Biosynthesis of Silver Nanoparticles from Sphagneticola trilobata and Investigating Antimicrobial Activity

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Abstract

Nanotechnology has emerged as a rapidly growing field, with silver nanoparticles gaining prominence for their multifunctional properties. This study synthesized silver nanoparticles using Sphagneticola trilobata extract through natural, heat-assisted, and non-heated methods, assessing their antimicrobial efficacy against pathogens like Salmonella, Streptococcus, and Candida auris. Heat-assisted synthesis yielded well-distributed nanoparticles with superior antimicrobial activity. Characterization was performed using UV spectrophotometry and High-Resolution Field Emission Scanning Electron Microscopy. The findings highlight the potential of Sphagneticola trilobata -derived Ag-NPs as sustainable antimicrobial agents, blending traditional medicinal knowledge with nanotechnology. Future research should optimize protocols, explore broader microbial applications, and investigate the mechanisms behind their activity.

Antimicrobial Activity, Pathogenic Microorganisms, Drug-Resistant Pathogens, Sustainable Antimicrobial agents.

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1 Introduction

Nanotechnology refers to the science and engineering involved in the design, synthesis, characterization, and application of materials and devices where at least one dimension is on the nanometer scale—one billionth of a meter. At this scale, the behavior of individual molecules and molecular assemblies becomes crucial, as controlling molecular structures enables manipulation of macroscopic chemical and physical properties. This unique capability has led to transformative advancements across multiple disciplines, including medicine, environmental science, and materials engineering. In medical and physiological applications, nanotechnology has enabled the development of materials and devices capable of interacting with the body at the molecular level, thereby facilitating highly specific targeting at cellular and tissue levels. Such innovations hold promise for targeted clinical therapies that maximize therapeutic efficacy while minimizing adverse side effects. The potential of nanotechnology to revolutionize medicine and other fields underscores its importance. Furthermore, nanotechnology fosters innovative methods for product development, replacing traditional manufacturing techniques and reformulating materials for enhanced performance. These advancements not only reduce material and energy consumption but also mitigate environmental harm and promote sustainable practices. The environmental applications of nanotechnology are extensive, addressing current challenges and offering solutions for more sustainable resource management and risk mitigation. These applications include water purification, pollution control, and the development of environmentally benign alternatives to traditional materials, thus bridging the gap between technological progress and ecological stewardship.[1,2]

Nanoscale materials are characterized by at least one dimension measuring less than 100 nanometers. A nanometer, equivalent to one millionth of a millimeter, is roughly 100,000 times smaller than the diameter of a human hair. At this scale, materials exhibit distinct optical, magnetic, electrical, and chemical properties that differentiate them from their bulk counterparts. These unique characteristics have broad implications for diverse

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fields, including electronics, energy, and biomedicine. Nanomaterials, due to their small size and high surface area, possess enhanced properties such as increased strength, superior electrical conductivity, and heightened catalytic activity. These attributes make them indispensable in applications ranging from energy storage and solar cells to drug delivery and environmental remediation. Nanomaterials contribute to technological advancements by enabling miniaturization and performance improvements in electronic devices while offering sustainable solutions for pressing global challenges such as renewable energy and healthcare. Their role in innovation and problem-solving places them at the forefront of modern scientific and technological endeavors.[3]

The synthesis of Nanomaterials involves two principal approaches: top-down and bottom-up. The top-down approach reduces larger structures to the nanoscale, employing techniques like micelle growth arrangement and surface functionalization. Conversely, the bottom-up approach assembles nanostructures atom by atom or molecule by molecule. Nanomaterials comprise three distinct layers: the core, which determines their intrinsic properties; the shell, chemically distinct from the core; and the surface layer, which is often functionalized with polymers or small molecules to enhance stability and reactivity. The ability to manipulate these structural layers allows for precise control over the size, composition, and functionality of Nanomaterials, broadening their application spectrum. A growing emphasis on green synthesis methods aims to produce Nanomaterials in an environmentally friendly manner, utilizing non-toxic precursors and mild conditions to avoid hazardous chemicals. These green approaches are particularly significant in the sustainable production of metal and metal oxide nanoparticles, which have applications in environmental remediation and other domains. The advancements in biosynthesis techniques and functionalization strategies underscore the potential for creating ecofriendly Nanomaterials, aligning technological progress with environmental sustainability.[4,5]

Antimicrobial properties of Nanomaterials have garnered significant attention, particularly in the face of rising antibiotic resistance. Antibiotic resistance is one of the most pressing

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global public health challenges, with over 70% of bacterial infections exhibiting resistance to commonly used antimicrobial agents. Approximately 79% of bacteria have demonstrated resistance to one or more antibiotics, leading to increased mortality, health complications, and heightened healthcare costs. Developing new antibiotics is a potential solution; however, this approach is often hindered by lengthy development timelines, high costs, and the eventual emergence of resistance to new drugs. Consequently, there is an urgent need for novel antimicrobial agents capable of addressing this growing threat. Nanoparticles have emerged as promising alternatives, offering innovative solutions to combat bacterial infections and overcome the limitations of traditional antimicrobials. Metals such as copper, silver, and zinc, historically valued for their antimicrobial properties, are now being harnessed through nanotechnology to create Nanomaterials with enhanced efficacy. These materials, including metal nanoparticles, metal oxides, and composite materials, benefit from large surface area-to-volume ratios, enabling diverse possibilities for antimicrobial applications. Nanoparticles derived from metals like gold, silver, titanium dioxide, zinc oxide, and copper oxide are already utilized in various fields, including cosmetics, device coatings, and food preservation, demonstrating their potential for addressing microbial infections.[6]

Among these, silver nanoparticles (Ag-NPs) have emerged as a particularly potent antimicrobial agent. The rise of nanotechnology has facilitated the creation of Nanomaterials like Ag-NPs, which are being explored to combat healthcare-associated infections and address antibiotic resistance. Several methods have been reported for synthesizing Ag-NPs, including physical and chemical approaches. Chemical synthesis, involving the reduction of silver salt solutions with reducing agents, is one of the most common, cost-effective, and straightforward methods. In this process, silver ions (Ag^+) are reduced to silver atoms (Ag^0), which aggregate into clusters to form colloidal Ag-NPs. However, colloidal stability is a significant challenge, as Ag-NPs tend to cluster due to their large surface area. To address this, stabilizing agents are employed during synthesis to control the size, shape, and stability of the nanoparticles. The physicochemical properties of Ag-NPs, including size and shape,

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play a critical role in determining their antimicrobial activity. Research has shown that smaller nanoparticles with higher surface area exhibit stronger antimicrobial effects due to increased interaction with microbial surfaces. [7]

The antimicrobial mechanisms of metal nanoparticles are multifaceted, involving physical disruption of microbial cell membranes, inhibition of cellular respiration, interference with DNA replication, and the generation of reactive oxygen species (ROS). These processes result in the leakage of intracellular components, ultimately leading to cell death. Factors such as particle size, shape, and surface charge influence the efficacy of metal nanoparticles. Smaller nanoparticles with greater surface area demonstrate enhanced antimicrobial activity due to their ability to interact more effectively with microbial surfaces. Metal nanoparticles are utilized in various applications, including wound healing, biomedical devices, and food preservation, where their antimicrobial properties contribute to infection control and contamination prevention. Moreover, the development of multifunctional Nanomaterials combining antimicrobial activity with additional properties, such as drug delivery or anti-inflammatory effects, holds great promise for advancing healthcare and other fields.[8]

Sphagneticola trilobata, commonly known as creeping oxeye, trailing daisy, or Singapore daisy, is a low-growing perennial herb native to Central and South America. This plant belongs to the Asteraceae family, which includes familiar species such as sunflowers, daisies, and chrysanthemums. Over time, Sphagneticola trilobata has expanded its distribution to tropical and subtropical regions worldwide, including Southeast Asia, parts of Africa, and the Pacific Islands. The plant is characterized by its bright yellow flowers and shiny, lobed leaves, making it a popular choice for landscaping and ground cover in tropical gardens. Its rapid growth and dense mat formation are advantageous for controlling soil erosion in certain areas. However, Sphagneticola trilobata has also gained notoriety as an invasive species in some regions, where its vigorous growth outcompetes native plants and disrupts local ecosystems. This ecological impact raises concerns about biodiversity loss and necessitates careful management in areas where the plant is introduced.

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Despite its potential to become invasive, Sphagneticola trilobata is valued for its hardiness, ease of cultivation, and aesthetic appeal. Its widespread presence and rapid growth make it an accessible and abundant resource for various applications, including its potential use in nanotechnology. The plant's phytochemical profile, rich in bioactive compounds, positions it as a promising candidate for the biosynthesis of nanoparticles. The utilization of plant-based systems for nanoparticle synthesis aligns with the principles of green chemistry, offering an environmentally friendly alternative to conventional chemical methods. The biosynthesis of silver nanoparticles using Sphagneticola trilobata represents a novel approach to harnessing the plant's natural properties for technological and biomedical applications. By leveraging the plant's inherent bio-reductive capabilities, researchers can produce silver nanoparticles with controlled size and shape, tailored for antimicrobial purposes. This approach not only addresses environmental concerns associated with chemical synthesis but also taps into the plant's potential as a sustainable resource for advanced material production.

In summary, the biosynthesis of silver nanoparticles from Sphagneticola trilobata offers a compelling intersection of nanotechnology, green chemistry, and biomedical innovation. This approach leverages the unique properties of nanoscale materials, the antimicrobial potential of silver nanoparticles, and the bio-reductive capabilities of Sphagneticola trilobata. By addressing the pressing challenges of antibiotic resistance and environmental sustainability, this research contributes to the development of innovative solutions that bridge scientific advancement with ecological responsibility. Biosynthesized silver nanoparticles hold great potential for applications in healthcare and environmental remediation. Utilizing plant-based synthesis presents a sustainable approach to nanotechnology, fostering significant and environmentally friendly progress.

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2 Experimental Procedure

The synthesized silver nitrate nanoparticles are characterised by the UV-Vis Spectrophotometry, Scanning Electron Microscopy.

2.1 Preparation of Sphagneticola trilobata solution

To prepare a sample of Sphagneticola trilobata extract, take 10 grams of Sphagneticola Trilobatas leaves and add it to 100 mL of distilled water. Using motor and pestle, grind the leaves along with distilled water. Once the extraction is complete, filter the mixture to remove the solid leaves particles using filter paper or a fine mesh sieve. After filtration, heat the filtrate gently to evaporate some of the water, concentrating the extract. Be cautious not to overheat, as excessive heat may degrade some of the sensitive compounds. The resulting concentrated Sphagneticola trilobata extract can then be used for further applications, such as the synthesis of silver nanoparticles or for medicinal purposes.

2.2 Preparation of Silver Nitrate solution

To prepare a silver nitrate solution, weigh 0.691 grams of silver nitrate ($AgNO_3$) using a precise balance. Add the measured silver nitrate to a clean 100 mL beaker. Slowly add 100 mL of distilled water to the beaker containing the silver nitrate. Stir the mixture gently using a glass rod until the silver nitrate is completely dissolved in the water. The resulting solution will be clear, with no visible solid particles remaining. This silver nitrate solution is now ready for use in various chemical reactions or experiments, such as the synthesis of silver nanoparticles or for laboratory analyses involving halide ions.

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2.3 Biosynthesis of silver nanoparticles

2.3.1 Natural synthesis

In this experiment, Sphagneticola trilobata solution was prepared and mixed with silver nitrate solution to facilitate the synthesis of silver nanoparticles. The Sphagneticola trilobata solution acts as a reducing and stabilizing agent, while silver nitrate provides the silver ions necessary for nanoparticle formation. After thoroughly mixing the two solutions, the mixture was placed in an incubator under controlled temperature conditions to ensure an optimal environment for the reaction. The incubation allows for the reduction of silver ions (Ag^{+}) to silver nanoparticles (Ag°) over time. This process is monitored for changes in color or other indicators signifying nanoparticle synthesis.

2.3.2 Heat assisted synthesis

In this procedure, 100 ml of Sphagneticola trilobata extract was combined with 100 ml of a pre-synthesized silver nanoparticle solution to study their interaction or to functionalize the nanoparticles. The mixture was transferred to a reaction vessel and subjected to controlled heating at a specific temperature to enhance the interaction between the Sphagneticola trilobata components and the silver nanoparticles. The heating process facilitates bonding, stabilization, or coating of nanoparticles with bioactive compounds from the Sphagneticola trilobata extract. Throughout the process, the reaction is monitored for any visible changes, such as color shifts or precipitation, indicating a successful reaction.

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3 Results and Discussions

3.1 UV-Vis Spectrophotometry of Silver nanoparticles

3.1.1 NATURAL SYNTHESIS (Day 1

The graph shows the optical density (OD) measurements of the synthesized silver nanoparticles using Sphagneticola Trilobata extract, recorded on Day 1 of natural synthesis. The absorbance values decrease significantly as the wavelength increases from 400 nm to 500 nm. The peak absorbance of 2.442 at 400 nm suggests the initial formation of silver nanoparticles, while the subsequent decline in OD indicates reduced nanoparticle concentration or aggregation at higher wavelengths. The lowest OD value of 2.204 at 500 nm shows minimal absorbance.

Wavelength	Absorbance
400	2.442
410	2.435
420	2.428
430	2
440	2.415
450	2.401
460	2.382
470	2.364
480	2.335
490	2.295
500	2.204

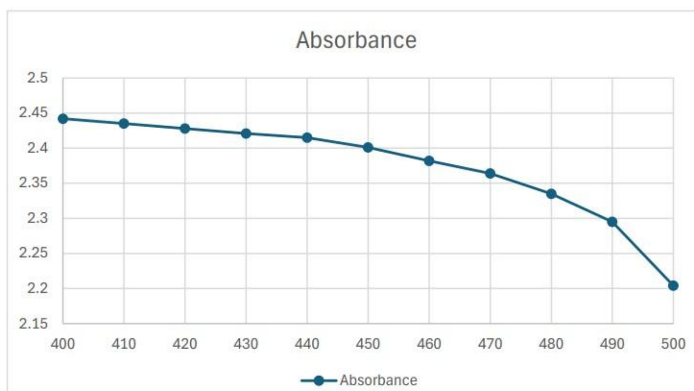

Figure 1: 1.1 Graph of Day 1 sample (Natural synthesis)

3.1.2 Natural Synthesis (Day 2

This graph shows the optical density of Synthesized Ag-NPs recorded on Day 2 of Natural synthesis. Here we get the peak value at 430 nm which shows the synthesis of silver nanoparticles using S.trilobata. Then it decreases into 1.382 at 500nm.

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Wavelength	Absorbance
400	2.63
410	2.684
420	2.761
430	2.775
440	2.745
450	2.67
460	2.571
470	2.317
480	1.997
490	1.668
500	1.382

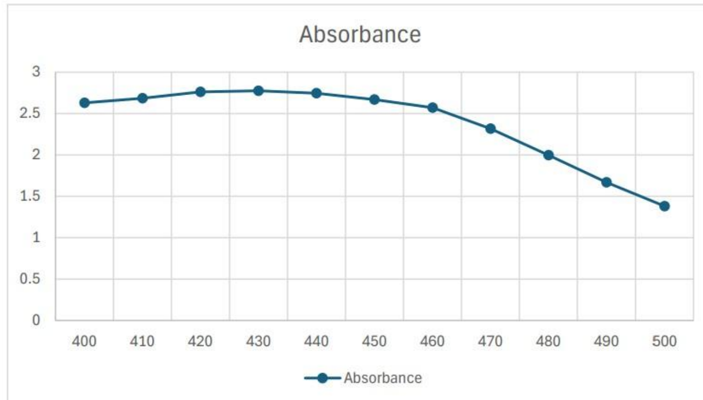

Figure 2: 1.2 Graph of Day 2 sample (natural synthesis)

3.1.3 Heat Assisted Synthesis (Day 1

The graph illustrates the optical density measurement of the synthesized nanoparticles using Sphagneticola trilobata extract, recorded on Day 1 with applying heat (heat assisted). The value peaks at 400nm and OD is 2.457 and the OD decreases as the wavelength increases. OD at 500nm is 2.314. There is no peak value in between the wavelength. As the wavelength increases, absorbance decreases.

Wavelength	Absorbance
400	2.457
410	2.457
420	2.449
430	2.442
440	2.435
450	2.428
460	2.421
470	2.405
480	2.389
490	2.358
500	2.314

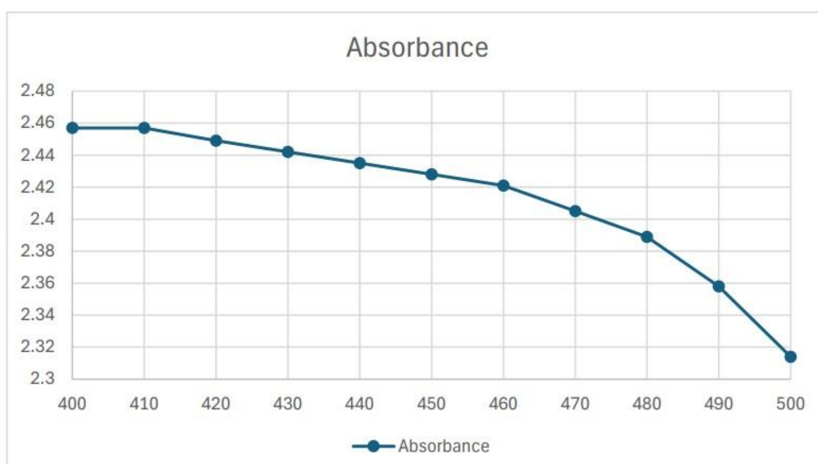

Figure 3: 1.3. Graph of Day 1 sample (heat assisted)

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3.1.4 Heat Assisted Synthesis (Day 2

The graph shows significant peak value in Day 2 of sample with heat assisted. At 400nm, the OD was 2.725 and at 500nm the OD was 1.594. Absorbance decreased from 400nm to 500nm with a peak value at 430nm is 2.86. This peak value shows the synthesis of silver nanoparticles from Sphagneticola trilobata .

Wavelength	absorbance
400	2.725
410	2.8
420	2.847
430	2.86
440	2.813
450	2.757
460	2.704
470	2.505
480	2.201
490	1.881
500	1.594

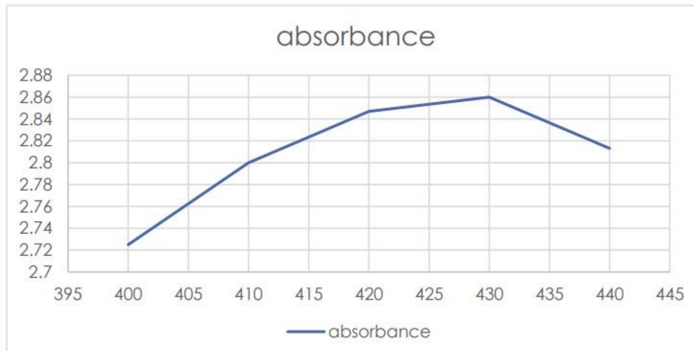

Figure 4: 1.4. Graph of Day 2 sample (heat assisted)

The spectrometry analysis of silver nanoparticles biosynthesized with Sphagneticola trilobata showed significant changes in absorbance over time and under various synthesis conditions. On the first day, both natural and heat-assisted synthesis methods exhibited the UV-Vis decreasing trend in absorbance values, indicating that nanoparticle formation was occurring in the reaction mixture containing 50 mL of silver nitrate and 50 mL of leaf extract.

After diluting the samples with double, the amount of distilled water the next day, a clear peak appeared at 430 nm for both synthesis methods. This peak indicates the surface plasmon resonance (SPR) of silver nanoparticles, confirming their successful formation.

These results suggest that both natural and heat-assisted synthesis methods are effective for producing silver nanoparticles using Sphagneticola trilobata. The peak at 430 nm emphasizes the unique optical properties of the synthesized nanoparticles, which supports their potential use in various fields such as medicine, catalysis, and environmental science. Future research could focus on optimizing synthesis conditions and assessing the stability

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and functionality of the nanoparticles.

3.2 SEM Characterization of Silver Nanoparticles

The SEM analysis of the synthesized silver nanoparticles (AgNPs) using Sphagneticola trilobata leaves extract revealed a size range of 30–70 nm with predominantly spherical shapes and uniform distribution.

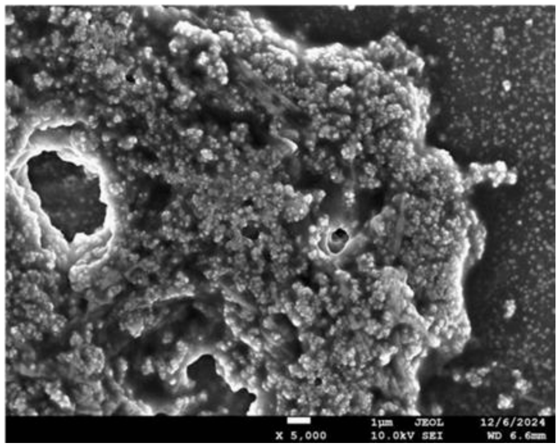

Figure 5: 2: SEM image of silver nanoparticle at 1micrometer scale

3.3 Zone of Inhibition

Zone of inhibition is the region where the clear area surrounding an antimicrobial agent in a culture of microorganisms, usually on an agar plate. The zone of inhibition is measured in mm.

In this study of zone of inhibition, we have taken the Gram-positive bacteria, Gram negative bacteria and a fungus inoculated on a petri plate of nutrient agar. We have taken Streptococcus as Gram positive bacteria and Salmonella as Gram negative bacteria. The zone of inhibition is measured of 24 hours of incubation. A drop of synthesized silver nanoparticles is introduced into the agar plate where the bacteria and fungi are inoculated to check for the antimicrobial and antifungal properties. A drop of natural synthesized AgNPs and a drop of heat assisted Ag-NPs were used. For the comparative study, we have used ampicillin and

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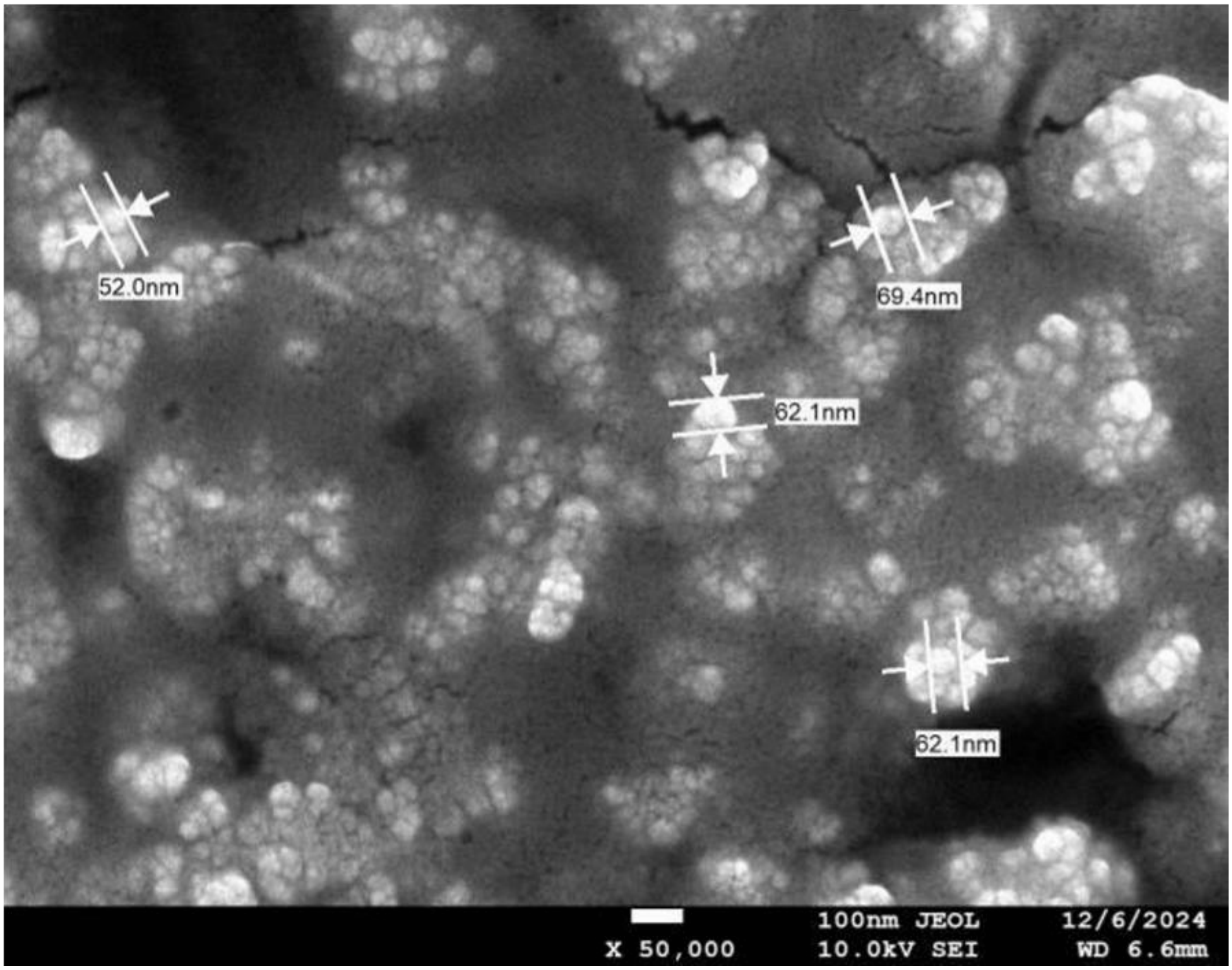

Figure 6: 3: SEM image clearly showing the presence silver nanoparticles of range 50-70nm

kanamycin tablets. As we could see the zone of inhibition is more in heat assisted Ag-NPs compared to natural synthesized Ag-NPs. And it had shown the clear zone of inhibition which depicts the significant antibacterial and antifungal properties.

Table 1: 4. Zone of inhibition Of Gram-positive bacteria (Streptococcus)

Antibacterial susceptibility test	Antibiotics (Diameter in mm)
Ampicillin	No clear zone
Kanamycin	40
Heat assisted Ag-NPs	25
Natural synthesized Ag-NPs	15

Fig.3.8. Antibacterial activity of Ag-NPs against Gram positive bacteria (Streptococcus)

Fig.3.9. Antibacterial activity of Ag-NPs against Gram negative bacteria (Salmonella)

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Table 2: 5. Zone of inhibition of Gram-negative bacteria (Salmonella)

Antibacterial susceptibility test	Antibiotics (Diameter in mm)
Ampicillin	No clear zone
Kanamycin	35
Heat assisted Ag-NPs	15
Natural synthesized Ag-NPs	15

Table 3: 6. Zone of inhibition of fungus

Antifungal susceptibility test	Antibiotics (Diameter in mm)
Ampicillin	No clear zone
Kanamycin	30
Heat assisted Ag-NPs	25
Natural synthesized Ag-NPs	20

Fig.3.10. Antifungal activity of Ag-NPs against fungus

3.4 Conclusion

Our study aimed at green synthesizing of silver nanoparticles from Sphagneticola trilobata, characterizing synthesized Ag-NPs using UV spectrophotometer and antimicrobial study of Ag-NPs using zone of inhibition test. It is simple, eco-friendly and cost-effective approach. The phytochemicals like Flavanoids, saponins, tannins etc. in the leaf extract acts as a reducing agent and eliminates harmful chemicals. The synthesized Ag-NPs showed significant Antibacterial and antifungal properties. It effectively inhibited the growth of both Gram positive, Gram negative and pathogenic fungi. This successfully showed antibacterial activity against Gram positive bacteria Streptococcus and Gram-negative bacteria Salmonella. And highlighted antifungal activity against pathogenic fungi. We conclude that, this study of Sphagneticola trilobata has potential of antimicrobial as well as antifungal properties. In future, antioxidant and anticancer studies will be done. And its application in medicine and industry will be studied in future.

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Figure 7: 7. Sample showing antibacterial and antifungal properties

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