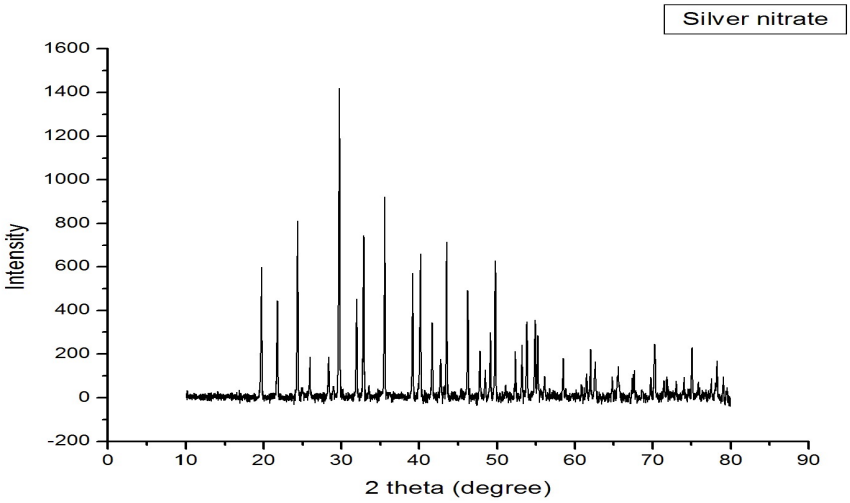

Figure 3 shows XRD pattern of Silver nitrate

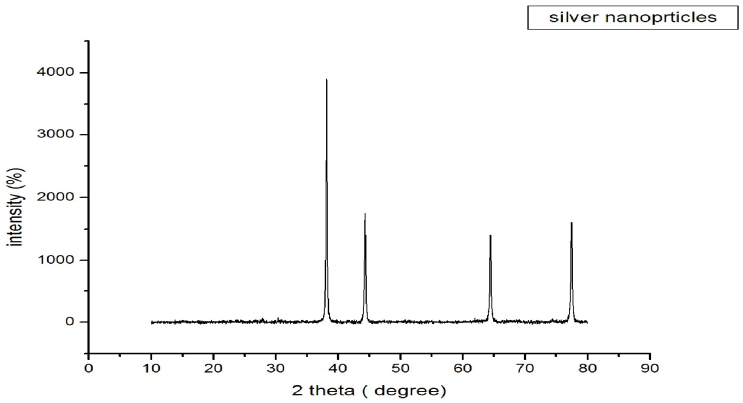

Figure 4 shows XRD pattern of Silver nanoparticles

Table.1 shows the XRD data of silver nanoparticles

2 Theta (degree)	Intensity (%)	d- spacing (A^0)	(h k l)
38.1234	3341	2.24801	111
44.3211	1522	1.44521	200
64.4256	1068	1.27436	220
77.3770	1146	1.23234	311

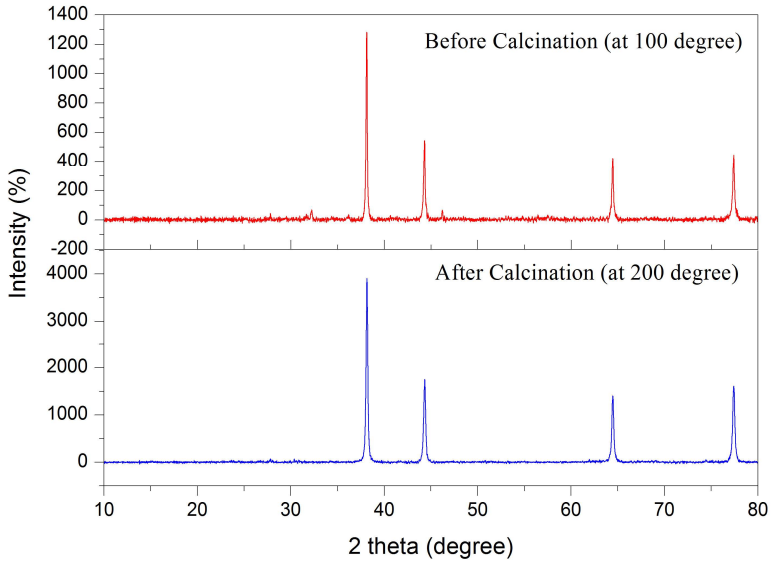

Figure 5 depicts the XRD patterns of silver nanoparticles before and after annealing

Table 2: The observed XRD peak intensity and their calculated grain size

2 Theta (degree)		Intensity	(%)	Grain size (D) nm	
100°C	200°C			At 100°C	At 200°C
38.1234	38.0956	100	100	0.04170	2.24801

44.3211	44.2798	45.56	42.38	1.88241	1.44521
64.4256	64.6578	31.99	28.07	2.93743	1.27436
77.3770	77.4109	34.31	30.56	2.96869	1.23234
			Mean	1.957	2.388

4.3 Fourier Transform Infrared Spectrum

FTIR analysis is used to detect the functional groups present in synthesized nanoparticles. The observed FT-IR spectrum of prepared silver nano particle is shown in the Figure 6. FT-IR spectra confirm the presence of Ag-O bonding.

FT-IR also predicts that the bimolecular compounds are responsible for the reduction and capping of silver nanoparticles. From FT-IR analysis it is clear that the immediate reduction and capping of silver ions into silver nanoparticles in the present analysis might be due to flavanoids and proteins. The flavanoids present in the peal extracts are powerful reducing agent.[18] Which may be suggestive for the formation of Ag-NP's by reduction of silver nitrate. The observed IR bands and their corresponding assignment of the silver nano particle are given in the Table3. The observed band at 3296 cm^{-1} is assigned to O-H stretching vibration [23,33]. The increase in O-H stretching vibration is due to the addition of AG within the silver nanoparticles. The C-H stretching vibration is observed in IR at 2926 cm^{-1}[25-27]. The C-C stretching vibration and C-O stretching vibrations are observed in IR at 1636 and 1021 cm^{-1} respectively [28-30]. The serious observed band at 778 and 725 cm^{-1} is assigned to the C-Cl vibration [31]. The NO2 deformation of the silver nano particle is observed in IR at 587cm^{-1} and the bending of C-O-O vibration is observed at 540 cm^{-1}[24].

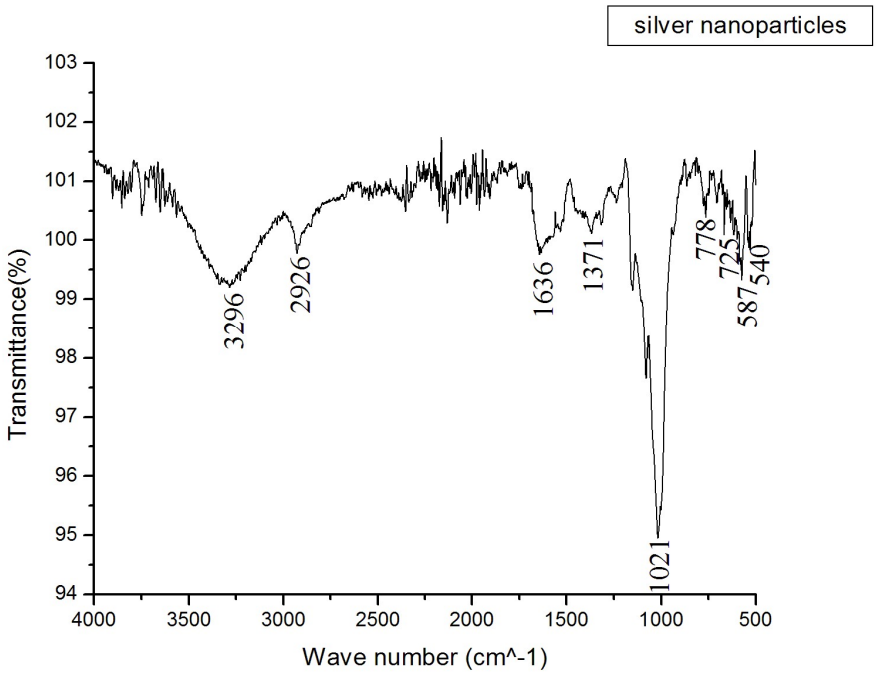

Figure 6 shows FT-IR spectra of silver nanoparticles.

Table.3 FT-IR data of silver nanoparticles

wave number (cm^{-1})	Functional group identification
540 cm^{-1}	Strong C-OO bending [22]
587 cm^{-1}	NO_2 deformation of aromatic nitro compounds [23]
725 cm^{-1}	C-CL stretching vibration in alkyl group [24]
778 cm^{-1}	C-CL stretching vibration of alkyl group [25]
1021 cm^{-1}	C-O Phe1nolic compound [26]
1371 cm^{-1}	C-F of alkyl halides [27]
1636 cm^{-1}	C=C Stretching vibration(alkenes) [28]
2926 cm^{-1}	alkyl C-H stretching vibration [29]
3296 cm^{-1}	stretching vibration of O-H bonds [30]

4.4 Scanning Electron Microscope

Scanning electron microscope can used to study the surface morphology and the particle size determination of the nanoparticles. SEM measurement has been used to demonstrate various shapes and sizes of metal nanoparticles. Thus the prepared silver nano particle is more or less spherical in shape. SEM image shows the agglomeration of silver nanoparticles and showed the average particle size of about ~ 17-25 nm regions.

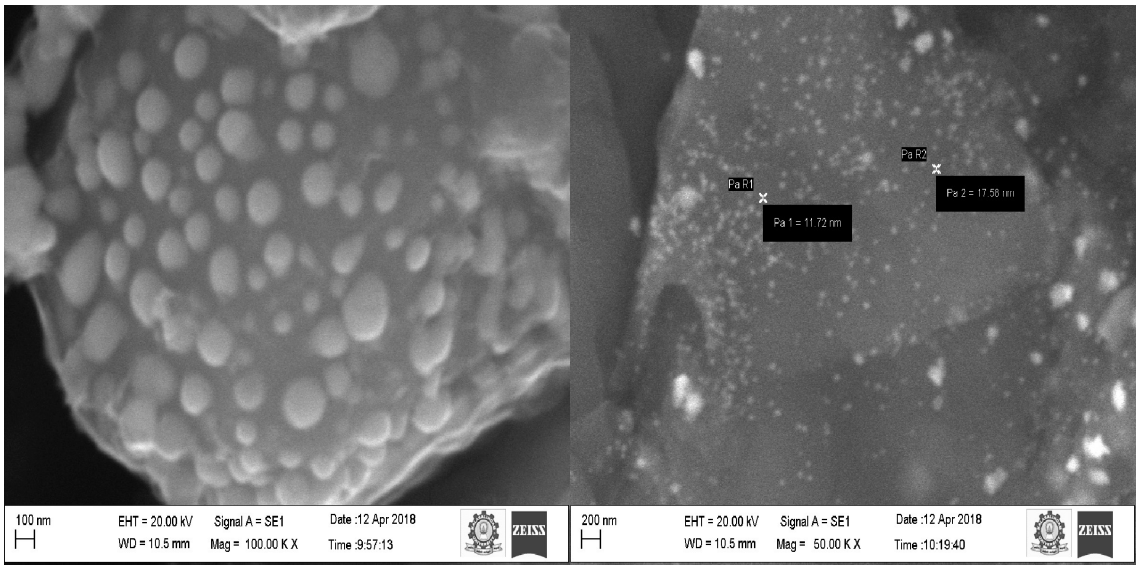

Figure 7 shows SEM images of silver nanoparticles.

4.5 Antibacterial Activity

The antibacterial activity of synthesized Ag NP's was performed by disc diffusion method. Antibacterial activity of the synthesized AgNPs was evaluated against Classmodium, Klebsilla and Pseudomonas bacterial pathogens. Silver nanoparticles displayed antibacterial activity against both gram positive and gram negative bacteria, as confirmed by the diameter of inhibition zone shows the antibacterial activity of prepared silver nanoparticles. The zone inhibition layer showed that silver nanoparticles can have more effective antibacterial activity

against Pseudomonas and Klebsilla. Classmodium pathogen showed less antibacterial activity when compared to Pseudomonas and Klebsilla. From antibacterial studies the investigated silver nanoparticles showed remarkable antibacterial activity against klebsilla sp., classmodium, and pseudomonas. These bacteria can form the zone inhibition of 0.4 mm for Classmodium pathogens and for 0.44 mm for Klebsilla pathogens [34] and 0.2 mm for Pseudomonas.[34] The antibacterial studies show that the prepared nanoparticles can be a better entrant for biomedical application. The experimental observation of anti microbial activity of Ag-NP's against Classmodium, Pseudomonas and Klebsilla pathogens are shown in the figure 8

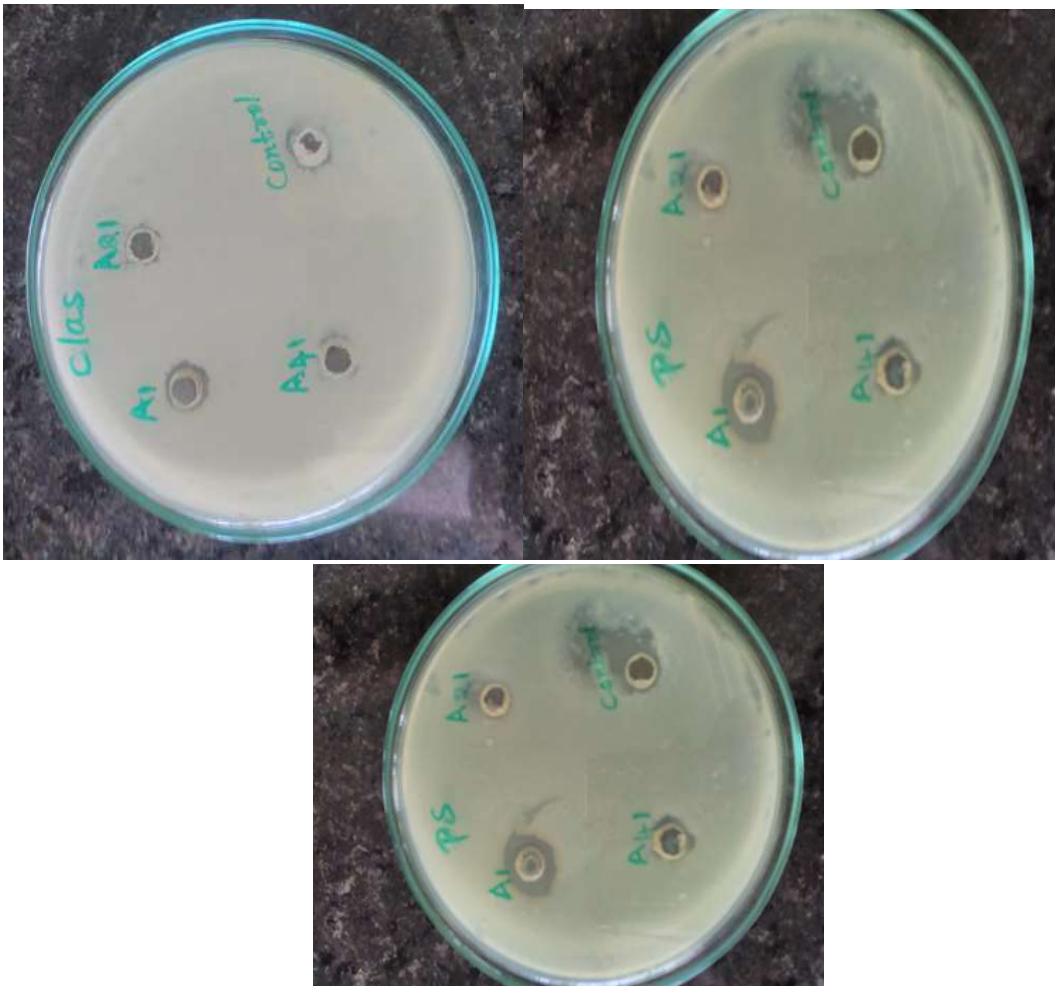

Figure 8 shows antibacterial activity of silver nanoparticles.

5. Conclusion

In this present study, Ag nanoparticles were prepared using Elachi seed peal extract (green synthesis). This work reports simple and eco-friendly approach for the synthesis of silver nanoparticles (Ag-NP's). The seed peal extract acts as both reducing and stabilizing agent. XRD diffraction pattern shows that the prepared silver nanoparticle is highly crystalline in nature and it is a Face Centered Cubic Structure. The average particle size is measured to be 27 nm by X-Ray Diffraction analysis. UV-Visible Spectrum shows absorption peak in the region of 440nm.the appearance of peak in the UV-Visible spectra shows the characteristics of surface plasmon resonance of silver nanoparticles. Surface morphology can be viewed by using

SEM image were spherical in shape and exhibited the size range between 25nm to 17.8nm. Flavonoids present in peal extract are powerful reducing and capping agent for synthesis of silver nanoparticles. The silver nanoparticles showed antibacterial activities against Classmodium, Pseudomonas and Klebsilla microorganisms. Overall, this green approach could be a competitive option to other alternative physical or chemical methods. Hence the synthesis of Ag NP's via green synthesis using elachi seed peel extract showed these characteristics is of significant interest and will offer great help in biomedical applications.

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Highlights

- Synthesis the silver nano particles using elachi seed peel extract
- Structure of the prepared silver nano particle was confirmed using XRD analysis.
- The UV Visible, FT-IR, and SEM analysis were done to characterize the prepared samples.
- Most of the results were agreed with the previous study result.
- The activity of the molecule also identified using the antibacterial analysis