



# *Chlorella minutissima*-assisted silver nanoparticles synthesis and evaluation of its antibacterial activity

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## Abstract

The conventional methods of nanoparticles synthesis led to the production of highly toxic by-products and the use of toxic chemicals that are highly expensive in nature. Thus, the recent past has witnessed a surge in green synthesis of nanoparticles as a sustainable alternative. The present study outlines the biogenic silver nanoparticles (Ag-NPs) synthesis from an aqueous extract of *Chlorella minutissima*. The effect of certain parameters such as the reaction mixture's pH and precursor metal solution to algal extract ratios were explored and optimized. The UV spectrophotometric analysis of Ag-NPs gave surface plasmon response maximally at 426 nm. The developed Ag-NPs were characterized using zeta potential, indicating their high stability (-21.2 mV) with a mean diameter of 73.13 nm. Results from field emission-scanning electron microscopy (FE-SEM) showed that the particles were spherical in shape. Ag-NPs synthesized using *Chlorella minutissima* extract could significantly inhibit the growth of both Gram-positive and Gram-negative bacterial species. The study highlights that using *C. minutissima* extract for Ag-NPs synthesis is a convenient and fast process for controlling the growth of Gram-positive as well as Gram-negative bacteria.

**Keywords** Green synthesis · Nanoparticles · Particles · Nano size · *Chlorella minutissima* · Antibacterial activity

## Introduction

The indiscriminate over exploitation of antimicrobials (antibiotics) over the past few decades has led to a fast-paced emergence of antimicrobial resistance (AMR) among a range of microbial populations. Resistance among these pathogens have led to huge burden on healthcare system and economy [1]. It has been predicted that by 2025, deaths caused by AMR will be more than deaths caused by cancer [2]. Pathogens have developed various resistance mechanisms such as alteration of the target molecules, active efflux of the antimicrobial agents from the bacterial cells, modification of the antimicrobial agents, decreased penetration of the antimicrobial agents into the bacterial cells, degradation of antimicrobial agents and resistance due to global adaptation [3, 4, 5]. Events such as horizontal gene transfer and conjugation also leads to the transfer of resistant genes

among the bacterial species leading to widespread resistance against the existing antimicrobial agents [6]. There have also been reports of bioaccumulation and biomagnification of antibiotics in crops and animals such as fish due to excessive usage of antibiotics [7, 8]. Problems caused by AMR has steered the research to discover novel and effective antimicrobial agents. Over the years nanoparticles have gained focus as an antimicrobial agent because they demonstrate bactericidal effects by directly interacting with bacterial cells and consequently overcome the mechanism of antibiotic resistance adopted by bacterial species [9, 10]. Nanoparticles have various advantages over conventional antimicrobial agents such as preventing the emergence of antibacterial resistant strains, economical, rapid synthesis and environment friendly. The microbicidal activity of nanoparticles can mainly be attributed to the following mechanisms: inducing oxidative stress by elevating the production of reactive oxygen species (ROS) in the bacterial cells, release of Ag<sup>+</sup> ions which interact with cellular components such as DNA, RNA and proteins, and cell membrane disruption [11].

The conventional methodology of nanoparticle synthesis consists of physical and chemical methods such as ball milling, ultrasonication, electrochemical synthesis, laser

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ablation, chemical reduction and sputtering. These conventional methods have several limitations such as production cost, release of harmful by-products and hazardous waste [12]. Thus, the recent past has witnessed a paradigm shift towards the green or biogenic synthesis of nanoparticles. Green synthesis methods are cost-effective, environment friendly as well as are highly sustainable [13]. In the case of biological or green synthesis approach, the extract obtained from organisms such as plants, algae, bacterial and fungal species acts as reducing as well as capping agent for the synthesis and stabilizing of nanoparticles. The reduction of metal ions occurs due to the presence of various biomolecules such as amino acids, proteins, enzymes, polysaccharides, vitamins and other primary or secondary metabolites [14]. Among all the potential candidates of nanoparticles synthesis, plants and bacterial species have been intensively researched upon for their potential of nanoparticles synthesis. Algal species have not received much focus but are promising candidates due to their limited requirement of nutrition, ability to grow on wastewater, limited space requirements and availability of a diverse variety of biomolecules which can act as capping and reducing agents. Several algae such as *Caulepra*, *Bifurcaria*, *Chlamydomonas*, *Chlorella* and *Sargassum* have been recently used for NPs synthesis for applications such as antimicrobial agents and for environmental remediation [15, 16, 17]. Algal mediated synthesis of NPs are obtained using following steps: (1) selection and identification of algal species, (2) harvesting algal biomass, (3) preparation of algal extract by boiling biomass in water or suitable solvent, (4) selection of suitable precursor metal salt solution, (5) preparation of molar salt solution, (6) incubation of algal extract and metal solution for certain period, at suitable temperature and pH [18].

In the recent times, the synthesis of silver nanoparticles (Ag-NPs) has gained new insights due to inherent antimicrobial properties of silver. The application of these Ag-NPs is not limited to therapeutics only and have many biotechnological applications such as to produce biohydrogen, biosensors and as a biocatalyst [19, 20, 21]. Currently the use of Ag-NPs in therapeutics is in developmental phase. Various groups have explored the potential of these Ag-NPs for its antimicrobial properties. Amin in his study demonstrated the use of Ag-NPs synthesized using *Ulva lactuca* as an alternative antimicrobial agent against bacterial and fungal pathogens such as *Bacillus subtilis*, *Klebsiella* sp., *Staphylococcus aureus* with comparable activities as commercially available agents [22]. Ag-NPs derived from *Chlorella* sp has been used as antimicrobial agents against *Staphylococcus aureus*, *Escherichia coli* with zone of inhibition 15 mm and 20 mm respectively [16]. Ag-NPs prepared using methanol extract of *A. millefolium* demonstrated a zone of inhibition of 14.33 mm and 13.67 mm against *S. aureus* and *Pseudomonas aeruginosa* respectively [20]. Fatima et al.,

synthesized Ag-NPs using *Portieria hornemannii* extract and demonstrated its antibacterial activity against fish pathogens *Vibrio harveyi* and *V. parahaemolyticus* [23]. Ag-NPs have also been demonstrated to have potent antiviral effects against SARS-CoV-2 and MS2 phage [24, 25]. In the recent years' scientists have developed "nanoparticles dressing" by embedding Ag-NPs in wound dressing that promotes wound healing as well as controls the growth of microorganisms [26].

The present study aims to synthesize Ag-NPs using algal extract of *Chlorella minutissima*. *C. minutissima* belongs to genus *Chlorella* and phylum Chlorophyta. It is a species of green microalgae which grows profusely over a wide range of light intensity and temperature. *C. minutissima* has been used for wastewater treatment and lipid extraction for biofuel production [27]. Depending upon nutrient availability and environmental conditions, the algal species demonstrates varied mode of nutrition including autotrophic, heterotrophic and mixotrophic modes. The species is a store house of various biochemicals such as carotenoids, amino acids, polysaccharides such as starch, polyphenols, vitamins, astaxanthins, proteins and enzymes [28]. These components serve as both reducing and stabilizing agent during the synthesis of nanoparticles [14]. Ag-NPs synthesized using *C. minutissima* extract were characterized and their antibacterial activity against Gram-positive (*Bacillus cereus*, *Staphylococcus aureus*) and Gram-negative (*E. coli*, *Klebsiella* sp. and *Salmonella* sp.) bacterial species were tested. The impact of certain parameters such as pH and extract to precursor metal salt solution on the synthesis of Ag-NPs has been studied. This study will lead to establishment of algal derived silver nanoparticles-based treatment of antimicrobial resistance.

## Methodology

### Growth media selection

*C. minutissima* culture was obtained from Centre for Conservation and Utilisation of Blue Green Algae, Indian Agricultural Research Institute (IARI), New Delhi-India. The culture of *C. minutissima* was inoculated at 10% ratio in five different growth media in 500 mL flask respectively, BG11: NaNO<sub>3</sub> (17.6 mM), CaCl<sub>2</sub>·2H<sub>2</sub>O (0.24 mM), MgSO<sub>4</sub>·7H<sub>2</sub>O (0.3 mM), K<sub>2</sub>HPO<sub>4</sub> (0.23 mM), Citric acid (0.031 mM), C<sub>6</sub>H<sub>8</sub>FeNO<sub>7</sub>-Ferric ammonium citrate (0.021 mM), Na<sub>2</sub>EDTA (0.0027 mM), Na<sub>2</sub>CO<sub>3</sub> (0.91 mM), Trace element solution (1 mL/L) [16], Zarrouk's medium: NaHCO<sub>3</sub> (162 mM), Na<sub>2</sub>CO<sub>3</sub> (38 mM), K<sub>2</sub>HPO<sub>4</sub> (2.9 mM), NaNO<sub>3</sub> (29.4 mM), K<sub>2</sub>SO<sub>4</sub> (5.74 mM), NaCl (17.1 mM), MgSO<sub>4</sub>·7H<sub>2</sub>O (0.27 mM), CaCl<sub>2</sub>·2H<sub>2</sub>O (0.27 mM) [29], Bold's basal medium (BBM): NaNO<sub>3</sub> (2.94 mM), CaCl<sub>2</sub>·2H<sub>2</sub>O (0.17 mM), MgSO<sub>4</sub>·7H<sub>2</sub>O (0.3 mM), K<sub>2</sub>HPO<sub>4</sub>

(0.43 mM),  $\text{KH}_2\text{PO}_4$  (1.29 mM), NaCl (0.43 mM), Trace element solution [30], Bristol medium (SE Media):  $\text{NaNO}_3$  (2.94 mM),  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  (0.17 mM),  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  (0.3 mM),  $\text{KH}_2\text{PO}_4$  (1.29 mM), NaCl (0.43 mM) [31] and Bold 3N medium:  $\text{NaNO}_3$  (8.82 mM),  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  (0.17 mM),  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  (0.3 mM),  $\text{K}_2\text{HPO}_4$  (0.43 mM),  $\text{KH}_2\text{PO}_4$  (1.29 mM) and NaCl (0.43 mM) [32]. The inoculated medium was incubated in white light chamber with 12 h of illumination followed by 8 h of dark period. The flasks were shaken periodically for proper mixing of algal cells and nutrients. Growth rate in each medium was studied at the interval of 3 days using optical density measured at 600 nm over a span of 18 days and a growth curve was prepared. The medium depicting highest growth rate was selected for the large-scale batch cultivation of *C. minutissima*.

### Preparation of *C. minutissima* extract

*C. minutissima* was cultivated in BG11 medium for 28 days and the cells in the stationary phase were harvested using centrifugation and used for the preparation of aqueous algal extract by boiling with 100 mL deionized water at 80 °C for 10 min. The extract was centrifuged to remove any cell debris. 10 mM silver nitrate ( $\text{AgNO}_3$ ) solution in deionized water was prepared. All reagents used were of analytical grade.

### Synthesis of silver nanoparticles (Ag-NPs) using *C. minutissima* extracts

Microalgal mediated Ag-NPs synthesis is dependent upon various factors such as pH of the reaction mixture, ratio of the algal extract to precursor metal solution, temperature of incubation, presence, or absence of source of illumination, strength of precursor metal salt solution, period of incubation [33]. In the present study, we have optimized a few parameters of synthesis such as pH of the solution and ratio of algal extract to precursor metal solution. The algal extract was incubated with 10 mM  $\text{AgNO}_3$  [16, 17] solution for biogenic-NP synthesis in the ratio of 1:10, 1:1 and 10:1. The pH of the solution was varied from 4–10. The mixture was incubated in light at 3000 Lux at 37 °C. Most of the bio-reduction of  $\text{AgNO}_3$  ions occurred within initial hours of incubation. A change in color of the medium from colorless or pale-yellow to orangish-brown was observed with the progression of time. The final synthesis was performed on the optimized parameters for larger yield.

### Characterization of Ag-NPs

The synthesis of Ag-NPs was confirmed to be a time dependent process through the development of orangish-brown

coloration of the reaction mixture over a period. The initial stage characterization of Ag-NPs was carried out using UV spectrophotometric analysis (Lambda 365, Perkin Elmer) was performed at regular intervals by taking aliquots of the reaction mixture and analyzing at the wavelengths of 200–700 nm. The morphology of Ag-NPs was examined through FE-SEM (ZEISS microscope). The mean diameter and stability of Ag-NPs were confirmed through zeta potential analysis (Zetasizer nano series ZS-Malvern PANalytical) of the Ag-NPs synthesized in the study.

### Antibacterial activity of Ag-NPs synthesized by *C. minutissima* extract

Gram-positive (*Bacillus cereus*, *Staphylococcus aureus*) and Gram-negative (*E. coli*, *Klebsiella* sp. and *Salmonella* sp.) cultures were employed to determine the antibacterial activity of the synthesized Ag-NPs. All the cultures used for study were taken from stock cultures maintained in Luria–Bertani (LB) broth with sterile glycerol. Overnight grown cultures were used to perform the studies. The disc diffusion assay with synthesized Ag-NPs was performed on nutrient agar (NA) plates (biological and technical triplicates were performed) spread with 0.1 mL of overnight grown bacterial cultures. The filter paper discs were supplemented with 10, 20, 30, 40 and 50  $\mu\text{L}$  of the synthesized Ag-NPs respectively, with algal extract and silver nitrate solutions loaded onto the disc as control. The plates were then incubated for 24 h at 37 °C and the zone of inhibition (in millimeters) around the discs were measured and the mean value of triplicated were recorded.

## Results and discussion

The present study aimed to determine the antibacterial activity of Ag-NPs synthesized through the aid of *Chlorella minutissima* extract. The green/biosynthesized Ag-NPs used in this study were characterized using UV–visible spectroscopy, zeta potential analyzer, field emission-scanning electron microscopy (FE-SEM) and X-ray diffraction pattern. Growth medium optimization for *C. minutissima* cultivation and certain parameters such as pH of the reaction mixture and algal extract to precursor metal salt solution has been optimized in the present study and has been presented in the following sections.

### Optimization of growth medium for *C. minutissima*

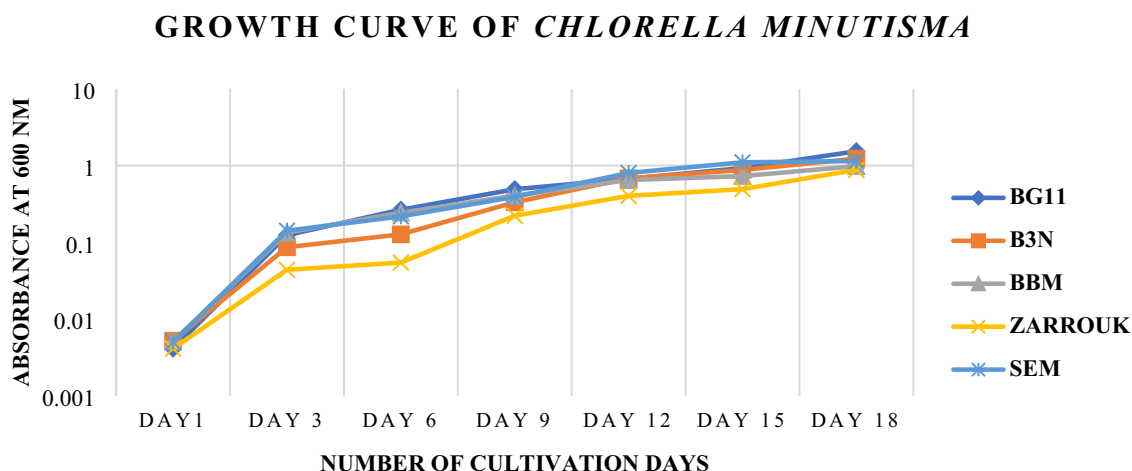
In the present study, growth of *C. minutissima* was assayed on five different media namely BG11, Zarrouk's medium, Bold's basal medium (BBM), Bristol medium (SE Media) and Bold 3N medium. The growth on each media was

analyzed spectrophotometrically at 600 nm on an interval of 3 days continuous for 18 days. The bulk cultivation of *C. minutissima* was done on the medium in which highest growth rate was observed. The growth curve of *C. minutissima* on various media is illustrated in Fig. 1. It was observed that *C. minutissima* demonstrated highest growth rate on BG11 as compared to other media under consideration. Similar results have been demonstrated by Sharma et al. in their study to optimize media for high biomass productivity [34].

### Optimization of Ag-NPs synthesis parameters using *C. minutissima*

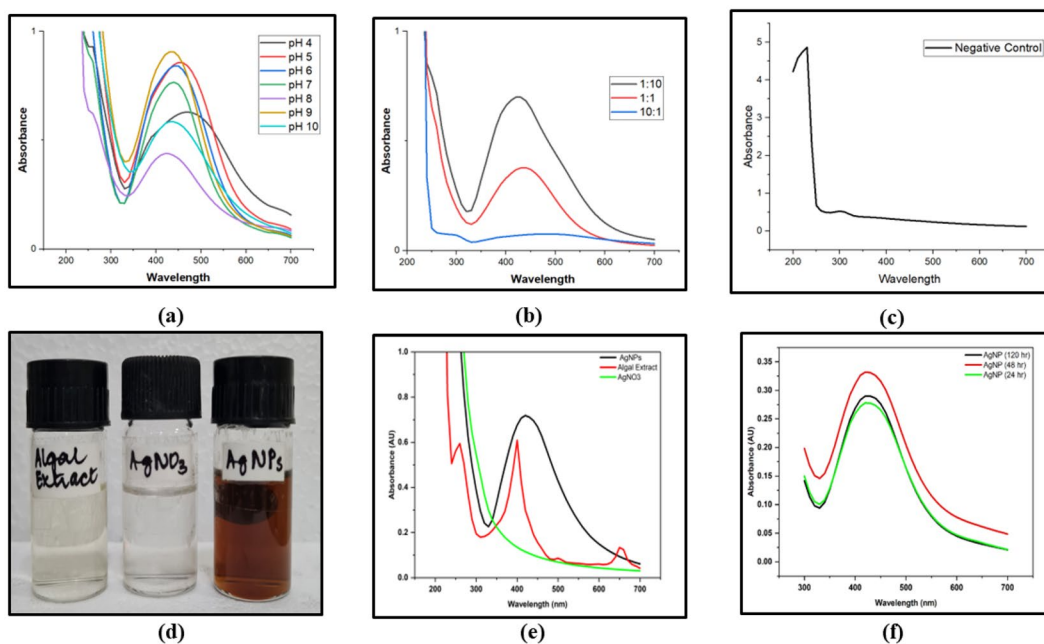
In the present study, different parameters for the synthesis of Ag-NPs such as pH of the solution and ratio of algal extract to precursor metal salt solution were studied and optimized. The screening for optimal synthesis conditions were performed by optimizing one condition at a time. The final bulk synthesis of Ag-NPs was performed at combination of optimized conditions to obtain high concentrations and high stability of Ag-NPs. UV–Vis spectroscopy was employed to track the synthesis and stability of Ag-NPs formed at different conditions and the spectrum thus obtained is illustrated in Fig. 2. The pace of synthesis of Ag-NPs were slower at the initial hours but Ag-NPs were completely synthesized within 24 h of upon incubating aqueous extract of *C. minutissima* with precursor salt solution. The visual color change of the mixture from pale or light green (due to extract) to gradually orangish-brown was found to intensify with the increase in the incubation temperatures due to the excitation of surface plasmon response (SPR) vibrations. The change in color of the solution indicated the synthesis of Ag-NPs [23, 35, 36]. Similar results were shown for the synthesis of Ag-NPs

using microalga *Chlorella sorokiniana* [16]. Factors such as pH of the solution, ratio of algal extract to precursor metal salt solution plays an important role in determining the morphology and stability of Ag-NPs. The spectroscopic analysis at different reaction condition demonstrated a strong SPR between 400 and 450 nm as illustrated in Fig. 2a, b. the available literature has also shown the occurrence of strong SPR in the range of 400–450 nm and thus indicating the presence of Ag-NPs in the solution mixture [17, 37, 38, 39, 40, 41]. pH of the reaction mixture affects the final morphology and structure of the nanoparticles under the synthesis process [16, 17]. The pH range selected for optimization was from 4 to 10. Figure 2a represents the absorbance spectrum of Ag-NPs synthesized at different pH. Ag-NPs formed at the pH 8, 9 and 10 were stable and within the range of 400–450 nm while those formed at pH 4, 5, 6 and 7 were highly unstable and were beyond the range of 400–450 nm absorbance. Among the stable pH 8, 9 and 10; Ag-NPs synthesized at pH 9 demonstrated a high SPR indicating that the reducing agents, capping and stabilizing agents present in the *C. minutissima* extract were highly active at pH 9 [42]. During Ag-NP synthesis, algal biomolecules acts as both reductant and stabilizer. The stabilization of Ag-NPs can be attributed to natural polymers formed by these biomolecules. Lower pH can cause the disruption of these natural polymers and thus destabilizing Ag-NPs [43]. Lower pH of the reaction mixture facilitates large-sized Ag-NPs which degrades easily while the higher pH of the reaction mixture leads to the formation of small-sized Ag-NPs faster which are also highly dispersed throughout the medium [44]. At higher pH, the ionization of algal functional group and reduction rate is higher. The organic functional groups present on the surface of Ag-NPs undergoes higher degree of deprotonation at elevated pH which in turn increases the overall negative charge



**Fig. 1** Growth curve of *Chlorella minutissima* on five different media: BG11, Zarrouk’s medium, Bold’s basal medium (BBM), Bristol medium (SE Media) and Bold 3N (B3N) medium accessed using absorbance at 600 nm





**Fig. 2** Formation of Ag-NPs by *C. minutissima* at different conditions. **(a)** Absorption spectrum of Ag-NPs synthesized at different pH (4–10), **(b)** absorption spectrum of Ag-NPs synthesized by mixing precursor metal solution to algal extract in ratios of 10:1, 1:1 and

1:10, **(c)** negative control: BG11 + AgNO<sub>3</sub> after 24 h of incubation, **(d)** algal extract AgNO<sub>3</sub> and Ag-NPs, **(e)** absorption spectrum of algal extract of AgNO<sub>3</sub> and Ag-NPs and **(f)** stability of Ag-NPs as observed after 24, 48 and 120 h of synthesis

on the surface of Ag-NPs. This increase of negative charge increases repulsion between the Ag-NPs leading to smaller aggregation diameters as compared to Ag-NPs formed in acidic environment [33].

The rate of synthesis of Ag-NPs is directly proportional to the concentration of precursor metal salts, but at the same time the aggregation of Ag-NPs also increases with the increase in the concentration of precursor metal solution. For the synthesis of stable nanoparticles, optimal concentration of the precursor metal solution should be incubated with algal extract solution [16]. In the present study, different ratios of precursor metal salt to algal extract (1:10, 1:1 and 10:1) was incubated and the formation of Ag-NPs were observed through UV–Vis spectroscopy. The formation of highly stable Ag-NPs was observed at 1:10 ratio as evident from the high absorbance peak in between 420–440 nm as depicted in Fig. 2 (b). This observation was due to the rapid scale reduction of Ag<sup>+</sup> ions present in the solution due to the optimal concentration of algal reducing agents present in the solution [16, 17]. there is a decrease in the size of nanoparticles with the increase in the algal extract amount [44].

The Ag-NPs were synthesized at the optimized conditions (Fig. 2d, e) and the stability of the Ag-NPs recorded after 24, 48 and 120 h of synthesis using UV–Vis spectrophotometer and it was noted that the particles formed at these optimized conditions were highly stable as depicted in Fig. 2f. As a negative control, to eliminate the possibilities

of BG11 contributing to the formation of Ag-NPs, 1 mL of 10 mM AgNO<sub>3</sub> was incubated with 10 mL BG11 and the absorbance was recorded after 24 h as depicted in Fig. 2c. The absorbance spectrum suggested no Ag-NPs formation due to BG11. The effect of the incubation period on the biosynthesis of Ag-NPs was also assessed using UV–visible spectrophotometric analysis and the peak of Ag-NPs became sharper with time. The SPR band of the synthesized Ag-NPs at 426 nm even after 120 h of incubation with only a slight variation resembles its stability (Fig. 2f). The increase in intensity can be attributed to the increase in the number of Ag-NPs formed after reduction of Ag<sup>+</sup> ions present in the solution [23, 33, 45]. The optimized condition for the synthesis of Ag-NPs using *C. minutissima* extract was precursor metal salt to extract ratio 1:10, with the pH of the mixture set at pH 9.

### Characterization of Ag-NPs synthesized using *C. minutissima*

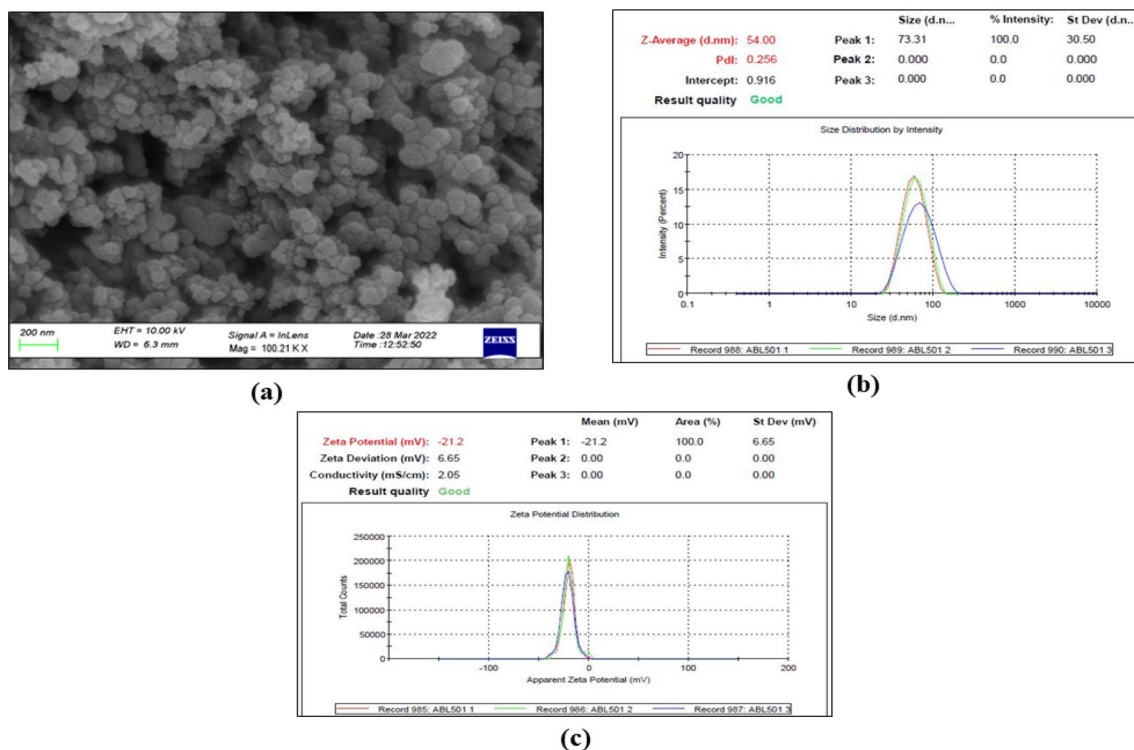
The *C. minutissima* extract-mediated Ag-NPs synthesized at optimum conditions were characterized initially using a UV–visible spectrophotometer. A color change of the reaction mixture was observed from pale green to orangish-brown, which intensified as the reaction proceeded. The absorption spectrum of Ag-NPs demonstrated a broad spectrum at 426 nm, which falls in the range of the characteristic

band of Ag-NPs. Additionally, the synthesized Ag-NPs were characterized using FE-SEM and Zetasizer (zeta potential) as depicted in Fig. 3a, b, c. The FE-SEM imaging further provided insights into the morphology of Ag-NPs. The synthesized Ag-NPs were spherical in nature (Fig. 3a). The mean diameter and stability of Ag-NPs were determined using zeta potential analysis. The mean diameter of Ag-NPs was 73.13 nm and the particles were highly stable, which was indicated by the  $-21.2$  mV value of zeta potential (ZP) (Fig. 2b, c). ZP indicates the stability and size of Ag-NPs contained in the aqueous suspension [16]. ZP is a measure of degree of repulsion between the charged particles and its values ranges between  $+100$  to  $-100$  mV. Higher the ZP, higher is the repulsion which prevents the aggregation of particles. Lower ZP indicates attraction between the particles, leading to coagulation of the dispersed particles [16, 17]. The ZP values between  $\pm 30$  mV is considered to be stable due to attraction/repulsion between the particles of the colloidal solution [46].

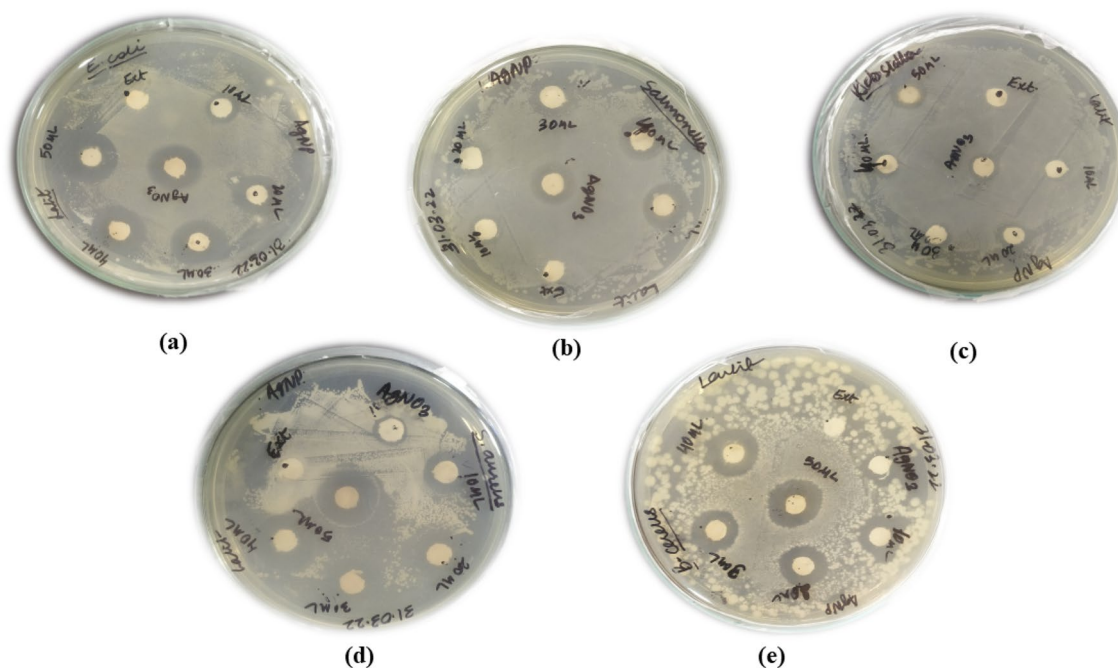
### Antibacterial effect of Ag-NPs synthesized using *C. minutissima*

In the present study, the antibacterial activity of *C. minutissima* extract-derived Ag-NPs was tested against *E. coli*, *Salmonella* sp., *Klebsiella* sp., (Gram negative), *Bacillus*

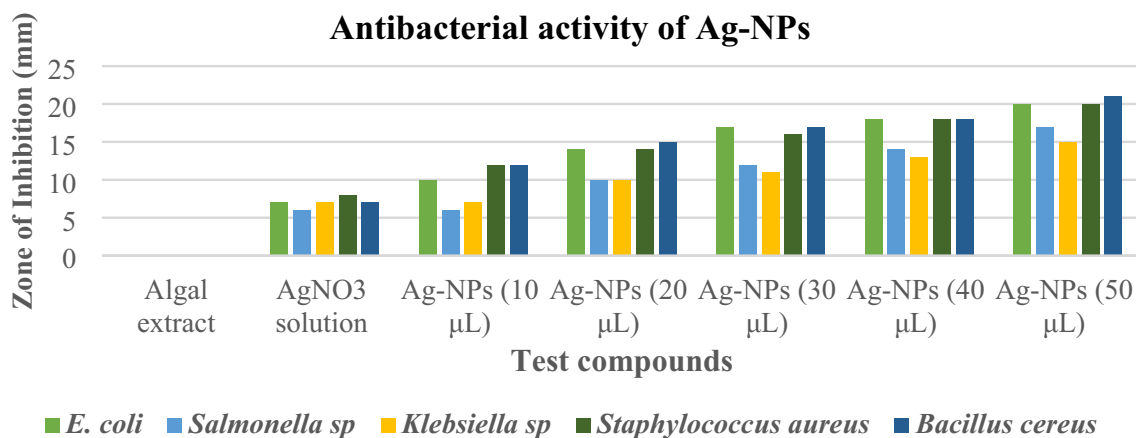
*cereus* and *Staphylococcus aureus* (Gram positive), which are potential human pathogens using disc diffusion assay. The results from disc diffusion assay showed clear zones of inhibition against Gram-positive and Gram-negative bacterial species. The zone of inhibition increased with an increase in the volume of Ag-NPs loaded onto the filter paper disc as depicted in Fig. 4. The maximum zone of inhibition was produced when 50  $\mu$ L of Ag-NPs was loaded. Among the five test bacterial species, Ag-NPs produced a maximum zone of inhibition against *B. cereus* ( $21 \pm 1$  mm), followed by *S. aureus* (20 mm) and *E. coli* (20 mm). Ag-NPs were found to be moderately effective against *Salmonella* spp. ( $17 \pm 1$  mm). *Klebsiella* spp. was found to be least susceptible toward Ag-NPs (Fig. 5). This can be attributed to the presence of the acidic capsule around the bacterial cell wall, which might have prevented the diffusion of Ag-NPs inside the cell and thus the death of bacterium [47]. The algal extract demonstrated no killing of bacterial species and the zone of inhibition was observed against AgNO<sub>3</sub>, which could be attributed to the release of Ag<sup>+</sup> ions by the dissociation of the compound [19, 48]. The effective killing of Gram-positive bacterial species as compared to the negative ones could be explained, due to the complex cell wall composition of Gram-negative cells, which makes the penetration of nanoparticles more difficult to the cytoplasm and thus prevent cell death [11].



**Fig. 3** a FE-SEM analysis of Ag-NPs indicating the spherical nature of Ag-NPs; b size distribution of Ag-NPs observed using zeta potential and c stability of Ag-NPs



**Fig. 4** Nutrient agar plates representing zone of inhibition caused by variable concentration of Ag-NPs, AgNO<sub>3</sub> and algal extract against (a) *E. coli*, (b) *Salmonella* sp., (c) *Klebsiella* sp., (d) *Staphylococcus aureus* and (e) *Bacillus cereus*



**Fig. 5** Graph representing antibacterial activity assessed as zone of inhibition (mm) of algal extract, AgNO<sub>3</sub> and Ag-NPs (10, 20, 30, 40 and 50 μL) against *E. coli*, *Salmonella* sp., *Klebsiella* sp., *Staphylococcus aureus* and *Bacillus cereus*

The bactericidal activity of Ag-NPs can be accredited to the nanoscale diameter of the particles, which can easily penetrate the bacterial cells and kill them. The smaller the nanoparticles, the greater is the surface area, which enables enhanced contact with the cell and its components, leading to enhanced bactericidal activity of the nanoparticles. Nanoparticles employ one or a combination of the following mechanisms to cause cell death: (1) increased production of ROS (reactive oxygen species)—nanoparticles upon crossing the outer membrane may cause an alteration in

electron transport chain located on the inner membrane of the bacterial cell by interaction with the thiol groups of ETC enzymes, leading to increased formation of ROS and other free radicals, which ultimately leads to apoptosis; (2) membrane penetration—nanoparticles tend to penetrate the outer bacterial membrane and get accumulated in the inner membrane, whereupon they destabilize and damage the membrane by increasing the permeability and thus inducing cellular leakage, ultimately leading to cell death; and (3) interaction with cellular components—upon gaining entry

into the cell, nanoparticles interact with the phosphorus and sulfur groups present in DNA, proteins and other subcellular components, leading to an altered structure and function and ultimately to cell death [12, 49, 50]. Ag-NPs can be used in synergy with antibiotics due to the following reasons: help in evading the resistance mechanism adopted by the AMR species, obstruct the formation of biofilms and have various mechanisms of killing and ability to carry antibiotics along with them for enhanced effects [51, 52, 53]. Studies have shown that nanoparticles are efficient in their bactericidal effect against multidrug-resistant (MDR) pathogens such as MRSA (methicillin-resistant *Staphylococcus aureus*) and thus can be highly effective in the treatment of infections caused by MDR strains [51, 52, 53, 54].

## Conclusion

The present study explored the use of *Chlorella minutissima* (green microalgae) extract for the synthesis of Ag-NPs. Stable and high yield of Ag-NPs was achieved at pH 9, with precursor metal salt solution to algal extract ratio of 1:10 and AgNO<sub>3</sub> concentration of 10 mM. A color change in the mixture of algal extract and precursor metal solution to orangish-brown color visually confirmed the synthesis of Ag-NPs. The UV–visible spectrum of Ag-NPs synthesized at optimal conditions depicted a strong SPR at 426 nm, which falls in the range of the characteristic Ag-NPs band. FE-SEM analysis revealed that the Ag-NPs synthesized were spherical in shape with a mean diameter of 73.13 nm, as depicted by zeta potential analysis. The green synthesized Ag-NPs using algal extract obtained from *C. minutissima* proved to be an excellent antibacterial agent against *E. coli*, *Salmonella* sp., *Klebsiella* sp., (Gram negative) *Staphylococcus aureus* and *Bacillus cereus* (Gram positive). Hence, the green synthesis approach using algal extract proves to be an economical, effective and sustainable alternative to conventionally used physical and chemical methods for the synthesis of Ag-NPs. *C. minutissima* can be used for the large-scale production of Ag-NPs. These Ag-NPs can be used for the treatment of wastewater effluent and lowering microbial load. Algae-based synthesis of silver nanoparticles for fight against antimicrobial resistance is a green, cost-effective and sustainable approach.

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**Data Availability** The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

## Declarations

**Conflict of interest** The authors declare no financial and non-financial conflict of interest.

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