

Research Article

Phytogenic Silver Nanoparticles: Green Synthesis for their Dual Bioactivity against Microbes and Malaria Parasites

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Abstract

Keywords

Silver Nanoparticles
(AgNPs),
Green Synthesis,
Chloris virgata,
Anti-microbial activity,
Anti-plasmodial activity,
Phytogenic synthesis,

Chloris virgata, a resilient grass of the Poaceae family, is widely distributed in warm climates and traditionally used for wound healing and livestock fodder. Its unique phytochemical composition and stress-tolerant nature have recently drawn interest for nanobiotechnology. In the present study, an aqueous extract of *C. virgata* was employed for the green synthesis of silver nanoparticles (AgNPs). Reduction of Ag⁺ ions from silver nitrate was achieved using thermal and microwave treatment, evidenced by a colour change from light red to dark brown. Characterization through UV-Vis, FTIR, XRD, SEM, and HR-TEM confirmed the formation of crystalline, predominantly spherical AgNPs with sizes ranging from 5.7 to 52.5 nm. The nanoparticles exhibited antimicrobial activity against Gram-positive and Gram-negative bacteria, fungi, and *Plasmodium falciparum*, producing inhibition zones of 8–28 mm with MIC values of 62.5–1000 µg/mL. Though less potent than standard drugs, their broad-spectrum efficacy highlights *C. virgata*'s promise as a sustainable source for nanomedicine.

Introduction

Chloris virgata is a grass species appreciated by livestock; it provides good fodder, but is not productive. Medicinal: Crushed leaves of *Chloris virgata*, which are then soaked in water, are applied to wounds to prevent infection. *Chloris virgata* belonging to the Poaceae family, is a weed of warm weather in the eastern part of Australia, and its occurrence can be easily observed throughout the mainland of Australia. Resistance or tolerance to glyphosate, dispersal through flooding and wind, and greater production of seeds are factors that result in the prevalence of *Chloris virgata* in both non-cropping and cropping seasons (Mahajan G et al. 2021, Rojas-Sandoval 2022). Different transcriptomic analyses were performed on *Chloris virgata*, which reported the major genes responsible for its fastest growth and germination, especially in the major grasslands of Mongolia (Bolortuya B 2021). *Chloris virgata* is an alkali-resistant halophyte that thrives under conditions of high buffer capacity and pH (pH 9.6). Therapeutically, it has shown an equal zone of inhibition against *Escherichia coli*, *Salmonella typhi*, *Proteus mirabilis*, and *Klebsiella pneumonia* due to the availability of different phyto compounds. After soaking in water, the crushed leaves of *Chloris virgata* can be applied to wounds to prevent infection. According to the literature survey, details about the authentication, use of this plant as a source of phyto compounds, or antioxidant activity are available. (Dolly Kumari et al. 2024)

Nanoparticles are defined as particles with at least one dimension ranging from 1 to 100 nanometers. Owing to their extremely small size, nanoparticles exhibit unique physical, chemical, and biological properties that differ significantly from those of their bulk counterparts (Kumar et al., 2019; Bhushan, 2017). These distinct characteristics primarily arise from the increased surface-area-to-

volume ratio and quantum effects, which become prominent at the nanoscale.

The enhanced surface area of nanoparticles, sometimes up to 35–45% greater than that of larger particles, enables superior reactivity and interaction with biological and chemical environments (Saqib et al., 2022; Azam et al., 2020). This attribute is particularly beneficial in various scientific fields, such as molecular biology, physics, organic and inorganic chemistry, medicine, and materials science. Owing to these advantageous properties, nanoparticles have been extensively applied in areas such as drug delivery, imaging, catalysis, environmental remediation, and electronic device fabrication (Vu et al., 2021; Rajapaksha et al., 2023; Doan et al., 2023). Recent developments in nanotechnology have focused not only on producing nanoparticles with precise sizes and shapes, but also on employing environmentally sustainable, cost-effective, and scalable synthesis methods that can be customized for specific end uses.

Silver nanoparticles (AgNPs) are highly versatile nanomaterials with unique physical, chemical, and biological properties. Their well-documented anti-microbial activity makes them widely used in medicine, textiles, water purification, and wound healing applications. (Lu, 2016; M.C. Gangorde, 2024) Beyond anti-bacterial uses, AgNPs play key roles in drug delivery, cancer therapy, biosensing, and diagnostics, offering benefits like improved drug stability, targeted delivery, and controlled release. Their optical and electrical properties also support applications in electronics, such as conductive inks and sensors, as well as in consumer products, such as cosmetics and food packaging. (Emelda Orlando Simbine et al., 2019; Nemčková K et al., 2025; Y.Z.N. Htwe, 2022; Gomeceria, 2024; Gajbhiye et al., 2016) Among various metallic nanoparticles, silver nanoparticles (AgNPs) have garnered significant attention due to their exceptional anti-microbial, optical, and catalytic properties. Multiple

synthetic routes have been established for the production of AgNPs, broadly categorized into chemical, physical, photochemical, and biological (or green synthesis) methods (Barabadi et al. 2017b; Yaqoob et al., 2020).

In this research, we have represented the green synthesis of silver nanoparticles by reducing the silver ions present in the solution of silver nitrate (AgNO_3) by an aqueous rootless plant extract of *Chloris Virgata*. Silver nanoparticles (AgNPs) were successfully designed and generated by treating AgNO_3 solution at 70°C for 30 min. and then placed in a microwave for 5 min at 2450 MHz power levels ranging from 350 W to 850 W. The colour change of the plant extract of *Chloris virgata* treated solution from light brown to dark brown confirmed the formation of silver nanoparticles, and the nature of the synthesized nanoparticles was characterized by UV-Vis spectroscopy, FT-IR(Fourier Transform Infrared spectroscopy), X-ray diffraction, SEM(Scanning

Electron Microscopy), and HR-TEM(High Resolution Transmission Electron Microscopy). AgNPs exhibit excellent anti-bacterial properties. This study provides a significant basis for applying AgNPs as antimetabolites and medicated AgNPs to play a major role in the field of nanomedicine.

Materials and Methods

Collection of the material

Chloris virgata is widely found in the Dang and Tapi districts. Plant samples were collected from the campus of the Government Science College, Songadh, Tapi district. (Lat-21.12 and Long. 73.40.) the whole plant was used without roots, and the plant was washed, shade-dried, and powdered in a kitchen grinder. Silver nitrate was purchased from Sigma–Aldrich. Here fig.1 indicates *Chloris virgata* plant.



Fig. 1 *Chloris virgata* plant

Method

Preparation of aqueous plant extract.

Fresh whole plants without roots were collected and washed with double distilled water, shade-dried, and pulverized using a kitchen grinder. Powdered plants (Shawn in fig.2) were stored in airtight containers for future use in the green



Fig.2 Powder form of *Chloris virgata* plant



Fig.3 Extract reaction apparatus

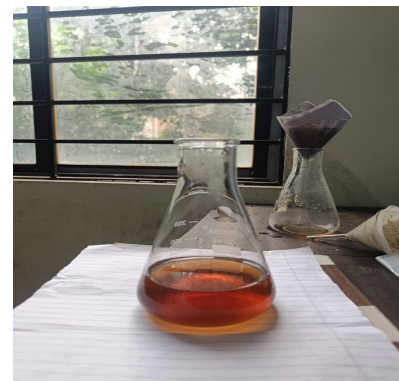


Fig.4 Plant extract

Synthesis of AgNPs using of aqueous plant extracts

For the bio-mediated synthesis of silver nanoparticles, 40 ml of 1 mM AgNO_3 salt solution was slowly added to 25 ml of the plant extracts. After completion of the addition, the reaction mixture was kept on a magnetic stirrer with a hot plate for 30 min. at 70°C and then placed in a microwave for 5 minutes at 2450 MHz power levels ranging from 350 W to 850 W. The colour change was observed between reaction solutions after adding AgNO_3 . The colour change indicated that AgNPs were generated. Here, the colour changed from red to dark brown. The reduction of Ag^{+1} to Ag^0 was mediated by the biomolecules present in the plant, which acted as reducing and capping agents. (Kabeya et al., 2025; Do et al., 2025; Liknaw et al., 2025; Abd El Aty et al., 2025; Plesnicute et al., 2025; Singh et al., 2025) and in vitro anti-plasmodial activity. (Kojom Foko et al., 2025; Nafa et al., 2025)

synthesis of the silver nanoparticles. Plant powder (15 g) was added to double-distilled water and shaken thoroughly. The prepared mixture was refluxed for 2 h 30 min at 60°C (Shawn in fig.3) and the hot mixture was filtered through Whatman filter paper no. 1. The aqueous extract (Shawn in fig.4) was refrigerated at 4°C and used as a stock solution for AgNPs synthesis.

Further confirmation of AgNPs was performed using UV–Vis spectral analysis with absorbance peaks from 420 to 460 nm. After this confirmation, the reaction mixture was subjected to centrifugation to isolate the silver nanoparticles at 10000 rpm for 30 min to isolate the AgNPs. The separated nanoparticles settled at the bottom, were collected, washed with double-distilled water, and then dried in a hot air oven at 60°C for 1 h 30 min. Finally, the powdered AgNPs were stored at 4°C for further characterization and identification of an anti-microbial zone of inhibition.

Anti-microbial activity

Accurate evaluation of anti-microbial efficacy is essential for pharmaceutical development and clinical microbiology. The choice of method for anti-microbial testing depends on the objective of the study and nature of the test compound. (Hamid LL et al. 2024; CLSI, 2020; R.M. Varghese et al., 2024; S K Sahu et al. 2025)

Zone of Inhibition (ZOI) for Anti-bacterial and Anti-fungal Activity

The Agar Cup Diffusion Method was employed to assess the anti-microbial efficacy of the synthesized compounds. (CLSI 2023; De Mel S, et al. 2025; Ahmad N, et al. 2024; Singh RK, et al. 2025) This is a standardized, non-automated in vitro method widely used to evaluate the susceptibility of microorganisms to anti-microbial agents by measuring the zone of inhibition (ZOI) (in mm), which corresponds to the area around the well where microbial growth is inhibited. (Iwuji C, et al. 2024; Keerthiga G, et al. 2025) The standard microbial strains for gram positive bacteria as *Staphylococcus aureus* (MTCC 86) and *Streptococcus pyogenes* (MTCC 443) for Gram-negative *Escherichia coli* (MTCC 442) and *Pseudomonas aeruginosa* (MTCC 441) were used for evaluating anti-bacterial and for Anti-fungal activity *Candida albicans* (MTCC 227), *Aspergillus niger* (MTCC 282) and *Aspergillus clavatus* (MTCC 1323) were used as test organisms. All strains were procured from the **Microbial Type Culture Collection (MTCC)** at the Institute of Microbial Technology, Chandigarh.

In Vitro Antimalarial Screening

All the synthesized compounds were screened for antimalarial activity in the Microcare Laboratory & TRC, Surat, and Gujarat.

The in vitro antimalarial assay was carried out in 86-well microtiter plates according to the microassay protocol of Rieckmann et al. with minor modifications. The cultures of *P. falciparum* 3D7 were maintained in medium RPMI 1640 supplemented with 25 mM HEPES, 1% D-glucose, 0.23% sodium bicarbonate, and 10% heat-inactivated human serum. (Srinivasa SB et al. 2025; Bestgen B et al. 2025) for observations of the in vitro antimalarial screening of the mean number of rings, trophozoites, and schizonts recorded per 100 parasites from duplicate wells after incubation for 38 h and percent maturation inhibition with respect to the control group.

Results and Discussion

The formation of AgNPs was indicated by a colour change of the solution from light red to dark brown. (Fig.5)

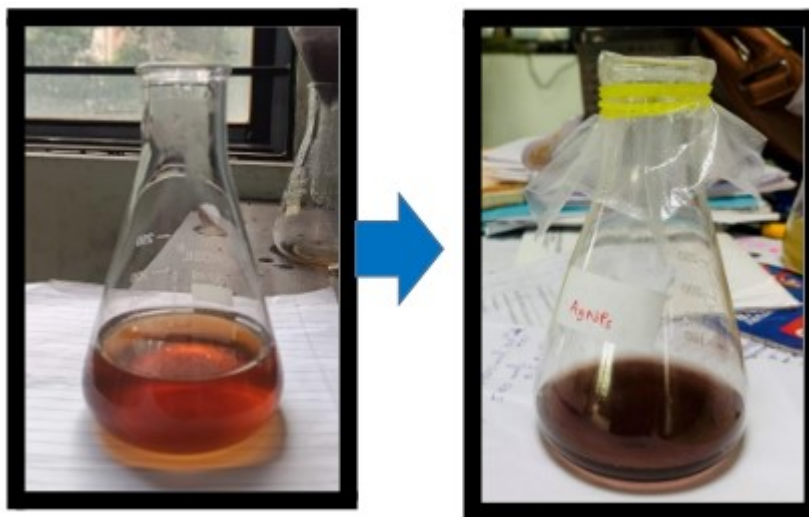


Fig. 5 Difference in the colour change before and after the formation of AgNPs.

UV-Visible Spectroscopy

UV-Visible Spectroscopy analysis was performed using a Varian Cary 50 UV-Vis spectrophotometer. The absorption spectra of the synthesized silver nanoparticles were recorded in distilled water. Fig. 6a shows the UV-visible spectra of AgNO_3 soln., which has an absorption peak at 214 nm. Fig. 6b shows the UV-visible spectra of the plant extracts with an absorption peak at 375 nm. Fig. 6c shows the UV-visible

spectra of silver nanoparticle formation and the colour change of the solution from dark red to dark brown, depending on the concentration of the extract and inclusion of the silver nitrate solution. With time, the concentration of AgNPs increased, and the peak value increased accordingly and became sharper from 420 nm to 465 nm. These results are similar to those of other researchers (Sana et al. 2018; Maarebia et al. 2018; Bali et al. 2025)

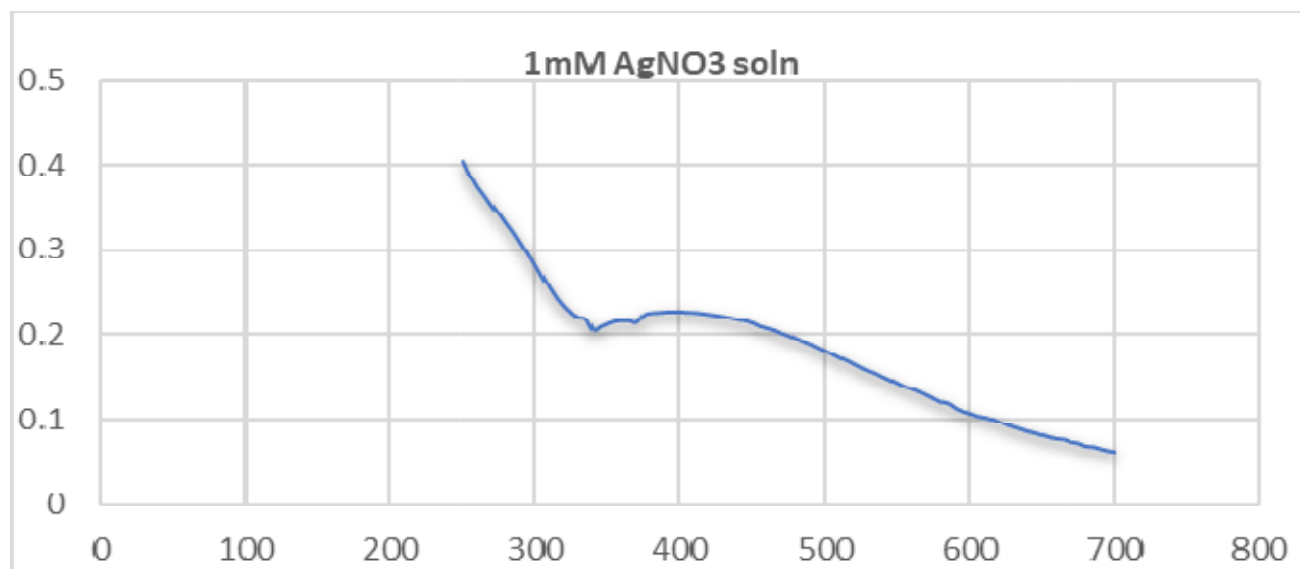


Fig. 6a shows the UV-Visible spectra of AgNO_3 soln.

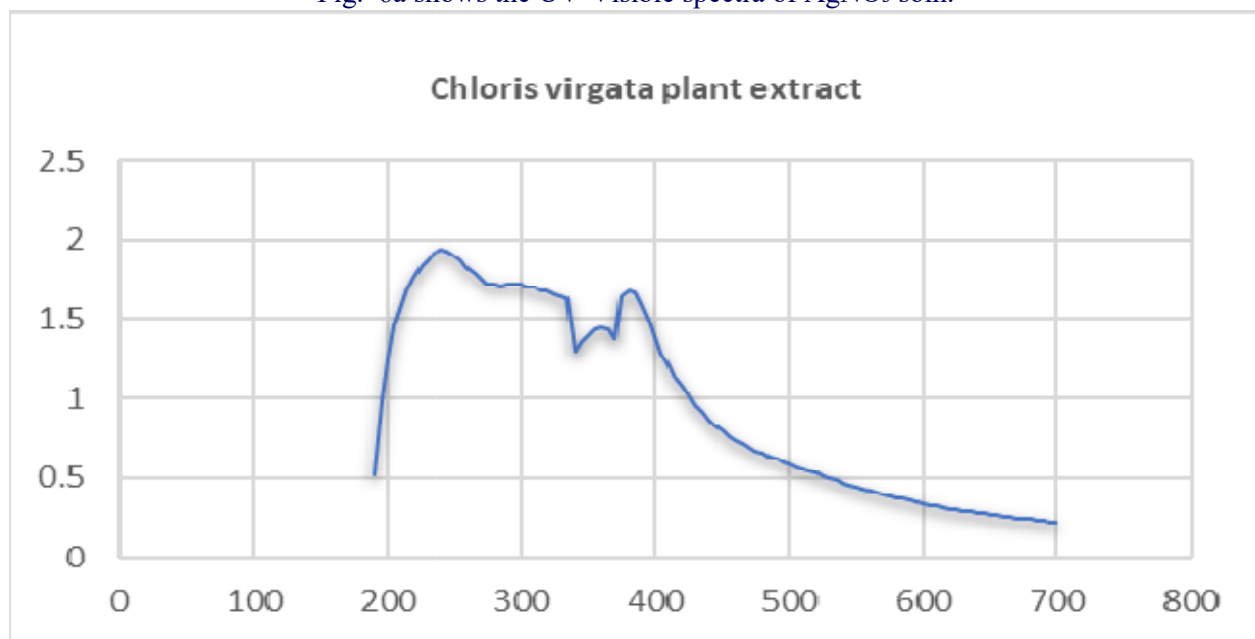


Fig. 6b shows the UV- Visible spectra of plant extracts

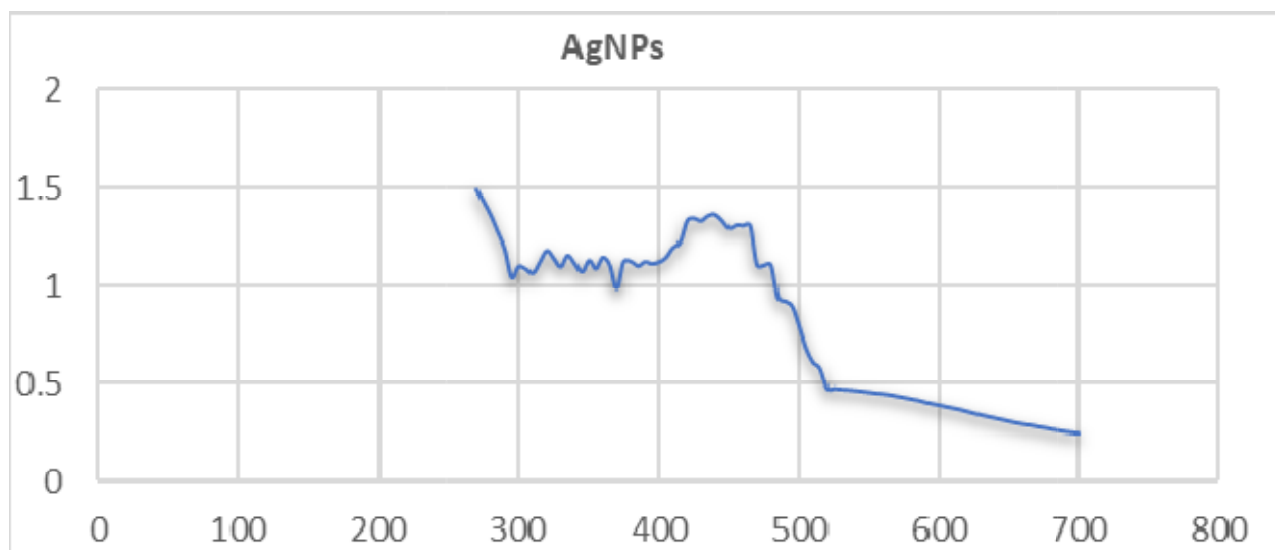


Fig. 6c shows the UV Visible spectra of AgNPs

Fourier Transform Infrared spectroscopy (FTIR)

FTIR Analysis for AgNPs

FT-IR analysis was performed using a Bruker, Germany, Model 3000 Hyperion Microscope with a Vertex 80 FTIR System, ranging from 80 cm^{-1} to 25000 cm^{-1} . Approximately 0.1 ml, or a single drop, of AgNPs was used for preparing liquid

samples in FTIR. The most common method involves sandwiching a thin layer of the liquid between two salt plates (like NaCl or KBr) and subjecting it to FT-IR spectroscopy measurement range set from 400 cm^{-1} to 4000 cm^{-1} . Fig. 7a and 7b show the FT-IR spectra. As like Silver nanoparticles FTIR reference research papers (Riaz et al., 2018; Mamand et al., 2025; Pasiczna-Patkowska et al., 2025).

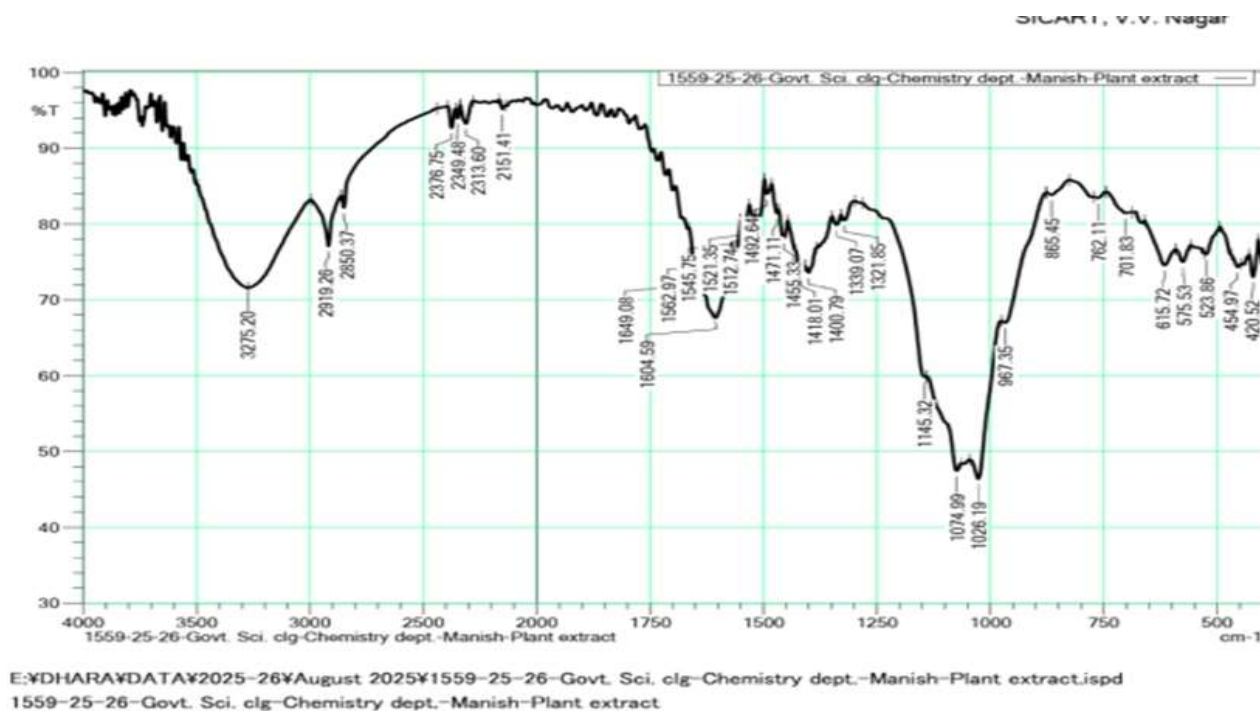


Fig.7a FTIR of Plant Extract

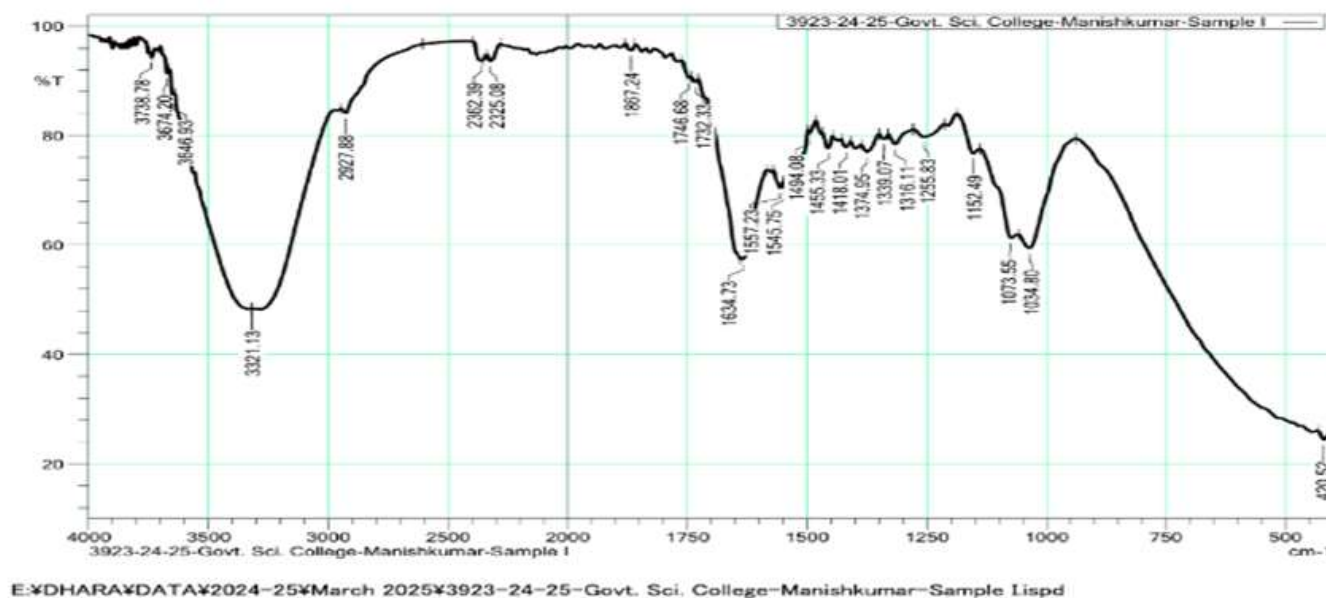


Fig.7b FTIR of AgNPs

Synthesis	Functional Group	Wave number (cm^{-1})
Synthesized AgNPs using <i>Chloris virgata</i>	O-H stretching vibration	3646 cm^{-1}
	typically indicates the presence of O-H or N-H stretching vibrations, often associated with alcohol (O-H) or amine (N-H) functional groups	3321 cm^{-1}
	indicates the presence of C-H stretching vibrations, specifically from alkanes, alkenes, and carboxylic acids	2827 cm^{-1}
	Indicates the presence of amide I band or C=O stretching vibrations, often associated with carbonyl groups in proteins, peptides, or amides.	1634 cm^{-1}
	indicates the presence of C-O stretching vibrations, often associated with alcohols, phenols, and ethers	1073 cm^{-1}
	indicates the presence of Si-O stretching vibrations or C-N bond vibrations	1034 cm^{-1}
	indicates the presence of a Silver-oxygen (Ag-O) bond	420 cm^{-1}

X-Ray Diffraction

X-ray Diffraction (XRD) measurements of the bio-reduced silver nanoparticles was performed using a Bruker Model D8 Advance analytical instrument at 200°C. The sharp diffraction patterns obtained from the XRD spectra indicated that the AgNPs were purely crystalline. Fig. 8 indicates 3 peaks at 2θ. The measurement profile values of 26.718°, 35.686°, and 57.466° correspond to the (hkl) values of the (1,1,1), (1,1,0), and (1,2,2) planes, respectively, which confirm the presence of a crystalline structure in AgNPs. Here, the AgNP hkl values are (1,1,1), which indicates an octahedral crystal form; (1,1,0), which indicates a rectangular or rhombic

shape; and (1,2,2), which indicates a tetragonal crystal structure and 25.23 nm size on the XRD data and calculation using the Scherrer equation.

$$D = ((K \times \lambda)) / ((\beta \times \cos \theta))$$

Where, D: is the average crystallite size (often in nanometers).

K: is the Scherrer constant, a shape factor that depends on the crystallite shape (typically around 0.8 for spherical or equiaxed crystallites).

λ: is the wavelength of the X-ray radiation used.

β: is the full width at half maximum (FWHM) of the diffraction peak, after instrumental broadening is accounted for.

θ: is the Bragg angle of the diffraction peak.

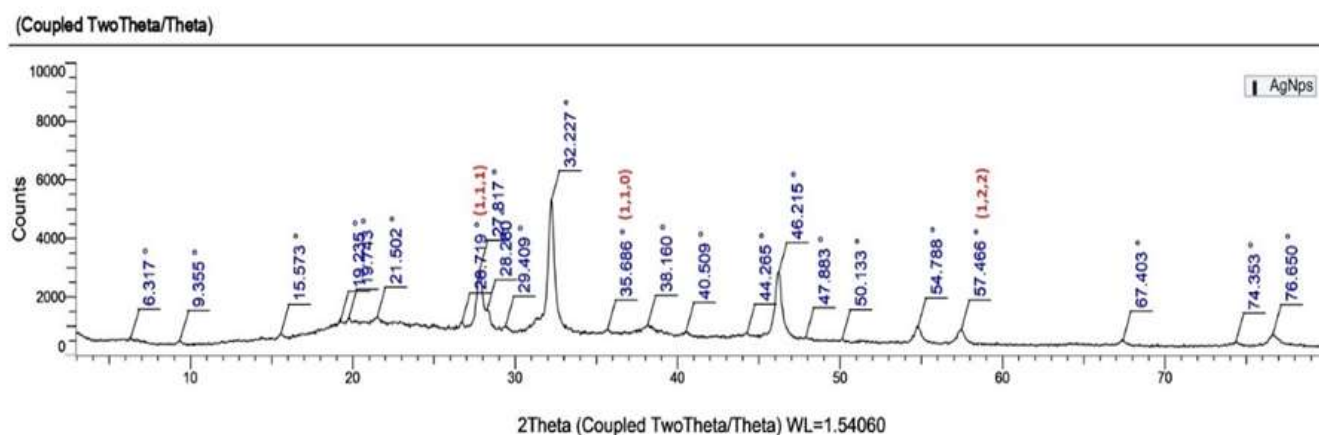


Fig.8 shows XRD of AgNPs

This technique is used to study the structure of materials, particularly crystalline materials. This involves the interaction of X-rays with a sample, resulting in a characteristic diffraction pattern that reveals information about the crystal structure, phase, and other properties of the sample. (R.N. Rai et al.; Karam et al., 2022)

Scanning Electron Microscopy Analysis

Scanning Electron Microscopy Analysis Field Emission Gun- Scanning Electron Microscopic (FEG-SEM) analysis was performed using the

JSM-7600F machine and operating at a voltage of 0.1 to 30 kV; for operation, a very small amount of dry powder sample was required to obtain the types of images shown in Figs. 10a, 10b, and 10c. Scanning electron microscopy analysis was carried out to determine the shape and size of the synthesized AgNPs. (Carter et al., 2025; Aigoïn et al., 2025) Here, see an image of silver nanoparticles, which are not pores or hollow in nature; they possess a completely solid shape. The obtained NPs were spherical in shape and size, ranging from approximately 8.72 nm, according to the XRD data.

FEG-SEM is a type of scanning electron microscope (SEM) that uses a field emission gun (FEG) as its electron source, enabling a higher

resolution and improved imaging capabilities compared to traditional SEMs.

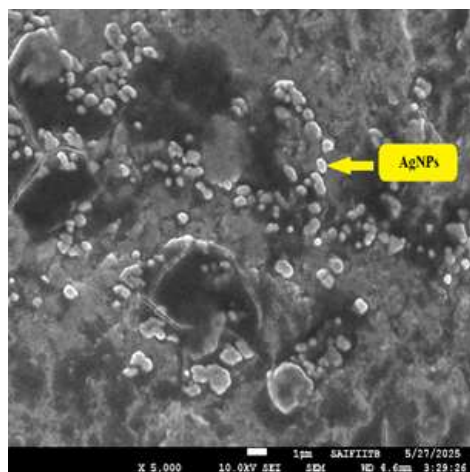


Fig. 9a shows under 1 μ m range AgNPs with 5K resolution

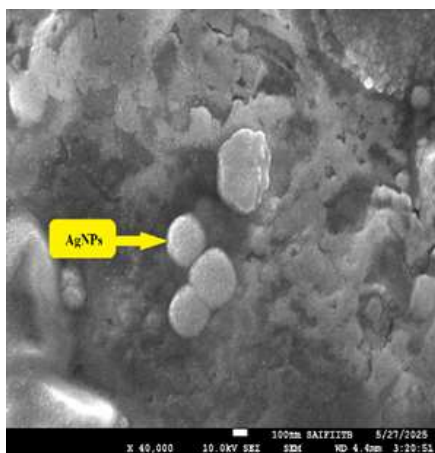


Fig. 9b shows under 100 nm range AgNPs with 40K resolution

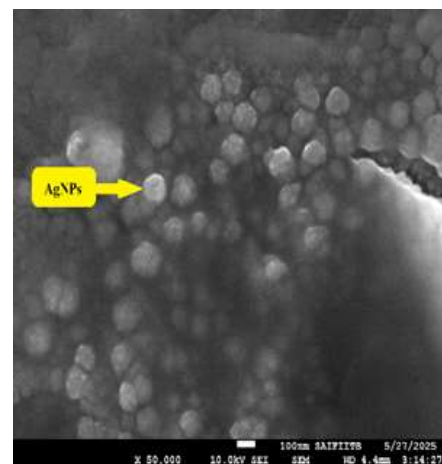


Fig. 9c shows under 100 nm range AgNPs with 50K resolution

High Resolution Transmission Electron Microscopy (HR-TEM) Analysis

This technique uses a focused beam of electrons to create high-resolution images of the internal structure of a material, particularly at the atomic level. (Wang et al., 2015) Here show HR TEM images of silver nanoparticles, which are round in shape, show a completely solid shape in nature.

FEG-TEM at 300 kV (Field Emission Gun Transmission Electron Microscope 300kv) on AgNPs was performed using FEI Tecnai G2 and model number F30. Images of AgNPs synthesized using an aqueous plant extract of *Chloris virgata* shown in Fig.10a, 10b, 10c, 10d, and 10e. The synthesized AgNPs were almost spherical in shape with an average diameter of 5.77 nm minimum value as determined by XRD.

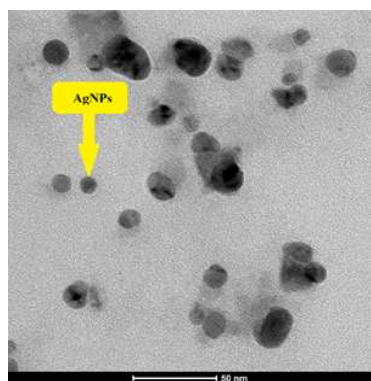


Fig. 10a shows under 50 nm

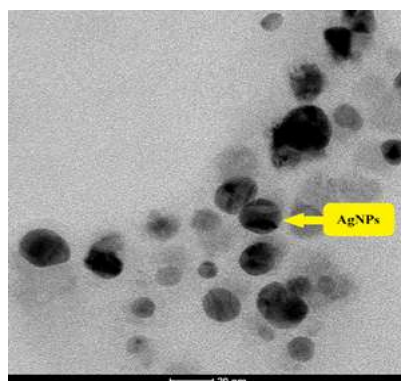


Fig. 10b shows under 20 nm

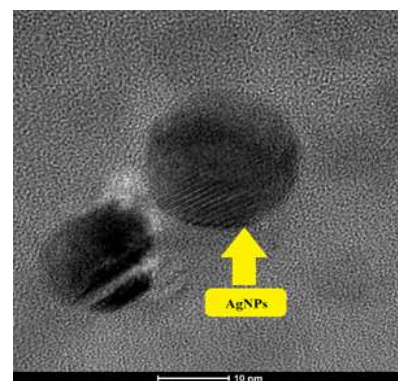


Fig. 10c shows under 10 nm

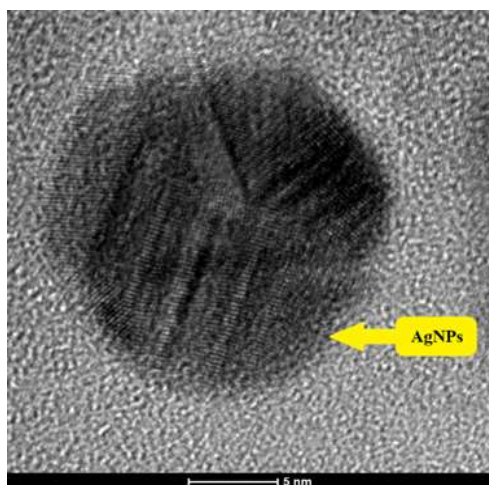


Fig. 10d shows under 5 nm

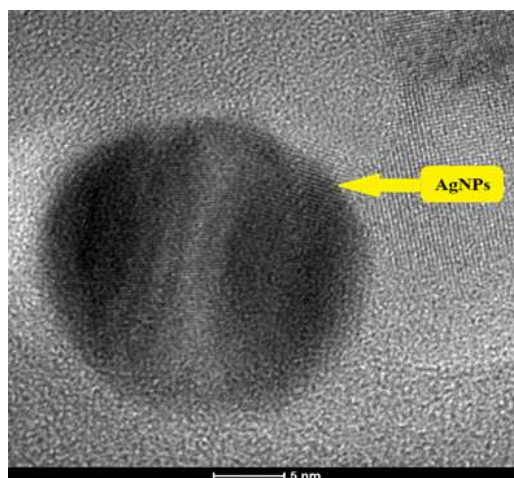


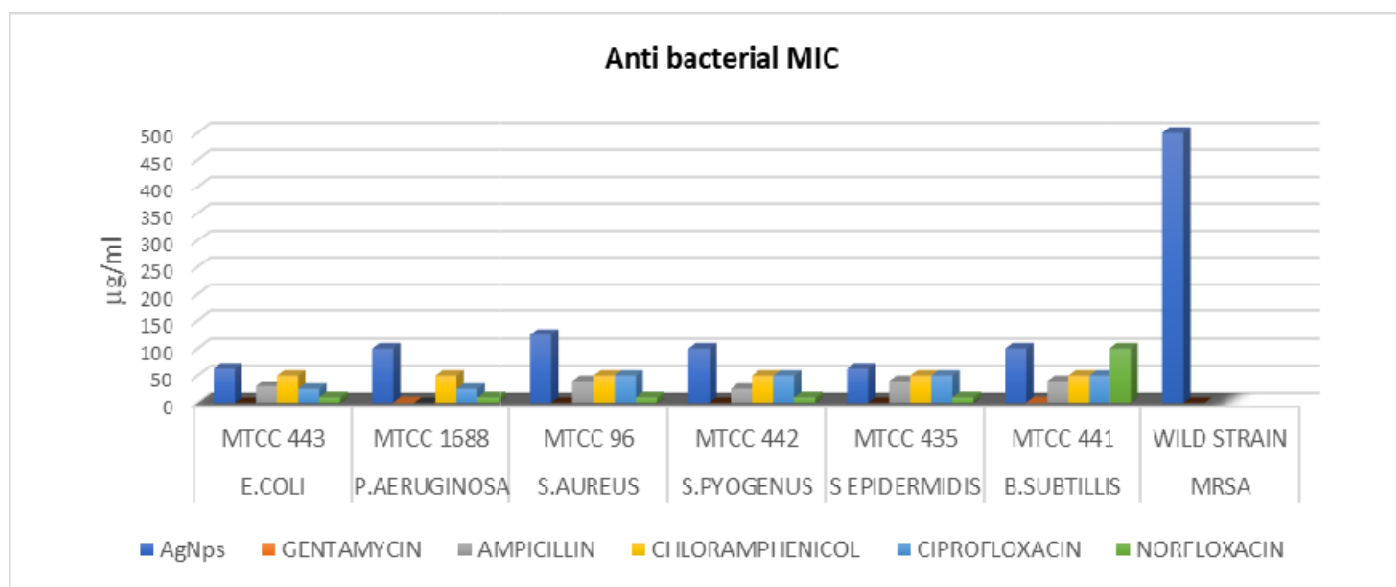
Fig. 10e shows under 5 nm

Biological activities

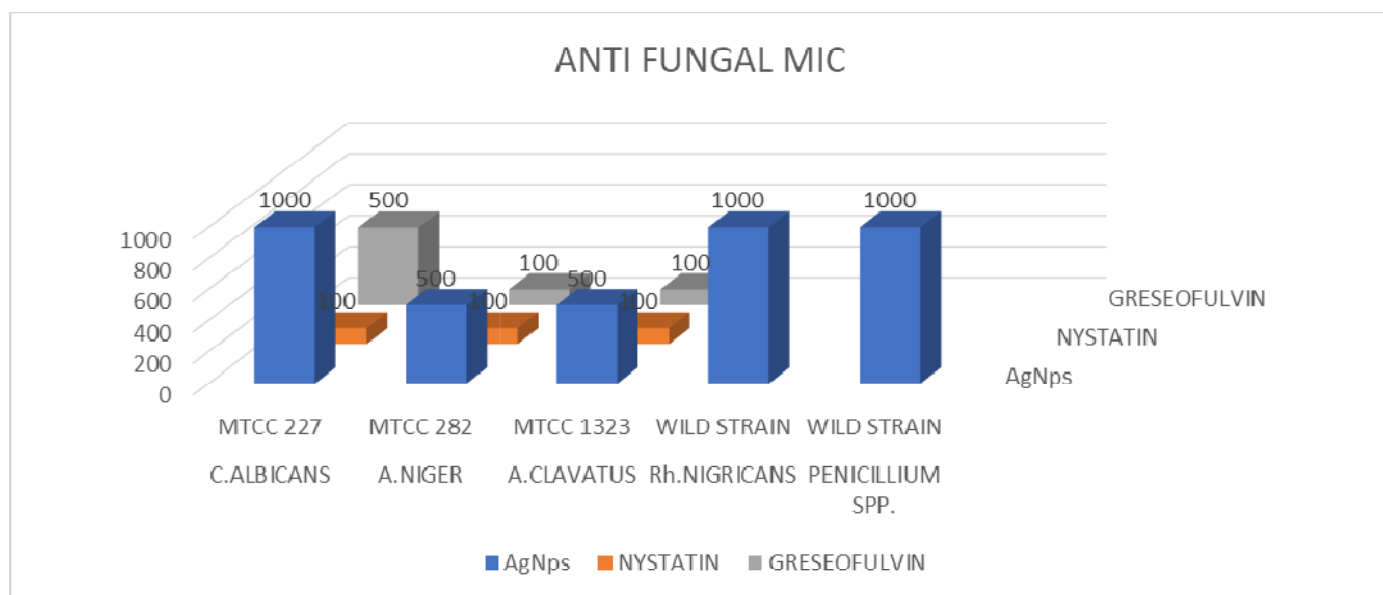
1. Anti-microbial activity

The minimum inhibitory concentration (MIC) was the highest, with at least 88 percent inhibition

zone. The outcome was significantly influenced by the inoculum size. The test mixture contained 10^8 organism/ml.

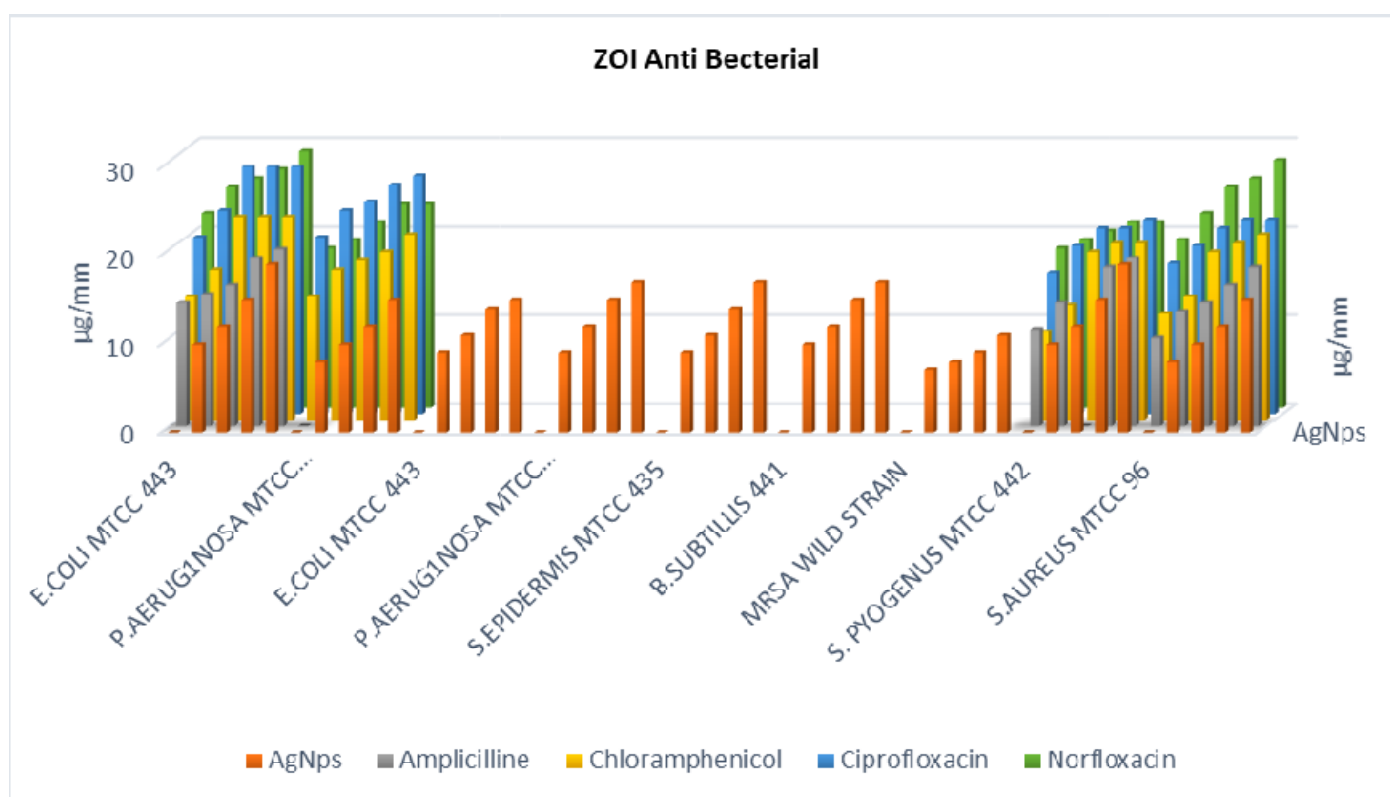


Graph 1: Anti-Bacterial MIC

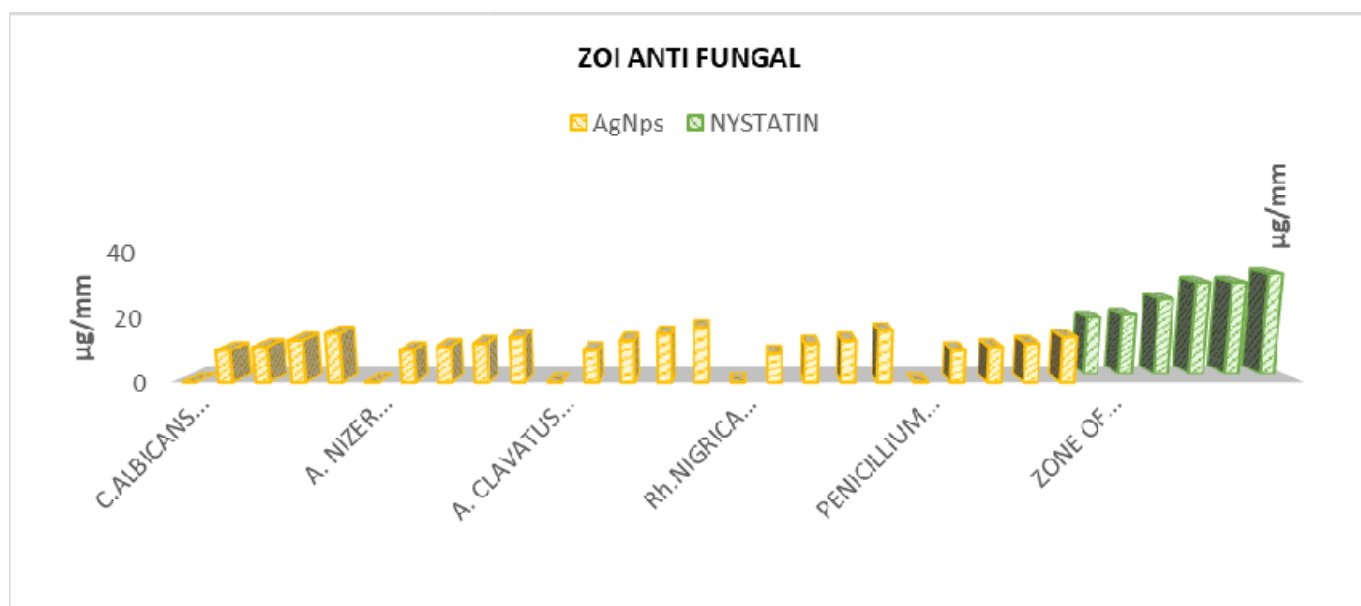


Graph 2: Anti-Fungal MIC

2. Zone of Inhibition (ZOI) for Anti-bacterial and Anti-fungal Activity

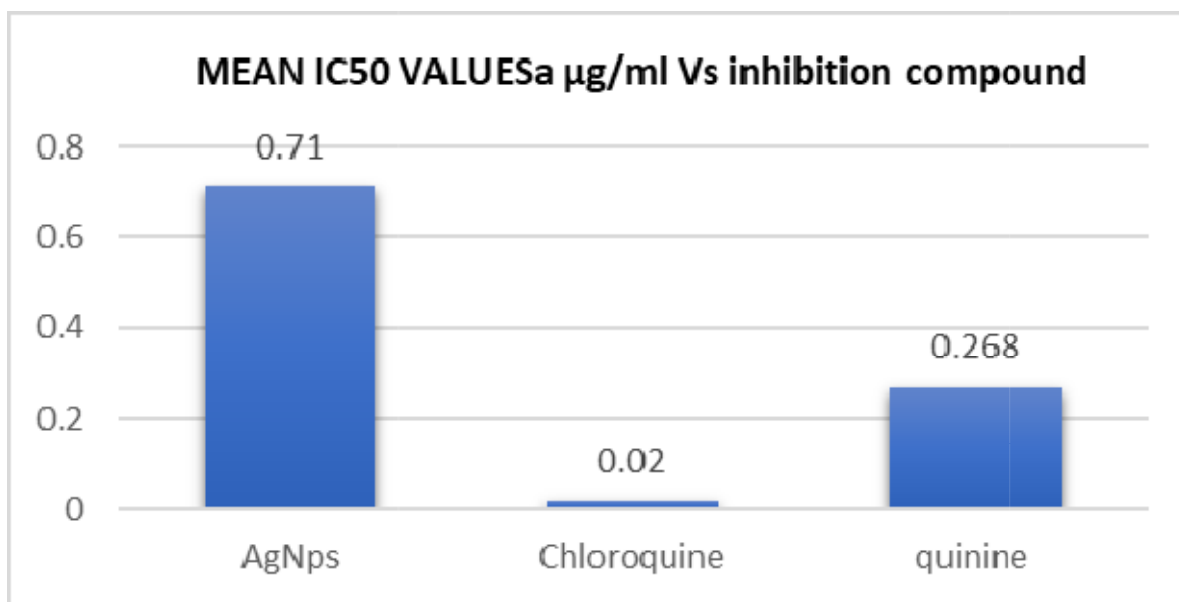


Graph 3: ZOI Anti-Bacterial



Graph 4: ZOI Anti-fungal

3. In Vitro Antimalarial Screening



Graph 5: Anti-plasmodial activity

A safe and easy method for the eco-friendly synthesis of silver nanoparticles using an aqueous plant extract of *Chloris virgata* as a reducing agent under laboratory conditions has been used without the use of poisonous inducers, hazardous chemical additives, and/or elaborate chemical reactions. The formation of AgNPs was detected

visually from a colour change and confirmed using UV-visible spectroscopy (420-465 nm) (Fig.6c) and further authenticated by SEM (Fig. 9a -9c, and XRD (Fig.10). In addition, using FT-IR Spectroscopy, the characteristics of AgNPs were confirmed, as shown in Fig.7b, which showed peaks at 420.52 cm^{-1} wave numbers

confirming the formation of Ag-O bonds. The particle size was determined c.a. as 25.23 nm. This was further confirmed by TEM studies; moreover, the almost spherical shape of the particles was known using transmission electron microscopy, Fig.10a-10e. The silver nanoparticles exhibited greater anti-bacterial activity and Anti-fungal activity; Graphs 1 and 2, Graph 3, Graph 4, and Graph 5 sequentially show ZOI Anti-bacterial, ZOI Anti-fungal, and in vitro anti-plasmodial activity.

In this study, biologically active nanomaterials were designed and developed as potent anti-microbial agents using aqueous extracts from *Chloris virgata* plant, which is an eco-friendly process. The benefit of using this method is that it is less time-consuming, from simple materials obtained from nature to a relatively easy way to synthesize of silver nanoparticles. The Silver Nanoparticles (AgNPs) were characterized by UV-Visible Spectroscopy, FTIR, XRD, SEM, TEM, and their in vitro anti-microbial, ZOI anti-bacterial, ZOI anti-fungal, and anti-plasmodial activities were investigated. UV-Visible spectroscopy results confirmed the formation of the particles. The change in colour of the solution from dark red to dark brown indicated the reduction of Ag^{+1} to Ag^0 . FTIR analysis revealed stretching and vibration bands, confirming the presence of various bonds in the sample. The particle size of the silver nanoparticles is 25.20 nm, which was confirmed by XRD and FEG-SEM analyses. Spherical in shape and in the size range of 5.77–52.58 nm of AgNPs were confirmed by HR-TEM analysis, and all were consistent.

Results for Anti-bacterial Activity using **Minimum Inhibitory Concentration (MIC)** – **Graph 1** shows the efficacy of various anti-microbial agents, including silver nanoparticles (AgNPs), against several bacterial strains. AgNPs exhibited higher MIC values (62.5–500 $\mu\text{g/mL}$), indicating comparatively lower potency than conventional antibiotics. For MRSA (wild-type

strain), AgNPs showed the highest MIC value (500 $\mu\text{g/mL}$), suggesting reduced effectiveness against resistant strains. Conventional antibiotics like Gentamycin and Norfloxacin showed significantly lower MICs (as low as 0.05–1 $\mu\text{g/mL}$), indicating higher potency. Despite this, AgNPs still demonstrated anti-microbial activity against all tested bacteria, albeit at higher concentrations. With **Zone of Inhibition (ZOI)** – **Graph 3** results reinforce the MIC findings, AgNPs produced moderate inhibition zones (8–28 mm), suggesting reasonable anti-bacterial activity. The highest ZOI by AgNPs was against *S. aureus* MTCC 86 (28 mm), whereas the lowest was against MRSA (7 mm). In comparison, standard antibiotics such as Norfloxacin and Ciprofloxacin produced larger ZOIs in many strains, confirming their superior efficacy. Interestingly, AgNPs performed comparably in some strains, such as *P. aeruginosa* and *S. pyogenus*, indicating their potential as supplementary treatments. For Anti-fungal Activity with **Minimum Inhibitory Concentration (MIC)** – **Graph 2** AgNPs exhibited varying Anti-fungal activity across fungal strains. MIC values for AgNPs ranged from 500 to 1000 $\mu\text{g/mL}$, with *C. albicans* and wild strains such as *Penicillium spp.* showing the least susceptibility (1000 $\mu\text{g/mL}$). Nystatin and Griseofulvin had significantly lower MICs (100–500 $\mu\text{g/mL}$), confirming their strong Anti-fungal properties. With **Zone of Inhibition (ZOI)** – **Graph 4** data further confirmed the lower Anti-fungal potency of AgNPs; AgNPs produced ZOIs between 8 and 17 mm, while nystatin showed higher inhibition zones (up to 32 mm). For *A. clavatus* and *R. nigricans*, AgNPs showed promising inhibition, suggesting their potential for targeted applications. However, overall, the Anti-fungal activity of AgNPs was inferior to that of the standard Anti-fungal agents. Results for Anti-Plasmodial Activity show that the anti-plasmodial efficacy was evaluated using IC_{50} values: (Graph 5) AgNPs demonstrated an IC_{50} of 0.71 $\mu\text{g/mL}$, which is relatively higher compared to Chloroquine (0.02 $\mu\text{g/mL}$) and

Quinine (0.268 µg/mL). This indicates that while AgNPs possess anti-plasmodial activity, their potency is significantly lower than that of established anti-malarial drugs. Nevertheless, the observed activity suggests a potential role in combination therapies or improved drug delivery systems.

Conclusion

AgNPs exhibit broad-spectrum anti-microbial activity, although with reduced efficacy compared to conventional antibiotics, Anti-fungal s, and anti-plasmodial drugs. Their moderate ZOI and MIC values indicate their potential as alternative or adjunctive therapeutic agents, especially in light of their increasing anti-microbial resistance and broad-spectrum efficacy. Further optimization of nanoparticle synthesis and formulation could enhance their bioactivity and clinical relevance. These findings support the growing importance of **nanotechnology** in anti-microbial research but also emphasize the need for **further optimization and targeted delivery**.

Acknowledgments

This work is a heartfelt tribute to the author's family, especially to their parents, whose unwavering support and blessings formed the foundation of this achievement. Gratitude is extended to Dr. Kespi Aspi Pithawala for valuable suggestions throughout the research.

Declaration of Funding Acquisition: No funding was received.

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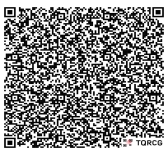
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	Website: www.ijarm.com
	Subject: Nanotechnology
Quick Response Code	
DOI: 10.22192/ijamr.2025.12.11.002	

How to cite this article:

M.C. Gangorde and Vatsal M. Patel. (2025). Phytogenic Silver Nanoparticles: Green Synthesis for their Dual Bioactivity against Microbes and Malaria Parasites. *Int. J. Adv. Multidiscip. Res.* 12(11): 17-34

DOI: <http://dx.doi.org/10.22192/ijamr.2025.12.11.002>