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ORIGINAL PAPER

Synthesis of silver nanoparticles from *Turbinaria ornata* and its antibacterial activity against water contaminating bacteria

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Abstract

Advancement of environmentally friendly synthetic methods of nanoparticles is growing in the field of nanotechnology. *Turbinaria ornata* is a brown alga which is highly abundant in coastal region of Sri Lanka and an excellent source of bioactive compounds. The aim of this study is to synthesise silver nanoparticles (AgNPs) with *Turbinaria ornata* to use in antibacterial activities. Sources contain Ag nanoparticles are being used for various applications such as photocatalytic degradation and antimicrobial activities. The parameters such as temperature and concentration of the silver nitrate and pH were chosen to find the efficient synthesis of AgNPs. Extracts were prepared at 70 °C was added with 1, 2, 3, 4 and 5 mM $AgNO_3$ and incubated for 24 h at different pHs to prepare the AgNPs. The synthesised nanoparticles were characterized by the surface plasmon resonance band at 430 nm. Highest absorbance band for nanoparticles synthesised from algal extract was obtained at 70 °C and 5 mM silver nitrate at pH 11. The characteristic bands at 3300.93, 2107.30, 1640.74 and 1051.32 cm^{-1} in FT-IR suggested that the distinct functional groups, O–H, N–H and C = C are responsible for the reduction and capping of AgNPs. The synthesised nanoparticles were also characterized with X-ray diffraction, energy-dispersive X-ray spectroscopy and scanning electron microscopy. The X-ray diffraction study revealed the average size of the nanoparticles was 8.15 nm obtained at pH 7, 51.11 nm at pH 9 and 40.64 nm at pH 11 and the peaks confirmed the face-centred crystalline lattice of the AgNPs. AgNPs were further confirmed by EDX and SEM images. The synthesised nanoparticles were tested with gram positive *Staphylococcus aureus*, *Enterococcus faecalis* and *Bacillus circulans* and gram-negative *Escherichia coli* and *Pseudomonas aeruginosa.* The extract with AgNPs showed the highest antibacterial activity against *Staphylococcus aureus*. Hence, the AgNPs from *Turbinaria ornate* was facile and innocuous synthesis which can serve as efficient antimicrobial agent against human pathogens as well as water contaminating bacteria.

Keywords Antibacterial activity · Characterization · Plant mediated synthesis · Silver nanoparticles · *Turbinaria ornata*

Introduction

Nanotechnology deals with production and distribution of chemical, physical and biological systems with structural features between single atoms or molecules to submicron dimensions and combining the resultant structures into larger systems (Nasrollahzadeh et al. 2019). Nanomaterials exhibit specific performances when compared to its bulk materials. Nanoparticles exhibit improved performance due to their large surface to volume ratio, increased percentage of atoms at grain boundaries and electronic properties, all of which are connected to the size of the particles (Mansoori and Soelaiman 2005).

Large surface to volume ratio enhances the use of wide spread nanomaterials for variety of applications. Reduction of the surface energy results in the interaction of the nanoparticles between its neighbours through chemical bonds while physical attractions lead to agglomeration. Individual size of the nanoparticles determines the attractive forces. Smaller the size the greater the van der Waals forces between each particle. Stabilizing agent plays a major role in the synthesis of nanoparticles which prevent the agglomeration of the nanoparticles (Roy et al. 2019).

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Several physical, biological and chemical methods are widely used to synthesise nanoparticles. These physical and chemical methods have drawbacks due to usage of inert gases, high pressure, toxic precursors and toxic solvents and due to generation of toxic by-products (Ijaz et al. 2020; Ahmed et al. 2016). Advancement of science in the field of nanotechnology introduce several eco-friendly routes which includes usage of biological agents such as plant extracts, bacteria and fungi to reduce the metal salts into their respective nanoparticles (Kuppusamy et al. 2016; Pattanayak et al. 2017). These agents function as in vitro reducing and capping agents (Roy et al. 2019).

General synthesis of the nanoparticles is done by the reduction of metal complexes in the dilute solutions. Most commonly accepted reducing agents such as hydrazine hydrate and sodium borohydride are not preferred because of their additional effects of undesired toxicity embedded with the nanoparticles which will affect the application of the nanoparticles (Roy et al. 2019). Therefore, great advantages could be harvested by using green materials such as extracts of plant, microbes and algae. Green synthesis of plant mediated nanoparticles gather greater attention nowadays due to their unique features such as process stability, wide ranging with fast rate of synthesis and variable morphological properties, compared to micro-organism-based green synthesis (Rajendran and Sengodan 2017).

In plant mediated synthesis of AgNPs phytochemical compounds such as polysaccharides, polyphenols, amides, vitamins, alkaloids, terpenoids, organic acids and aromatic dicarboxylic acids which is present in the plant, reduce the Ag^+ to Ag nanoparticles (Heinlaan et al. 2008; Qu et al. 2011; Jha et al. 2009) and functioned as capping agent (Raveendran et al. 2003). Capping is more advantageous in many ways such as prevention of agglomeration of nanoparticles, reduces toxicity (Roy et al. 2013) as well as it improves antimicrobial action(Panja et al. 2016; Mandal et al. 2014; Ahmed et al. 2016).

Various plant materials have been used for the synthesis of AgNPs via green synthesis method. Leaf extracts of *Azadirachta indica* (Shankar et al. 2004), *Aloe vera* (Chandran et al. 2006), *Camellia sinensis* (Vilchis-Nestor et al., 2008), *Cinnamomum camphora* (Huang et al. 2007), *lemon grass* (Shankar et al. 2004), *Krishna tulsi* (Philip and Unni 2011), *Psidium gujava* (Raghunandan et al. 2011), *Hibiscus rosa sinensis* (Philip 2010), and *Allium sativum* (Von White et al. 2012) have been used for the synthesis of AgNPs.

AgNPs became the centre of the attention because of the antimicrobial activity against multidrug resistant pathogens from virus to prokaryotes (Gong et al. 2007). AgNPs have been widely used in the medical devices (He et al. 2013), wound dressing (Wilkinson et al. 2011), water purification due to their antimicrobial property (Jain et al. 2005) (Lin et al. 2013), textile materials (Vigneshwaran et al. 2007; Xu et al. 2013), pharmaceuticals (Kumar et al. 2011), detection of DNA molecules (Thompson et al. 2008), sensing food adulterants (Ping et al. 2012) and adsorption of metals and pesticides (Das et al. 2012). Owing to the optoelectronic properties and physiochemical properties AgNPs have been attracted many researchers. AgNPs have showed noxious property against various micro-organisms. Due to this antibacterial property AgNPs have been widely used in ion exchange fiber, coating of medical devices and dental resins (Krishnaraj et al. 2010, Sondi et al. 2004).

T. ornata is a brown alga and widely distributed in the tropical and subtropical seas, which was selected for the synthesis of AgNPs. This brown algae composed of many bioactive compounds such as saponins, polyphenols, tannins and alkaloids(Deepak et al. 2017). These secondary metabolites contain functional group which can reduce the silver ions to silver and act as stabilizing agent for nanoparticles(Morales-Lozoya et al. 2021). Therefore, this study focuses on synthesis of AgNPs from *T. ornata* and investigates the antibacterial activity following water contaminating bacteria such as *Bacillus circulans, Staphylococcus aureus, Enterococcus faecalis, Escherichia coli and Pseudomonas aeruginosa.*

Materials and methods

Materials

All the reagents used in the experiments are in analytical grade and used without purification. Silver nitrate, hydrochloric acid, sodium hydroxide, Muller–Hinton agar and nutrient agar were purchased commercially. Streptomycin; gram positive and gram negative bacterial stains were obtained from microbiology laboratory, Department of Botany, University of Jaffna. Brown algae, *T. ornata* (Fig. 1), were collected from the coastal region of Jaffna, Sri Lanka and removed sand and debris by washing with tap water for several times followed by deionized water. They were dried in the shady environment for 3 weeks and powdered.

Preparation of *T. ornata* extract

Dried powder of *T. ornata* (2 g) was added to 100 mL of deionized water at room temperature (30 °C) and stirred for 30 min. The extracts were filtered through Whatman No 1 filter paper. The procedure was repeated at 50, 60, 70 and 100 °C and the extracts were stored at 4 °C for the synthesis of AgNPs.

Biosynthesis of AgNPs

The reaction mixture was prepared in a clean flask by adding 10 mL of aqueous extract (prepared at 50, 60, 70 and 100 °C)

Fig. 1 Turbinaria ornata

and 90 mL of 1 mM aqueous $AgNO_3$. This procedure was repeated with different concentration (2 mM, 3 mM, 4 mM and 5 mM) of $AgNO_3$ solution (Vijayan et al. 2014). All these reaction mixtures were kept under continuous stirring for 2 h, 4 h, 6 h and 24 h in order to get the higher amount of AgNPs. The bio-reduction of the Ag^+ ions was monitored by UV–visible spectrophotometer. The AgNPs were centrifuged at 9400 RCF for 10 min. The supernatant solution was discarded, and residue was rinsed with deionized water for twice by repeating the centrifugation steps. The biosynthesized AgNPs were kept in a desiccator.

Effect of pH on the synthesis of AgNPs

The influence of pH on the synthesis of AgNPs was carried out by changing the pH of the reaction mixture. Ten mL of, 5 mM $AgNO_3$ was added to the 90 mL of filtrate of *T.ornata* obtained at 70 °C. The different pH was maintained (pH 3, 5, 7, 9 and 11) to examine the effect of pH on synthesis of AgNPs. The pH of the solution was changed using 1 M HNO_3 and 1 M of NaOH. After 24 h of incubation, the absorbance was measured.

Characterization of AgNPs

The absorbance of the bio-reduction of Ag^+ ion was determined by the UV–vis spectrophotometer (Jasco V-570 UV/VIS/NIR) of the wavelength from 350–700 nm (Bakht Dalir SJ et al. 2020). Plant extract and the AgNPs were studied using FT-IR in the range of 4000–700 cm^{-1} to identify functional groups. The XRD patterns were obtained from X-ray diffraction (XRD, PANalytical-AERIS). The diffraction pattern was collected using Cu Kα radiation (λ = 1.5408 Å) at ambient temperature, under the following operational conditions: accelerated voltage 40 kV; emission current of 44 mA; scan range (2θ) between 10° and 90° with a step size of 0.0027 ° and a scan speed of 4°/min. The nano dimensional size of the synthesized AgNPs was ensured by employing Sigma Scanning Electron Microscope (Sigma-SEM) with EDS detector from Oxford instrument with Nano Analysis, Concord, MA, USA at 4 kV.

Antibacterial activity

In Vitro antibacterial activity was tested against *Bacillus circulans, Staphylococcus aureus, Enterococcus faecalis, Escherichia coli* and *Pseudomonas aeruginosa* by the agar well diffusion method. Bacterial suspensions were prepared separately in sterile saline water and their concentrations were maintained as $X \times 10^8$ cells/mL with the 0.5 M McFarland standard using dilution techniques. The bacterial suspension (0.1 mL) was spread uniformly on the surface of the Muller–Hinton agar (MHA) plate. Nine mm diameter wells were made by using a sterile cork borer.

Biosynthesised AgNPs at pH 11 and $AgNO_3$ (5 mM) were diluted by tenfold with deionised water and pH was adjusted to 7. Each well was eluted with 100 µL of biosynthesized AgNPs, equal $AgNO_3$ solution, algal extract, streptomycin (30 µg/mL) and deionized water separately. All the plates were incubated at 37 °C for 24 h and the diameter of the clear zone (zone of inhibition) was measured. Each experiment was carried out in triplicates and mean ± SD of the zone of inhibitions were taken for calculating the antibacterial activity of the extracts.

Results and discussion

UV–visible spectra of AgNPs

After the incubation time of 24 h, the colour change of the solutions from yellow to dark brown colour can be seen as evidence of Ag^+ reduction to AgNPs. The intensity of colour was increasing with concentration of silver nitrate and with the algal extract prepared at higher temperature. The formation of AgNPs was monitored by UV–visible spectrum. The parameters such as concentration of silver nitrate, pH of the solution and temperature for preparing the algal extract were optimizing the conditions. UV–visible spectroscopy is an effective tool to monitor the formation and the stability of the nanoparticles, which depends on the surface plasmon spectroscopy (Huong and Ngoc Thang 2021). As shown in Fig. 2 a-c the UV–visible spectra of AgNPs displayed peaks around 430 nm, which is due to the surface plasmon resonance effect of

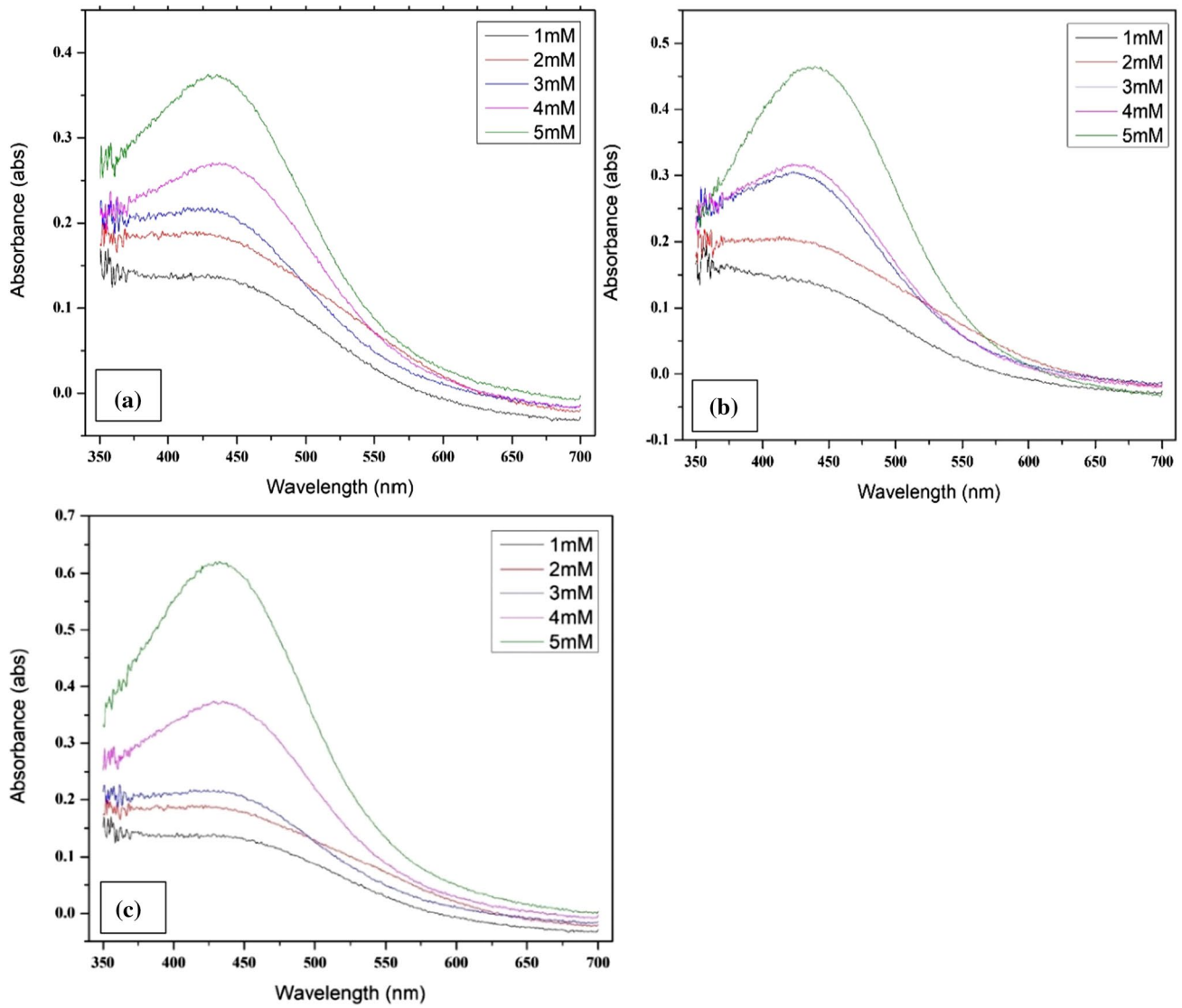

Fig. 2 UV/Visible spectra of synthesised AgNPs with different concentrations of $AgNO_3$ from extract prepared at **a** 50 °C **b** 60 °C **c** 70 °C

the AgNPs. Excitation of the electron to the conduction band of the nanoparticles surface is known as surface plasmon resonance (Gul et al. 2016). With the increase of the concentration of silver nitrate solution added (1, 2, 3, 4 and 5 mM) the absorbance of the graph was found to be increasing which shows that the synthesised nanoparticles were increased with the concentration of the $AgNO_3$ solution. The algal extracts were prepared at different temperature from 30 to 100 °C. Higher absorbance due to the nanoparticles was obtained at 70 °C compared with other solutions. Higher amount of molecules extracted from algal at 70 °C may be the reason for the highest absorbance in Fig. 2c compared with Fig. 2a and b. With the increase of the concentration of $AgNO_3$ and the temperature the absorbance peak becomes narrower which indicates smaller polydispersity and with low concentration of $AgNO_3$ the peak becomes broader which indicates the larger polydispersity (Morales-Lozoya et al. 2021; Gul et al. 2016). The formation of AgNPs at different time intervals was also monitored by UV–vis spectroscopy and maximum absorption peak was observed at 24 h of incubation.

The colour of the solutions of nanoparticles synthesised at different pH has shown at Fig. 3. With the increase of the pH, the colour of the solution becomes more intense. The intensity of UV–visible spectra of the synthesised nanoparticles solutions at different pH such as pH 3, 5, 7, 8, 9 and 11 is shown in Fig. 4 which indicated the higher amount of AgNPs occurred at pH 11. Similar effects of pH have been reported (He et al. 2017).

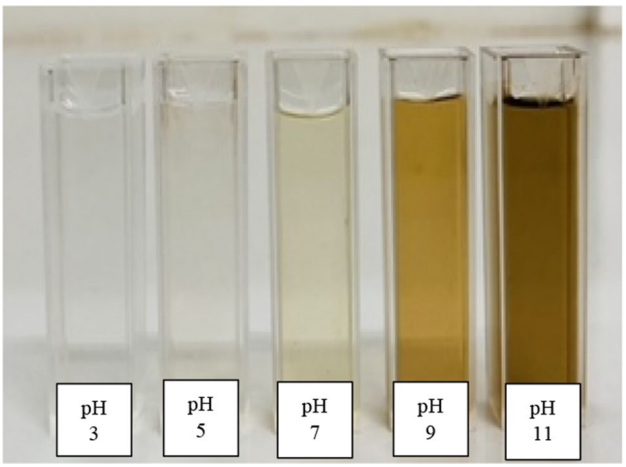

Fig. 3 AgNP solutions prepared at pH = 3, 5,7,9 and 11

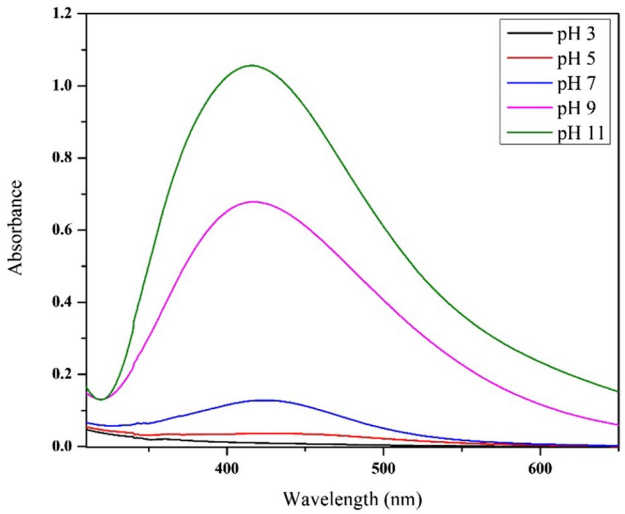

Fig. 4 UV/Visible spectra of AgNPs at different pH

FT-IR analysis of the biosynthesized AgNPs

FT-IR analytical techniques are an important tool for detection of the organic functional groups present in the algal extract of *T.ornata*. These functional groups are responsible for the reduction of silver and the stabilization of the nanoparticles. FT-IR spectrum of algal extract and biosynthesized AgNP is shown in Fig. 5. The peaks at 3300.93 cm^{-1} corresponds to the O–H stretch of free hydroxyl alcohol and phenol, peak at 2107.30 cm^{-1} due to the aromatic C = C stretching frequency, the band of 1640.74 cm^{-1} represents the stretch of N–H bending of primary amines. Peak at 1051.32 cm^{-1} represents the C-N stretch of aliphatic amines (Vallepu N et al., 2021). The FTIR spectrum of the extract with AgNPs also exhibit the same wave number but a significant reduction in the peak intensity confirming the

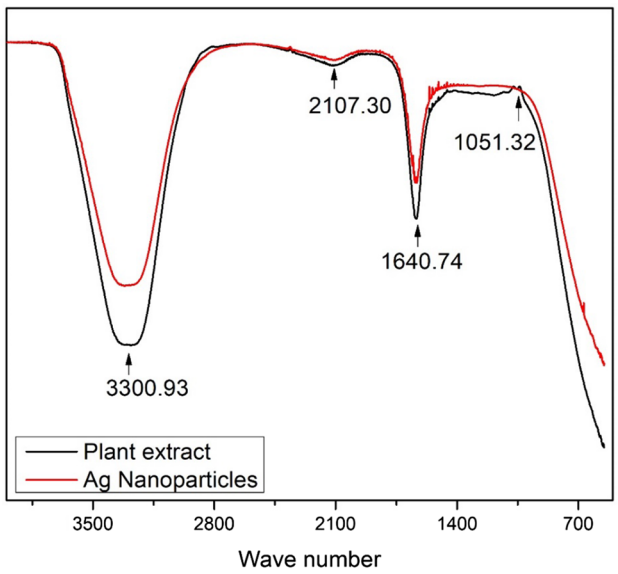

Fig. 5 FT-IR spectra of AgNP and algal extract

possibility of the above functional group act as reducing and stabilizing agent for the AgNPs (Roy et al. 2019). Proteins can bind to the metal nanoparticles through cysteine residues contain free amino group and it has stronger capability to bind with the AgNPs and functioned as the capping ligand and thus preventing the aggregation of the synthesised nanoparticles (Gole et al. 2001). *T. ornata* has rich sources of bioactive secondary metabolites such as polysaccharides, flavonoids, phenolic acids, bromophenols and tannins (Deepak et al. 2017).

X-Ray diffraction analysis

The crystalline nature and the size of the AgNPs at different pH solutions were confirmed by the X-ray crystallography pattern shown in Fig. 6. The main peaks in the X-ray crystallography 38.39°, 44.62°, 64.93°, 78.21° and 82.38° which corresponds to the (111), (200), (220), (311) and (222) crystallographic planes, respectively. The above peaks confirm the crystallographic planes of the face-centred cubic silver crystals. A few peaks marked with the star marked are due to the crystallization of the secondary metabolites of the algal extract on the surface of the AgNPs (Vanaja and Annadurai 2013). The size of the nanoparticle was measured from the Scherrer equation,

$$D = \frac{\kappa\lambda}{\beta\cos\theta}$$

where θ-diffraction angle, D-crystalline domain size, β-width of its peak at half of its height (FWHM), λ-X-ray wavelength and κ-Scherrer constant. The crystallinity size

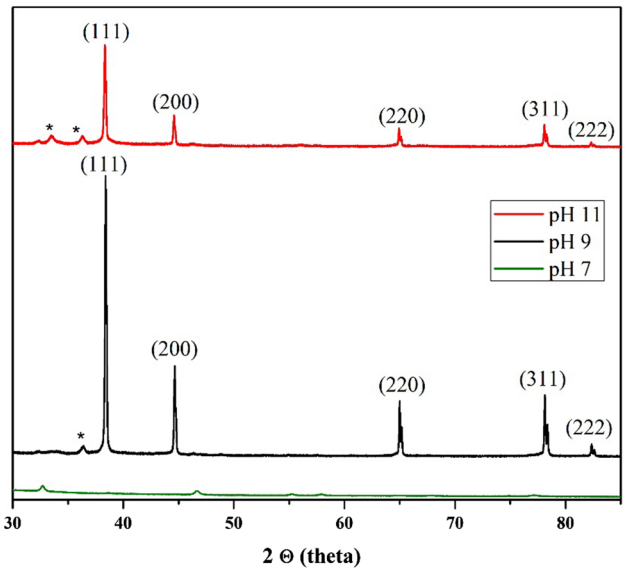

Fig. 6 XRD pattern of synthesised AgNPs at pH 7, 9 and 11

of biosynthesised AgNPs varied with pH of the solution. The size of AgNPs was 40.64, 51.11 and 8.15 nm at pH 11, 9 and 7, respectively.

Scanning electron microscopy

The surface morphological analysis of the biosynthesized AgNPs was examined by Sigma Scanning Electron Microscope (Sigma-SEM). Based on the results of the SEM image, the size of the AgNPs prepared at pH 11 was around 30–40 nm which is in accordance with XRD results. AgNPs show spherical shaped aggregated particles (Fig. 7). Elemental dispersive spectra of AgNPs show the presence of metallic Ag and other chemical elements such as silicon from silicon wafer as a substrate, carbon from carbon tape as adhesive and C, O are part of the composition of the aqueous solution (Fig. 8).

Antibacterial activity

Antibacterial activity of *T.ornata* extract and the synthesised AgNPs produced from this algae were tested against gram positive bacteria namely *S.aureus*, *B.circulans* and gram-negatives *E.coli, E.faecalis* and *P.aeruginosa* by agar well diffusion method (Fig. 9). As shown in Figs. 9 and 10, synthesised AgNPs were more effective in antibacterial activity compared to $AgNO_3$ solution and algal extract to all tested bacteria and no clear zone was observed with deionised water. The standard streptomycin exhibited the highest activity. The extract of AgNPs showed higher antibacterial activity against gram positive *S.aureus* than compared to gram negatives. Previous study indicated that AgNPs was more effective against gram negative bacteria compared to the positives (Bakht Dalir SJ et al. 2020).

The AgNPs treated bacteria undergo lysis, their cell wall break down, resulting in the release of their cellular contents in to the surrounding environment, and finally the cytoplasm was emptied. Several studies reported that AgNPs passed over cell wall of bacteria and acted with cell membrane to damage the enzyme and interfered with normal metabolism of cell. Subsequently, the AgNPs entered into bacterial cells and condensed on Deoxyribonucleic acid (DNA) to prevent the replication and reproduction. Finally resulting in the death of bacteria, researchers mentioned that the silver cations from the AgNPs appear to attach to the negatively charged bacterial cell wall and rupture it, leading to protein denaturation and causing cell death. The attachment of

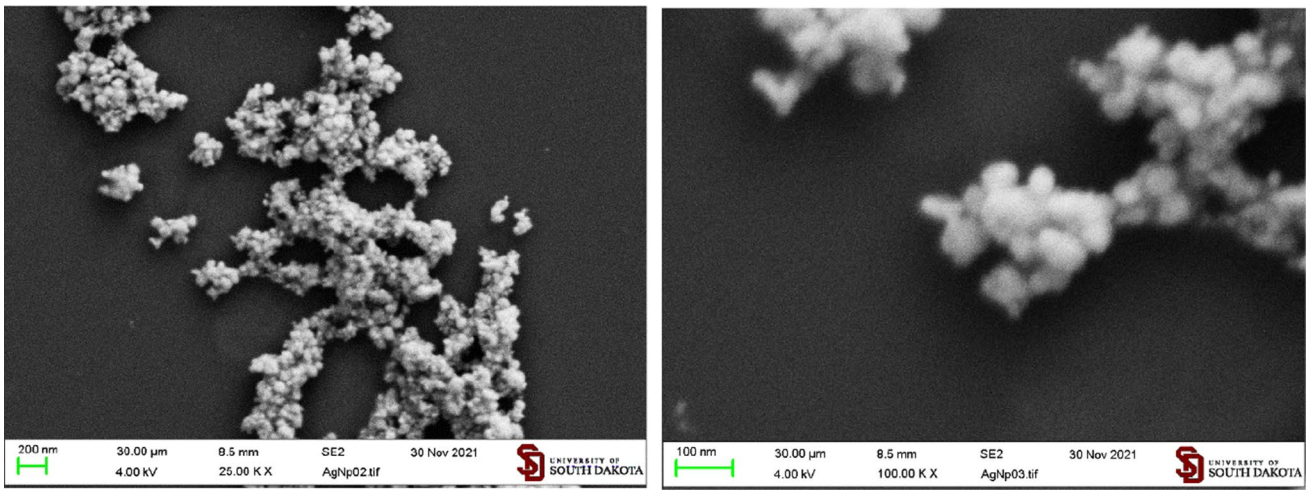

Fig. 7 SEM image of biogenically synthesised AgNPs at pH 11

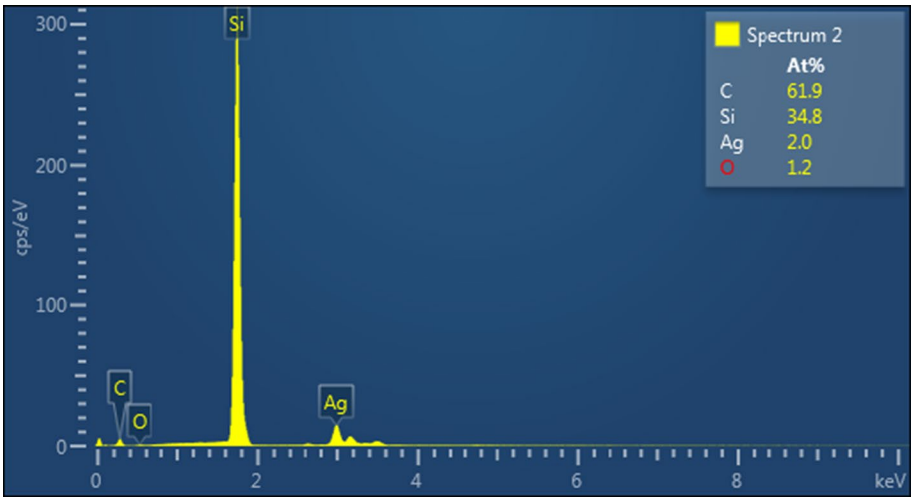

Fig. 8 Elemental dispersive spectra of AgNPs synthesised at pH 11

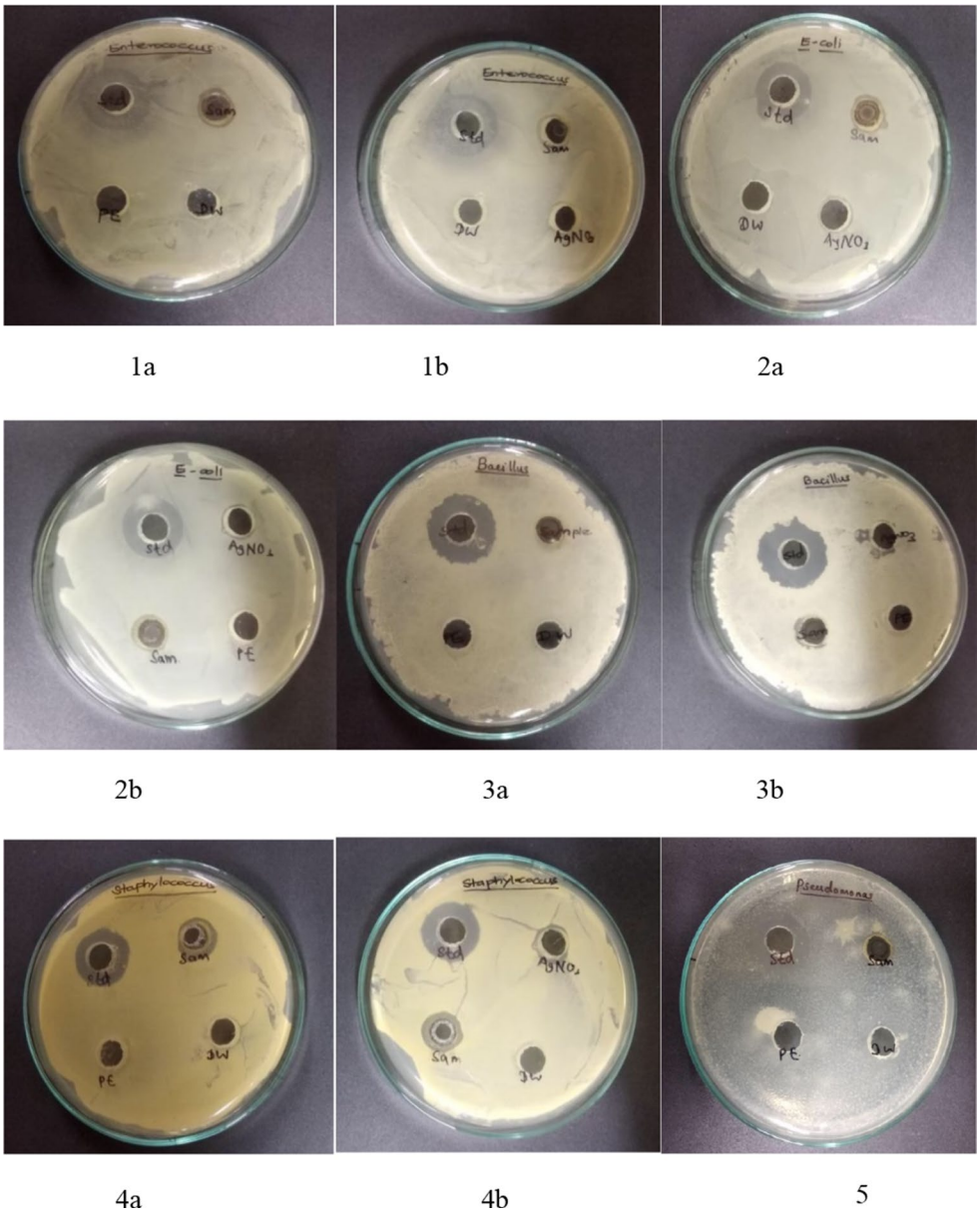

Fig. 9 Inhibition zones of 1a, 1b) *E. faecalis* 2a,2b) *E. coli* 3a,3b) *B. circulans* 4a, 4b) *S. aureus* 5) *P. aeruginosa* [DW-deionized water, $AgNO_3$, PE-algal extract, sam- AgNPs]

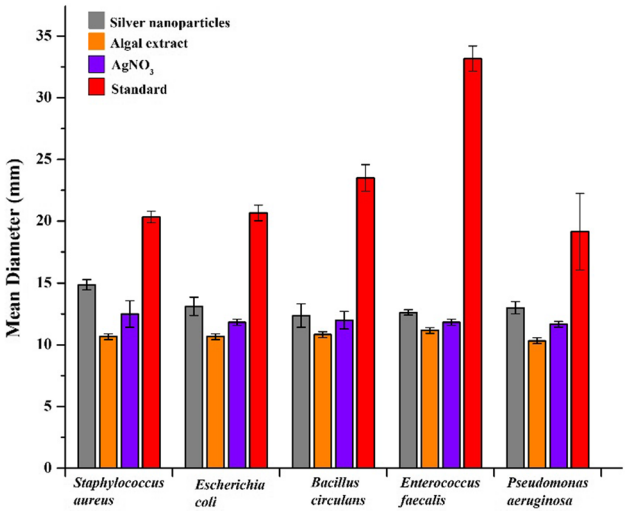

Fig. 10 The histogram showing the inhibition zone of *E. faecalis, E. coli, B. circulans, S. aureus* and *P. aeruginosa*

silver cations or nanoparticles on to the cell wall leads to an increase in protein precursors, which results in the loss of the proton motive force and finally cell death (He et al. 2017; Li et al. 2011).

There is a report on green synthesis of AgNPs and the application in water purification which stated the silver-nano biohybrid material adsorbed pesticides such as organophosphorus and bacterial pathogens. It was reported that silver-nano biohybrid material possess significant efficient of 85–99% of organophosphorus (parathion and chlorpyrifos) adsorption. After the treatment the *E.Coli* was undetectable and concentration of parathion (< 1 µL) and chlorpyrifos (1.2 µL) was decreased to the permissible level (Das et al. 2012). Another study was reported water treatment of paper impregnated with AgNPs and these sheets exhibited antibacterial activity against *Escherichia coli* and *Enterococcus faecalis* (Dankovich et al. 2011).

Conclusion

The present study revealed the synthesis of AgNPs with algal extract, *T.ornata* in reducing silver ions and stabilizing the AgNPs. The most, efficient way to synthesize nanoparticles was addition of 5 mM silver nitrate into aqueous extract obtained at 70 °C and maintained at pH 11. The moderate temperature used for the extraction of the algal extract yields higher the number of chemical compounds extracted. *T.ornata* may have rich source of the amino acids residues of the protein acts as a reducing agent for the synthesis of AgNPs. The AgNPs were characterised using UV–visible, FT-IR, XRD and SEM spectroscopies. The highest peak (0.62) was observed at 432 nm in UV–visible spectrophotometry. The size of the nanoparticles were varied at different pH of the solutions. The synthesized nanoparticle has an average size 8.15, 51.11 and 40.64 nm at pH 7, 9 and 11, respectively, and they showed face-centred cubic crystals. The synthesised nanoparticles have antibacterial activity against water contaminating bacteria. It showed an effective inhibition on both gram negative and gram positive bacteria.

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Data availability Data sharing is not applicable. It will be shared if needed.

Declarations

Conflict of interest The author declare that they have no competing interest.

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