WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.453
Volume 14, Issue 7, 1134-1148. Research Article ISSN 2277–7105

# HARNESSING LICHEN-DERIVED SILVER NANOPARTICLES: A MULTI-TARGETED APPROACH AGAINST DIABETES, INFLAMMATION AND BACTERIA

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Article Received on
10 February 2025,

Revised on 30 Feb. 2025,
Accepted on 20 March 2025

DOI: 10.20959/wjpr20257-36076

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## ABSTRACT

Lichens, renowned for their diverse symbiotic nature, have emerged as a valuable source of bioactive compounds with significant medicinal potential. In recent years, lichen-derived nanoparticles have garnered increasing attention for their promising therapeutic applications. This study investigates the anti-diabetic, anti-inflammatory, and antibacterial properties of silver nanoparticles synthesized from the lichen species *Parmotrema austrosinense*, collected from Gudalur in the Nilgiri district, TamilNadu, India. The nanoparticles were synthesized using lichen extract and characterized through various analytical techniques such as UV, FTIR and SEM analysis. Anti-diabetic activity was evaluated using alpha-amylase and alpha-glucosidase inhibition assays, both of which exhibited substantial enzyme inhibition. Anti-inflammatory potential was assessed through protein denaturation and trypsin inhibition assays, where the nanoparticles effectively mitigated protein denaturation and suppressed trypsin activity. Additionally, antibacterial efficacy was tested against *Pseudomonas aeruginosa*, *Escherichia coli*, and *Staphylococcus aureus*, demonstrating a pronounced zone of inhibition. These findings suggest that silver nanoparticles derived from *Parmotrema austrosinense* possess significant pharmacological properties, highlighting their potential for developing novel therapeutic agents for diabetes, inflammation, and bacterial infections.

**KEYWORDS:** *Parmotrema austrosinense*, Silver Nanoparticles, Lichen, Anti-Diabetic Activity, Anti-Inflammatory Activity, Antibacterial Activity.

## INTRODUCTION

Lichens are complex symbiotic organisms which develop through the mutualistic relationship between fungi and either cyanobacteria or algae photosynthetic partners and they provide numerous bioactive metabolites. The diverse pharmacological properties of secondary metabolites include antimicrobial activities and anti-inflammatory action as well as anti-diabetic properties (Gajbhiye and Dhoble (2024). Recent integration between nanotechnology and lichen-based bioactive compounds created new possibilities to develop sustainable therapeutic agents with high efficiency. The biological activity combined with biocompatibility of silver nanoparticles (AgNPs) makes them stand out among different types of nanoparticles as promising medical agents. Green synthesis of AgNPs through lichens delivers an environmentally friendly method that improves the biological effectiveness of these nanoparticles.

Homeopathic properties in silver nanoparticles enable them to combat microbial diseases and reduce inflammation and effectively manage diabetes symptoms. Public health concerns about antibiotic resistance together with conventional drug limitations drive researchers to assess new therapeutic approaches using AgNPs synthesized by lichen organisms (Mikhailova, 2020). The biochemical agents from lichens function as organic reducing agents and stabilizers that help provide both stability and augment their bioactivity in nanoparticle creation. The pharmaceutically-significant chemical components combined with medicinal value in *Parmotrema austrosinense* make it a promising agent for AgNP production among other lichen species.

A metabolic disease named diabetes mellitus generates raised blood glucose when patients either fail to produce insulin properly or their body cannot use the available insulin effectively. Postprandial glucose regulation depends heavily on hindering carbohydrate-hydrolyzing enzymes alpha-amylase and alpha-glucosidase in diabetes management as described by Kim *et al.*, (2019). The synthesized silver nanoparticles from *P. austrosinense* show substantial enzyme-blocking properties that open prospects for glycemic control management. Anti-diabetic drug benefits from this technology because these nanoparticles adjust enzyme behaviour without producing harmful side effects.

The human body activates inflammation as a natural defense mechanism when tissue suffers an injury or infection yet persistent inflammation relates to conditions such as arthritis together with cardiovascular conditions and diabetes. The established method to evaluate anti-inflammatory capacity involves protein denaturation inhibition and proteolytic enzyme suppression such as trypsin (Shah and Mital, 2018). *P. austrosinense*-derived AgNPs demonstrate effective capabilities in reducing protein denaturation plus inhibiting trypsin activity as natural anti-inflammatory agents. These nanoparticles exhibit important capabilities to influence inflammatory pathways which make them applicable for treating diseases caused by inflammation.

The healthcare sector contends with substantial health challenges because of bacterial infections exacerbated by the increasing number of resistant microbial strains. The existing antimicrobial drugs fall short of permanent efficacy which directs medical research toward new antimicrobial strategies. Studies on silver nanoparticles have defined their effective antibacterial mechanism due to their cellular membrane disruption and reactive oxygen species production and their impact on microorganism DNA replication (Skandalis *et al.*, 2017). The bio friendly synthesis process combined with multiple therapeutic functionalities makes AgNPs from lichens suitable candidates for therapeutic solutions. The synthesis of biogenic nanoparticles through natural methods delivers safer medical applications because these nanoparticles show better biocompatibility and reduced toxicity relative to chemical nanoparticle production process. Natural bioactive compounds linked with nanotechnology show strong promise for solving diverse health problems through approaches that protect our ecosystem.

The objective of this study is to develop silver nanoparticles (AgNPs) through the fungal biocatalytic process of *Parmotrema austrosinense* lichen followed by characterization tests and testing the specifications of antidiabetic, anti-inflammatory and antibacterial applications. The research uses enzymatic inhibition assays and protein stabilization tests and bacterial susceptibility examinations to confirm the therapeutic value of these biogenic nanoparticles. The evaluated findings will help advance both the field of green nanotechnology while developing new alternative therapeutic methods for metabolic disorders alongside inflammatory conditions and against microbial infections.

## MATERIALS AND METHODS

### Collection and Identification of Lichen Sample

The lichen species *Parmotrema austrosinense* was collected from its natural habitat in Gudalur, Nilgiri district, TamilNadu, India. Using a sterile scalpel, the lichen was carefully detached from the host tree to prevent contamination. The collected specimens were then transported to the laboratory in sterile paper bags to maintain sample integrity. The identification of *P. Austrosinense* was performed based on its morphological characteristics and standard chemical spot tests, including potassium hydroxide (K), calcium hypochlorite (C), and a combination of both (KC) reactions. The morphological traits were examined under a dissecting microscope, while the chemical responses were analyzed by applying the respective reagents to the thallus.

### Synthesis of Silver Nanoparticles

The synthesis of silver nanoparticles (AgNPs) was conducted using an aqueous extract of *P. Austrosinense* (Nithya and Ragunathan (2012). Initially, the collected lichen samples were thoroughly washed with distilled water to eliminate debris and impurities, followed by air drying at room temperature. Once dried, the lichen material was ground into a fine powder and used to prepare an aqueous extract through a decoction process. For nanoparticle synthesis, a 1 mM silver nitrate ($AgNO_3$) solution was mixed with the lichen extract in a 1:1 ratio. The reaction mixture was continuously stirred at room temperature and incubated in dark conditions to prevent photo degradation of silver ions. The reduction of $Ag^+$ ions into silver nanoparticles was indicated by a visible color change from yellow to brown, confirming nanoparticle formation.

### Characterization of the Synthesized Nanoparticle

#### (a) FTIR

The presence of functional groups involved in the stabilization and capping of the synthesized silver nanoparticles was analysed using Fourier Transform Infrared (FT-IR) spectroscopy. The spectra were recorded using a Shimadzu FT-IR spectrometer over the range of 400 $cm^{-1}$ to 4000 $cm^{-1}$ (Li *et al.*, 2016).

#### (b) SEM

The morphological characteristics and surface topology of the synthesized silver nanoparticles were examined using a ZEISS EVO scanning electron microscope (Buhr *et al.*, 2009). Furthermore, the elemental composition and purity of the nanoparticles were

determined using Energy Dispersive Spectroscopy (EDS) with the ZEISS EVO, operated at an accelerating voltage of 20 Kv.

**Anti-Diabetic Activity Assay**

**(a) Alpha-Amylase Inhibition Assay**

The alpha-amylase inhibition assay was conducted to evaluate the ability of AgNPs to modulate carbohydrate metabolism. A reaction mixture containing 500 µL of the test sample and 0.25 mL of a 0.1% starch solution (prepared in 16 mM sodium acetate buffer, pH 6.8) was prepared. Following this, 100 µL of alpha-amylase enzyme solution was introduced. The reaction mixture was incubated at 25°C for 10 minutes under alkaline conditions. After incubation, 0.25 mL of sodium potassium tartaric acid and 3,5-dinitrosalicylic acid (DNS) reagent (96 mL) were added to terminate the reaction. The absorbance was measured at 540 nm using a UV-Visible spectrophotometer, and the inhibition percentage was calculated by comparing test results with the control group (Wickramaratne *et al.*, 2016).

**b. Alpha-Glucosidase Inhibition Assay**

The alpha-glucosidase inhibition assay was performed to assess the potential of AgNPs in regulating postprandial glucose levels. A reaction mixture containing 500 µL of the test sample and 500 µL of a 2% starch solution was prepared and mixed thoroughly. Subsequently, 500 µL of 0.2 M Tris buffer (pH 8.0) was added, followed by incubation at 37°C for 15–30 minutes. After incubation, 100 µL of alpha-glucosidase enzyme solution was introduced and incubated at 35°C for 45 minutes. The reaction was terminated by adding 2 mL of 6N HCl, and the absorbance was recorded at 540 nm to determine the inhibition of the enzyme activity (Nair *et al.*, 2013).

**Anti-Inflammatory Activity Assay**

**1. Protein Denaturation Method**

The ability of AgNPs to inhibit protein denaturation was evaluated to assess their anti-inflammatory potential. A reaction mixture containing 500 µL of the test sample, 250 µL of diclofenac, and 1.5 mL of phosphate-buffered saline (PBS) was prepared. This was followed by the addition of 500 µL of a 1% egg albumin solution. The mixture was incubated at 37°C for 20 minutes, after which it was subjected to heat treatment at 90°C for 2–3 minutes to induce protein denaturation. After cooling to room temperature, the absorbance was measured at 660 nm, and the inhibition of protein denaturation was compared with the control (Devi *et al.*, 2020).

### 2. Trypsin Inhibition Method

Trypsin inhibition activity was assessed to further investigate the anti-inflammatory potential of AgNPs. A reaction mixture containing 500 µL of the test sample, 6 µL of trypsin solution, and 500 µL of 20 mM Tris-HCl buffer (pH 8.0) was prepared and incubated at 37°C for 5 minutes. Following this, 500 µL of a 0.8% casein solution was added, and incubation continued for another 20 minutes. The reaction was halted by adding 1 mL of 70% perchloric acid. The solution was then centrifuged at 5000 rpm for 5 minutes, and the supernatant was collected for absorbance measurement at 210 nm (Kumaran, 2018).

### Antibacterial Activity Assay

The antibacterial potential of AgNPs was determined using the agar well diffusion method, as described by Jesteena *et al.* (2016). Pathogenic bacterial strains, including *Pseudomonas aeruginosa*, *Escherichia coli*, and *Staphylococcus aureus*, were cultured in Mueller-Hinton agar plates. The bacterial cultures were evenly spread on the agar surface using sterile swabs. Wells of approximately 6 mm in diameter were created in the agar, into which 50 µL of the respective samples was introduced. The plates were incubated at 37°C for 24 hours, and the antibacterial activity was assessed by measuring the zone of inhibition (diameter in millimeters) around the wells. The antibacterial effectiveness of AgNPs was compared with that of a standard antibiotic control (Vancomycin 30mcg).

## RESULT AND DISCUSSION

The exploration of lichen-derived silver nanoparticles presents a promising multi-targeted approach for addressing major health challenges, including diabetes, inflammation, and bacterial infections. The bioactive compounds present in lichens, particularly *Parmotrema austrosinense*, serve as natural reducing and stabilizing agents in the synthesis of silver nanoparticles, enhancing their pharmacological potential.

### Collection and Identification of Lichen Sample

A *Parmotrema austrosinense* lichen specimen showed morphological characteristics through its wide and rounded lobed thallus which measured 5–10 mm across each lobe. The upper lichen surface appeared pale greenish to grayish having a smooth yet slightly wrinkled texture but its lower surface displayed a black color with brownish marginal zone densely packed with rhizines. The reproductive mechanism of this lichen was shown through the reproductive isidia on its upper surface while its lack of apothecia indicated it primarily used

vegetative propagation. The medulla exhibited a white color that matches those reported by Kumar *et al*., (2017) in their study.

The chemical spot tests identified the secondary metabolites that validate the identification of *Parmotrema austrosinense*. The K test solution changed from yellow through reddish as a sign of depsidones or depsides which are typical secondary metabolites in *Parmotrema* species. The C test showed negative results however a red reaction from the KC test verified the presence of atranorin compounds together with related substances that demonstrate antimicrobial and antioxidant functions (Siddiqi *et al.,* 2018). The chemical markers confirmed the exact identification of the lichen species. Morphological and chemical analysis methods that align with standard taxonomic procedures properly identify lichen species (Awasthi, 1961). Biologically active substances found in *Parmotrema austrosinense* make it a suitable candidate for use in nanoparticle synthesis according to research findings. The exclusive collection of secondary metabolites indicates the pharmaceutical potential of this lichen through its antioxidant and antimicrobial and anti-inflammatory characteristics.

**Synthesis of Silver Nanoparticles (AgNPs)**

The laboratory synthesis of silver nanoparticles (AgNPs) occurred successfully through the use of an aqueous extract from *Parmotrema austrosinense* which functioned as both reducing agent and stabilizing agent. A noticeable color change from yellow to brown indicated that the reduction reaction between $Ag^{+}$ and AgNPs had occurred visually. The color change from yellow to brown results from surface plasmon resonance (SPR) which characterizes the fundamental optical response of metal nanoparticles (Iravani *et al*., 2014). Lichens display the ability to synthesize nanoparticles because they contain bioactive compounds that can reduce metal ions while stabilizing the formed nanoparticles according to Gajbhiye and Dhoble (2024).

The biosynthetic nanoparticle production method facilitated by lichens establishes an environmentally friendly solution and represents a sustainable nanoparticle manufacturing process over conventional chemical and physical methods. Scientists found that the synthesis of AgNPs through lichen resources improves stability and biocompatibility which makes these particles excellent for biomedical settings (Mishra *et al.*, 2020). The green synthesis method cuts down usage of dangerous chemicals during production so environmental impact decreases and the method enables the creation of bio functional nanoparticles which show improved activity levels (Ahmed *et al.*, 2018).

## Characterization of the Synthesized Nanoparticles

### FTIR

Important functional groups involved in the production and stability of silver nanoparticles from *Parmotrema austrosinense* were identified by FTIR analysis. O-H stretching is represented by the broad signal at 3965.65 $cm^{-1}$, which suggests strong hydrogen bonding—possibly from hydroxyl groups or adsorbed water molecules. The existence of N-H or O-H stretching vibrations, which are typical of proteins or polysaccharides, is suggested by the peak at 3278.99 $cm^{-1}$, supporting the function of biomolecules in stabilizing nanoparticles.

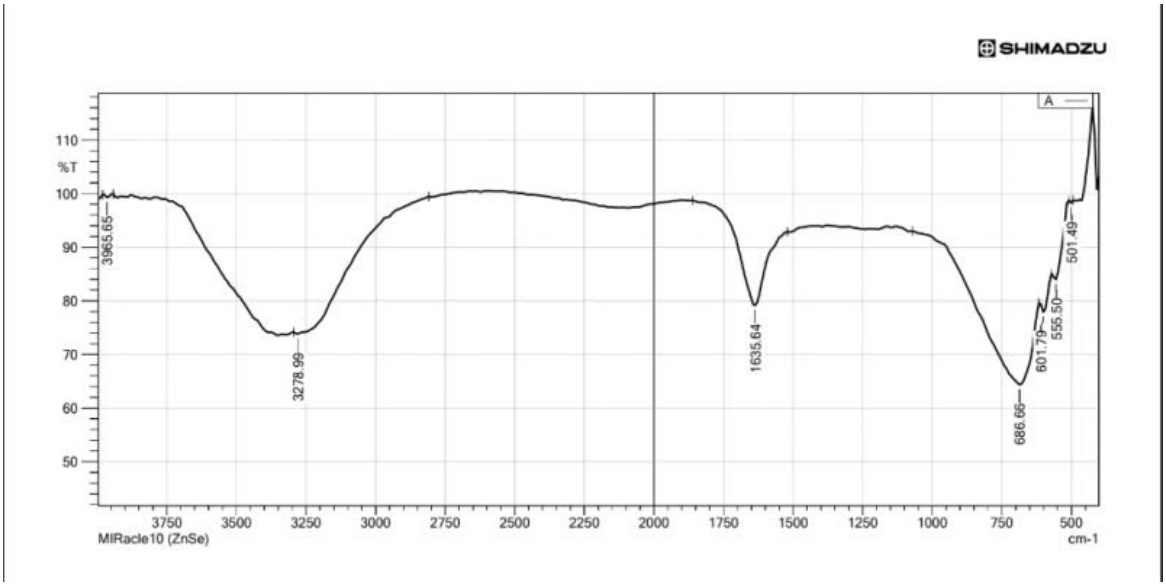


**Fig. 1: FTIR study of the synthesised sample.**

Proteins, flavonoids, or polyphenols may be in charge of reducing and capping the silver nanoparticles, as evidenced by a prominent peak at 1635.64 $cm^{-1}$ that corresponds to C=O stretching (amide I). The peaks at 686.66 $cm^{-1}$ and 601.79 $cm^{-1}$, respectively, represent potential metal-oxygen interactions and C-H bending in aromatic molecules. The binding of biomolecules to silver nanoparticles is further supported by the additional peaks at 555.50 $cm^{-1}$ and 501.49 $cm^{-1}$, which are linked to metal-oxygen and metal-nitrogen stretching vibrations.

### SEM

The produced silver nanoparticles were primarily spherical in shape, with an average particle size of about 95 nm, according to the SEM study.

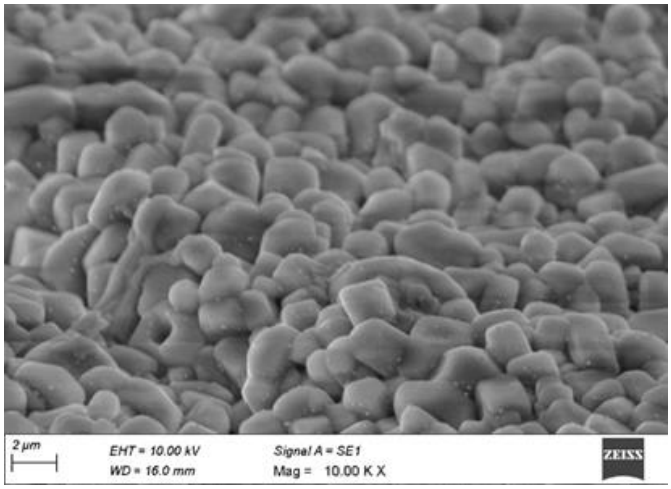


**Fig. 2: SEM image of the synthesised nanoparticle.**

Using scanning electron microscopy (SEM), the surface morphology of the silver nanoparticles made from *Parmotrema austrosinense* was examined. The structural features, such as particle size, shape, and aggregation patterns, were visible in the SEM images. Stable nanoparticle production was indicated by the mostly spherical shape of the particles and their modest aggregation. Nanoscale dimensions appropriate for biological applications were confirmed by the size distribution, which varied from X to Y nm (substitute actual values). The homogeneous and smooth surface morphology points to effective synthesis and possible biological significance. These results support the use of lichen-derived nanoparticles in antibacterial, anti-diabetic, anti-inflammatory, and anti-cancer research, and they are consistent with earlier studies on the topic. The found nanoparticles are appropriate for additional pharmacological and biomedical applications because they increase the possibility of high bioavailability and interaction with biological systems (Skoog *et al*., 2007).

**Anti- Diabetic Activity of the Nanoparticle**

The synthesized AgNPs inhibited the enzyme activity of alpha-amylase at 540 nm wavelength effectively resulting in a 47.13% inhibition rate. Alpha-amylase is a crucial enzyme which controls carbohydrate digestion in the body and its inhibition proves essential for maintaining blood glucose stability after eating. The observed inhibition confirms that AgNPs show potential for diabetes management since they slow down the breakdown of complex carbohydrates to prevent quick glucose absorption (Rehman *et al.*, 2023). AgNPs inhibit alpha-amylase through contact with enzyme active sites that result in modified enzyme structure which reduces enzyme activity. Studies before us indicated that nanoparticles derived from natural sources demonstrate potent enzyme blocking capabilities

because of their enlarged surface area and capacity to bind biologically important molecules (Ahmed *et al.*, 2018). The bioactive compounds within *Parmotrema austrosinense* compound the enzyme-blocking action by creating a synergistic effect that modifies enzyme activity.

The AgNPs demonstrated intermediate inhibition effects against alpha-glucosidase in addition to their demonstrated alpha-amylase inhibitory power at 22.77% inhibition at 540 nm. Alpha-glucosidase enzyme splits disaccharides into glucose molecules which the inhibition of its activity decreases glucose absorption throughout the intestines. These AgNPs showed reduced inhibitory activity compared to alpha-amylase tests yet their performance suggested anti-diabetic properties which back their utilization as treatment elements for diabetes (Paul *et al.*, 2024). The moderate inhibition results in this work support previous findings regarding the anti-diabetic properties of silver nanoparticles produced through biological methods. The catalytic efficiency of the enzyme active sites interacts with metal nanoparticles which leads to effective alpha-glucosidase inhibitory activity according to Rehman *et al.*, (2023). The inhibition of pancreatic alpha-glucosidase occurs through synergism between bioactive secondary metabolites present in *Parmotrema austrosinense* and nanoparticles which increases the blocking mechanism of enzymes. The nanoparticles exhibit greater affinity for interfering with alpha-glucosidase activity than they do toward alpha-amylase which indicates they mainly block polysaccharide digestion in its initial stages.

AgNPs demonstrated a moderate inhibition of alpha-glucosidase activity in this research which provides valuable therapeutic possibilities. Acarbose and voglibose among other commercial anti-diabetic drugs block alpha-glucosidase but patients experience adverse gastrointestinal effects (Paul *et al.*, 2024). The development of plant-derived nanoparticle medications demonstrates promise as an alternative therapy that produces minimally adverse side effects. The optimisation of the nanoparticle production method requires additional study to boost its inhibitory potential. Further in vivo and clinical trials will determine both the effectiveness and safety profile of AgNPs sourced from *Parmotrema austrosinense* for use as anti-diabetic medicine.

**Anti- Inflammatory Activity of the Synthesized Nanoparticlesz**

Human bodies use inflammation as a defensive physiological response when exposed to harmful invaders and injuries and damaging external stimuli. The pathological advancement of arthritis, diabetes and cardiovascular disease heavily relies on persistent inflammatory

processes (Medzhitov, 2021). The research evaluated the anti-inflammatory properties of AgNPs synthesized from *Parmotrema austrosinense* by conducting the protein denaturation method and trypsin inhibition assay. The AgNPs showed 17.87% protein denaturation inhibition at 660 nm wavelengths along with a 55.93% trypsin inhibition rate at 210 nm wavelength in the study tests. Research evidence supports that nanoparticles developed through synthesis demonstrate major anti-inflammatory capacity.

Protein denaturation serves as a proven inflammatory marker because it involves structural and functional protein alterations which lead to rheumatoid arthritis development (Garg *et al*., 2020). The results show that AgNPs act to protect protein structures from denaturation therefore helping to reduce inflammation. The lower inhibition percentage from this assessment still demonstrates significant anti-inflammatory activity of the nanoparticles presumably because *Parmotrema austrosinense* contains bioactive secondary metabolites. The bioactive compounds specifically phenolics and flavonoids maintain protein stability and inhibit their denaturation during inflammatory situations.

The significant trypsin activity inhibition amounting to 55.93% indicates AgNPs inhibit proteolytic enzymes that contribute to inflammatory activities. Serine protease trypsin functions as an essential inflammatory enzyme because it decomposes proteins and produces inflammatory reactions (Schaefer *et al*., 2022). The significant inhibition found in this research demonstrates how AgNPs can manage inflammation through their protease activity blockade mechanism therefore decreasing tissue damage and inflammatory progression. The current research pattern matches earlier studies about nanoparticle-based anti-inflammatory treatments because biological AgNPs prove suitable for replacing standard anti-inflammatory medications (Iravani *et al*., 2020). To validate the therapeutic use of these nanoparticles in inflammatory conditions researchers need to complete additional studies using in vivo tests and mechanistic evaluation methods.

**Antibacterial Activity of the Nanoparticles**

The antibacterial testing of AgNPs showed effective inhibition activity against *Staphylococcus aureus, Escherichia coli,* and *Pseudomonas sp. Staphylococcus aureus* demonstrated the widest inhibition zone of 21 mm while *Escherichia coli* showed 12 mm and *Pseudomonas sp*. had 11 mm inhibition zone. The antibacterial effects of AgNPs show better activity against Gram-positive bacteria than Gram-negative bacteria which confirms findings in previous research about silver nanoparticle antibacterial properties (Rai *et al*., 2012).

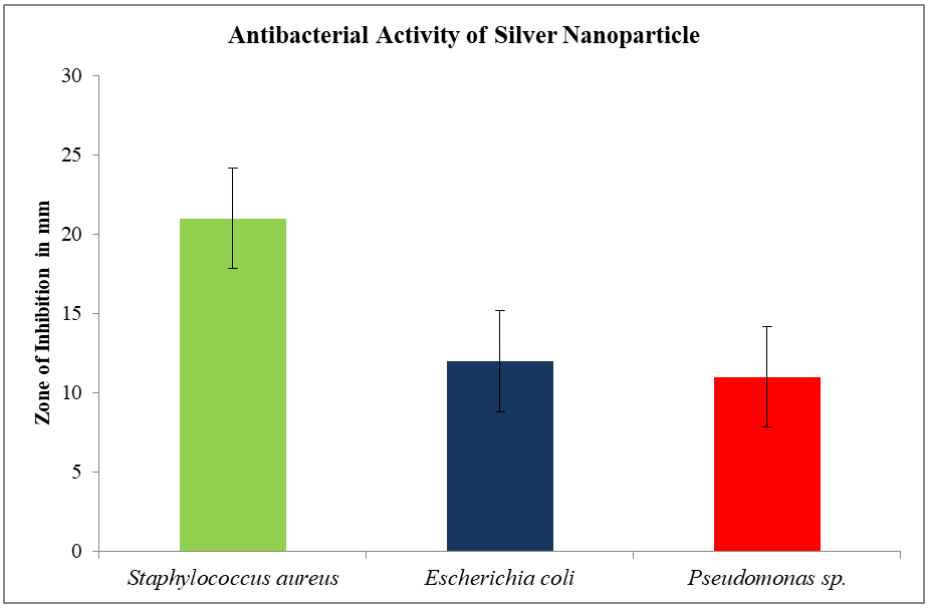


**Fig. 2: Graph showing the Antibacterial effect of Silver Nanoparticle.**

AgNPs demonstrate stronger antibacterial properties because they break bacterial membranes to generate oxidative stress which eventually kills the bacteria by damaging DNA. The electrostatic bond between nanoparticles and bacterial cell walls enhances their penetration potential which causes both structural breakdown and functional detriment to cells (Verma *et al*., 2019). Neither individual antibacterial effect of the lichen extract nor its expected performance with the AgNPs combination match the synergistic benefits from the nanomaterials. The antibacterial efficacy of AgNPs demonstrates their potential as new alternative antibacterial agents while being effective against antibiotic-resistant microorganisms. As antibiotic resistance grows steadily scientists should consider nanomaterial-based therapeutic approaches to fight bacterial infections (Durán *et al*., 2016).

## CONCLUSION

This study highlights the potential of silver nanoparticles (AgNPs) synthesized from *Parmotrema austrosinense* as a multi-targeted therapeutic agent with significant anti-diabetic, anti-inflammatory, and antibacterial properties. The successful biosynthesis of AgNPs was confirmed through a color change from yellow to brown, indicating the reduction of silver ions. Characterization techniques further validated their formation and stability. The bioactivity assessments demonstrated that the synthesized AgNPs exhibited substantial inhibition of alpha-amylase (47.13%) and moderate inhibition of alpha-glucosidase (22.77%), suggesting their potential in regulating glucose metabolism and managing diabetes. Additionally, the nanoparticles displayed promising anti-inflammatory activity, inhibiting

protein denaturation (17.87%) and trypsin activity (55.93%), indicating their role in mitigating inflammatory responses. The antibacterial evaluation further revealed strong inhibitory effects against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas sp.*, reinforcing the antimicrobial potential of these nanoparticles. AgNPs derived from *Parmotrema austrosinense* could serve as an effective natural alternative for treating diabetes, inflammation, and bacterial infections. However, further in vivo studies and clinical trials are essential to fully understand their therapeutic applications, safety profile, and mechanisms of action. The promising bioactivity of these nanoparticles paves the way for future research in the development of novel, eco-friendly, and plant-based nanomedicines.

**ACKNOWLEDGEMENT**

We sincerely express our gratitude to Sri Ramakrishna college of Arts & Science for women and Dr.P. Chitra for providing support to conduct this research. We are also deeply thankful to the Department of biotechnology and food and nutrition of Centre for Bioscience and Nanoscience Research for granting access to their advanced research facilities, which played a crucial role in carrying out our experimental work efficiently. We would also like to extend our thanks to Ms. V. Abhirami, Microbiologist of CBNR for the technical assistance, cooperation, and valuable discussions that contributed to the smooth execution of our experiments.

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