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Research Article
Biopotential Application of Synthesis Nanoparticles as Antimicrobial Agents by Using *Laurencia papillosa*

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Abstract

Background and Objective: The synthesis of nanomaterials play significant role in nanotechnology. The pathogenic organisms are more resistant to the available antibiotics. The green synthesis of silver nanoparticles by using marine algae, which have a wide range of bioactive secondary metabolites is a need to find new antibiotics. **Materials and Methods:** The red alga, *Laurencia papillosa* was extracted with deionized water. The algal extract was added to 1.0 mM $AgNO_3$ solution. The aqueous solution was subjected to stirring for overnight. The reaction mixture was monitored to emphasize the formation of silver nanoparticles. The change in color of the solution was recorded by using UV-vis spectrophotometer. The biosynthesized nanoparticle solution was subjected to Fourier transform infrared and x-ray diffraction analysis. The morphology of the nanoparticles was observed by using scanning electron microscope. The antibacterial and antifungal activity of algal silver nanoparticles was tested by using agar well-diffusion method. The minimum inhibitory concentration of the solution was studied. **Results:** The UV-vis spectroscopic analysis confirmed the silver nanoparticle formation with the peak at 450 nm after 72 h. Fourier transform infrared measurement was carried out to identify the possible biomolecules responsible for the stabilization of the newly synthesized silver nanoparticles. The x-ray diffraction analysis showed that the silver nanoparticles synthesized from the algal extract were highly crystallized and purified. The scanning electron microscope image showed the predominant of cubic shape nanoparticles on the surface of the cell. The antimicrobial activity of synthesized silver nanoparticles (50-150 µL) was evaluated against pathogenic bacteria and fungi. The greatest antibacterial and antifungal activities were observed against *Bacillus subtilis* and *Aspergillus flavus*, respectively. The minimum inhibitory concentration for nanoparticles was ranged between 10 and 20 µg mL^{-1}. **Conclusion:** The results confirmed that the capping of algal extract with silver nanoparticles is capable of rendering antimicrobial efficacy and hence has a great potential in the preparation of therapeutic drugs.

Key words: Algal extract, silver nanoparticles, UV-spectroscopy, FT-IR, x-ray diffraction, SEM, antimicrobial activity, MIC

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

In modern sciences the scope of nanotechnology considered as one of the greatest effective fields of study. Nanotechnology is the newly emerging multidisciplinary research area with synthesis of nanosized material[1,2]. Nanotechnology is defined as the production of materials ranging in size from 1-100 nm scale[3,4]. In health care and medicine diagnostic and screening purposes, tissue engineering, antisense and gene therapy applications and drug delivery systems; the nanoparticles can play a topmost role[5]. Biological method of nanoparticles synthesis is a vast growing technique in the field of nanotechnology[6].

Nanoscience is the understanding of material properties on the nanoscale and search to supply improved materials[7]. Ahmed *et al.*[8] dealt with green nanoscience research and avoiding the disruption of natural ecosystems to develop environmentally friendly nanomaterials. Recently, biological materials such as algae were used to synthesis of nanoparticles. Algae are known as bionanofactories among the biological materials because of their live and dead dried biomass can be used for synthesis of metallic nanoparticles. It is low cost and environmentally effective and has the distinct advantage due to its high metal uptake capacity[9]. The rate and size of the nanoparticles were controlled by optimizing substrate concentration, incubation time, pH and temperature[10].

Marine algae can produce a great variety of secondary metabolites[11]. Most of the marine algae have antimicrobial activity[12] and antibiotic resistant post-operative infectious pathogens[13]. Marine algal compounds have fungicidal, bactericidal and bacteriostatic activities[14]. The bioactive compounds which recorded this activity were phlorotannins, terpenoids, steroids, phenolic compounds, alkanes, ketones, acrylic acid, amino acids and fatty acids. Aqueous extract of marine algae contains carbohydrates, proteins, tannin and steroids as bioactive compounds[15].

Nanoparticles have an enlarged contact area with microorganisms that facilitates their biological and chemical activity[16-18]. The presence of resistant bacterial strains against antibiotics leads to increase in the bactericidal nanomaterials studies. Antimicrobial properties and the attachment of the nanoparticles with the microbe have been investigated in the green synthesized silver nanoparticles[19,20]. Synthesized silver nanoparticles showed antimicrobial activity against fungi and bacteria as reported by Kim *et al.*[21].

Since algae act as reducing agents and produce nanoparticles from metal salts without producing any toxic product, they can be considered as a good source of biomolecules. Thus the present study aimed to give the possibility of biomedical applications of silver nanoparticles synthesized by algae. The antimicrobial activity of silver nanoparticles synthesized by using the red alga *Laurencia papillosa* in aqueous solution were assessed. Characterization of silver nanoparticles by UV-vis, x-ray diffraction, Fourier transform infrared spectroscopy and scanning electron microscopy was investigated.

MATERIALS AND METHODS

Algae material: The marine alga, *Laurencia papillosa* was collected from the coastal area of the Red Sea of Jeddah, Saudi Arabia. The sample was washed in running taps water and finally in distilled water. The samples were allowed to dry in the air. The dried sample was grounded into fine powder.

Algae extract: Algae powder (10 g) was mixed with 100 mL deionized water in a conical flask, kept on shaker at 50°C for 24 h and then filtered using filter paper (Whatman No. 1). The supernatant stored in a refrigerator until analysis.

Biogenesis of silver nanoparticles: Silver nitrate solution ($AgNO_3$) was utilized for synthesis of silver nanoparticles. The algal extract (10 mL) was added to 1.0 mM $AgNO_3$ solution (90 mL). The aqueous solution was subjected to stirring for overnight to facilitate absolute reduction using a magnetic stirrer at room temperature. The reaction mixture was continuously monitored for its colour change from yellow to brown to emphasize the formation of AgNP's.

Characterization of synthesized silver nanoparticles

UV-visible spectroscopy: The change in color of the solution was noted through visual inspection and UV-vis spectrophotometer (Thermo Scientific Evolution 300) at wavelength of 350-900 nm.

Fourier transform infrared analysis: The aqueous algal extract before and after synthesis of silver nanoparticles were lyophilized and subjected to FT-IR analysis in the range of 4000-500 cm^{-1} (Tensor 27, Bruker).

X-ray diffraction analysis: Crystalline nature of the purified silver nanoparticles was measured by using the x-ray diffractometer (Rigaku-Ultima IV). The solution of AgNP's was centrifuged at 12.000 rpm for 10 min, dispersed into deionized water for 3 times and then the filtrate was dried. The x-ray powder diffraction patterns were recorded in the

range of 2θ = 20-80° on the diffractometer operating at 20-60 kV with Cu Kα (λ = 1.5405 Å) radiation. The size of AgNP's was calculated from the PXRD peak position using Bragg's law.

Scanning Electron Microscope (SEM): The morphology of the biosynthesized nanoparticle was observed by using SEM (JEOL-JSM7600F).

Antimicrobial activity: The antimicrobial activity of *Laurencia papillosa* silver nanoparticles was tested by using agar well-diffusion method against pathogenic Gram-positive bacteria (*Bacillus subtilis*, *Staphylococcus aureus* and *Streptococcus pneumoniae*), Gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*) and fungi (*Aspergillus flavus*, *Aspergillus fumigatus* and *Aspergillus niger*). The bacterial (10^8 cells mL^{-1}) and fungal suspensions (2×10^5 cells mL^{-1}) were streaked uniformly onto the Mueller-Hinton agar for bacteria and Sabouraud dextrose agar plates for fungal strains. Wells of 8 mm diameter were made by using a sterile borer. The nanoparticles solution (50 µL) at different concentrations, 50, 100 and 150 µL were poured onto each well. The plates were incubated at 37°C for bacteria and 28°C for fungi. About 0.1 mg mL^{-1} ampicillin and miconazole was used as positive control for bacteria and fungi, respectively. The negative control was aqueous algal extract. The antimicrobial activity was examined by measuring the diameter of inhibition zone (mm) after 24 h for bacteria and 48 h for fungi. The results were recorded as Mean±SD of triplicate experiments.

Minimum Inhibitory Concentration (MIC): The MIC leading to the microbial inhibition was tested as described by Ter Laak *et al.*[22]. The samples were diluted with 100 µL Mueller-Hinton broth inoculated with the tested bacteria and Sabouraud dextrose broth for fungal strains at a concentration of 10^6 CFU mL^{-1} plus some drops of phenol red. The plates were incubated and the growth was examined by change of the color of phenol red indicator from red to yellow. Minimum Inhibitory Concentrations (MICs) are defined as no change in color of phenol red.

Statistical analysis: The experiments were done in triplicate (n = 3). All data was expressed as Mean±SD and analyzed by using analysis of variance (ANOVA). Significant differences between the means of parameters was determined.

RESULTS AND DISCUSSION

Visual and UV-vis spectral analysis: The UV-vis spectroscopy is an important technique to ascertain the formation and stability of nanoparticles in aqueous solution. The color of the algal extract becomes turbid after the addition of aqueous $AgNO_3$ solution signifying the initiation of reaction (Fig. 1a). The formation of silver nanoparticles was visually identified by color change from white to brown in the aqueous solution of reaction mixture (Fig. 1b-d). The deep brown color of silver nanoparticles occurred after 72 h indicating that the change in the color depends on the time of incubation. Rajeshkumar *et al.*[23] reported that the silver nanoparticles synthesis of *Sargassum longifolium* was completed at 64 h of incubation indicating that the increasing of color intensity is directly proportion to the time of incubation. The brown color formed due to the oscillation of free electrons in the reaction mixture. However, Chandran *et al.*[24] found that the silver nanoparticles of *Aloe vera* extract formed after 24 h of incubation.

The UV-vis spectroscopy is a preliminary, indirect and appropriate method for characterization of silver nanoparticles, based on optical properties called surface plasmon resonance[25]. The UV-vis absorption spectra (Fig. 2) recorded the formation of nanoparticles in the reaction mixture of algal extract and $AgNO_3$ at different time intervals (24, 48 and 72 h). Figure 2 showed no peak formed at the initial stage which indicated that there was no synthesis for silver nanoparticles. After 24 h of incubation, the surface

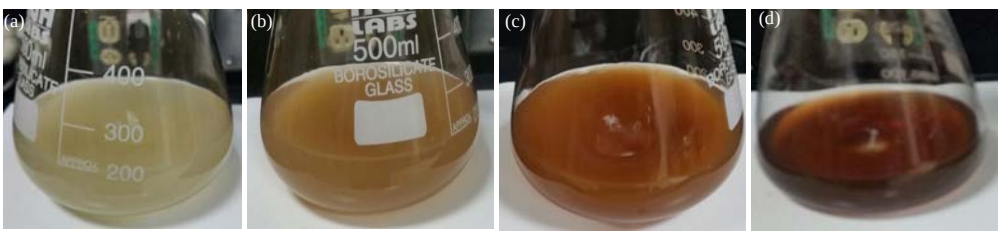

Fig. 1(a-d): Visual observation of silver nanoparticles (AgNPs) green synthesis of *Laurencia papillosa* aqueous extract, (a) Initial color, (b) Color after 24 h, (c) Color after 48 h and (d) Color after 72 h

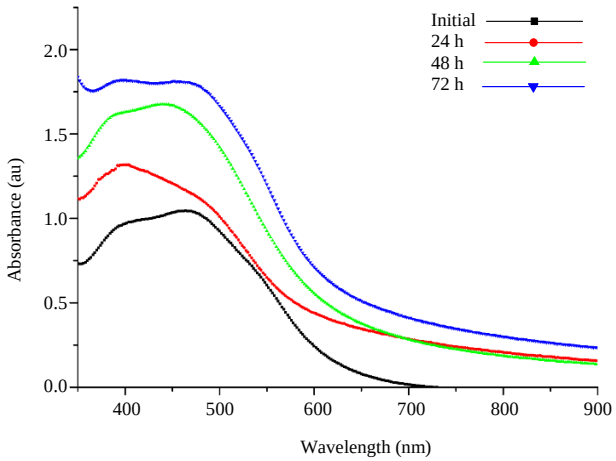

Fig. 2: UV-visible spectra of silver nanoparticles (AgNPs) prepared using aqueous extract of *Laurencia papillosa* after incubation for 72 h at room temperature

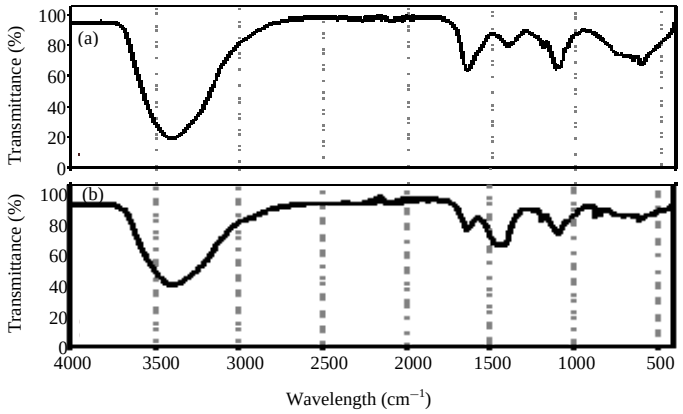

Fig. 3(a-b): FT-IR spectra of aqueous extract of *Laurencia papillosa* (a) Before and (b) After synthesis of silver nanoparticles

Plasmon resonance band for silver nanoparticles was found at 450 nm and the synthesis was steadily increase with increasing time of incubation without change in the peak position after 72 h. The recorded band in this range has been related to silver nanoparticles and confirming the formation of spherical silver nanoparticles with narrow size distribution[26,27]. Formation of broad band with no peak variation in the position indicated the presence of poly-dispersity nanoparticles. Basavaraj *et al.*[28] and AbdelRahim *et al.*[29] showed that the increase in absorption indicates formation of more nanoparticles. The UV-vis spectral analysis of formed silver nanoparticles showed that the surface plasmon absorbance did not change even after three months, which means the stability of nanoparticles.

FT-IR spectrum: The results in Fig. 3a showed that, FT-IR peak at 3400 cm^{-1} attributable to OH stretching vibration of polyphenols or proteins or polysaccharides. The peak at 1660 cm^{-1} refers to amide group (NH) C=O of protein. The peak at 1480 cm^{-1} specify the bending vibration of alkyl groups. The peak observed at 1420 cm^{-1} assigned to OH group in plane bending vibration. The band at 1100 cm^{-1} suggest the presence of C-O stretching vibration. The species of *Gracilaria* have been found to contain carbohydrates, minerals, amino acids, fatty acids, vitamins and phenolic compounds[30]. Of which, phenolic compounds especially polyphenols and tannin have reported to have antimicrobial, anti-carcinogenic and antioxidant properties[31].

The FT-IR spectrum of the synthesized silver nanoparticles showed changes in the intensity and position of absorption bands at 3400, 1660, 1420 and 1100 cm^{-1} (Fig. 3b). This indicates the involvement of OH group and amide group (NH) C=O in stabilizing of silver nanoparticles by the complexation with silver ions. Satyavani *et al.*[32] showed that

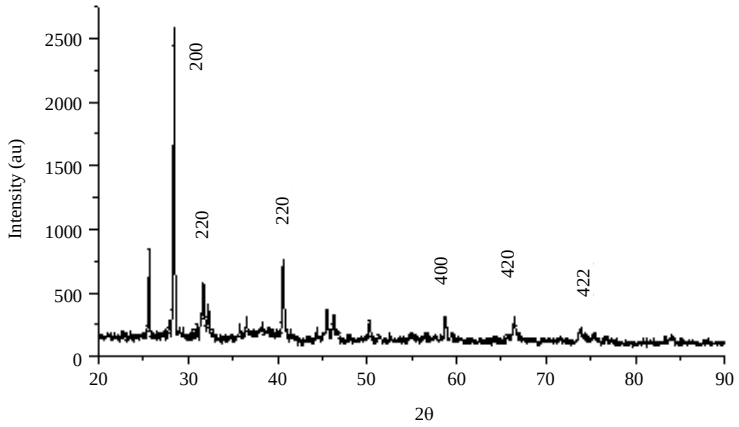

Fig. 4: XRD spectrum of silver nanoparticles (AgNPs) synthesized by aqueous extract of *Laurencia papillosa* after incubation for 72 h at room temperature

Table 1: Antibacterial activity and MIC of silver nanoparticles (AgNPs) synthesized by *Laurencia papillosa* aqueous extract against pathogenic bacteria

	Zone of inhibition (mm)				
Pathogens	50 µL	100 µL	150 µL	Positive control*	MIC (µg mL^{-1})
Gram-positive bacteria					
Bacillus subtilis	17±0.4	19±0.3	21±0.5	24±0.7	10±0.2
Staphylococcus aureus	16±0.2	19±0.2	19±0.3	21±0.6	12±0.3
Streptococcus pneumoniae	15±0.3	17±0.5	18±0.2	20±0.8	11±0.4
Gram-negative bacteria					
Escherichia coli	14±0.4	16±0.7	17±0.2	21±0.5	14±0.3
Klebsiella pneumoniae	15±0.3	17±0.3	18±0.5	20±0.4	16±0.5
Pseudomonas aeruginosa	13±0.2	15±0.2	16±0.3	19±0.3	15±0.6

*Ampicillin, Mean±SD of triplicate experiments

ions of silver may be bind to phenolic compounds resulting in the formation of silver nanoparticles.

XRD analysis: The XRD analysis explained the crystalline nature of silver[33]. The structural characters of silver nanoparticles were determined for the whole spectrum of 2θ values, from 20-80° by using x-ray diffractometer. The crystalline behavior observed for green synthesized silver nanostructure by employing algal extract was established by the characteristic peaks in the XRD image (Fig. 4). The various diffraction peaks of the 2θ values of 25.59, 28.39, 31.75, 40.49, 50.30 and 66.44° could be assigned the plane of Miller indices 110, 111, 200, 211, 220 and 311, respectively. The results indicated that the synthesized AgNP's were crystalline in nature and face-centered cubic (fcc) with lattice parameters, a = b = c = 6.2465 (8) Å. The synthesized silver nanoparticles were compared with pure silver particles and standard silver nitrate which were reported by Joint Committee on Powder Diffraction Standards (File No. 04-0783 and 84-0713). The XRD showed that the recognized planes of synthesized AgNP's from the algal extract were highly crystallized and purified. Leff *et al.*[34] and Rajeshkumar *et al.*[23] found that the existence of intensive peaks of AgNP's assigned to 111, 200 and 220, which were recorded as silver face-centered cubic (fcc) crystalline phase.

SEM analysis: Scanning Electron Microscope (SEM) image showed the morphology of the algae mediated synthesized bio-nanoparticles at different magnifications (Fig. 5). The SEM image showed high density of silver nanoparticles distributed on the surface of the algae cells. The predominant of cubic shape nanoparticles on the surface of the cell was observed at magnification of 1 µm x7500. The polydispersed silver nanoparticles were adhered on the surface of the biomolecules of the algal cells due to weakly dislodging of bound silver nanoparticles during silver nitrate reaction[35,36]. Similar results were obtained in the gold nanoparticles using algal extract of *S. marginatum* with the size ranging from 40-85 nm[37] and also by using the *T. conoides* algae[38]. It reveals the involvement of the polyphenolic or secondary capping agent in the nanoparticles formation.

Antimicrobial activity of AgNP's: The silver nanoparticles (AgNP's) synthesized by *G. corticata* extract were examined against Gram-positive and Gram-negative bacteria at different concentrations 50, 100 and 150 µL (Table 1). The

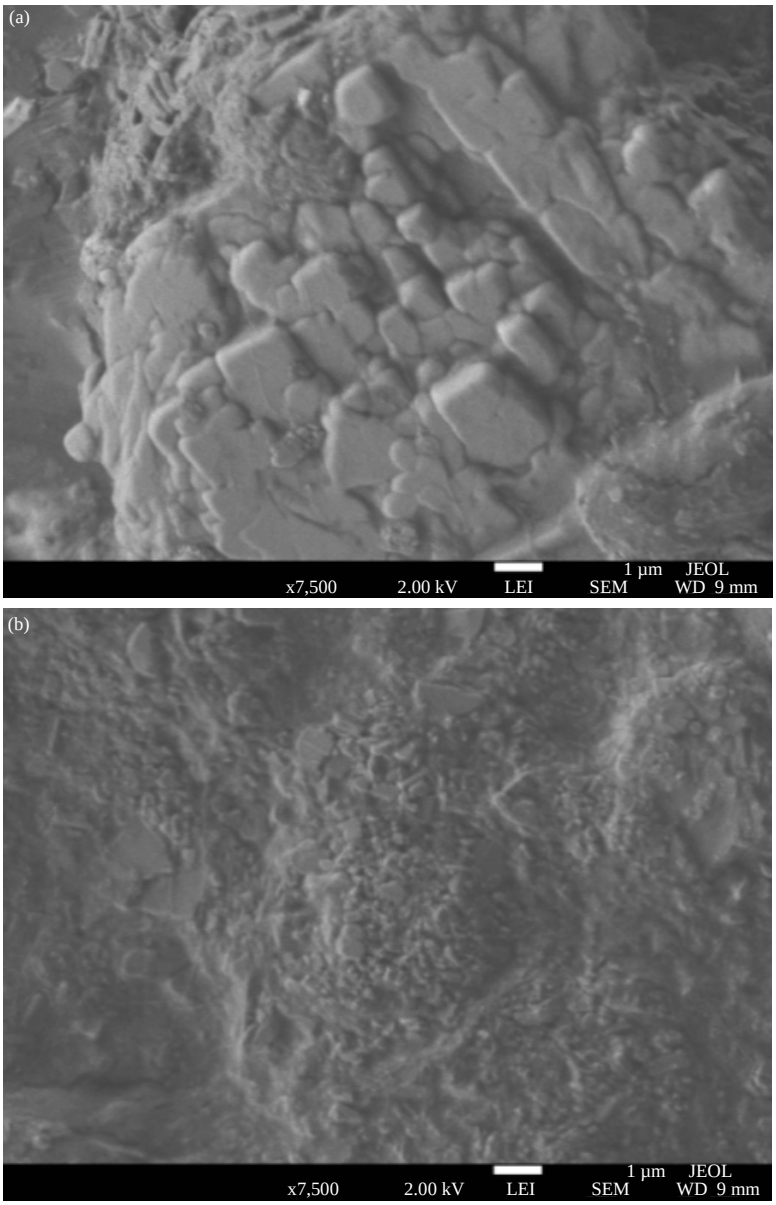

Fig. 5(a-b): Scanning electron microscope image of silver nanoparticles (AgNPs) synthesized by aqueous extract of *Laurencia papillosa* showing nanoparticles at 1 µm x7500, (a) Algal extract and (b) Algal extract nanoparticles after 72 h of incubation at room temperature

results showed that the diameter of inhibition zone were gradually increased with increasing AgNP's concentration. The highest antibacterial activity for both types of bacteria was found at 150 µL AgNP's. The greatest inhibition zone at 150 µL AgNP's was noticed with the Gram-positive; *B. subtilis* (21±0.5 mm), *S. aureus* (19±0.3 mm) followed by *S. pneumoniae* (18±0.2 mm). However, the minimum activity at 150 µL AgNP's was observed with Gram-negative, *P. aeruginosa*, *E. coli* and *K. pneumoniae* with inhibition zone of 17±0.2, 18±0.5 and 16±0.3 mm, respectively. The positive control ampicillin showed zone of inhibition ranged from 19±0.3 to 24±0.7 mm against the tested bacterial pathogens (Table 1). Table 1 shows, the MIC for AgNP's was lower when tested against Gram-positive bacteria viz., *B. subtilis* (10±0.2 µg mL^{-1}), *S. aureus* (12±0.3 µg mL^{-1}) and *S. pneumoniae* (11±0.3 µg mL^{-1}) as compared to Gram-negative bacteria viz., *E. coli* (14±0.3 µg mL^{-1}), *K. pneumoniae* (16±0.5 µg mL^{-1}) and *P. aeruginosa* (15±0.6 µg mL^{-1}).

Table 2: Antifungal activity and MIC of silver nanoparticles (AgNPs) synthesized by *Laurencia papillosa* aqueous extract against pathogenic fungi

	Zone of inhibition (mm)				
Pathogens	50 µL	100 µL	150 µL	Positive control*	MIC (µg mL^{-1})
Aspergillus flavus	12±0.2	14±0.4	15±0.5	18±0.2	17±0.3
Aspergillus fumigatus	11±0.5	12±0.7	13±0.3	17±0.4	18±0.2
Aspergillus niger	10±0.3	11±0.4	12±0.4	16±0.6	20±0.5

*Miconazole, Mean±SD of triplicate experiments

Silver nanoparticles showed antibiofilm activity against Gram-positive and Gram-negative pathogenic bacteria. Among the bacterial test strains, the Gram-positive bacteria were more susceptible to the AgNP's than Gram-negative bacteria. This may be due to the more complex structure of cell wall of Gram-negative bacteria[39,40]. Nanoparticles have the capability to accumulate, penetrate the membrane of bacterial cell and disturb its permeability and respiration causing change in the structure of the cell and finally death[41-44]. The antimicrobial activity is highly affected by the shape, size and nanoparticles concentration[45]. The significant antibacterial activity of synthesized AgNP's may be returned to their effect on the biochemical process of the bacterial cell by interacting with thiol, amino groups and nucleic acids of cell wall[46]. In addition, this may cause interaction between AgNP's and microorganism which lead to the discharge of hydroxyl radicals and singlet oxygen of highly reactive oxygen species[47]. Lok *et al.*[48] suggested that the free uptake of free silver ions may cause disruption of DNA replication and ATP production.

In the present study the AgNP's exhibited the highest antifungal activity at the concentration of 150 µL (Table 2). Among the tested fungi, the highest antifungal activity was detected at 150 µL AgNP's against *A. flavus* followed by *A. fumigatus* and finally *A. niger* with inhibition zone of 15±0.5, 13±0.3 and 12±0.4 µL, respectively. The standard reference with antifungal drug (Miconazole) recorded zone of inhibition ranged between 16±0.6 and 18±0.4 mm. The MIC values of AgNP's for inhibition of tested fungi were ranged from 17±0.3 to 20±0.5 µg mL^{-1} (Table 2). The results clarified that the pathogenic fungi were less affected by AgNP's as compared with tested bacteria. In accordance with our results, Nabikhan *et al.*[49] showed that the antifungal activity of synthesized AgNP's was less distinct than antibacterial activity. Ghorbani *et al.*[7] recorded that the antimicrobial activity of AgNP's is likely to be well correlated with its decreased shape and size owing to increase in the surface area with enhancing the antimicrobial activity. Further, Panacek *et al.*[50] reported that the fungal cells require higher concentrations of ionic silver due to the complexity of their cell. Negatively charged cell membranes of microbes may attach with positively charged silver by electrostatic attraction[51]. Exposure of the fungal cells to silver nanoparticle solution caused formation of pits on the membrane surface lead to destruction of its integrity[52]. The increase in hydroxyl radicals by silver nanoparticles induced apoptotic cell death in *Candida albicans*[53]. The silver nanoparticles may penetrate the bacterial and fungal cells and react with phosphorus and sulphur containing compounds such as DNA, causing their damage[54]. Kim *et al.*[55] explained that silver nanoparticles inhibit the conidial germination on fungi. Finally, the silver nanoparticles have a great potential to control the spore producing fungi.

CONCLUSION

The developing of nanoparticles as antibiofilm factor is growing area of research. The nanoparticles are tremendous promising and wonderful biomedical agents because of their small size and large surface to volume ratio. The biological molecules of algal cell undergo highly controlled making them suitable for the metal nanoparticle synthesis and can form a great effect in next years. The algal silver nanoparticles can be used as a promising therapeutic agent against biofilms as the bacteria are less likely to develop resistance against nanoparticles. Our findings propose that the AgNP's can act as a new antimicrobial agent.

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REFERENCES

1. Amin, M., F. Anwar, M.R.S.A. Janjua, M.A. Iqbal and U. Rashid, 2012. Green synthesis of silver nanoparticles through reduction with *Solanum xanthocarpum* L. berry extract: Characterization, antimicrobial and urease inhibitory activities against *Helicobacter pylori*. Int. J. Mol. Sci., 13: 9923-9941.

2. Al-Houri, H.J., N.M.S. Moubayed and S.I. Irsan, 2015. Silver nanoparticles biosynthesis using *Spirulina platensis* used as antioxidant and antimicrobial agent. Der Pharmacia Lettre, 7: 9-21.
3. Rajesh, S., D.P. Raja, J.M. Rathi and K. Sahayaraj, 2012. Biosynthesis of silver nanoparticles using *Ulva fasciata* (Delile) ethyl acetate extract and its activity against *Xanthomonas campestris* pv. *malvacearum*. J. Biopest., 5: 119-128.
4. Mohanpuria, P., N.K. Rana and S.K. Yadav, 2008. Biosynthesis of nanoparticles: Technological concepts and future applications. J. Nanoparticle Res., 10: 507-517.
5. Kubik, T., K. Bogunia-Kubik and M. Sugisaka, 2005. Nanotechnology on duty in medical applications. Curr. Pharm. Biotechnol., 6: 17-33.
6. Sinha, S., I. Pan, P. Chanda and S.K. Sen, 2009. Nanoparticles fabrication using ambient biological resources. J. Applied Biosci., 19: 1113-1130.
7. Ghorbani, P., M. Soltani, M. Homayouni-Tabrizi, F. Namvar, S. Azizi, R. Mohammad and A.B. Moghaddam, 2015. Sumac silver novel biodegradable nano composite for bio-medical application: Antibacterial activity. Molecules, 20: 12946-12958.
8. Ahmed, S., M. Ahmad, B.L. Swami and S. Ikram, 2016. A review on plants extract mediated synthesis of silver nanoparticles for antimicrobial applications: A green expertise. J. Adv. Res., 7: 17-28.
9. DaVis, T.A., B. Volesky and A. Mucci, 2003. A review of the biochemistry of heavy metal biosorption by brown algae. Water Res., 37: 4311-4330.
10. Gericke, M. and A. Pinches, 2006. Microbial production of gold nanoparticles. Gold Bull., 39: 22-28.
11. Salvador, N., A.G. Garreta, L. Lavelli and M.A. Ribera, 2007. Antimicrobial activity of Iberian macroalgae. Scientia Marina, 71: 101-113.
12. Lavanya, R. and N. Veerappan, 2011. Antibacterial potential of six seaweeds collected from Gulf of Mannar of Southeast Coast of India. Adv. Biol. Res., 5: 38-44.
13. Ravikumar, S., L. Anburajan, G. Ramanathan and N. Kaliaperumal, 2002. Screening of seaweed extracts against antibiotic resistant post operative infectious pathogens. Seaweed Res. Utilisation, 24: 95-99.
14. Mtolera, M.S.P. and A.K. Semesi, 1996. Antimicrobial activity of extracts from six green algae from Tanzania. Curr. Trends Mar. Bot. Res. East Afr. Reg., 18: 211-217.
15. Sangeetha, S., N.B. Dhayanithi and N. Sivakumar, 2014. Antibacterial activity of *Sargassum longifolium* and *Gracilaria corticata* from Gulf of Mannar against selected common shrimp pathogens. Int. J. Pharm. Bio Sci., 5: 76-82.
16. Prema, P. and S. Thangapandiyan, 2013. *In-vitro* antibacterial activity of gold nanoparticles capped with polysaccharide stabilizing agents. Int. J. Pharm. Pharm. Sci., 5: 310-314.
17. Priyadarshini, S., V. Gopinath, N.M. Priyadharsshini, D. MubarakAli and P. Velusamy, 2013. Synthesis of anisotropic silver nanoparticles using novel strain, *Bacillus flexus* and its biomedical application. Colloids Surf., B: Biointerf., 102: 232-237.
18. Puiso, J., D. Jonkuviene, I. Macioniene, J. Salomskiene, I. Jasutiene and R. Kondrotas, 2014. Biosynthesis of silver nanoparticles using lingonberry and cranberry juices and their antimicrobial activity. Colloids Surf. B: Biointerf., 121: 214-221.
19. Sharma, S., A.K. Manhar, P.J. Bora, S.K. Dolui and M. Mandal, 2015. Evaluation of antioxidant and antibacterial activity of various aspect ratio gold (Au) nanorods. Adv. Mat. Lett., 6: 235-241.
20. Jha, A.K. and K. Prasad, 2016. Green synthesis and antimicrobial activity of silver nanoparticles onto cotton fabric: An amenable option for textile industries. Adv. Mater. Lett., 7: 42-46.
21. Kim, J.S., E. Kuk, K.N. Yu, J.H. Kim and S.J. Park *et al*., 2007. Antimicrobial effects of silver nanoparticles. Nanomed. Nanotechnol. Biol. Med., 3: 95-101.
22. Ter Laak, E.A., A. Pijpers, J.H. Noordergraaf, E.C. Schoevers and J.H. Verheijden, 1991. Comparison of methods for *in vitro* testing of susceptibility of porcine *Mycoplasma* species to antimicrobial agents. Antimicrob. Agents Chemother., 35: 228-233.
23. Rajeshkumar, S., C. Malarkodi, M. Vanaja and K. Paulkumar, 2014. Green-chemical fabrication of silver nanoparticles by marine macro algae and its fungicidal activity. Int. Res. J. Pharm. Biosci., 1: 1-7.
24. Chandran, S.P., M. Chaudhary, R. Pasricha, A. Ahmad and M. Sastry, 2006. Synthesis of gold nanotriangles and silver nanoparticles using *Aloevera* plant extract. Biotechnol. Prog., 22: 577-583.
25. Bhainsa, K.C. and S.F. D'Souza, 2006. Extracellular biosynthesis of silver nanoparticles using the fungus *Aspergillus fumigatus*. Colloids Surf. B: Biointerfaces, 47: 160-164.
26. Henglein, A., 1993. Physicochemical properties of small metal particles in solution: Microelectrode reactions, chemisorption, composite metal particles and the atom-to-metal transition. J. Phys. Chem., 97: 5457-5471.
27. Kumar, P., S.S. Selvi and M. Govindaraju, 2013. Seaweed-mediated biosynthesis of silver nanoparticles using *Gracilaria corticata* for its antifungal activity against *Candida* spp. Applied Nanosci., 3: 495-500.
28. Basavaraj, U., P. Naik, S.T. Savadatti, R. Sharma and S. Balgi, 2012. Synthesis and characterization of silver nanoparticles. Int. J. Pharm. Biol. Sci., 2: 10-14.
29. AbdelRahim, K., S.Y. Mahmoud, A.M. Ali, K.S. Almaary, A.E.Z.M.A. Mustafa and S.M. Husseiny, 2017. Extracellular biosynthesis of silver nanoparticles using *Rhizopus stolonifer*. Saudi J. Biol. Sci., 24: 208-216.

30. Yang, J.I., C.C. Yeh, J.C. Lee, S.C. Yi, H.W. Huang, C.N. Tseng and H.W. Chang, 2012. Aqueous extracts of the edible Gracilaria tenuistipitata are protective against H_2O_2-induced DNA damage, growth inhibition and cell cycle arrest. Molecules, 17: 7241-7254.
31. Arunkumar, K., S.R. Sivakumar and R. Rengasamy, 2010. Review on bioactive potential in seaweeds (Marine macroalgae): A special emphasis on bioactivity of seaweeds against plant pathogens. Asian J. Plant Sci., 9: 227-240.
32. Satyavani, K., S. Gurudeeban, T. Ramanathan and T. Balasubramanian, 2011. Biomedical potential of silver nanoparticles synthesized from calli cells of *Citrullus colocynthis* (L.) schrad. J. Nanobiotechnol., Vol. 9. 10.1186/1477-3155-9-43.
33. Jegadeeswaran, P., R. Shivaraj and R. Venckatesha, 2012. Green synthesis of silver nanoparticles from extract of *Padina tetrastromatica* leaf. Digest J. Nanomater. Biostruct., 7: 991-998.
34. Leff, D.V., L. Brandt and J.R. Heath, 1996. Synthesis and characterization of hydrophobic, organically-soluble gold nanocrystals functionalized with primary amines. Langmuir, 12: 4723-4730.
35. Sastry, M., A. Ahmad, M.I. Khan and R. Kumar, 2003. Biosynthesis of metal nanoparticles using fungi and actinomycete. Curr. Sci., 85: 162-170.
36. Rajeshkumar, S., C. Malarkodi, K. Paulkumar, M. Vanaja, G. Gnanajobitha and G. Annadurai, 2014. Algae mediated green fabrication of silver nanoparticles and examination of its antifungal activity against clinical pathogens. Int. J. Metals, Vol. 2014. 10.1155/2014/692643.
37. Rajathi, F.A.A., C. Parthiban, V.G. Kumar and P. Anantharaman, 2012. Biosynthesis of antibacterial gold nanoparticles using brown alga, *Stoechospermum marginatum* (kutzing). Spectrochimica Acta Part A: Mol. Biomol. Spectrosc., 99: 167-173.
38. Vijayaraghavan, K., A. Mahadevan, M. Sathishkumar, S. Pavagadhi and R. Balasubramanian, 2011. Biosynthesis of Au(0) from Au(III) via biosorption and bioreduction using brown marine alga *Turbinaria conoides*. Chem. Eng. J., 167: 223-227.
39. Pesendo, D. and B. Caram, 1984. Screening of marine algae from the French mediterranean coast for antibacterial and antifungal activity. Bot. Mar., 27: 381-386.
40. Reichelt, J.L. and M.A. Borowitzka, 1984. Antimicrobial activity from marine algae: Results of a large-scale screening programme. Hydrobiologia, 116: 158-168.
41. Luo, C., J. Sun, B. Sun, D. Liu and L. Miao *et al*., 2016. Facile fabrication of tumor redox-sensitive nanoassemblies of small-molecule oleate prodrug as potent chemotherapeutic nanomedicine. Small, 12: 6353-6362.
42. Ling, Z., L. Yonghong, S. Changqing, L. Junfeng, Z. Li, J. Chunyu and L. Xianqiang, 2016. Preparation, characterization and pharmacokinetics of tilmicosin and florfenicol-loaded hydrogenated castor oil-solid lipid nanoparticles. J. Vet. Pharmacol. Therapeut., (In Press). 10.1111/jvp.12356.
43. LewisOscar, F., S. Vismaya, M. Arunkumar, N. Thajuddin, D. Dhanasekaran and C. Nithya, 2016. Algal Nanoparticles: Synthesis and Biotechnological Potentials. In: Algae-Organisms for Imminent Biotechnology, Thajuddin, N. and D. Dhanasekaran (Eds.). Chapter 7, InTech Publisher, Croatia, ISBN 978-953-51-2431-3, pp: 157-182.
44. Omolo, C.A., R.S. Kalhapure, M. Jadhav, S. Rambharose, C. Mocktar, V.M.K. Ndesendo and T. Govender, 2016. Pegylated oleic acid: A promising amphiphilic polymer for nano-antibiotic delivery. Eur. J. Pharm. Biopharm., 112: 96-108.
45. LewisOscar, F., D. MubarakAli, C. Nithya, R. Priyanka, V. Gopinath, N.S. Alharbi and N. Thajuddin, 2015. One pot synthesis and anti-biofilm potential of copper nanoparticles (CuNPs) against clinical strains of *Pseudomonas aeruginosa*. Biofouling, 31: 379-391.
46. Powers, C.M., A.R. Badireddy, I.T. Ryde, F.J. Seidler and T.A. Slotkin, 2011. Silver nanoparticles compromise neurodevelopment in PC12 cells: Critical contributions of silver ion, particle size, coating and composition. Environ. Health Perspect., 119: 37-44.
47. Yang, H., C. Liu, D. Yang, H. Zhang and Z. Xi, 2009. Comparative study of cytotoxicity, oxidative stress and genotoxicity induced by four typical nanomaterials: The role of particle size, shape and composition. J. Applied Toxicol., 29: 69-78.
48. Lok, C.N., C.M. Ho, R. Chen, Q.Y. He and W.Y. Yu *et al*., 2006. Proteomic analysis of the mode of antibacterial action of silver nanoparticles. J. Proteome Res., 5: 916-924.
49. Nabikhan, A., K. Kandasamy, A. Raj and N.M. Alikunhi, 2010. Synthesis of antimicrobial silver nanoparticles by callus and leaf extracts from saltmarsh plant, *Sesuvium portulacastrum* L. Colloids Surf. B: Biointerf., 79: 488-493.
50. Panacek, A., M. Kolar, R. Vecerova, R. Prucek and J. Soukupova *et al*., 2009. Antifungal activity of silver nanoparticles against *Candida* spp. Biomaterials, 30: 6333-6340.
51. Sondi, I. and B. Salopek-Sondi, 2004. Silver nanoparticles as antimicrobial agent: A case study on *E. coli* as a model for Gram-negative bacteria. J. Colloid Interface Sci., 275: 177-182.
52. Nasrollahi, A., K. Pourshamsian and P. Mansourkiaee, 2011. Antifungal activity of silver nanoparticles on some of fungi. Int. J. Nano Dimension, 1: 233-239.

53. Hwang, I.S., J. Lee, J.H. Hwang, K.J. Kim and D.G. Lee, 2012. Silver nanoparticles induce apoptotic cell death in *Candida albicans* through the increase of hydroxyl radicals. FEBS J., 279: 1327-1338.
54. Govindaraju, K., V. Kiruthiga, V.G. Kumar and G. Singaravelu, 2009. Extracellular synthesis of silver nanoparticles by a marine alga, *Sargassum wightii* Grevilli and their antibacterial effects. J. Nanosci. Nanotechnol., 9: 5497-5501.
55. Kim, S.W., K.S. Kim, K. Lamsal, Y.J. Kim and S.B. Kim *et al*., 2009. An *in vitro* study of the antifungal effect of silver nanoparticles on oak wilt pathogen *Raffaelea* sp. J. Microbiol. Biotechnol., 19: 760-764.