Synthesis of nano-silver from *Navicula cincta* and the evaluation of its antimicrobial activity

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Green synthesis of silver nanoparticles is cost effective and encourages the use of non-toxic chemicals. The biological sources of reductants have trouble free protocols and when applied for human health associated field, is an easy approach for maintaining aseptic environment during the synthesis of nanoparticles. Microalgae are used as a source of natural colours and exhibit extensive applications as natural colorants in nutraceuticals, cosmetics and pharmaceutical industries. The use of *Navicula cincta* microalgae in the biogenic synthesis of silver nanoparticles is unexplored and underexploited. In this work, silver nanoparticles synthesised using algal extract by sunlight method were characterised by UV–Visible spectroscopy, X-ray diffraction, scanning electron microscopy, energy dispersive spectroscopy, Fourier transform infrared spectroscopy and particles size analyser. The antimicrobial activity of nanosilver against seven bacteria, *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Shigella flexneri, Vibrio cholera* and *Salmonella typhi* and four fungi *Aspergillus flavus, Aspergillus niger, Aspergillus fumigatus and Rhizopus stolonifer* are investigated using agar well diffusion technique and compared with standard antibiotics namely Chloramphenicol and Nystatin.

1. Introduction: A new branch of nanotechnology is nanobiotechnology which combines biological principles with physical and chemical procedures to generate nano-sized particles with specifics functions [1]. Metal nanoparticles have received considerable attention in recent years because of their unique properties such as magnetic, electronic configuration, optical and so on. Metallic nanoparticles are most promising biomedical agents. Among these, Au, Ag and Zn are most popular metals in bio-nanomaterial synthesis. Silver nanoparticles (AgNPs) are used for numerous physical, biological and pharmaceutical applications. AgNPs are synthesised by different physical and chemical methods like sol–gel technique, solvo-thermal synthesis, chemical reduction, laser ablation, inert gas condensation and so on. Biosynthesis of nanoparticles using biological materials is an emerging field that highlights the intersection of nanotechnology and biotechnology and has attracted increased attention because of clean and eco-friendly green environment [2]. Biosynthesis of AgNPs is becoming popular day-by-day using microorganisms like bacteria, fungi and algae [3]. Algal mediated synthesis of AgNPs is now being extensively carried out by researchers because of the high rate of synthesis and efficacy, eliminates the elaborate process of maintaining cell culture and exhibits best compatibility [4]. Potential antimicrobial AgNPs synthesised using microalgae against human pathogenic bacteria is reported [5]. The genus *N. cincta* is often one of the most species-rich genera in spring habitats [6, 7]. *N. cincta* has distinct, size, central area and proximal raphe endings and this species was found to be characteristic of medium–high conductivity freshwaters rich in sulphate and chloride, often nitrate enriched. The use of *Navicula cincta* micro-algae in the synthesis of AgNPs is unexplored and underexploited. Synthesis of AgNPs using extracts of *N. cinta*, a diatom, is reported for the first time. The protocol of synthesis is also eco-friendly, and bio-compatibility and also find the potential medical applications in future.

2. Materials and methods

2.1. Collection of algal sample: The microalgae was collected from a fresh water pond located at Vettaikaran pudur village, Pollachi, Coimbatore, Tamil Nadu and stored in a sterile airtight polythene bag. The sample was powdered and stored at −4°C for further analysis. The growth potential of the algae was maintained through regular sub-culturing under laboratory condition at 28°C, in a 16/8 h light/ dark cycle, under cool fluorescent light. The sample was identified in Botanical Survey of India, Coimbatore, Tamil Nadu. The authentication number is SI/SRC/5/23/2017/Tech/3488.

2.2. Preparation of algal extract: The algal powder (5 g) was soaked in 100 ml sterile deionised distilled water for 24 h and heated at 70°C for 20 min. The resulting infusion was filtered through Whatman filter paper No.1 until no insoluble material appeared in the algal extract.

2.3. Biosynthesis of AgNPs: The aqueous solution of silver nitrate was prepared in different concentrations (1–5 mM) and used for the synthesis of AgNPs. The volume of algal extract (1–5 ml) was added to each 10 ml $AgNO_3$ solution (1–5 mM) and kept at sunlight at different time intervals (0–4 min). The biosynthesis of AgNPs was determined by the colour change in the reaction mixture at optimised conditions. A control was maintained throughout the experiment.

2.4. Phytochemical screening of algal extract: Phytochemical colour tests as per standard procedures was carried out for the algal extracts to identify the primary and secondary metabolites [8].

2.5. Characterisation of synthesised AgNPs: AgNPs synthesised using microalgae were characterised by UV–Visible spectroscopy, Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD) analysis, scanning electron microscopy (SEM), energy dispersive spectroscopy (EDS) and particles size analyser. The formation of nano-silver shows visible colour change which was monitored by UV–Visible absorption spectroscopy (Double beam spectrophotometer 2202-Systronics) and FTIR was recorded in the range of 4000–400 cm^{-1} on a Shimadzu FTIR–8400S spectrophotometer. XRD analysis (X' Pert Pro PANalytical's) was used

to analyse the size of NPs. Morphology, size and elements of AgNPs were investigated using scanning electron microscope and energy dispersive X-spectroscopy using NOVA-450 instrument. The particle size range of the synthesised AgNPs was determined using a particle size analyser using Nanopartica SZ100-Horiba.

2.6. In-vitro antimicrobial activity of synthesised AgNPs: Antimicrobial activity was carried out for biosynthesised AgNPs by agar-well diffusion method against seven bacterial and the four fungal strains. Seven bacterial strains (*Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Shigella flexneri, Vibrio cholera and Salmonella typhi*) and four fungal strains (*Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus* and *Rhizopus stolonifer*) were inoculated into the nutrient broth and potato dextrose broth under aseptic conditions separately and incubated at 37°C for 24 h (bacteria) and room temperature for 5 days (fungi). Muller Hinton agar (bacteria) and Rose Bengal chloramphenicol agar media (fungi) were prepared aseptically and each were poured into petriplates and allowed to solidify. Five wells were bored onto the plates and they were swabbed with the respective bacterial and fungal cultures. To each well 20 µl algal extract and the sample were added. Chloramphenicol (positive control for bacteria) and nystatin (positive control for fungi) were added separately. The plates were incubated at 37°C for 24 h and at room temperature for 5 days to assess the antibacterial and antifungal activity.

2.7. Minimum inhibitory concentration (MIC) of synthesised AgNPs: Broth micro-dilution assay was used to determine the MIC of the synthesised AgNPs. The bacterial and fungal cultures (99 µl) were added into microtitre plates separately and a control was maintained. The synthesised AgNPs 50–0.32 µg/ml were added to the wells with 1 µl of different concentrations and serially diluted. The sterile microtiter plate was incubated at 37°C for 24 h (bacteria) and for 5 days at room temperature (fungus).

3. Results and discussion

3.1. Algal mediated synthesis of AgNPs: Various operational parameters such as concentration of $AgNO_3$ (1–5 mM) (Fig. 1*a*), algal extract (1–5 ml) and incubation time (0–40 min) were set for effective NPs formation (Fig. 1*d*). When 5 ml of algal extract was mixed with 10 ml $AgNO_3$ (5 mM), the colour of the reaction mixture changed from light green to reddish brown within 30 min (Figs. 1*b* and *c*) under sunlight. This might be due to the excitation of surface plasmon vibrations and it provides a convenient spectroscopic signature to indicate the formation of AgNPs. As the time interval was increased from 0 to 40 min, the colour change in the reaction mixture was observed at 30 min after which there was no remarkable change in the colour. This indicates that the formation of NPs to be high during the initial stage of the reaction and there after it decreases. Fig. 1*d* indicates the colour change in the reaction mixture at different time intervals.

3.2. Phytochemical screening: The phytochemical screening assays showed (Table 1) the presence of metabolites like carbohydrates, amino acids, phenols, alkaloids and so on, in the algal extract and AgNP embedded in diatom solution. Secondary metabolites exhibit several biological activities such as anti-oxidants, anti-microbial, anti-ageing and anti-inflammation. Most of these biological activities have been associated with their intrinsic reducing capability towards pro-oxidants [9].

3.3. UV–Vis spectral analysis: AgNPs are known to exhibit a UV–Vis absorption maximum in the range of 380–450 nm due to their size-dependent optical properties [10]. Fig. 2 shows the absorption spectra of extract, $AgNO_3$ and AgNPs. The eco-friendly synthesis of AgNPs exhibit a characteristic absorption peak at 445 nm corresponding to n–π* transition [11], which indicates the formation of AgNPs by residual phyto-molecules of the algal extract.

Table 1 Qualitative screening of the phytochemical constituents in the algal extract and synthesised AgNPs

Phytochemicals	Algal extract	AgNPs
alkaloids	+	+
flavonoids	+	+
tannins	—	—
carbohydates	+	+
saponins	+	+
terpenoids	+	—
phenol	+	+
quinone /anthraquinone	—	—
glycosides	+	+
starch	+	+
amino acids	+	+
celluose	+	+
steroids	+	—
anthocyanin	—	—

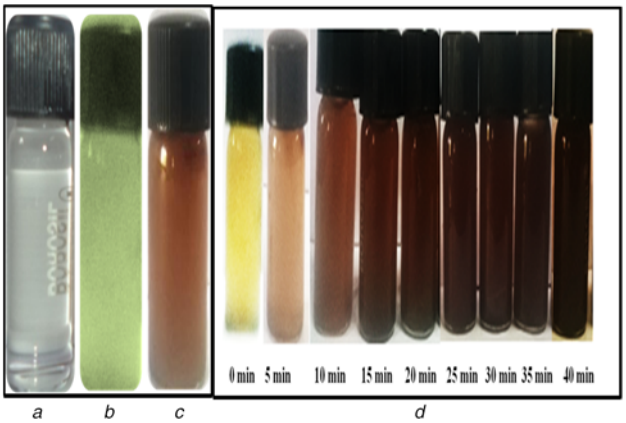

Fig. 1 *Photograph of*
a $AgNO_3$ solution
b Algal extract
c Biosynthesis of AgNPs at optimised conditions
d Synthesised AgNPs at various time intervals

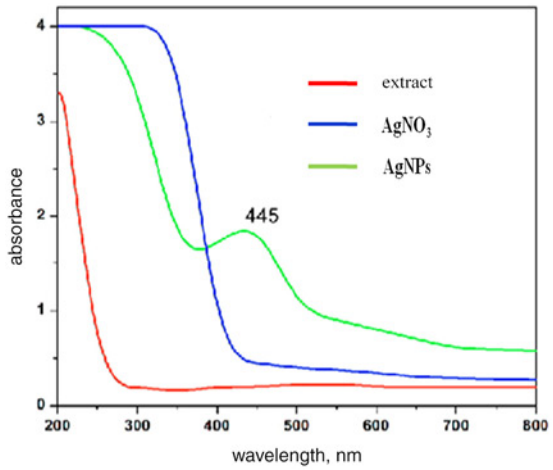

Fig. 2 *UV–Visible spectra of $AgNO_3$, algal extract and synthesised AgNPs*

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3.4. FTIR analysis: The dual role of algal extract as a green reducing and capping agent is quite obvious from the FTIR spectra of algal extract and synthesised AgNPs (Fig. 3). FTIR spectra of algal extract exhibits absorption peaks at 2239 and 1093 cm^{-1} and synthesised AgNPs exhibit several absorption peaks at 3625, 3440, 1890 and 970 cm^{-1}. The peak at 3340 cm^{-1} was assigned to the stretching vibrations of hydrogen bonded –OH stretch which may be due to the alcoholic or phenolic compounds. The peak appearing in the region 1890 cm^{-1} is characteristic of [NH] C=O group, which is the characteristic group of proteins. The peak at 3625 cm^{-1} is that of free amine groups or cysteine residues.

From the results of phytochemical studies, it is clear that the functional groups of these diverse metabolites have reacted with metal ions and reduced their size into nano-range. Recent studies have shown that the phytochemicals such as amino acids [12, 13], flavonoids, phenols, alkaloid and saponins present in the plant extracts play a major role in the bioreduction of silver ions and capping of the synthesised nano-particles [14]. A review of literature has revealed one or two phytochemical constituents to be responsible for the conversion of silver salt to silver nano-particles [15].

3.5. X-ray diffraction analysis: XRD measurements carried out to confirm the crystalline structure, phase composition and preferential orientation of formed AgNPs and dried powder algal extract are given in Figs. 4*a* and *b*. Fig. 4*b* indicates sharp diffraction lines at low angles ranging from 10 to 80°. The pattern clearly showed the main peaks at (2θ) 20.47, 28.49, 39.08, 53.54 and 71.34 corresponding to lattice planes (220), (122), (231), (241) and (331) evidently indicating the formation of face centred cubic structure of synthesised AgNPs. Furthermore, remaining small peaks are observed and these peaks are due to crystallisation of bioorganic compounds present in the synthesised AgNPs.

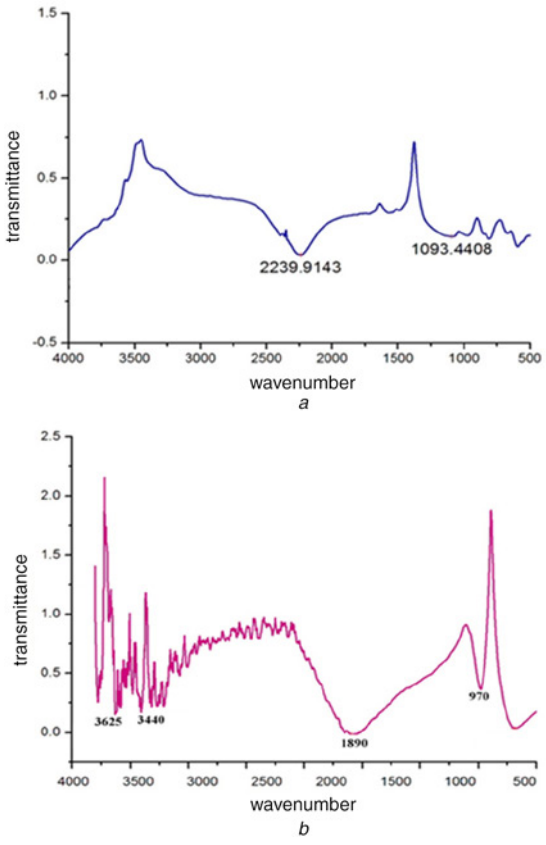


Fig. 3 *FTIR spectra of*
a Navicula cincta
b Synthesised AgNPs

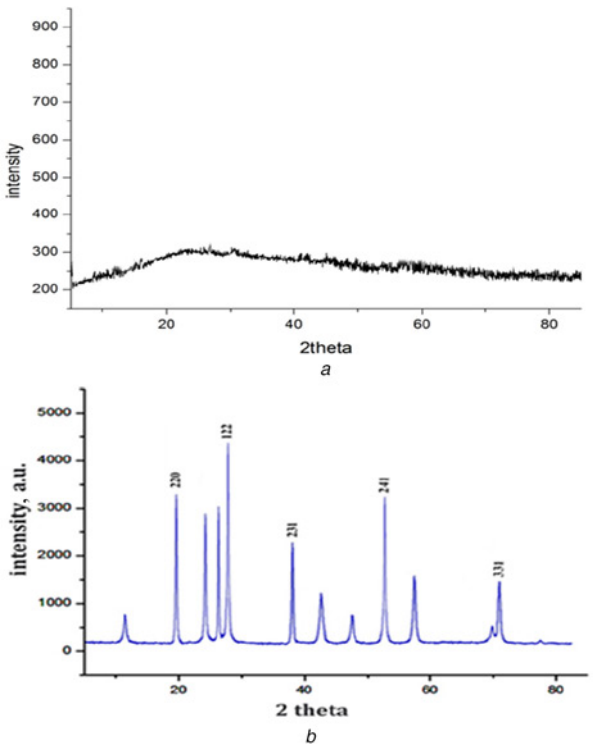


Fig. 4 *XRD patterns of*
a Navicula cincta
b Synthesised AgNPs

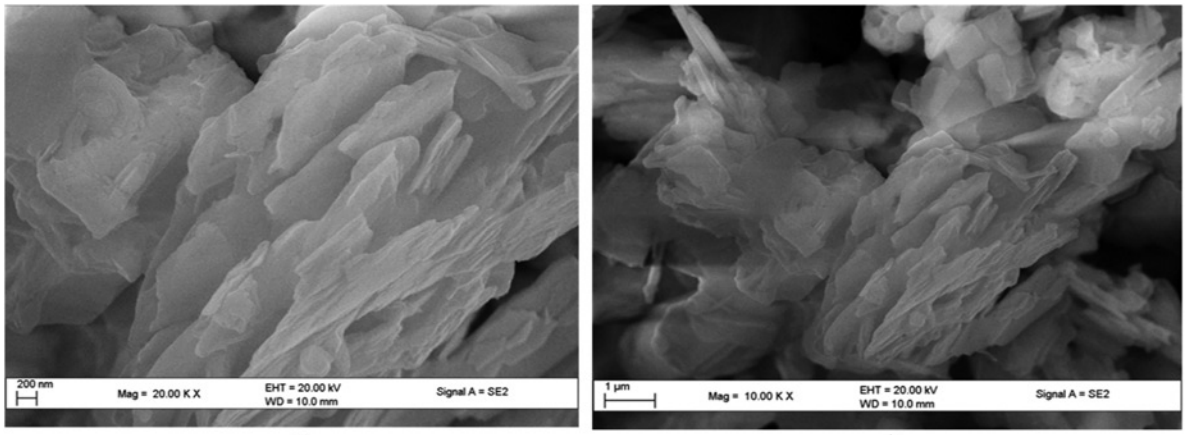
3.6. Scanning electron microscopy: Scanning electron microscope analysis was carried out to understand the topology of *Navicula cincta*, Fig. 5*b* shows the synthesis of polydisperse spherical AgNPs. AgNPs seems to be trapped within the cellular structures and evenly distributed throughout the biomass without aggregation (Fig. 5*b*).

3.7. Energy dispersive spectroscopy: The EDS spectrum (Fig. 6) reveals elemental silver at 3 keV. N and O peaks reveal plant extract embedded in the AgNPs.

3.8. Particle size determination: Particles size distribution was analysed using the results obtained from dynamic light scattering analysis with a maximum intensity at 32 nm having narrow intensity (Fig. 7). This is due to algal extract for reducing $AgNO_3$, causing nucleation, growth and agglomeration of AgNPs [16].

3.9. Anti-microbial activity of synthesised AgNPs: Silver ions have strong inhibitory and broad spectrum of antimicrobial activities. The anti-microbial activities of AgNPs are related to their size and shape. It is observed that smaller AgNPs with a larger surface area have a high bactericidal effect.

3.9.1. Anti-bacterial activity of the biosynthesised AgNPs: The AgNPs in the present study showed good activity against the pathogenic bacteria namely *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Klebsiella pnemoniae, Pseudomonas aeruginosa, Shigella flexneri, Vibrio cholera* and *Salmonella typhi* (Table 2 and Fig. 8). The results clearly indicate that the biomass of *Navicula cincta* synthesised AgNPs had the highest antibacterial activity against *E. coli* (25 mm), *P. aeruginosa* (24 mm), moderate activity against *S. aureus* (22 mm) and *B. subtilis* (20 mm) a weak activity towards *K. pneumonia* (15 mm), *S. flexneri* (13 mm), *V. cholera* (14 mm) and *S. typhi* (12 mm). The silver nitrate solution ($AgNO_3$) exhibited a remarkable zone of inhibition



a *b*

Fig. 5 *SEM micrographs of*
a Navicula cincta
b Synthesised AgNPs

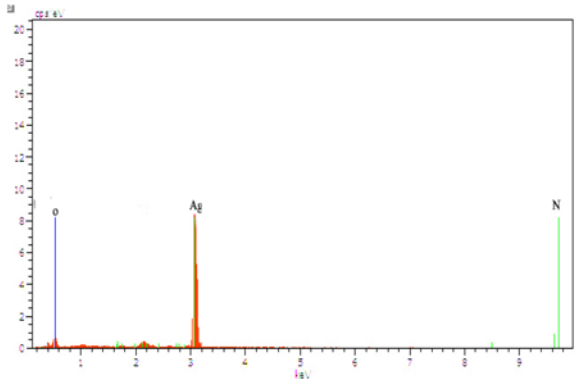


Fig. 6 *EDS spectra of synthesised AgNPs*

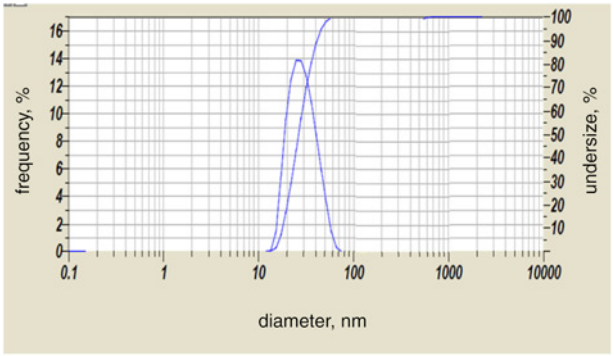


Fig. 7 *Particles size determination*

against *Escherichia coli* (20 mm), *Staphylococcus aureus* (18 cm), *Bacillus subtilis* (17 cm) followed by *Vibrio cholera* (12 mm), *Shigella flexneri* (10 mm), *Salmonella typhi* (9 mm).

Blank experiments were also conducted with doubly distilled water and algal extract. AgNPs are more effective against Gram-negative bacteria, which is related to differences in the construction of cell walls of both groups of bacteria. Gram-negative bacteria have lipopolysaccharide layer positioned on the outside of the cell wall beneath a thin layer of peptidoglycan. A negative charge, located on lipopolysaccharides attracts the positively charged particles of AgNPs and $Ag+$ ions, deposited on the surface. However, only a thin layer of peptidoglycan, which creates a 3D rigid structure, is the main component of cell walls of Gram-positive bacteria. Its structure comprises polysaccharide cross-linked short chains of protein molecules. The stiffness and geometry of these layers are not conducive to penetration of AgNPs through the cell wall of Gram-positive bacteria. However, it has been proved that AgNPs are able to penetrate both types of cell wall and enter into the cell, resulting in the uncontrolled tyrosine phosphorylation [17, 18]. Thus, the antibacterial effect of nanoparticles is attributed to the nanoparticle modulated, phosphotyrosine profile of bacterial peptides that affects the signed transduction and hence the microbial inhibition [19].

3.9.2. Anti-fungal activity of biosynthesised AgNPs: The MIC values of (Table 3) synthesised AgNPs against *Aspergillus flavus, Aspergillus niger, Aspergillus fumigatus* and *Rhizopus stolonifer* revealed significant zone of inhibition (Table 3 and Fig. 9). The synthesised nanoparticles revealed higher appreciate zone of inhibition against *Aspergillus flavus* higher than the control Nystatin. Silver nitrate exhibited the zone of inhibition ranging

Table 2 MIC and inhibition zone of synthesised AgNPs against bacterial isolates

Bacteria isolates	Conc., mg/µl	Zone of inhibition, mm			
		$AgNO_3$	Algal extract	Chloramphenicol	AgNPs
Escherichia coli	6.02	20	-	28	25
Pseudomonas aeruginosa	6.02	18	1	30	24
Staphylococcus aureus	6.02	18	-	28	22
Bacillus subtilis	12.05	17	-	30	20
Klebsiella pneumoniae	6.02	13	-	30	15
Vibrio cholera	6.02	12	-	30	14
Shigella fiexneri	6.02	10	-	28	13
Salmonella typhi	6.02	9	-	30	12

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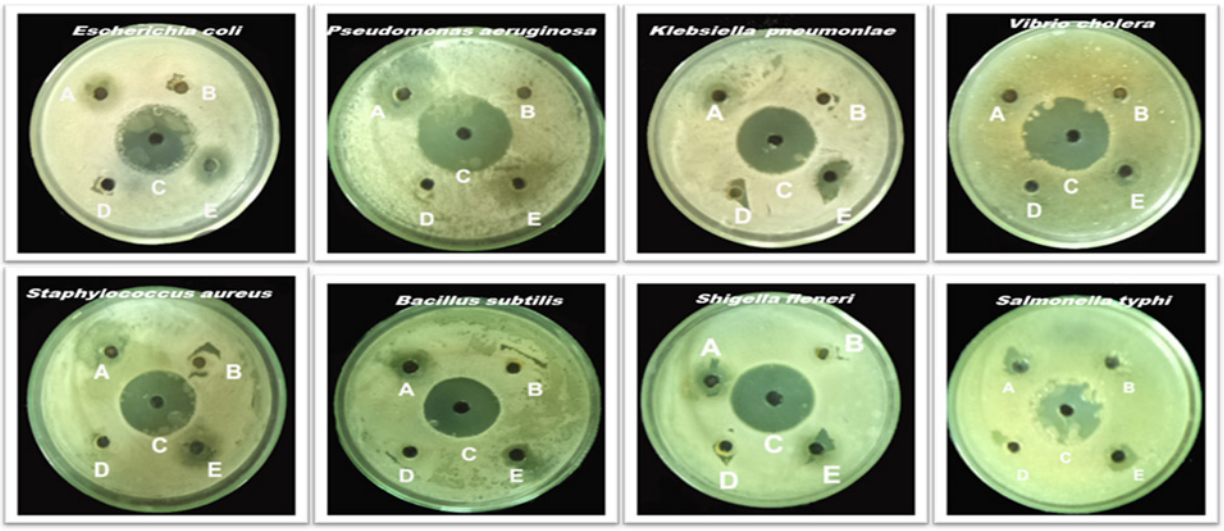

Fig. 8 *MIC of anti-bacterial activity of synthesised AgNPs: (A) $AgNO_3$, (B) algal extracts, (C) chloramphenicol, (D) distilled water, (E) AgNPs*

Table 3 MIC and inhibition zone of synthesised AgNPs against fungal isolates

Fungus isolates	Conc., mg/µl	Zone of inhibition, mm			
		$AgNO_3$	Algal extract	Nystatin	AgNPs
Aspergillus flavus	6.02	13	-	12	18
Aspergillus niger	6.02	12	-	17	16
Aspergillus fumigates	3.01	8	-	10	14
Rhizopus stolonifer	3.01	7	-	5	9

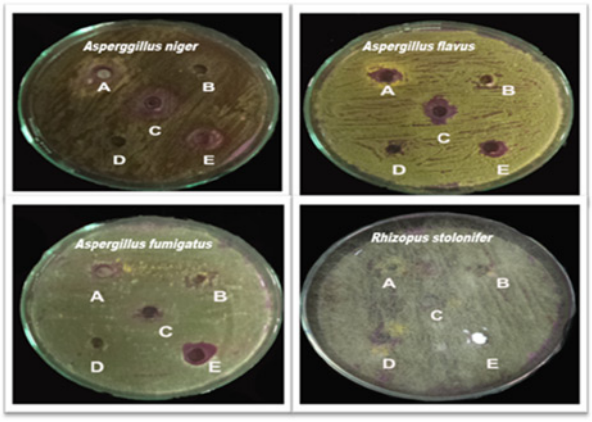

Fig. 9 *MIC of anti-fungal activity of synthesised AgNPs: (A) $AgNO_3$, (B) algal extracts, (C) nystatin, (D) distilled water, (E) AgNPs*

from 7 to 13 mm, while no zone of inhibition was found with algal extract and doubly distilled water. Thus, maximum antifungal activity was observed in AgNPs against *A. flavus*. The AgNPs possibly destroy the fungi cell membrane, as they are able to penetrate to the interior of the cell causing leakage of ions and other compounds, such as glucose and trehalose. Trehalose prevents inactivation and denaturation of proteins which may be caused by temperature changes or the effects of oxidising agents [20].

4. Probable mechanism of synthesised AgNPs: There are some literature available to explain the probable mechanism of bio-mediated synthesis of NPs [21]. In the present study, mechanism of AgNPs from algal extract consists of three stages: (i) reduction of Ag^+ ions, (ii) trapping/nucleation, (iii) formation of NPs. Bio-proteins may reduce Ag^+ ion to Ag nuclei and itself changed to its secondary structure and by further reduction of Ag nuclei grow up to AgNPs, which are capped and stabilised by reducing phytochemicals present in the algal extract [22]. Ag ions from AgNPs can penetrate into the microbial cell, which can oxidise cellular biomolecules, helps the formation of reactive oxygen species and damages the cell [23].

5. Conclusion: A facile method of synthesising nanosilver was carried out using the aqueous extract of *N. cincta* as green capping agents. The formation of AgNPs was confirmed and characterised by UV, FTIR, particle size analyser, XRD, SEM and EDS analyses. The biosynthesised AgNPs exhibited potent activity against all tested bacterial and fungal strains. The marine microalgae *N. cincta* can produce silver nanostructures through efficient green nanochemistry avoiding the presence of hazardous and toxic solvents and waste. Applications of these eco- friendly NPs with bactericidal and other medical applications will have high potentiality for large-scale synthesis in future.

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