

Biogenic synthesis of silver nanoparticles using *Artemisia nilagirica* leaf extract and their antimicrobial activity against pathogenic bacteria

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Abstract

Silver nanoparticles (AgNPs) were fabricated through green method using *Artemisia nilagirica* leaf extract as a reducing and capping agent. Here, rapid synthesis of AgNPs using leaf extract of *Artemisia nilagirica* has been claimed which is just 10 min at room temperature. The obtained nanoparticles were characterized by UV-visible (UV-Vis), XRD, FTIR and FE-SEM. The absorption band centered at 434 nm in UV-Vis reflects surface plasmon resonance (SPR) of AgNPs. XRD analysis revealed that biosynthesized AgNPs are crystalline in nature with the face centered cubic structure. FTIR showed nanoparticles capped with plant compounds. FE-SEM showed the spherical nature of the AgNPs within a size range of 20-30 nm.

The process of nanoparticles preparation is green, rapid, environmentally benign and cost effective. These biologically synthesized nanoparticles were also proved to exhibit excellent antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*. The synthesized AgNPs from leaf extract of *Artemisia nilagirica* shows 14 mm zone of inhibition against *E. coli* and 12 mm for *S. aureus*.

Keywords: Ag Nanoparticles, Biogenic synthesis, *Artemisia nilagirica* leaf extract, Antimicrobial activity, XRD analysis.

Introduction

Nanoparticles having distinctive structural and physico-chemical properties compared to bulk counterpart demonstrated unusual applications in current research activities and hence demand of nanomaterials is increasing day by day^{34,37}. The noble metal nanoparticles have tremendous applications in various fields such as medicine, electronics, energy and catalysis^{20,30}. Generally metal nanoparticles have been prepared by physical and chemical methods¹⁸. However, due to their hazardous and adverse impact on eco system, it is indeed important to replace the conventional chemical methods of nanomaterials synthesis by ecofriendly methods offering clean, non-toxic and environmentally acceptable method.

Furthermore, the interdisciplinary research work has broadened the boundary of material research. The increasing environmental pollution had motivated the researchers to introduce and implement new methods of nanomaterials

synthesizing. In this concern, several biological methods of nanoparticles synthesis have been reported which cover bacteria, fungi and plant extracts³⁸. Current research in the area of AgNPs synthesis is mainly focused on the use of fresh plant leaves extract^{6,28}. In this regard, Patil et al²⁷ reported the biosynthesis of nanoparticles using *Tridax procumbens* leaf extracts and its antioxidant potential. They studied antioxidant activities of AgNPs using *Tridax procumbens* leaf extract²⁷ whereas Huang et al¹¹ exploited the synthesis of silver and gold nanoparticles using the sun-dried *Cinnamomum camphora* leaf extract.

The field of nano biotechnology is one of the most active areas of research in modern material sciences. Nobel elements like Ag, Au, Pt and Pd have been synthesized by different methods including hard template using bacteria¹², fungi²⁹ and plants²⁶. Among these, silver as a non-toxic and efficient material discloses tremendous applications in the most commonly used metal for synthesizing nanoparticles. It is nontoxic, active at low concentrations and exhibits tremendous applications in the fields of catalysis, antimicrobials and therapeutics, sensitive to bio-molecules diagnostics and detection⁸. AgNPs have gained more popularity on account of their broad spectrum of antimicrobial and surface plasmon resonance (SPR) effect¹³. The physical and chemical methods are common for the synthesis of AgNPs, but these methods fail at economic and environmental fronts. Therefore, it is indeed important to develop ecofriendly techniques for AgNPs synthesis that sidestep requirement of hazardous chemicals^{38,39}.

In this concern, synthesis of AgNPs using plants extract is the best method because it is a non hazardous option and plants are more easily available and widely distributed². Plant contains abundant natural compounds such as phenols, flavonoids, alkaloids, saponins, steroids and tannins. These phytochemicals act as reducing and capping agent. The biomolecules like protein, phenol and flavonoids play an important role in reducing the ions and finally to obtain the nanoparticles¹.

India is rich in biodiversity and has great potential for bio prospecting. Each plant contains different types of metabolites which give rise to a wide diversity of nanoparticles. *Artemisia nilagirica* (Asteraceae) is an annual herb native to Asia and has been used for many centuries in traditional Asian medicine for the treatment and prevention of fever and chills⁴. It is highly aromatic annual herb and widely dispersed throughout the temperate region. Recent research has shown that it destroys malarial parasites¹⁵. A

variety of compounds have been extracted from *Artimisia nilagirica* such as sesquiterpenoids, flavonoids, coumarins, lipids, phenolics, purines, steroids, triterpenoids, aliphatics and artemisinin⁴. A sweetly aromatic herb with small, yellow flower heads, sweet wormwood contains the chemical artemisinin and its aerial parts are used in making anti-malarial drugs. Additionally, artemisinin is known to have antibacterial, antifungal, antileishmanial, antioxidant, antitumor and anti-inflammatory activity⁴.

In the present study, we are reporting one pot environmentally benign and rapid synthesis of AgNPs using *Artimisia nilagirica* leaf extract as a green reducing and stabilizing agent. The synthesized AgNPs nanoparticles were characterized by UV-visible, XRD, Fourier Transform Infra red Spectroscopy (FTIR) and Scanning field emission-scanning electron microscopy (FE-SEM). Further, these bioprospected plant mediated synthesized AgNPs were exploited as possible antibacterial agents against *Staphylococcus aureus* and *Escherichia coli*.

Material and Methods

Preparation of plant extract: Leaves of *Artimisia nilagirica* were collected from Kolhapur, India (Geographical location: 16°41'30"N 74°14'00"E). Collected fresh plant leaves were brought to the laboratory and washed using tap water to remove dirt from the leaf surface and then leaves were shade dried for 15 days under dust free environment. These dried leaves were ground into fine powder with grinder and were stored in plastic container at room temperature for further use. The powder was used for extract preparation. The extract was prepared by adding 10 gm of powder in 100 ml of double distilled water and boiled at 100°C for 20 min³. The extract was allowed to cool at room temperature and filtered using Whatmann filter paper no. 41 with the help of Buchner funnel. This extract was stored in refrigerator to protect it from fungal growth.

Preparation of AgNPs: For the biosynthesis AgNPs, 10 ml of leaf extract of *Artimisia nilagirica* was mixed with 100 ml of AgNO₃ solution (1 mM/ml) and incubated at 28 °C for 72 h. The reaction mixture was kept in dark room condition until the color changed from light brown to dark brown (Fig. 1). The solution of AgNPs was later centrifuged at 18,000 rpm for 25 min to collect the AgNPs.

Characterization of AgNPs

UV-Visible spectrophotometer: The formation of AgNPs was further analyzed by using UV-Vis spectrophotometer (Shimadzu, UV-3600) within the wavelength range of 220 nm –750 nm.

X-ray diffraction analysis: The X-ray diffraction analysis was performed between 2θ value 20° to 80° using X-Ray diffractometer (Bruker, AXS D8 Advances) identifying the phase structure of the synthesized particles.

Fourier transform infrared spectroscopy (FT-IR): FT-IR analysis was used to recognize the different functional groups of phytoactive biomolecules of *Artimisia nilagirica* involved in the reduction of silver nitrate (AgNO₃) to AgNPs. The obtained nanoparticles were analyzed by FT-IR spectroscopy. FTIR measurements were carried out from 4000 cm⁻¹ to 400 cm⁻¹ on a Perkin Elmer spectrometer in diffuse reflectance mode at a resolution of 4 cm⁻¹ using KBr pellets.

Scanning Field emission-scanning electron microscopy (FE-SEM): A thin film of dried samples was prepared on a carbon coated copper grid by dropping a very small amount of the samples on the grid. The films on the carbon coated copper grid (SEM grid) were allowed to dry by putting it under a mercury lamp for 5 min. The morphological images, size and structure of synthesized nanoparticles were analyzed and recorded.

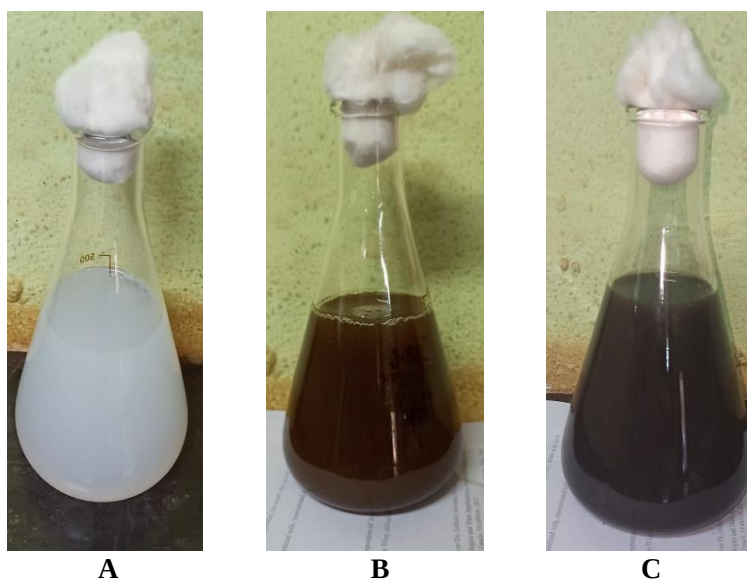


Fig. 1: Synthesis of AgNPs from *Artimisia nilagirica* A: AgNO₃ solution, B: AgNO₃ with plant extract, C: Color change after reduction reaction

Antimicrobial Assay: Antibacterial activities of synthesized AgNPs were analyzed by well diffusion method against two pathogenic bacteria *Escherichia coli* and *Staphylococcus aureus*¹⁴. The strains bacteria were sub cultured on nutrient agar (HiMedia) and were incubated at 37 °C for 24 h. Fresh overnight bacterial cultures were taken and spread on the nutrient agar plates using glass rod to cultivate bacteria. 6 mm diameter wells were made on nutrient agar plate with the help of cork borer. 25 µl of AgNPs and distilled water (as control) were inoculated to the well and then the plates were incubated in incubator at 37°C for 24 h. The antibacterial activity was measured based on the inhibition zone around the wells.

Results and Discussion

Visual observation and UV-Vis Studies: In the present study, AgNPs were synthesized using the leaf extract of *Artimicia nilagirica*. The colorless solution of AgNO₃ turns dark brown when it was mixed with the leaf extract of *Artimicia nilagirica* (Fig.1). It is well known that AgNPs show strong absorption band in visible region and generate specific color to the solution. The qualitative analysis of AgNPs was carried out by visual observations. After addition of *Artimicia nilagirica* leaf extract into the aqueous solution of AgNO₃, colour change was observed from brown to dark brown that indicates the reduction of ionic silver to metallic silver and eventually to AgNPs¹⁵. UV-Vis spectroscopy is a foremost technique to authenticate the formation and stability of AgNPs in an aqueous solution. The spectrum band at 434 nm (Fig. 2) corresponded to the surface plasmon resonance of the metallic AgNPs³³.

XRD Analysis: The XRD patterns of AgNPs clearly indicate that the prominent peak of AgNPs was formed in the range of 2θ (20 to 80) and the pattern showed the four different peaks at 38.2, 44.3, 64.4 and 77.4 with corresponding lattice plane value recorded at (111), (200), (220) and (311) of FCC silver crystal (Fig. 3) which matches to standard JCPD file no. 04-0783.

Thus, using XRD, the presence of AgNps was confirmed. The size of the nanoparticles was calculated with the help of the Scherrer eq. The average size of nano particles synthesized is 23.07 nm. A few unassigned peaks observed could be due to the presence of some bioorganic compounds/proteins^{22, 32}.

Fourier transform infrared (FTIR) spectroscopy: It was used to identify the secondary metabolites involved in the reduction and capping of AgNPs. FTIR spectra of *Artemesia nilagirica* and silver nanoparticles were recorded and shown in fig. 4. The prominent peaks were observed at 3280, 1623 (4a) and 1028 (4b). The peak at 3280 cm⁻¹ is due to stretching in alcohol and phenolic compounds present in the *Artimicia nilagirica* leaf extract¹⁵. The peak at 1623 cm⁻¹ may be attributed to aromatic C=C stretching vibrations¹⁵. The peak at 1028 cm⁻¹ responsible for the phenolic O-H group³². The peaks at 3280 cm⁻¹ and 1623 cm⁻¹ confirmed the involvement of flavonoids or polyphenolic compounds in silver nitrate bioreduction into AgNPs. FTIR spectroscopy clearly indicates that biomolecules present in *Artemesia nilagirica* are responsible for the synthesis of nanoparticles and their stabilization.

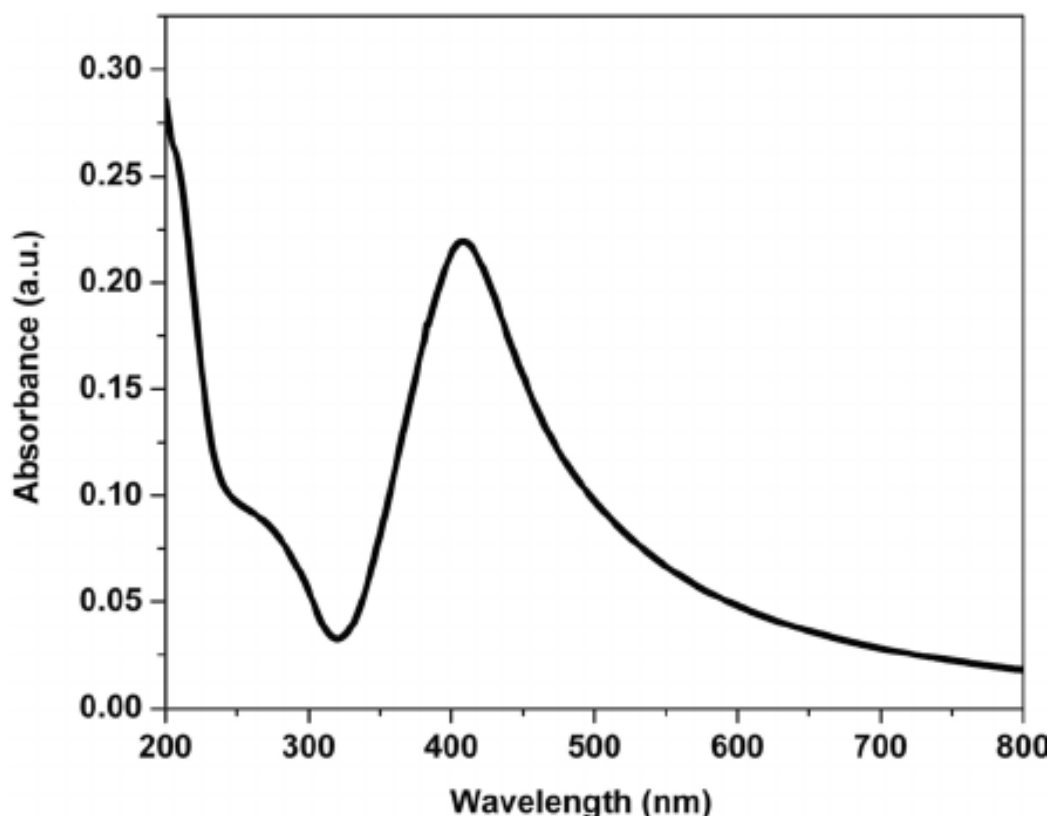


Fig. 2: UV-Vis spectrum of synthesized AgNPs from *Artimicia nilagirica* leaf extract.

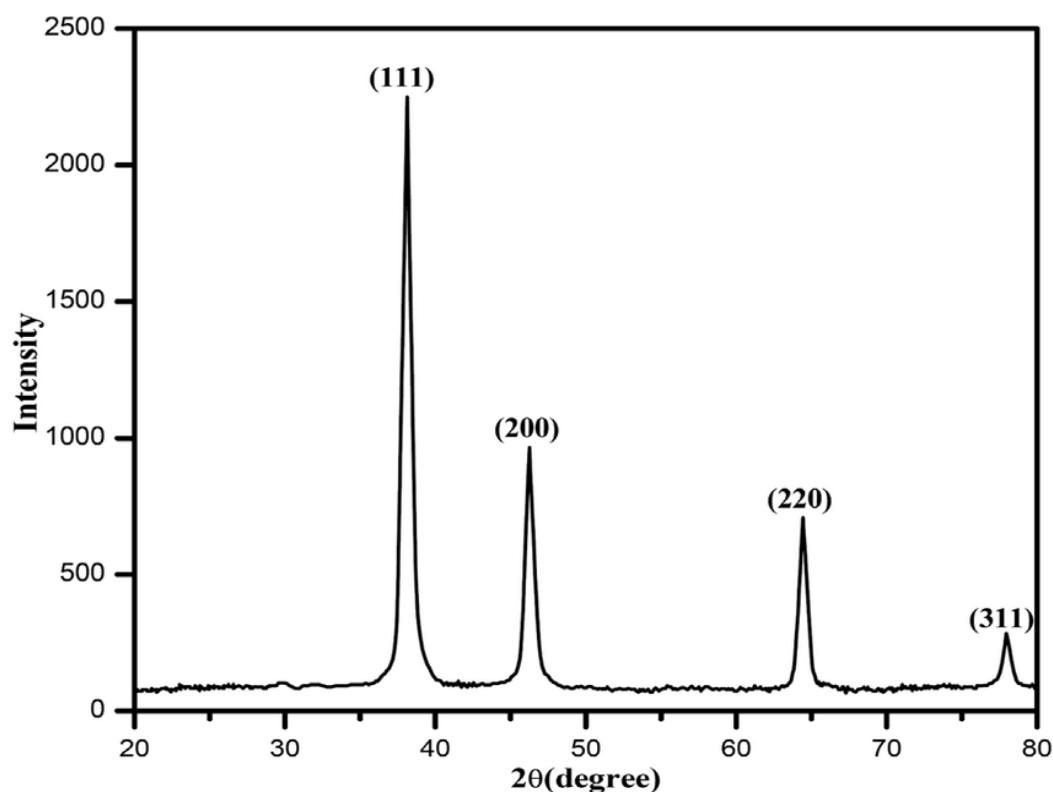


Fig. 3: XRD pattern of biogenically synthesized AgNPs.

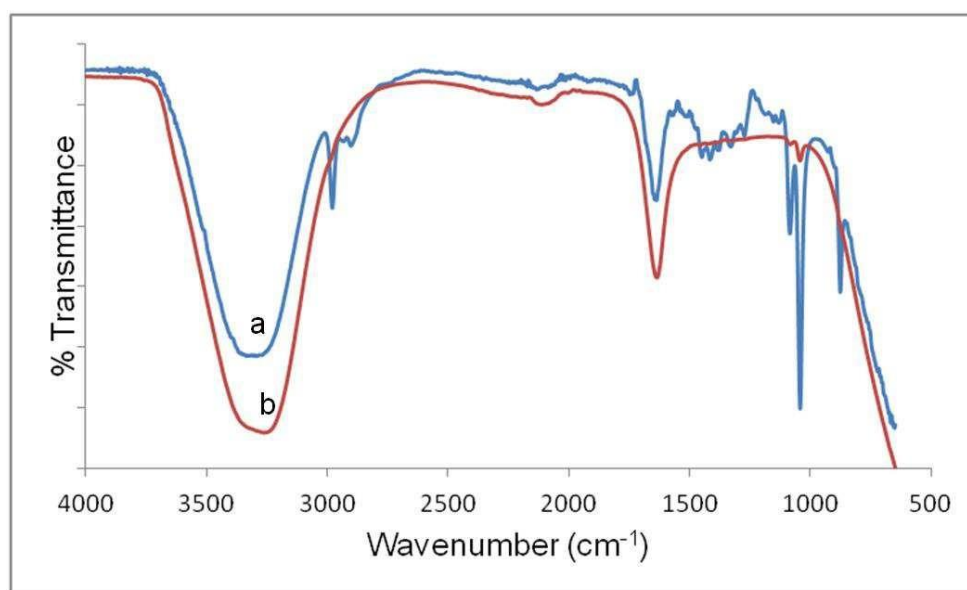


Fig. 4: FTIR spectra of (a) *Artemisia nilagirica* leaf extract; (b) FT-IR spectra of synthesized AgNPs by using *Artemisia nilagirica* leaf extract

FTIR spectra measurements revealed that the proteins possess a strong affinity to bind with metal and could be involved in the stabilization of the AgNPs synthesized by *Artemisia nilagirica* leaf extract, which were similar to the previous analysis by various researchers.^{5,7,19}

Scanning Field emission-scanning electron microscopy (FE-SEM): FE-SEM images were used to observe the surface morphology AgNPs. Fig. 5 shows various SEM images with different magnifications revealing the formation of AgNPs with various shapes (in the range 20-30

nm). The variation in particle size and shapes of AgNPs may be due to complex constituents of plant extract.^{16,32}

Antimicrobial Activity: AgNPs have been widely used in health, medicine and environmental applications. In this study, AgNPs prepared by using *Artemisia nilagirica* leaf extract were examined for antibacterial activity against the bacterial strains of *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) using well diffusion technique. The zone of inhibition around the well is shown in fig. 6. Maximum zone of inhibition 14 mm was seen

against *E. Coli* and 12 mm for *Staphylococcus aureus*. (Fig. 6 and table 1). No inhibition zone was observed for control prepared by the stock solution taken in well without AgNPs. The antibacterial activity of AgNPs should be referred with several mechanisms including (i) activity of Reactive Oxygen Species (ROS), (ii) the presence of Ag^+ ions in AgNPs in making bond with sulphhydryl groups for denaturation of proteins in the bacteria¹⁷ and (iii) release of Ag^+ ions from the AgNPs which simply penetrate into the cell wall and cause severe damage to the bacteria and kill them.

From fig. 6, it is observed that AgNPs show more antimicrobial activity on *E. coli* (Gram negative) than *S. aureus* (Gram positive) bacteria. The different antibiotic activity is due to variation in cell wall membrane of these bacteria. *E. coli* (Gram negative) have very thin layer cell wall membrane, its thickness ranged 7–8 nm and was made up of peptidoglycans and lipopolysaccharides. On the other

hand, *S. aureus* (Gram positive) bacteria have a very thick cell wall membrane, its thickness ranged from 20–80 nm and made up of large number of *mucopeptides*, lipoteichoic and acids murein²³. Several similar results were obtained in previous studies^{21,24,25,40,41}. Inhibition against *E. coli* and *Staphylococcus aureus* was observed in the case of AgNPs synthesized using extract from *Ayapana triplinervis*. Nanoparticles showed high toxicity to *E. coli* than *Staphylococcus aureus*⁸.

Conclusion

In summary, a green novel route for the rapid, ecofriendly and non-toxic synthesis of AgNPs at room temperature using easily available *Artimicia nilagirica* leaf extract has been reported, in which leaf extract acts as a green reducing as well as stabilizing agent. The synthesized AgNPs are found in various shapes with size of 20 to 30 nm and are also found to be very stable.

Table 1
Anti-microbial activity of AgNPs synthesized by the leaf extract of *Artimicia nilagirica*

Bacterial species	Diameter of inhibition zone (mm)	
	AgNp from leaf extract of <i>Artimicia nilagirica</i>	Control
<i>E. Coli</i>	14	0
<i>Staphylococcus aureus</i>	12	0

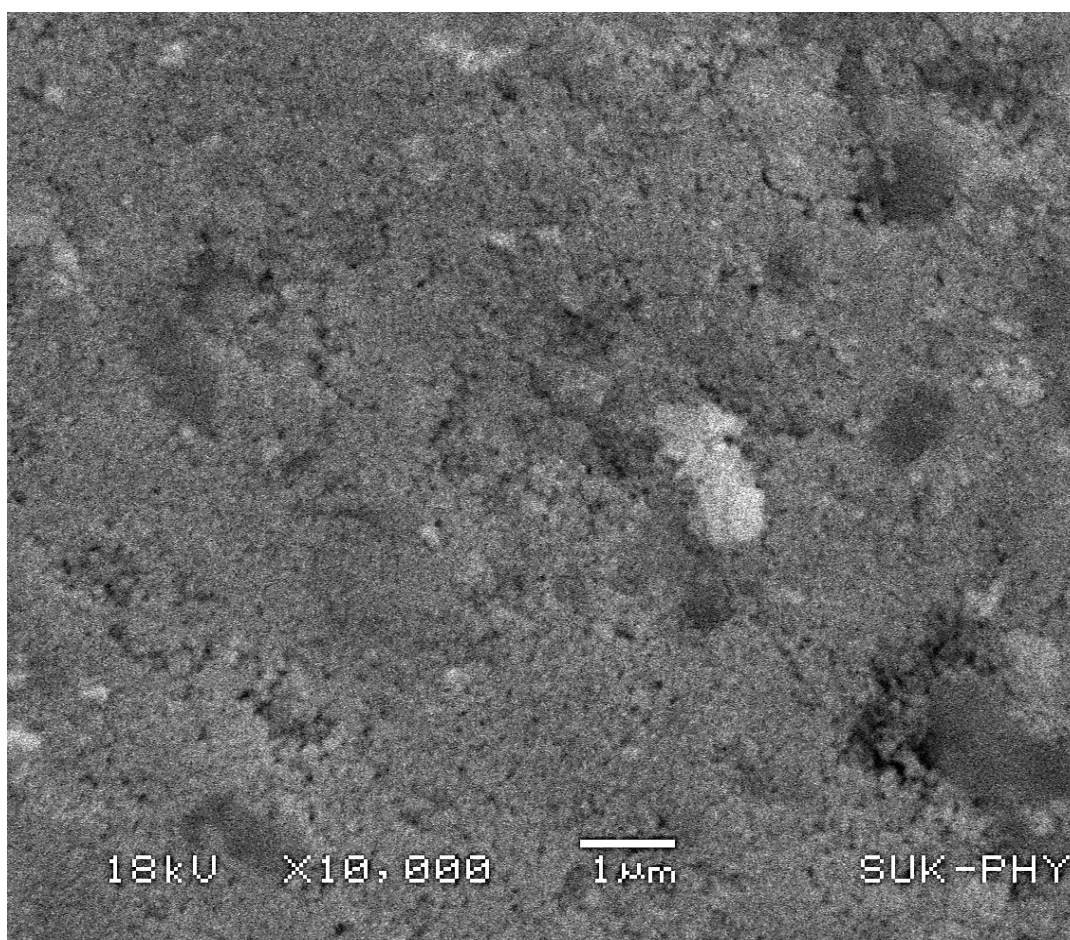


Fig. 5: FE-SEM image of synthesized AgNPs

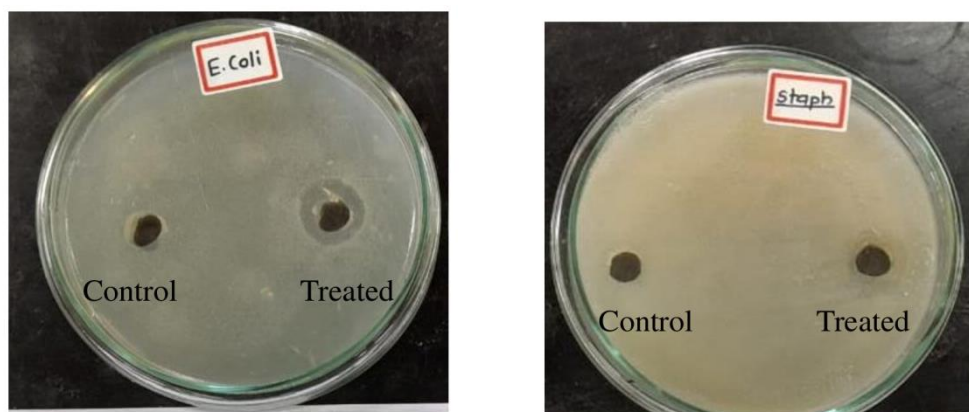


Fig. 6: Antimicrobial Effect of AgNPs (*Artimicia nilagirica*) on *E.coli* and *Staphylococcus aureus*

The biosynthesized AgNPs demonstrated significant antimicrobial activity against *E. coli* and *Staphylococcus aureus*³⁶. The reaction time for the synthesis of AgNPs in the present study was found to be significantly lower as compared to earlier reports. This biogenic synthesis may open door for the other noble metals also.

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