

Original Article

GREEN SYNTHESIS, CHARACTERIZATION OF SILVER NANOPARTICLES OF A MARINE RED ALGA *SPYRIDIA FUSIFORMIS* AND THEIR ANTIBACTERIAL ACTIVITY

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Received: 15 Jan 2017 Revised and Accepted: 31 Mar 2017

ABSTRACT

Objective: In the present system, the green synthesis of silver nanoparticles using marine the red alga *Spyridia fusiformis* and antibacterial activity was carried out.

Methods: The seaweed extract was used for the synthesis of AgNPs at room temperature. The silver nanoparticles were characterized by using UV-Visible spectroscopy, Fourier transform infrared spectroscopy, transmission electron microscope and X-ray diffraction (XRD) techniques. The antibacterial activity of biosynthesized silver nanoparticles was carried out by disc diffusion method against pathogenic bacteria.

Results: The UV-visible spectroscopy revealed surface plasmon resonance at 450 nm. The FT-IR measurements showed the possible functional groups responsible for the formation of nanoparticles. The X-ray diffraction analysis showed that the particles were crystalline in nature. TEM micrograph has shown the formation of silver nanoparticles with the size in the range of 5–50 nm. The silver nanoparticles synthesized from the *S. fusiformis* showed higher activity and proved their efficacy in controlling the pathogenic bacterial strains. The nanoparticles showed highest inhibition activity on *K. pneumoniae* and *S. aureus* up to 26 and 24±0.01 mm at 100 µg/ml of nanoparticles.

Conclusion: The synthesised AgNPs have shown the best antibacterial activity against human pathogens *E. coli*, *K. pneumoniae*, *S. aureus* and *P. aeruginosa*. The above eco-friendly AgNPs synthesis procedure could be a viable solution for industrial applications in the future and therapeutic needs.

Keywords: Antibacterial activity, Green synthesis, Marine algae, Silver nanoparticles, *Spyridia fusiformis*

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DOI: <http://dx.doi.org/10.22159/ijpps.2017v9i5.17105>

INTRODUCTION

Nanoparticles are being viewed as the fundamental building blocks of nanotechnology. The most important and distinct property of nanoparticles is that they exhibit larger surface area to volume ratio. Metal nanoparticles have tremendous applications in the area of catalysis, photo electronics and diagnostic biological probes and also in display devices. Among the metal nanoparticles, silver nanoparticles play a significant role in the field of biology and medicine.

In the current scenario, the biological synthesis of the metallic nanoparticle is gaining more interest, as it is reliable and eco-friendly. The previous literature survey revealed that the nanoparticle synthesis using biological sources like algae has been unexplored and unexploited [1-11].

Nanosilver (silver nanoparticle, AgNP) material has a wide range of applications. Because of the effective antimicrobial nature and low toxicity to mammalian cells, AgNPs have become one of the most commonly used nanomaterials in consumer products (104 out of 502 nanoproducts surveyed) [12]. The antibacterial activity of the silver-containing materials can be used in some medicines to reduce infections as well as to prevent bacterial colonization. The present study evaluated the biosynthesis and characterization of silver nanoparticles of *S. fusiformis* and investigated their antibacterial potential.

MATERIALS AND METHODS

Chemicals and bacterial cultures

The chemicals employed in the present study such as silver nitrate was AR grade and purchased from SRL (India) and used as such without further purification. All glasswares and quick fits used in the experimental work were made up of corning/borosil glass. These glasswares were washed thoroughly and dried in hot air oven before use. Bacterial cultures *Escherichia coli* (ATCC 10798), *Klebsiella*

pneumoniae (ATCC 31488), *Staphylococcus aureus* (ATCC 10832D-5), *Pseudomonas aeruginosa* (ATCC 207), were purchased from M/s. LGC Promochem India Pvt. Ltd, Bangalore, India.

Collection of seaweed

The marine red seaweed *Spyridia fusiformis* was collected from a depth of 2.5 meters in the rapid island Gulf of Mannar, near the Mandapam Coastal area in South India (co-ordinates: 9 ° 16' 48" N, 79 ° 7' 12" E).

Preparation of algal extract

Collected red seaweed was washed with seawater to remove the epiphytes and sand particles. After dried, 1 g of materials was cut into small pieces; ground with 50 ml of distilled water with mortar and pestle and these extracts were boiled for 5 min. The boiled extract was filtered through a high-quality Whatman1 filter paper and the supernatant was used and stored at 4 °C for further process.

Biosynthesis of silver nanoparticles

In the typical synthesis process of silver nanoparticles, 10 ml of aqueous algal extract was added with 90 ml of 1 mmol of silver nitrate solution in 250 ml conical flask. The reaction mixture was kept at room temperature under mechanical stirring [4-8].

Characterization of silver nanoparticles

Biogenic synthesis of nano silver was monitored using a UV-visible spectroscopy (UV-1601 Shimadzu spectrophotometer). After the complete reduction of silver ions by the *S. fusiformis* extract, it was analyzed by FT-IR spectroscopy. XRD pattern of dry nano silver powder was acquired by CuK α radiation (1.5406 Å; 45 kV, 30 mA). It was also analyzed to determine peak intensity, position and width. The size and shape of the biosynthesized nanoparticles were observed using a transmission electron microscope (TEM) (Hitachi, Model: S-3400N).

Antibacterial activity

Antibacterial activity of *S. fusiformis* assisted, silver nanoparticles were carried out using disc diffusion method against pathogenic bacteria. These bacterial cultures were freshly cultivated for 24 h in nutrient broth. Each bacterial culture was spread on the Muller Hinton agar plates. Sterile paper discs containing three different concentrations of silver nanoparticles were placed and incubated. After 24 h of incubation, the zone formation was recorded. The experiments were repeated for three times.

Statistical analysis

The data obtained in the present study were analyzed by using the one-way ANOVA with equal sample size by using SPSS 17.0.

RESULTS

Biosynthesis of silver nanoparticles

The shade-dried biomass of the marine red alga *S. fusiformis* was used for the synthesis of silver nanoparticles. Reduction of AgNO_3 was visually observed from the change in reaction mixture colour from transparent to brownish yellow after 30 min of reaction (Fig. 1). The intensity of brown colour gradually intensified in direct proportion to the reaction period. Also, the colour of the solution gradually intensified on heating, which indicates the formation of Ag nanoparticles. In the case of the negative control (silver nitrate solution only), no change in colour was observed [9-11].

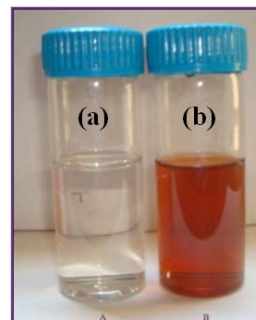


Fig. 1: The test tube containing the filtrate of the *S. fusiformis* biomass in aqueous solution of 1×10^{-3} M silver nitrate at the (a) beginning of reaction and (b) after 2 h of reaction

Characterization of silver nanoparticles

UV-visible spectroscopy

UV-Visible spectroscopy is an important technique to determine the formation and stability of metal nanoparticles in an aqueous solution. The extracellular synthesis of silver nanoparticles using *S. fusiformis* involved by the reduction of Ag^+ ions was identified by the UV-Visible spectroscopy. The characteristic absorption peak observed at 450 nm in UV-Visible spectrum for *S. fusiformis* (Fig. 2) indicated the formation of AgNPs.

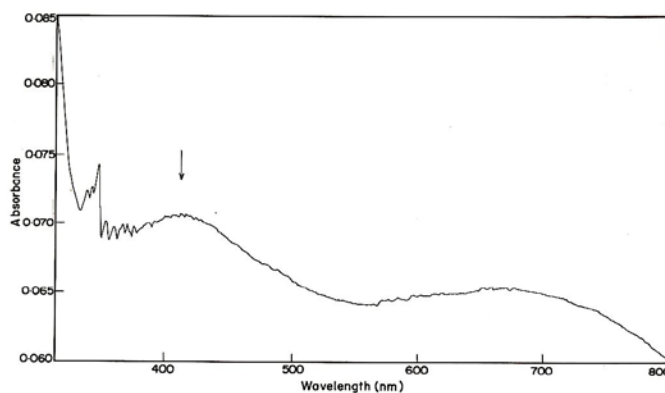


Fig. 2: UV-Visible-spectra of silver nanoparticles synthesized from extract of *S. fusiformis*

FT-IR spectroscopy

The FT-IR spectrum recorded for silver nanoparticles synthesized using *S. fusiformis* biomass, showed a variation in the intensity of bands in different regions (Table 1). A number of broad bands were observed in the region of 3907, 3779, 3410, 2927, 2853, 2593, 1644, 1416, 1170 and 749 cm^{-1} (Fig. 3).

The peak at 3907 and 3779 cm^{-1} was assigned to the vibrational modes of the -O-H stretching vibrations of the phytochemical molecules. The peak at 3410 cm^{-1} corresponds to -N-H stretching

vibrations. The peaks at 2927 and 2853 cm^{-1} were due to the stretching vibration of alkyl (-CH₂-) group. The peak at 2593 cm^{-1} was assigned to be a free SH. The presence of a weak broadband centred at 1644 cm^{-1} was characteristic of the amide bond in proteins and indicates a small concentration of protein in the silver nanoparticle solution synthesised using the *S. fusiformis* extract (Fig. 3). The band at 1416 cm^{-1} was due to the CH₂ bending. The band at 1170 cm^{-1} assigned to the secondary cyclic alcohols. The band at 749 cm^{-1} may be assigned as -CH=CH-(cis) bends (Table 1).

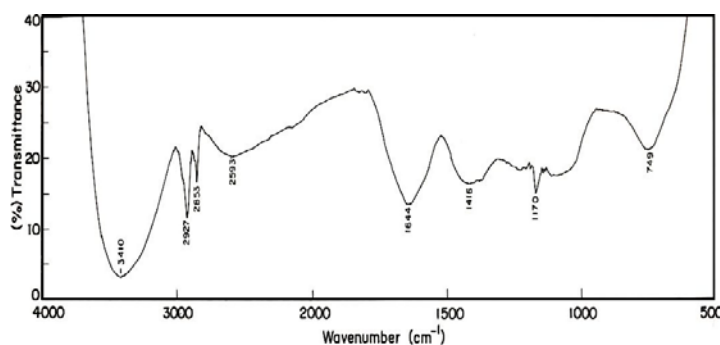


Fig. 3: FT-IR spectra of silver nanoparticles synthesized by the aqueous extract of *S. fusiformis*

Table 1: FT-IR spectral interpretation of silver nanoparticles in *S. fusiformis*

Assignments	Wavenumber (cm ⁻¹)
OH stretching vibrations	3907
OH stretching vibrations	3779
N-H Stretching vibrations	3410
CH ₂	2853
Free SH	2593
(C=OStretching vibrations) Amides	1644
CH ₂	1416
Secondary Cyclic Alcohols	1170
-CH=CH-(cis)	749

HR-TEM analysis

The HR-TEM measurements were recorded on drop coated films of the Ag nanoparticles synthesised using *S. fusiformis* (Fig. 4).

The various shape and size distributions of synthesised silver nanoparticles were clearly observed in the HR-TEM analysis. Fig. 4 shows the HR-TEM ascertained morphology and size of the red seaweed *S. fusiformis* treated with a 10⁻³M silver nitrate solution for

24 h. The HR-TEM images of *S. fusiformis* synthesised silver nanoparticles showed mostly large and small spherical, triangle, pseudo-spherical and some in rounded rectangle shapes.

The micrograph showed the nanoparticles with variable shape, most of them present in spherical shape within the size range of 5 to 50 nm. The size distribution of the silver nanoparticles is shown in fig. 5. The average size of the nanoparticles was found to be 32.70 nm as observed from the HR-TEM images.

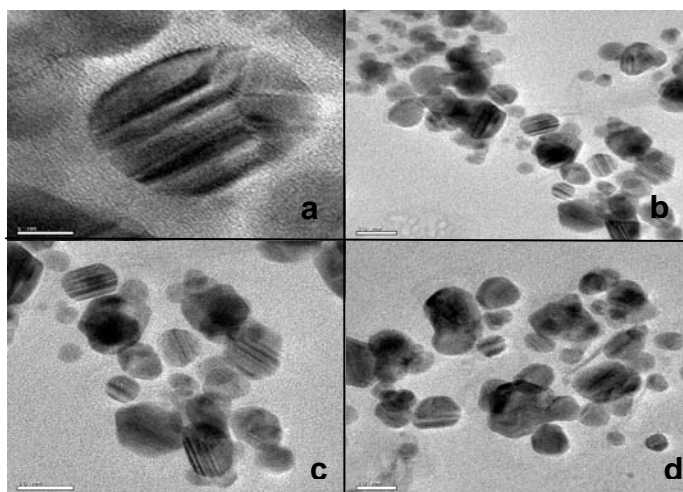


Fig. 4: HR-TEM images of the silver nanoparticles synthesized by using *S. fusiformis*

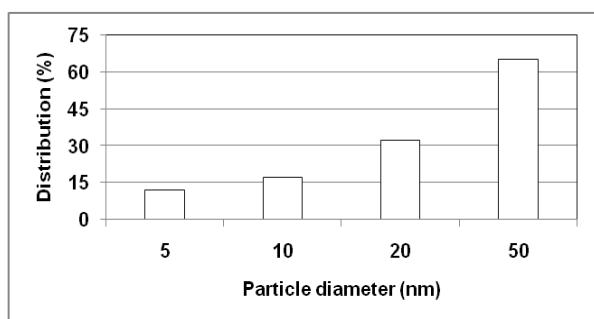


Fig. 5: A particle size distribution histogram of synthesized silver nanoparticles determined from HR-TEM images of *S. fusiformis*

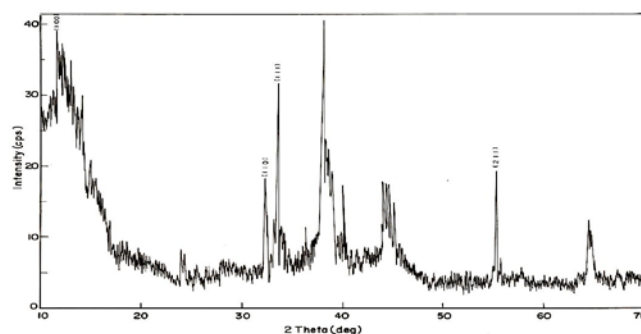


Fig. 6: X-ray diffraction pattern of the silver nanoparticles obtained from *S. fusiformis*

XRD analysis

XRD is a widely used technique to elucidate the structure, crystalline nature and to estimate the purity of nanoparticles synthesized.

X-ray diffractogram of the biosynthesized Nano silver exhibits Bragg reflections, corresponding to face-centered cubic (FCC) type bulk silver.

These XRD peaks indicate that the silver nanoparticles are crystalline in nature. In *S. fusiformis*, the XRD pattern of $2\theta = 11.62^\circ$, 32.19° , 34.12° and 55.12° (Fig. 6) are in agreement with the Joint Committee on Powder Diffraction Standards (file No. 04-0783), which further proves the formation of crystal silver nanoparticles. The presence of intense peaks of silver nanoparticles corresponding to the {1 0 0}, {1 1 0}, {1 1 1} and {2 1 1} planes have been indexed as crystalline silver face-centered cubic (FCC) phase.

Antibacterial activities of crude extract and silver nanoparticles

The antibacterial activity of the silver nanoparticle synthesized from *S. fusiformis* was tested against human bacterial pathogens (*E. coli*, *K. pneumoniae*, *S. aureus* and *P. aeruginosa*).

The antibacterial activity of the silver nanoparticles was carried out by the disc diffusion method and the zone of inhibition was measured.

The silver nanoparticles were synthesized by the marine red alga *S. fusiformis* at a higher concentration of 100 µg/ml which showed maximum zone of inhibition of 26 mm as observed in the *K. pneumoniae* followed by *S. aureus* (24 mm) when compared to the standard drug streptomycin (table 2; fig. 7), which shows only 18 mm of zone inhibition. In the case of the experimental alga *S. fusiformis*, the MIC was determined as 250 µg/ml and MBC was 125 µg/ml against the pathogen *K. pneumoniae*.

Table 2: Antibacterial activity of silver nanoparticles of *S. fusiformis*

S. No	Name of the microorganisms	Zone of inhibition in mm on human pathogen				
		25 µg/ml	50 µg/ml	75 µg/ml	100 µg/ml	Streptomycin (100 µg/ml)
1	<i>E. coli</i>	12±0.003	18±0.004	20±0.006	21±0.003	16±0.002
2	<i>K. pneumoniae</i>	17±0.001	20±0.006	23±0.003	26±0.007	18±0.003
3	<i>S. aureus</i>	15±0.002	17±0.001	22±0.003	24±0.004	16±0.001
4	<i>P. aeruginosa</i>	19±0.003	15±0.002	20±0.006	22±0.003	17±0.001

Values are expressed as mean±SEM, n=3

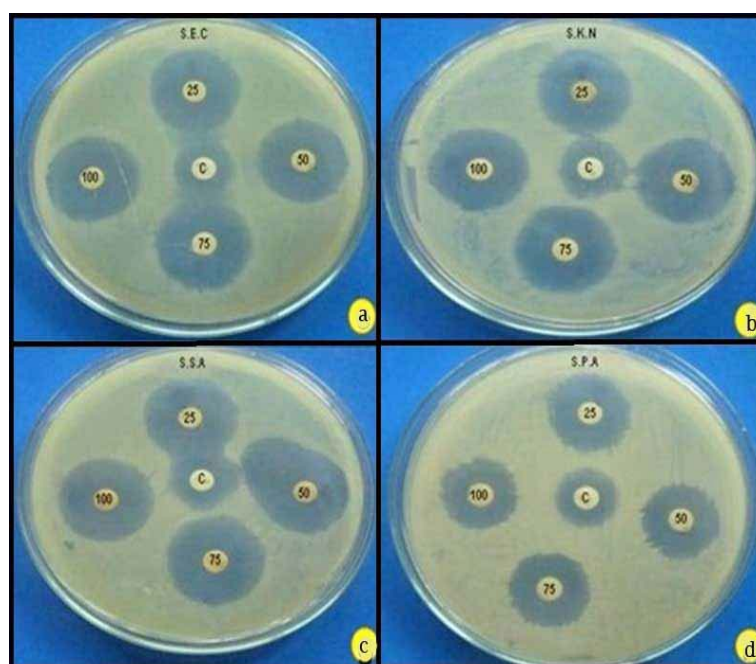


Fig. 7: Antibacterial activity of the silver nanoparticles synthesized from, *S. fusiformis*. a) *Escherichia coli* b) *Klebsiella pneumoniae*, c) *Staphylococcus aureus* and d) *Pseudomonas aeruginosa*

DISCUSSION

In the present study, a green synthesis of silver nanoparticles using *S. fusiformis* has been carried out. It is observed that the colour of the solution turned from colourless to brownish yellow after 2 h of the reaction, indicating the formation of silver nanoparticles. This observation indicates that the reduction of the Ag⁺ ions takes place extracellularly. The colour of the seaweed *S. fusiformis* extract becomes turbid after the addition of aqueous AgNO₃ solution signifying the initiation of the reaction [13]. The appearance of brown colour was due to the excitation of surface Plasmon vibrations, typical of silver nanoparticles [8, 14, and 15]. The UV-visible absorption spectrum of the reaction mixture was analyzed at different wavelengths ranging from 400 to 700 nm. The characteristic absorption peak at 450 nm in UV-Visible spectrum confirmed the formation of AgNPs [16, 17]. The broad peak indicates the presence of nanoparticles with a large size distribution as well as polydispersity nature of the particle [18]. The Plasmon resonance bands of silver nanoparticles are broad with an absorption tail in the longer wavelength that extends well into the near-infrared region

attributing the excitation of the in-plane SPR and indicates considerable anisotropy in the shape of silver nanoparticles. According to the Mie theory (1908), the small size and spherical shape of nanoparticles, which were formed in the reaction mixture were identified by forming a single SPR band for silver nanoparticles [19, 20]. The intensity of the band increased upon varying the time without any shift in peak position. The attribute surface Plasmon absorption bands are noticed between 430 to 450 nm and rising of nanoparticle size can also affect the SPR band broadening [21]. Based on the high intensity, we confirmed that the surface Plasmon resonance was eminent.

Similarly, Ghodake and Lee (2011) reported that the SPR band was located at 530 nm for gold nanoparticle synthesized using brown algae *Lonicera japonica* [22]. FT-IR analysis was carried out to identify the possible biomolecule responsible for the reduction of the silver ion and capping agent of bio-reduced silver nanoparticles by *S. fusiformis*. The shift in peak clearly attributes the reduction of silver ions into nanoparticles. These records indicate the formation of silver ions due to a bio-reduction and their possible stabilization

as nanoparticles. Since a member of (NH)C=O group within the cage of cyclic peptides is involved in stabilizing the nanoparticle, the shift of (NH)C=O band is quite small. Thus, the peptides play a major role in the reduction of Ag⁺ of Ag nanoparticles. Bands at 1016 cm⁻¹ are assigned as absorption peak of -C-O-C. The band at 1416 cm⁻¹ in the silver nanoparticle spectrum may be attributed to -C-O is stretching mode [23-26]. A broad IR band around 1512 cm⁻¹ in the spectrum of silver nanoparticle arose from the stretching vibrations of the C=C chain [27]. Hence, it may be inferred that these biomolecules are responsible for capping and efficient stabilization. The peak at 1644 cm⁻¹ indicates the presence of C=O [28, 29]. A strong absorption peak at 3,430 cm⁻¹ indicates the presence of phenols and alcohols with the free O-H group. The peak at 1,644 cm⁻¹ represents the presence of amide I group and may well arise due to the carbonyl stretch in proteins [17]. Absorbance bands were seen at 3410 cm⁻¹ were assigned to the stretching vibrations of primary and secondary amines, respectively. The result revealed that the capping ligand of the AgNPs may be an aromatic compound or alkanes or amines. These findings support the results of Gole *et al.*, [30] which states that proteins can bind to nanoparticles either through free amine groups or cysteine residues and therefore the stabilization of the silver nanoparticles by protein occurs [30]. The biological molecules such as secondary metabolites could possibly perform the dual functions of formation and stabilization of silver nanoparticles in the aqueous medium [31, 32]. The High-Resolution Transmission electron microscopy (HR-TEM) is the technique used to determine the size and size distribution of nanoparticle samples [33]. HR-TEM images clearly revealed the formation of spherical and rounded rectangle like structures. The size of the nanoparticle is around 5-50 nm. The similar results were observed by Murugesan *et al.*, when using red alga *Gracilaria edulis* as a reducing as well as capping agent [34]. The average size of resulting particles is about 32.70 to 38.70 nm. It is well known that the shape of metallic nanoparticles considerably changed their optical and electronic properties as reported earlier by [35, 36] Shipway *et al.*, (2000); Nie and Emory (1997). When compared to the previous reports of [37, 38] Noruzi *et al.*, and Raghunandan *et al.*, states that the algal-mediated silver nanoparticle shape varies as spherical triangle, and pseudo-spherical [35-38]. The coating material is believed to be a bio-organic component of *S. fusiformis*. The X-ray diffraction patterns obtained for the silver nanoparticles which are synthesized using *S. fusiformis* extract a number of Bragg reflections with 2θ values of 11.63 °, 32.20 °, 34.13 ° and 55.13 ° sets of lattice planes are observed which may be indexed in the {1 0 0}, {1 1 0}, {1 1 1} and {2 1 1} faces of silver and respectively, which confirms the face-centered cubic structure of the formed silver nanoparticles. X-Ray diffraction pattern thus clearly illustrates that the silver nanoparticles formed in this present study are crystalline in nature. The antibacterial behaviours of silver nanoparticles from marine red alga *S. fusiformis* provide the great expectations in pathogenic microbe controlling by advance technology. The antibacterial activity was conducted against the human pathogenic bacteria *E. coli*, *S. aureus*, *K. pneumoniae* and *P. aeruginosa*. The antibacterial effect of silver nanoparticles was studied by using the disc diffusion method. Pal *et al.*, (2007) reported that the shape of the silver nanoparticle could influence antimicrobial activity [39]. The zone of inhibition clearly indicates the promising effect of biosynthesized silver nanoparticles than the chemically synthesized nanoparticles. Stoimeno *et al.*, (2002) demonstrated that the highly reactive metal oxide nanoparticles exhibit excellent biocidal activity against Gram-positive and Gram-negative bacteria [40]. Aymonier *et al.*, (2002) showed that hybrids of silver nanoparticles with amphiphilic hyperbranched macromolecules exhibit effective antimicrobial surface coatings [41]. It is believed that DNA loses its replication ability and cellular proteins become inactivated on Ag⁺ treatment [42]. It was also shown that Ag⁺ binds to functional groups of proteins, resulting in protein denaturation [43]. Some studies have reported that the positive charge of the Ag ions is crucial for its antimicrobial activity through the electrostatic attraction between negatively charged cell membranes of microorganism and positively charged nanoparticles [40-42]. The nanoparticles were reacted with the bacterial cell walls and inhibit the growth of the bacteria vigorously and formed the zone size. The present study shows that the algal assisted synthesis of silver nanoparticles from *S. fusiformis*

against the different bacterium *E. coli* and *K. pneumoniae* has a towering inhibition and got decreased gradually against *S. aureus* and *P. aeruginosa* respectively, whereas silver nanoparticles have more or less similar effect on the bacteria. The mode of the bactericidal activity of silver nanoparticle is depending on the source from which the particles derived [44].

The silver nanoparticle has various modes of action when invaded to microbes. It depends on different parameters like source, concentration and contact time, nature of microbe, temperature and pH. The exact mechanisms of silver nanoparticles against the bacterial culture are clearly known and the small surface area containing nanoparticles having interaction with the large surface area may attach the cell membrane of the bacteria and involve the process of upsetting the respiration and permeability. The adsorption on the bacterial surface and intracellular enzyme activity is the main reason for the antibacterial reactions.

CONCLUSION

The extract of marine red seaweed *S. fusiformis* is capable of producing silver nanoparticles as observed from our investigation. The synthetic method using algal sources is an environmentally benign process and the algal extracts can be used as an effective capping as well as a reducing agent for the synthesis. The AgNPs showed potential antibacterial activity against human pathogens like *E. coli*, *S. aureus*, *K. pneumoniae* and *P. aeruginosa*. Applications of these eco-friendly nanoparticles with bactericidal and other medical applications will have a high potential for large-scale synthesis in future.

ACKNOWLEDGMENT

The financial support received from University Grants Commission, New Delhi (Grant No. F1-17.1/2011-12/RGNF-SC-TAM-5342/(SA-III/Website) dated 6/6/12) is gratefully acknowledged.

AUTHOR CONTRIBUTION

S. Murugesan contributed for overall work design, results in interpretation and manuscript preparation

S. Bhuvaneshwari contributed for algae collection, extraction and nanoparticles synthesis and biological studies

V. Sivamurugan contributed for spectral characterisation, interpretation of results and manuscript preparation

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest

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How to cite this article

- Subbiah Murugesan, Sundaresan Bhuvanewari, Vajiravelu Sivamurugan. Green synthesis, characterization of silver nanoparticles of a marine red alga sphyridia fusiformis and their antibacterial activity. Int J Pharm Pharm Sci 2017;9(5):192-197.