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***Tetrademus obliquus*- *Lagosinema tennisi* Consortium-mediated Silver Nanoparticles for Evaluation of Antimicrobial and anti-inflammatory**

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Abstract: The present work demonstrates an innovative, eco-friendly approach towards biosynthesis of silver nanoparticles (AgNPs) utilizing the consortium of two different microorganisms viz. *Tetrademus obliquus* (algae) and *Lagosinema tenuis* (cyanobacterium). The synergistic interaction between these two microorganisms enhances their metabolic efficiency, enabling more effective NPs synthesis than utilizing alone species. This consortium-based approach provides an environmentally friendly, scalable, biocompatible, and economically viable alternative for the biosynthesis of AgNPs with potential applications in biomedical, antimicrobial, and environmental fields. The biosynthesized AgNPs was analysed using various spectroscopic techniques like, XRD, EDS, FTIR, SEM, and TEM, to confirm its development, crystallinity, structure, element identity, particle's shape and sizes. The XRD pattern indicated the cubic crystal lattice of AgNPs, whereas FTIR spectrum showed strong absorptions for O–H, N–H, and C–O or C–N stretching, due to association of proteins and polysaccharides molecules with biosynthesized NPs. The SEM study reveals nearly monodispersed agglomerated spherical morphology having sizes 50-180 nm, while TEM shows monodispersed spherical shape particles having sizes of 8.70 nm. The fabricated consortium-mediated AgNPs was assessed for antibacterial potential and showed better efficacy, producing the highest inhibition zones of 15±0.15 mm at concentrations of 100 µg/mL against *K. pneumoniae*, whereas consistent zones against *P. aeruginosa* and *S. aureus*. Furthermore, AgNPs exhibits significant of antioxidant, anti-inflammatory, and anti-diabetic potential. These results show the effectiveness of the consortium association of microalgal and cyanobacterial species in producing a cheap, eco-friendly green NPs.

Keywords: Green synthesis; AgNPs; Algal-cyanobacterial consortium; Electron spectroscopy; Antimicrobial activity; Anti-inflammatory.

1. Introduction:

Nanoparticles (NPs) play a significant role in modern science and nanotechnology owing to their distinctive physicochemical properties, such as a high surface-area-to-volume ratio, enhanced catalytic activity, and tunable optical and electrical characteristics [1]. In particular, AgNPs demonstrate pronounced antimicrobial and antioxidant activities, in addition to their catalytic properties [2]. As per the review of literature, AgNPs have been produced using hazardous chemical reagents and energy-consuming methods, leading to the formation of resulting in toxic by-products and associated safety concerns [3]. One of the most demanding topics in the pursuit of sustainable alternatives is the use of green synthesis that employ biological systems such as plants, protozoa, bacteria, fungi, algae, and cyanobacteria [4, 5]. However, consortium of microalgae and cyanobacteria have demonstrated exceptional capabilities as a biological nanofactories, reducing metal ions into stable, biocompatible NPs through their intrinsic metabolic and enzymatic pathways [6].

Beyond AgNPs, microalgae and cyanobacteria have been extensively utilized for the biosynthesis of various metal NPs, include gold (Au) [7], platinum (Pt) [8], copper (Cu) [9], cadmium (Cd) [10], zinc (Zn) [11], chromium (Cr), and many more. This indicates how well these organisms are generally suited for biomanufacturing of metallic NPs [12]. For instance, polysaccharide-mediated biosynthesis of Au NPs using macroalgae species such as *Fucus vesiculosus* and *Chondrus crispus*, and similarly Pt NPs was successfully synthesized utilizing cyanobacteria including *Plectonema boryanum*, *Anabaena flos-aquae*, *Calothrix pulvinata*, and *Spirulina platensis* [13]. Cyanobacteria and microalgae are capable of transforming metal ions into their corresponding metallic NPs *via* intra- and extracellular pathways [14]. Besides, the diverse set of bioactive compounds such as amides, enzymes, proteins, pigments, polysaccharides, phenolic compounds, organic acids, etc. have a great role, they acted as a reducing and stabilizing agents during the processes of NPs biosynthesis [15].

Basically, cyanobacteria and microalgae are the photoautotrophic species that are widely distributed across diverse ecosystem and exhibit unique metabolic traits, including nitrogen fixation mediated by nitrogen reductase enzymes [16]. These metabolic properties allow cyanobacteria and microalgae to effectively bio-transform and eliminate heavy metal ions. Furthermore, their secondary metabolites, proteins, enzymes, and pigments also play an important role in the formation and stabilization of NPs [17]. Their rapid growth and high biomass production further support their

potential for scalable NPs synthesis. Owing to their biological efficiency and eco-friendly nature, cyanobacteria and microalgae represent cost-effective and promising nanobiofactories for green nanotechnology applications [18]. Microbial consortia formed by synergistic combinations of bacteria, fungi, microalgae, and cyanobacteria, function as robust biological nanofactories capable of producing NPs, through collective enzyme cascades and metabolite secretions. These collective processes enable the reduction of a wide range of metal ions into stable, polydisperse NPs with higher yield and improved uniformity compared to monocultures [19]. In particular, bacterial consortia involving *Lactobacillus sp.* and *Bacillus sp.* have been shown to synthesize smaller (7.97-14.3 nm) and highly pure Au NPs through enhanced protein-mediated reduction and capping pathways. Likewise, algae-cyanobacteria consortia have been reported to outperform better in that Ag and TiO₂ NPs. These NPs exhibited enhanced antibacterial, antifungal, antioxidant, and anti-inflammatory properties which are primarily attributed to the synergistic activity between polysaccharides and pigments during NPs synthesis and stabilization [20].

Moreover, metabolic complementarity within microbial consortia confers multiple advantages, including the promotion of scalable green synthesis, reduced NPs aggregation, and customized functionality for biomedical and environmental application [21]. These cooperative interactions improve NPs yield, crystallinity, and stability, while allowing precise control over particle size and morphology through dynamic metabolic regulation [22]. Earlier reports demonstrated successfully silver ions biologically reduced into stable silver NPs utilizing algal and fungal cultures [23-24]. Microalgae act primarily as bioreductants by producing phenolic and carbohydrate metabolites capable of reduce metal ions, and cyanobacteria supply enzymatic systems and extracellular biomolecules that contribute to NPs stabilization. Keeping this scenario, the present work is based on a consortium comprising *Tetradismus obliquus* and *Lagosinema tenuis* for fabrication of AgNPs, which enhance functionality, smaller particle size, and biological performance. The resulting physicochemical properties of such NPs are of high quality, as they are produced via a biosynthetic process that integrates the reducing, stabilizing, and enzymatic activities of both partners. Consequently, algal-cyanobacterial consortia represent a novel, scalable, cost effective, and ecologically responsible platform for creating multifunctional nanomaterials (NMs). The present study is demonstrate the green synthesis of AgNPs using algal-cyanobacterial consortium, highlighting their potential as effective biocontrol agents. To the best of our knowledge, this study represents the first consortium of microalgae and cyanobacteria for biosynthesis of AgNPs, highlighting their high biological efficiency and promising application potential. As biosynthesized AgNPs was characterized by several spectroscopic techniques to confirm its phase, structure,

element and morphology. Subsequently, the synthesized AgNPs were assessed for their antimicrobial, antifungal, antibiofilm, anti-inflammatory, antioxidant, and anti-diabetic activities.

2. Materials and Methods:

2.1. Materials:

Analytical-grade silver nitrate (AgNO_3), α -amylase, 2,2-diphenyl-1-picrylhydrazyl (DPPH), bovine serum albumin (BSA) and 3,5-dinitrosalicylic acid (DNS), and BG-11, Mueller Hinton Agar (MHA), Potato dextrose Agar (PDA) were purchased from Hi Media Laboratories, Nagpur, India.

2.2. Algal and Cyanobacterial Culture:

The strains of algae *Tetradesmus obliquus* (PP085426) and cyanobacteria *Lagosinema tenuis* (PX863881) previously reported in earlier work for the biosynthesis of PtNPs [25,26], were utilized from the Postgraduate Department of Microbiology, Seth Kesarimal Porwal College Kamptee, Nagpur, India. Sterile BG-11 nutrient medium (Hi Media, Nagpur) was used to harvest cultures incubated at 24-34 °C with a 12hrs light/12hrs darkness photoperiod at 1500-1700 lux (Digital Lux Meter HI97500, Hanna). Optimized conditions were maintained for growth and biomass creation during the exponential stage of the growth cycle.

2.3. Formation of Algal-Cyanobacterial Consortium:

For the synthesis of AgNPs, the culture of *T. obliquus* and *L. tenuis* harvested on sterile BG-11 nutrient medium was and incubated at room temperature with pH 6.5 ± 0.2 for a 12hrs light/12hrs darkness photoperiod at 1500-1700 lx (Digital Lux Meter HI97500, Hanna). These conditions supported optimal growth during the exponential phase of the growth cycle. Following cultivation, the cultures were diluted in the new deionized water and inoculated in fresh BG-11 medium at a ratio of 1: 10 (v/v) ratio. The inoculated samples were maintained under identical environmental conditions to ensure homogeneous growth and metabolic stability. Consortium formation was initiated after the cultures attained active growth under the same conditions. An algal-cyanobacterial consortium was established by mixing equal volumes (1:1) *T. obliquus* and *L. tenuis* in modified BG-11 medium. To promote co-cultivation and metabolic interaction, the consortium culture was continuously aerated and gently agitated. This co-culture served as a biological matrix for the green synthesis of AgNPs.

2.4. Synthesis of AgNPs using *T. obliquus*-*L. tenuis* consortium:

The schematic representation of synthesis of AgNPs shown in Fig.1. In typical synthesis, 1.6987g of (0.01 M) aqueous silver nitrate was added to 1 L Erlenmeyer flask containing the algal-cyanobacterial consortium (500 mL of *T. obliquus*+500 mL *L. tenuis*). The reaction mixture was maintained at room temperature under a 12hrs light/12hrs dark photoperiod with an illumination intensity of 1500-1700 lux minimal stirring, and pH optimization over a range of 5.5 to 7.5, with optimal NPs formation observed at pH 6.5 ± 2 . After 7 days of incubation the reduction of Ag^+ ions to AgNPs were evidenced by a visible colour change from green to brown, indicating bio-reduction mediated by cellular metabolites. The resulting precipitate was harvested by centrifugation at 10,000 rpm for 15 min and repeatedly washed with deionized water followed by ethanol to remove residual impurities. The purified precipitate was oven-dried at 80 °C for 24 hrs and subsequently calcined at 500 °C for 3 hrs in a muffle furnace to obtain desired materials. This biosynthetic approach exploits the biochemical potential of the algal-cyanobacterial consortium for the sustainable production of stable, crystalline AgNPs with potential biomedical applications.

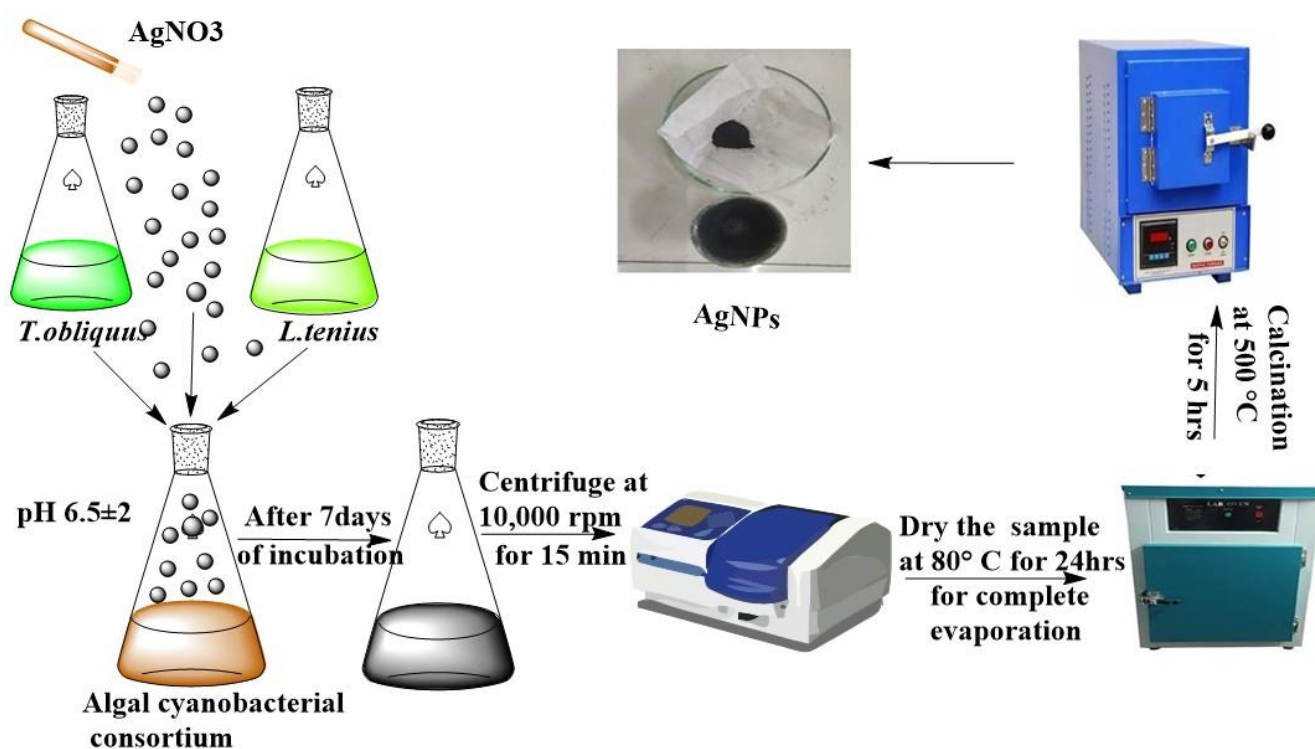


Fig. 1. Biosynthesis of AgNPs using algal-cyanobacterial consortium.

2.5. Antibacterial Activity of biosynthesized AgNPs:

The antimicrobial properties of the AgNPs derived from an algal-cyanobacterial consortium was evaluated using the agar well diffusion method against the Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*) and the Gram-positive *Staphylococcus*

aureus [27-29]. *Ciprofloxacin* served as the positive control, while dimethyl sulfoxide (DMSO) was used as negative control bacterial cultures were grown in nutrient broth at 37 °C and adjusted up to 0.5 McFarland (1.5×10^8 CFU/mL) after 24 hrs incubation. Subsequently, 100 µL of bacterial suspension was evenly spread on Muller-Hinton agar plates, and wells of 8 mm diameter were aseptically punched using a sterile borer. The AgNPs suspensions at concentrations of 100, 50, and 25 µg/mL were prepared in DMSO, and 20 µL of each concentration was dispensed into the respective wells. The plates were incubated at 37 °C, for 24 hrs, after which the zone of inhibition were measured millimetres (mm). The results demonstrated a concentration dependent antibacterial activity the AgNPs against all pathogens [30-31].

2.6. Antifungal Activity of biosynthesized AgNPs:

The antifungal activity of AgNPs were evaluated against *Candida tropicalis* and *Candida dubliniensis* using agar well-diffusion technique. Ketoconazole was used as the positive control while DMSO served as negative control. Fungal cultures were grown in broth medium up to 28° C and adjusted to a 0.5 McFarland (1.5×10^8 CFU/mL) after 48-hours incubation. Thereafter, 100 µL of each fungal suspension were evenly spread on the surface of the Sabouraud Dextrose Agar (SDA) plates using sterile cotton swabs. Wells of approximately 8 mm in diameter were aseptically punched into the Sabouraud Dextrose Agar plates using a cork borer. The AgNPs suspensions at concentrations of 100, 50, and 25 µg/mL were prepared in DMSO, and 20 µL of each concentration was dispensed into the respective wells. The plates were incubation at 28° C, for 48 hours, after which the zone of inhibition were measured in mm [32].

2.7. Anti-biofilm Activity of biosynthesized AgNPs:

Qualitative tube and microscopic methods employing crystal violet staining were used to evaluate the inhibitory effect of AgNPs on biofilm formation by (*P. aeruginosa* and *K. pneumoniae*)

2.7.1. Tube Method:

In the tube assay, bacterial cultures were inoculated into 5 mL of Brain Heart Infusion (BHI) broth and incubated at room temperature for 24 hrs. Different concentrations (100, 50, and 25 µg/mL) of AgNPs were prepared in DMSO. Subsequently 1 mL of each AgNPs suspension was added to tubes containing 3 mL of BHI broth and 1 mL of bacterial culture. Tubes without AgNPs were served as a control. All tubes were incubated at 37° C for 42 hrs after which the contents were gently decanted. The tubes were washed with phosphate-buffered saline (PBS, pH 7.4) to remove planktonic cells, air-dried, and stained with 0.1% w/v crystal violet. Excess stain was removed by

rinsing with deionized water, and the Tubes were dried in inverted position. Biofilm formation was indicated by a visible violet ring lining the inner surface of the tubes, were as its absence indicated antibiofilm activity mediated by AgNPs [33,34].

2.7.2. Coverslip Method:

For the microscopic analysis 3 mL of BHI broth, was mixed with 1 mL of bacterial suspension, 1 mL of AgNPs solution at different concentrations (100, 50, and 25 µg/mL) prepared in DMSO and a sterile coverslip placed inside the tubes. The mixtures were incubated at 37 °C for 48 hrs. After incubation, the cover-slips, were gently washed with phosphate-buffered saline (PBS) to removed loosely adhered cells and stained with 0.1 % w/v crystal violet for 15min. Excess stain was removed by washing with distilled water, and the slides were air-dried for 30 min. biofilm coherence was examined under a light microscope [35-37].

2.8. Anti-inflammatory Activity of biosynthesized AgNPs:

The anti-inflammatory potential of AgNPs was evaluated by heat-induced BSA denaturation inhibition assay. Briefly, the reaction mixture contained 1 mL of 1% BSA and 2.5 mL of phosphate buffer (pH 7.4) with various concentrations of Ag NPs (50-250 µg/mL). The mixtures were first incubated at room temperature for 10 min, followed by heating at 70 °C for 10 min in water bath to induce protein denaturation. After cooling to room temperature, the absorbance was recorded at 660 nm using Elico SL-177 visible spectrophotometer. Phosphate buffer containing BSA alone served as the control, and *diclofenac* was used as a reference anti-inflammatory drug [38]. The percentage inhibition of protein in denaturation was used to determine anti-inflammatory activity using following formula:

$$\% \text{ of Protein Denaturation Activity} = \frac{A_c - A_t}{A_c} \times 100$$

Where, A_c = Absorbance of the control and A_t = Absorbance of the test samples.

2.9. Anti-diabetic Activity of biosynthesized AgNPs:

The inhibitory effect of AgNPs on the enzyme α -amylase activity was determined using a colorimetric assay based on the DNS method. The AgNPs at varying concentrations (50-250 µg/mL) were incubated with sodium phosphate buffer (pH 6.9), containing 200 µL of (0.01 g) α -amylase enzyme solution (1 U/mL). Thereafter, 250 µL of 2% starch solution was added, and the reaction mixtures were allowed to process at room temperature for 10 minutes. The reaction was terminated

by the addition of 450 μ L of DNS reagent, followed by heating in a water bath for 5 min. After cooling, absorbance was recorded at 540 nm using a UV-visible spectrophotometer. A control reaction without AgNPs was maintained under identical conditions. Acarbose, a standard antidiabetic drug, was used as the positive control. The assay enabled the evaluation of the α -amylase inhibitory potential of AgNPs, indicating their possible antidiabetic activity [39]. The inhibitory activity of α -amylase was calculated using the formula:

$$\% \text{ of Inhibition} = \frac{Ac - At}{Ac} \times 100$$

Where, Ac= Absorbance of the control and At= Absorbance of the test samples.

2.10. Antioxidant Activity of biosynthesized AgNPs:

The free radical scavenging activity of the free radical scavenging test was used to identify the antioxidant ability of AgNPs was evaluated using the DPPH assay, a widely accepted method for assessing antioxidant potential [40]. The DPPH is a stable free radical characterized by a deep violet colour with a strong absorption maximum at 518 nm. Upon reduction by an antioxidant, DPPH undergoes discoloration due to the formation of its non-radical form. The 25 to 125 μ g/mL concentrations of both AgNPs and ascorbic acid (standard reference) were prepared using a 1 mg/mL stock solution, and 0.1 mM DPPH solution added to each test solution, and then the volume was adjusted to 4 mL with ethanol. The reaction mixtures were incubated at room temperature in the dark condition (30 min in the dark to prevent photo-induced degradation). Subsequently, the absorbance was recorded at 518 nm using a UV-visible spectrophotometer. The radical scavenging activity was determined by comparing the absorbance of the sample with that of the control. The antioxidant activity (%) was calculated using below equation:

$$\% \text{ of Inhibition} = \frac{Ac - At}{Ac} \times 100$$

Where, Ac= Absorbance of the control and At= Absorbance of the test samples.

3. Results and Discussion:

3.1. Mechanism of Algae-Cyanobacterial Consortium-mediated AgNPs:

The AgNPs biosynthesized using a consortium of algae and cyanobacteria exploit the complementary metabolic and biochemical capabilities of both partners, resulting in enhanced NPs formation and stability. The green microalga *T. obliquus* exhibits a higher capacity for metal ions hyperaccumulation and mediates their reduction through a diverse array of secondary metabolites,

pigments, and extracellular polymeric substances (EPS), which contain functional groups like amino, hydroxyl, carboxyl, thiol, and phosphate that work as natural reducing and capping agents [41,42]. Cyanobacteria, particularly, contribute through intracellular and extracellular pathways, where cell surface enzymes, pigments, and EPS facilitate the bio reduction of NPs [43,44]. The physicochemical characteristics of the biosynthesized AgNPs, including particle size, shape, morphology, crystallinity, phase, and surface properties, are significantly influenced by mixed microbial communities, which can perform complex metabolic functions that are difficult or impossible for individual microorganisms to achieve. In addition, operational parameters such as pH, temperature, metal ion concentration, and incubation time also play a crucial role in determining the properties of the biosynthesized AgNPs [45] (Fig. 2). Optimization of these parameters governs nucleation kinetics and crystal growth, thereby improving NPs uniformity and yield. The integrated biosynthesis strategy offers a sustainable, eco-friendly, and scalable approach for the production of the AgNPs with enhanced biomedical potential [46].

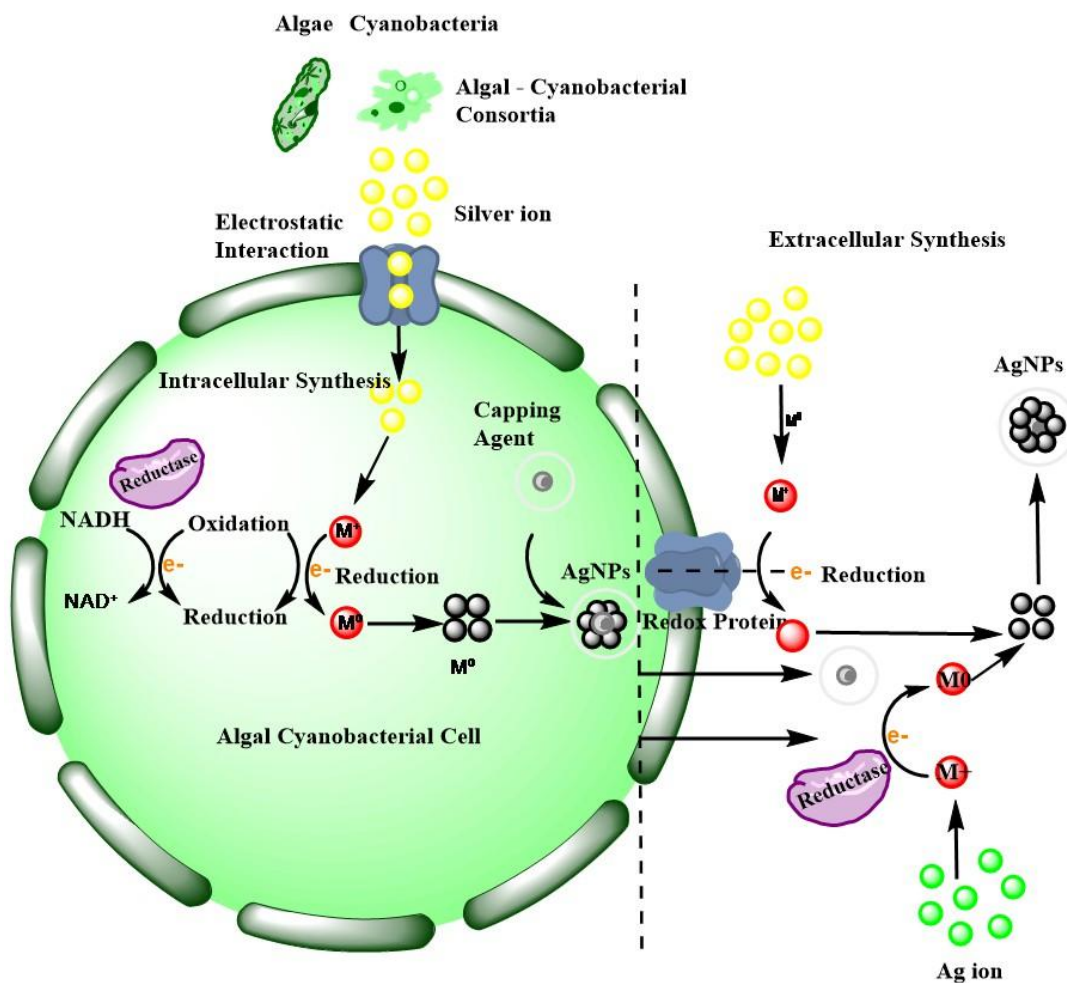


Fig. 2. Mechanism of formation of AgNPs from algae-cyanobacterial consortium.

3.2. Structural and Morphological Investigation of Algae-mediated AgNPs:

3.2.1. XRD and EDS Analysis of AgNPs:

Algal-cyanobacterial consortia-mediated AgNPs were comprehensively characterized to confirm their structural integrity, phase purity, and dimensional properties using XRD, EDS, and FTIR analyses. The XRD pattern (Fig. 3a) exhibited distinct diffraction peaks at 2θ values of 38.11° , 44.29° , 64.44° and 77.44° , corresponding to the (111), (200), (220), and (311) crystallographic planes, respectively, which are characteristic of a face-centered cubic Ag structure. The XRD pattern matches with reference JCPDS card number 65-2871. The intensity of peak position reflections confirmed the crystallite nature of the AgNPs. However, some small peaks appear in the XRD spectra due to the certain sulphur-related impurities remain with calcined material [47]. Furthermore, EDS analysis (Fig. 3b) validated the elemental existence of Ag, supporting the XRD data. In addition, EDS spectrum shows extra peaks which may arise due to the secondary metabolites/bioactive molecules which are associated with AgNPs [48]. These findings collectively confirm the successful synthesis of highly crystalline AgNPs *via* the algal-cyanobacterial consortium-mediated approach.

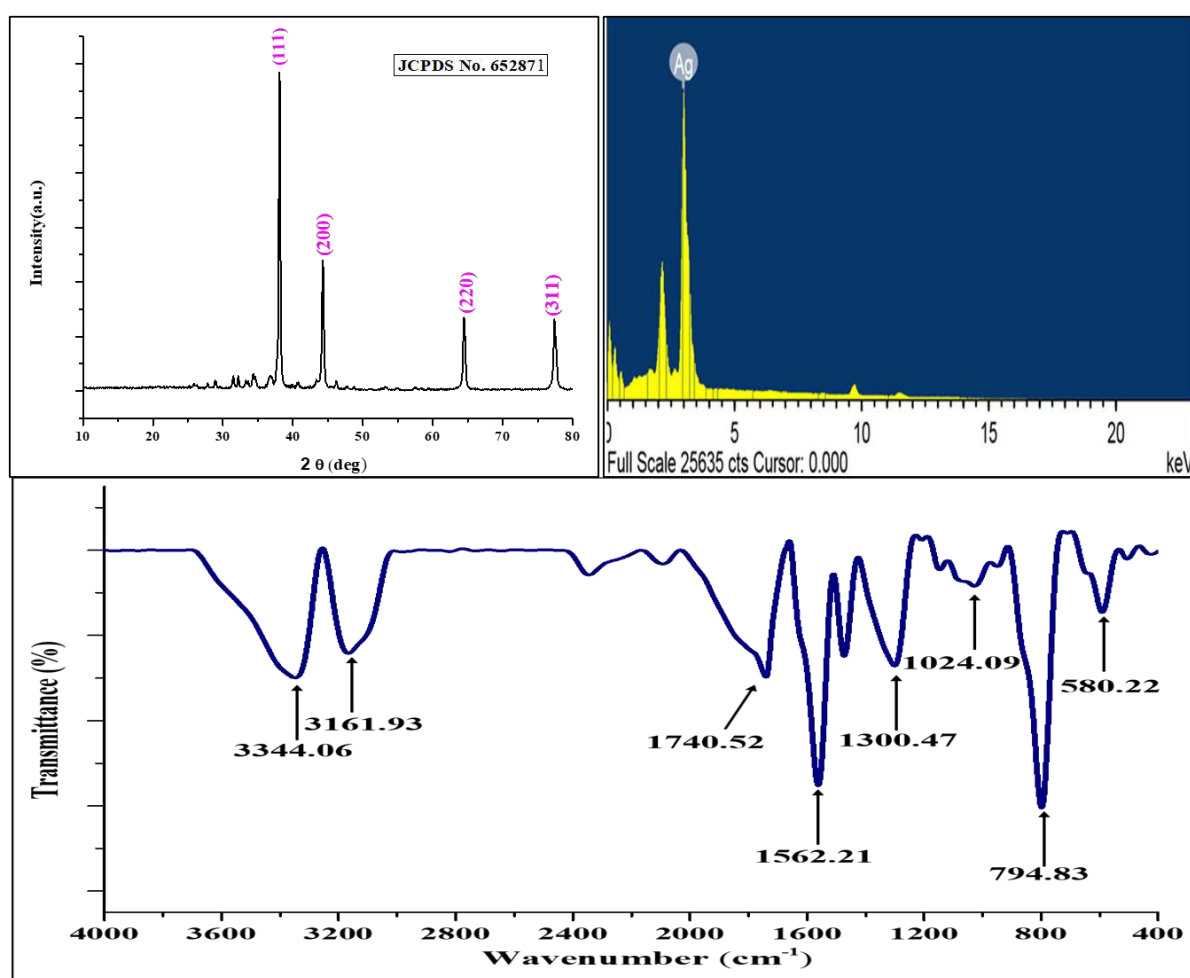


Fig. 3. (a) XRD, (b) EDS, and (c) FTIR spectra of algal-cyanobacterial-mediated AgNPs.

3.2.2. FTIR Analysis of AgNPs:

FTIR spectroscopy was employed to elucidate the nature of the surface functional groups associated with the AgNPs. The FTIR spectrum displayed in Fig. 3c, shows a two prominent and broad absorption band at 3344.06 and 3161.93 cm^{-1} corresponding to O-H and N-H stretching vibrations, indicative of hydroxyl and amine groups derived from protein and polysaccharides [49,50]. A distinct band observed at 1740.52 cm^{-1} was attributed to C=O stretching vibrations of peptide linkages, confirming the involvement of proteinaceous components in NPs stabilization [51]. Further, the absorption peak observed in the range 1562.21 to 1024.09 cm^{-1} due the presence of C-C, C-O, C-N, and C=C groups, indicating polysaccharides and amino-containing biomolecules are associated with AgNPs. Additionally, peaks at 794.83 and 580.22 cm^{-1} may be attributed to Ag-O vibrations interactions, indicating the presence of strong interactions between AgNPs and the biomolecules.

3.2.3. Morphological Analysis of AgNPs:

The surface morphological characteristics of AgNPs were examined using SEM, and depicted in Fig. 4a-d. From the SEM micrographs at low resolution (Fig. 4a) revealed that the biosynthesized AgNPs possesses an irregular, highly agglomerated morphology appearing like broccoli shaped, which is composed of densely packed nearly monodispersed microscale balls with diameter size 85-200 nm (Fig. 4b-c). However, at high resolution (Fig. 4c-d), image reveals that each nearly monodispersed microscale balls is consists of thousands of densely packed nearly monodispersed nanoscale spherical particles having sizes in the range of 37-58 nm (Fig. 4d). Further, these spherical structures are developed from hundreds of ultrafine particles having sizes about-7-9 nm, it is also supported from TEM (Fig. 5a-d). The surface is highly irregular with numerous rounded nanograins forming compact clusters. The primary NPs appeared to be nearly spherical to quasi-spherical and are fused together, giving rise to larger secondary agglomerates. The AgNPs pronounced compactness are attributed to abundant surface-active biomolecules, particularly hydrophilic exopolysaccharides and

secondary metabolites which facilitate NPs clustering and aggregation during biosynthesis [52,53]. This improved cohesion and uniformity results from effective capping and stabilization by biomolecules released by algae and cyanobacteria, which results in controlled reduction of silver ions to AgNPs.

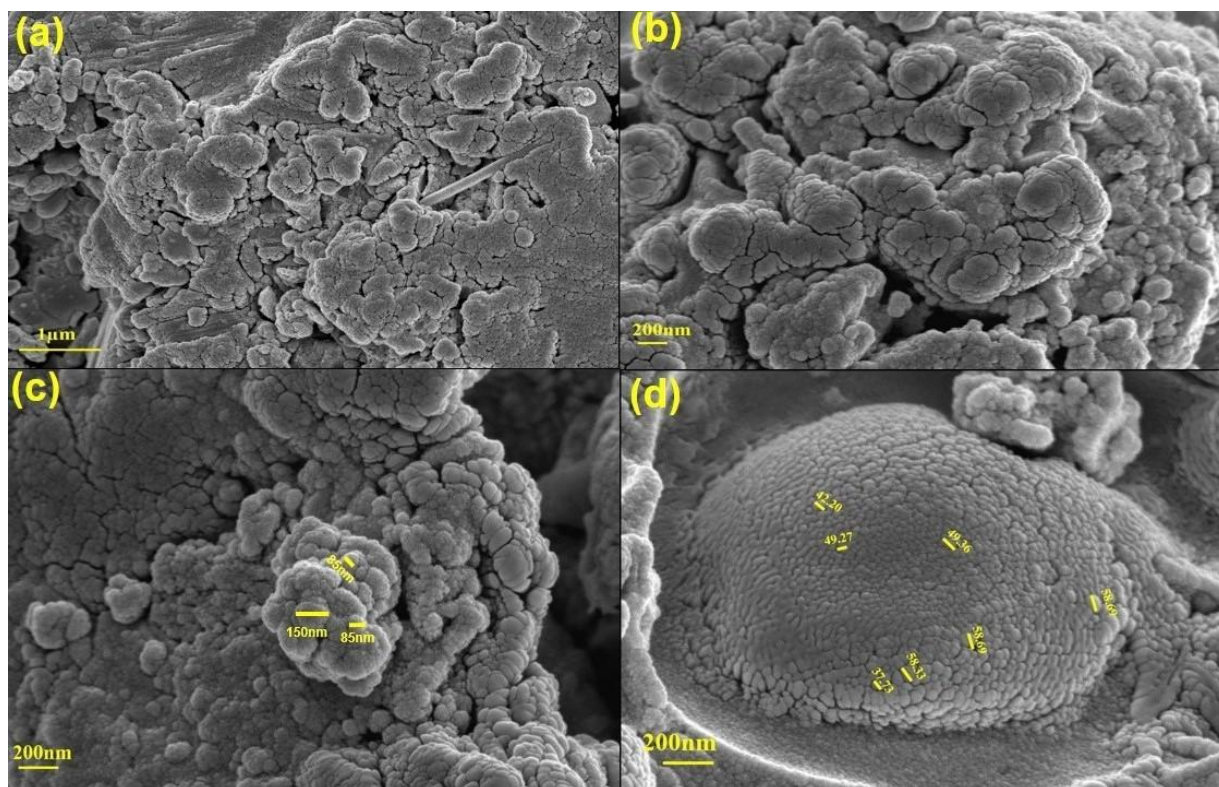


Fig. 4. (a-d) Scanning electron microscopic image of AgNPs.

Next, the size distribution of AgNPs was authenticated using TEM study (Fig. 5a-f). From Fig. 5b-d, it is clearly exposed that, biosynthesized AgNPs are monodispersed spherical shape having sizes 7-9 nm (Fig. 5c). Moreover, the histogram images show a fairly uniform size distribution with average particle size in the range of 8.70 nm (Fig. 5e). This confirms the successful synthesis of nanoscale AgNPs. Further, SAED pattern (Fig. 5f) shows distinct pattern of rings of AgNPs, and suggested the polycrystalline nature. These results are in good accordance with XRD data. Nucleation and growth dynamics strongly depend on physicochemical factors such as pH, ionic strength, enzymatic reducing potential, and incubation time. These factors are widely documented in research on the production of NPs mediated by cyanobacteria and algae [54]. Changes in these variables affect the balance of reduction rate and stabilization efficiency, which in turn influences particle size. Many studies on the production of NPs mediated by these organisms have shown similar molecular findings [55].

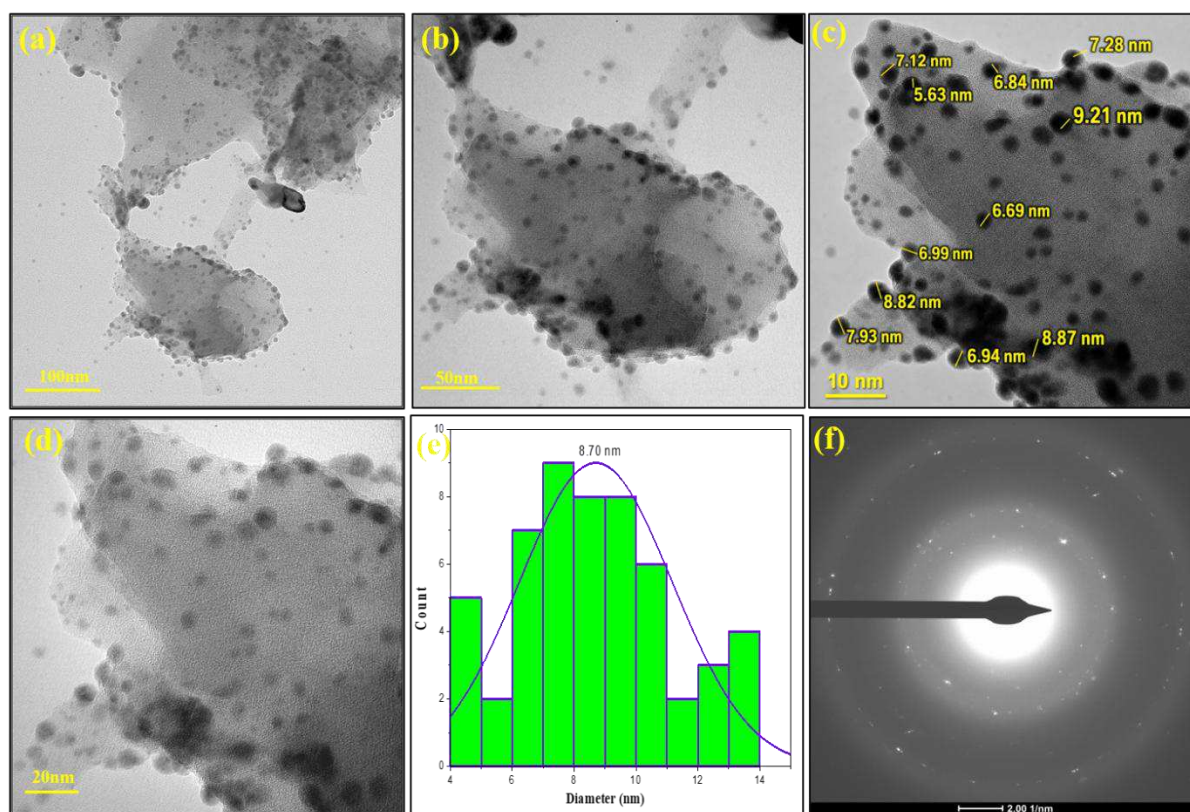


Fig. 5. (a-d) Transmission electron micrograph, (e) histogram and (f) SAED image of AgNPs.

3.3. Antibacterial Activity of AgNPs:

The AgNPs showed remarkable antimicrobial activity against Gram-negative (*K. pneumoniae*, *P. aeruginosa* and *E. coli*) and Gram positive (*S. aureus*) bacterial strains, as shown in Fig. 6. (a-d) The AgNPs illustrate better efficacy, producing the highest inhibition zones of 15 ± 0.15 mm, 13 ± 0.60 mm and 11 ± 0.49 mm at 100, 50, and 25 $\mu\text{g/mL}$ concentrations respectively, against *K. pneumoniae*. The findings of antibacterial activity is tabulated in Table 1. In comparison, *E. coli* showed limited inhibition with zones ranging from 11 ± 0.36 to 12 ± 0.05 mm. The conventional antibiotic, *Ciprofloxacin*, showed higher zones compared to AgNPs against Gram-positive and Gram-negative bacteria (Supporting Information Fig. S1), whereas, the DMSO as a negative control exhibited no antimicrobial effects.

The antimicrobial potency of the AgNPs can be primarily attributed to their reduced particle size and favourable surface characteristics, which are influenced by the unique metabolic profile of algae-cyanobacteria consortia. The small size of AgNPs provides a higher surface area-to-volume ratio, thereby facilitating increased interaction with bacterial cell membranes. Additionally, biofunctional moieties derived from algae and cyanobacteria metabolites may confer improved

surface reactivity, enhancing adhesion to bacterial cell walls and promoting membrane destabilization [56]. The antimicrobial activity of AgNPs is mediated through multiple complementary mechanisms involving both direct nanoparticle–cell interactions and the intracellular release of silver ions. Initially, AgNPs adhere to the bacterial cell surface and induces structural perturbations in the cell envelope, increases membrane permeability, and may result in partial detachment or disruption of the cytoplasmic membrane, thereby compromising cellular integrity [57-58]. Due to their high surface area-to-volume ratio, AgNPs can efficiently attach and penetrate the cell wall, forming membrane lesions or pore-like structures that ultimately lead to cytoplasmic leakage and cell death [59-60]. Subsequent to cellular internalization, the release of silver ions enhance antimicrobial efficacy. Silver ions interact with thiol (-SH) groups of vital respiratory enzymes, leading to enzymatic inactivation and disruption of the electron transport chain. This interference promotes the generation of reactive oxygen species (ROS), resulting in oxidative stress and impairment of adenosine triphosphate (ATP) synthesis. Elevated ROS levels contribute to lipid peroxidation, membrane disintegration, protein oxidation, and nucleic acid damage. Moreover, silver ions exhibit strong affinity for phosphorus- and sulfur-containing biomolecules, including DNA and associated proteins [61]. Binding of silver ions to these functional groups may interfere with DNA replication and transcription processes, thereby inhibiting cell division and microbial proliferation. In addition, silver ions can induce ribosomal denaturation within the cytoplasm, leading to suppression of protein synthesis and subsequent metabolic failure [62,63].

Table 1 Antibacterial activity of Ag NPs in comparison with Gram-Positive and Gram-negative bacteria.

Sr. No.	Strains	Zone of Inhibition with (mean $\pm$ SD) (n = 3)				
		<i>Ciprofloxacin</i>	Control	25 μ g/mL	50 μ g/mL	100 μ g/mL
1.	<i>E. coli</i>	17 $\pm$ 0.95	No zone	11 $\pm$ 0.36	11 $\pm$ 0.70	12 $\pm$ 0.05
2.	<i>K. pneumonia</i>	15 $\pm$ 0.55	No zone	11 $\pm$ 0.49	13 $\pm$ 0.60	15 $\pm$ 0.15
3.	<i>S. aureus</i>	15 $\pm$ 0.05	No zone	11 $\pm$ 0.49	11 $\pm$ 0.47	12 $\pm$ 0.55
4.	<i>P. aeruginosa</i>	16 $\pm$ 0.55	No zone	12 $\pm$ 0.17	12 $\pm$ 0.05	13 $\pm$ 0.11

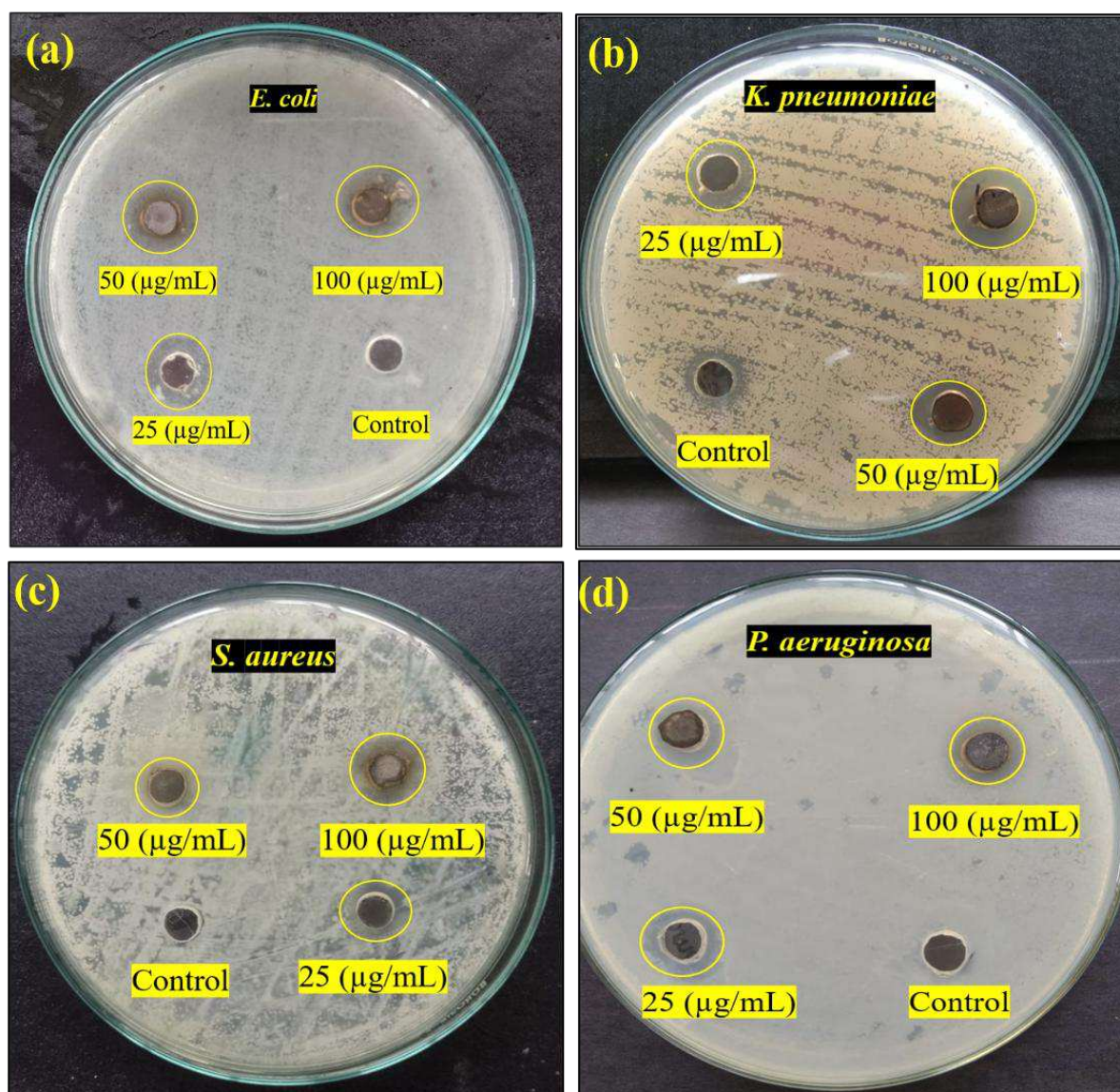


Fig. 6. (a-d) Antimicrobial activity of AgNPs against Gram-positive and Gram-Negative bacteria.

3.4. Antifungal activity of Ag NPs

The AgNPs showed excellent antifungal activity against *C. tropicalis* and *C. dubliniensis* in a concentration-dependent manner Fig. 7 (a-b). The AgNPs demonstrated stronger inhibition as presented in Table 2. The epic inhibition zones were noticed at 100 μg/mL against *C. dubliniensis* (15 ± 0.15 mm) and *C. tropicalis* (13 ± 0.60 mm). Positive controls (*Ketoconazole*) using conventional antifungals agents showed 11 ± 0.36 - 12 ± 0.17 mm zones, and DMSO exhibited no zone. An enhance antifungal activity of AgNPs exert their antifungal activity through multiple interconnected mechanisms. Upon entering the fungal cell, AgNPs stimulate the excessive production of reactive oxygen species (ROS), resulting in oxidative stress that damages essential cellular components, including the cell membrane, proteins, mitochondria, and DNA. The disruption of mitochondrial

function compromises ATP synthesis, leading to impaired energy metabolism and reduced cellular viability. In addition, AgNPs suppress the yeast-to-hyphal transition, a critical virulence factor of *Candida albicans*, thereby preventing its transformation into the invasive hyphal form and inhibiting biofilm development. Mechanistic investigations further reveal that the antifungal efficacy of AgNPs is mediated through ROS generation, membrane disruption, and mitochondrial dysfunction, which collectively contribute to fungal cell death and effective biofilm inhibition. [64,65].

Table 2 Antifungal activity of AgNPs.

Sr. No.	Strains	Zone of Inhibition with (mean $\pm$ SD) (n = 3)				
		<i>Ketoconazole</i>	Control	25 $\mu\text{g/mL}$	50 $\mu\text{g/mL}$	100 $\mu\text{g/mL}$
1.	<i>Candida Tropicalis</i>	11 $\pm$ 0.36	No zone	11 $\pm$ 0.70	12 $\pm$ 0.55	13 $\pm$ 0.60
2.	<i>Candida Dubliniensis</i>	12 $\pm$ 0.17	No zone	12 $\pm$ 0.17	13 $\pm$ 0.60	15 $\pm$ 0.15

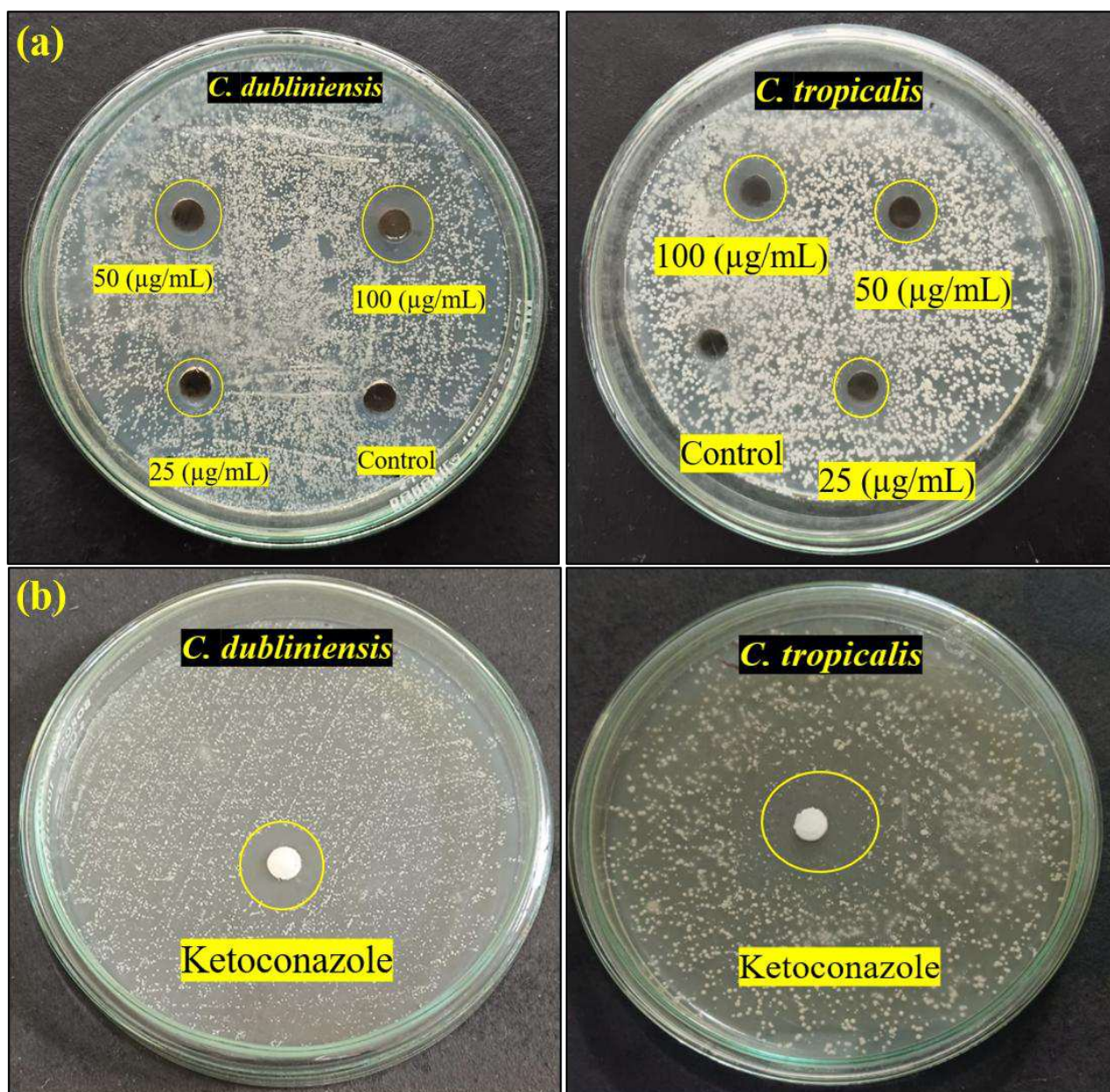


Fig. 7. (a-b) Antifungal activity of AgNPs against *C. tropicalis* and *C. dubliniensis*, (a) with AgNPs and (b) with positive control

3.5. Antibiofilm activity of Ag NPs

The anti-biofilm efficacy of AgNPs was evaluated using tube adherence and coverslip-based crystal violet staining assays against *K. pneumoniae* and *P. aeruginosa*. In the tube method, visible biofilm formation is typically indicated by the presence of a dense ring at the bottom and along the inner wall of the tube, reflecting strong bacterial adhesion and extracellular polymeric substance (EPS) production as shown in Table 3. The absence of this ring indicates inhibition of biofilm formation. These findings are consistent with previous reports demonstrating the antibiofilm

potential of AgNPs against clinically relevant Gram-negative pathogens [66,67]. The inhibitory effect was concentration-dependent, with the highest level of inhibition observed at 100 µg/mL (Fig. 8). The observed biofilm inhibition underscores the potential of AgNPs as effective agents for controlling biofilm-associated infections, particularly those involving persistent or drug-resistant bacterial strains.

The presence or absence of biofilm layers, with a marked reduction in biomass, served as an indicator of strong antibiofilm activity. The AgNPs disrupt bacterial adhesion and inhibit the synthesis of extracellular polymeric substances (EPS), which are critical for biofilm structural integrity [68]. In addition, NPs induce the production of ROS, leading to oxidative stress and bacterial cell death within the biofilm. Experiments demonstrate these mechanisms in numerous species of bacteria. The described methods of qualitative analysis can be included during the proper examination of the effectiveness of NPs in terms of the development of antibiofilm, which is fundamentally necessary to generate new antimicrobial therapy [69].

Table 3. Effect of AgNPs on the biofilm produced by the tested bacteria.

Sr. No.	Strains	Control	100µg/mL
1.	<i>P. aeruginosa</i>	+	-
2.	<i>K. Pneumonia</i>	+	-

Note: (+) biofilm formation, (-) no biofilm or its inhibition

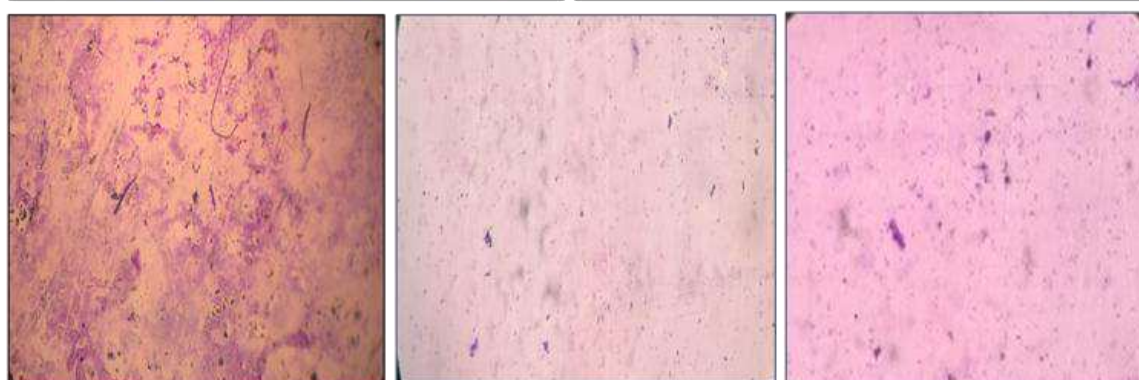
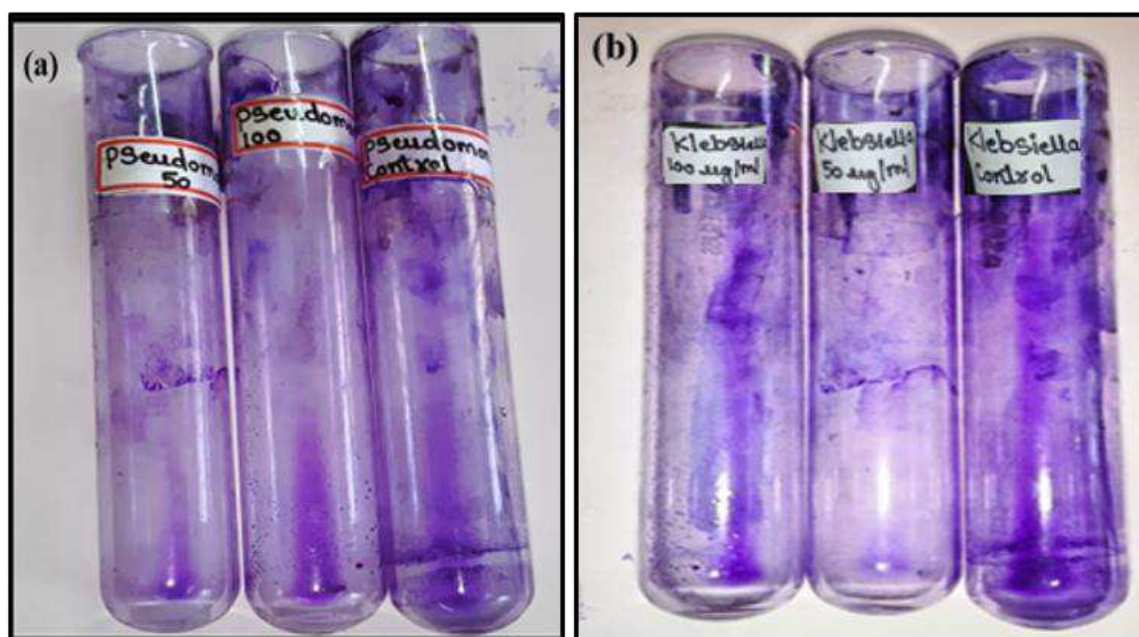


Fig.(c) *K. pneumoniae* control Fig.(d) *K. pneumoniae* 100 µg/mL Fig.(e) *K. pneumoniae* 50 µg/mL

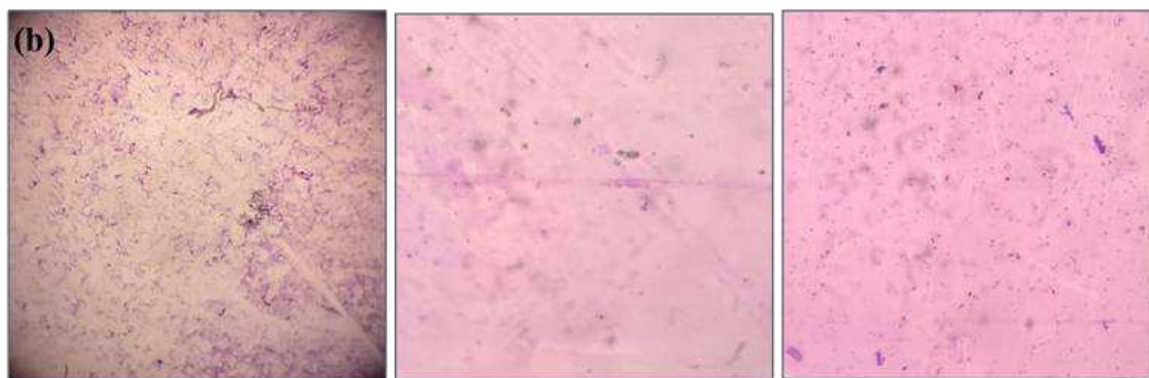


Fig.(f) *P. aeruginosa* control Fig.(g) *P. aeruginosa* 100 µg/mL Fig.(h) *P. aeruginosa* 50 µg/mL

Fig. 8. Antibiofilm Activity of AgNPS (Tube method) against (a) *K. pneumoniae* (b) *P. aeruginosa*, and Coverslip method against (c) *K. pneumoniae* control, (d) *K. pneumoniae* (100 µg/mL) and (e) *K. pneumoniae* (50 µg/mL), (f) *P. aeruginosa* control, (g) *P. aeruginosa* (100 µg/mL) and, (h) *P. aeruginosa* (50 µg/mL)

3.6 Anti-inflammatory of AgNPs:

The BSA denaturation inhibition assay is used to determine anti-inflammatory activity of AgNPs. The inhibitory activity of AgNPs increased significantly in a dose-dependent manner, depicted in Fig. 9a, and tabulated in Table 4. The AgNPs demonstrated superior inhibition (97.18 ± 0.99 %), closely matching the standard drug diclofenac (99.13 ± 0.55 %). The AgNPs are known for better biocompatibility and bioavailability as well as possible higher therapy effectiveness, supported by recent works that emphasized NPs size, uniformity, and surface chemistry are important to optimize the anti-inflammatory activity [70,71]. These results suggest that AgNPs, produced by algal-cyanobacterial consortium, can serve as a therapeutic candidate for the treatment of inflammatory disorders in biomedical applications.

Table 4. Values represent (SD) of anti-inflammatory activity.

% Inhibition (mean $\pm$ SD)			
Sr. no	Concentration	<i>Diclofenac</i>	AgNPs
1.	50 μ g/mL	89.13 ± 1.08	80.23 ± 0.58
2.	100 μ g/mL	91.62 ± 1.22	89.69 ± 0.57
3.	150 μ g/mL	95.07 ± 0.61	91.75 ± 0.55
4.	200 μ g/mL	96.08 ± 0.83	94.23 ± 0.56
5.	250 μ g/mL	99.13 ± 0.55	97.18 ± 0.99

3.7. Antioxidant Activity of AgNPs:

The antioxidant potential of AgNPs was evaluated using the DPPH radical scavenging assay, revealing strong and dose-dependent activity (Fig. 9b). At concentrations ranging from 25 to 125 μ g/mL, percentage inhibition varied from 82.27 ± 0.63 % to 94.51 ± 0.72 %, demonstrating substantial radical scavenging capacity as shown in Table 5. The antioxidant activity of the biosynthesized AgNPs was comparable to, and at higher concentrations even exceeded, that of standard ascorbic acid. The enhanced activity of AgNPs produced via algal–cyanobacterial consortia can be attributed to their smaller, uniform particle size and the presence of bioactive capping agents, including

phenolic compounds and proteins. These biomolecules not only stabilize NPs but also contribute to their reactivity by donating electrons or hydrogen atoms to neutralize free radicals. The interplay of NPs size, surface chemistry, and biological capping thus underpins the observed antioxidant efficacy, highlighting the potential of these AgNPs for biomedical applications [72,73].

Table 5. Values represent (mean $\pm$ SD) of anti-oxidant activity.

Sr. no	% Inhibition (mean $\pm$ SD)		
	Concentration	Ascorbic Acid	AgNPs
1.	25 μ g/mL	94.09 $\pm$ 0.95	82.27 $\pm$ 0.63
2.	50 μ g/mL	90.71 $\pm$ 0.76	87.34 $\pm$ 0.65
3.	75 μ g/mL	96.20 $\pm$ 0.56	90.71 $\pm$ 0.52
4.	100 μ g/mL	99.15 $\pm$ 0.58	93.24 $\pm$ 0.59
5.	125 μ g/mL	96.20 $\pm$ 0.60	94.51 $\pm$ 0.72

3.8. Anti-diabetic activity of AgNPs

The anti-diabetic potential of AgNPs synthesized via algal–cyanobacterial consortia was evaluated through α -amylase inhibition assays (Fig. 9c). The results demonstrated a concentration-dependent increase in inhibitory activity, with higher concentrations of AgNPs yielding greater enzyme inhibition. At 250 μ g/mL, as shown in Table 6. AgNPs exhibited 83.65 $\pm$ 1.27% inhibition, which is comparable to the standard anti-diabetic drug acarbose (85.57 $\pm$ 0.70%). The observed activity is attributed to bioactive compounds present in the algal-cyanobacteria consortium, which serve both as stabilizing agents for the nanoparticles and as facilitators of effective enzyme interaction. These compounds likely improve the binding affinity of AgNPs to the enzyme active site, enhancing α -amylase inhibition. Collectively, these findings suggest that biosynthesized AgNPs may serve as promising anti-diabetic agents and present a potential alternative to conventional therapeutic drugs [74].

Table 6. Value represent (mean $\pm$ SD) of anti-diabetic activity.

% Inhibition (mean $\pm$ SD)			
Sr. no	Concentration	<i>Acarbose</i>	AgNPs
1.	50 μ g/mL	70.83 $\pm$ 0.95	68.17 $\pm$ 0.53
2.	100 μ g/mL	79.16 $\pm$ 0.56	76.76 $\pm$ 0.57
3.	150 μ g/mL	80.53 $\pm$ 0.75	78.62 $\pm$ 0.64
4.	200 μ g/mL	84.00 $\pm$ 0.94	81.87 $\pm$ 0.98
5.	250 μ g/mL	85.57 $\pm$ 0.70	83.65 $\pm$ 1.27

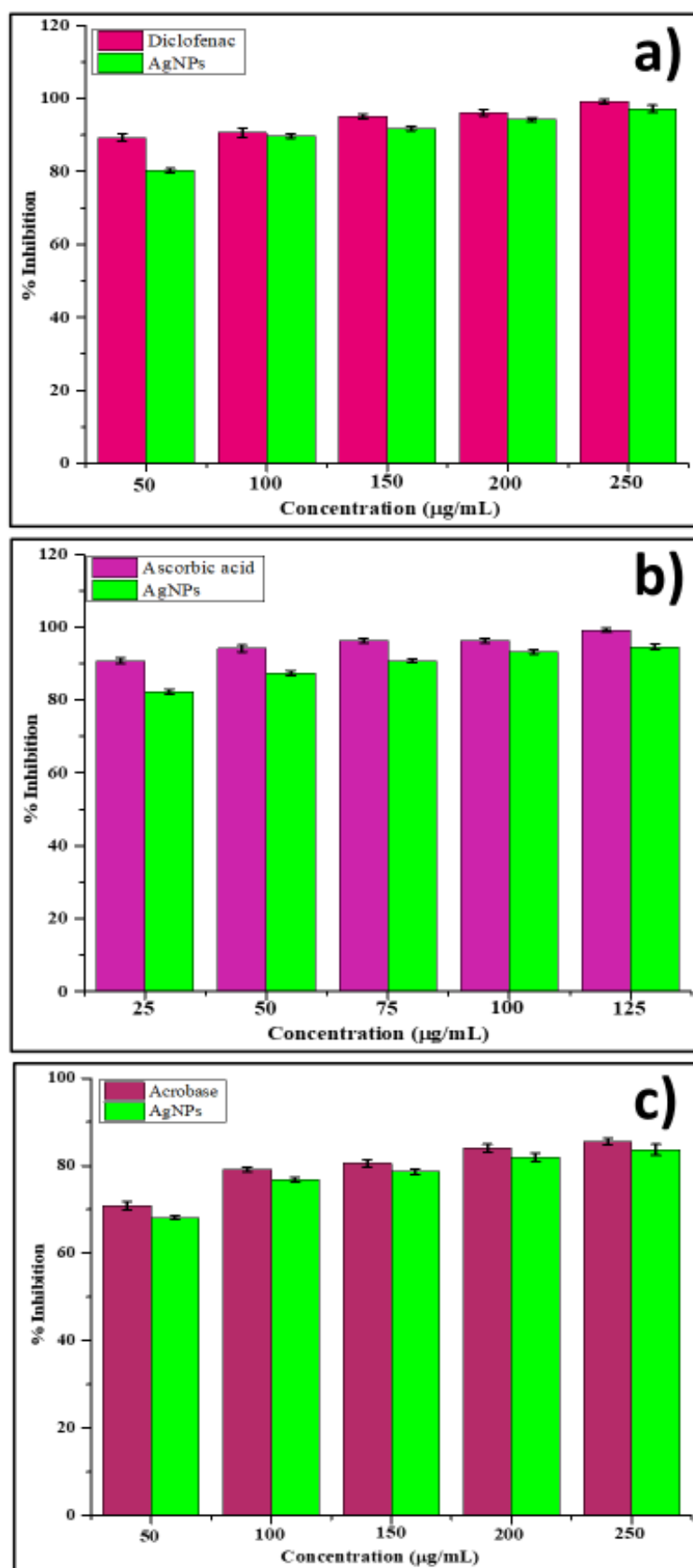


Fig. 9. (a) Anti-inflammatory, (b) Antioxidant, and (c) Anti-diabetic activity of AgNPs.

4. Conclusion

In a conclusion, the study supports successfully biosynthesis of AgNPs utilizing a algal-cyanobacterial consortium (*Tetradasmus obliquus* and *Lagosinema tenuis*). As-biosynthesized AgNPs is authenticated by various spectroscopic techniques. The XRD revealed a face-centered cubic, while FTIR suggested hydroxyl, amine, and carboxyl groups, due to association of biomolecules of proteins and polysaccharides with silver in chelating form. The SEM revealed broccoli structure of agglomerated nearly monodispersed microball, while TEM supports ultrafine particle formation with an average particle size of 8.70 nm. The AgNPs demonstrated strong antimicrobial activity against both Gram-negative and Gram-positive bacteria, with the highest inhibition zone observed against *Klebsiella pneumoniae*, and showed pronounced antifungal activity against *C. dubliniensis*. The nanoparticles also inhibited significant biofilm formation in susceptible bacterial strains and exhibited dose-dependent anti-inflammatory activity up to 97.18 ± 0.99 %, comparable to *diclofenac*. Furthermore, AgNPs displayed significant antioxidant activity (96.20 ± 0.56 % inhibition), and anti-diabetic potential, with 83.65 ± 1.27 % α -amylase inhibition at 250 $\mu\text{g/mL}$. Overall, the enhanced physicochemical homogeneity, reduced size, biocompatible and effective biomolecular capped AgNPs is highly effective, highlighting their potential for diverse therapeutic applications.

Author's contributions

P.V. and P.B.: writing original draft, methodology and data analysis; data evaluation; **S.K., V.U. and R.C.:** reviewed manuscript and formatting; **A.R.:** writing original draft and communications. All authors reviewed the manuscript.

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Competing interests

The authors declare no competing interests.

Data availability

The dataset used and/or analyzed during the current study available from the corresponding author on reasonable request.

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