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Synthesis, characterization and dose dependent antimicrobial and anticancerous efficacy of phycogenic (*Sargassum muticum*) silver nanoparticles against Breast Cancer Cells (MCF 7) cell line

Nookala Supraja [1a], J. Dhivya [1b], T.N.V.K.V. Prasad [*2] and Ernest David [1c]

[1] *Department of Biotechnology, Thiruvalluvar University, Serkkadu, Vellore 632 115, TN, India*
[2] *Nanotechnology Laboratory, Institute of Frontier Technology, Regional Agricultural Research Station, Acharya N G Ranga Agricultural University, Tirupathi 517 502, AP, India*

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Abstract. In the present study silver nanoparticles (AgNPs) were successfully synthesized using aqueous extract of *Sargassum muticum*. The aqueous extract (10%) treated with 1 mM silver nitrate solution resulted in the formation of AgNPs and the surface plasmon resonance (SPR) of the formed AgNPs was recorded at 360 nm using UV-Visible spectrophotometer. The molecules involved in the formation of AgNPs were identified by Fourier transform infrared spectroscopy (FT-IR), surface morphology was studied by using scanning electron microscopy (SEM), SEM micrograph clearly revealed the size of the AgNPs was in the range of 40-65 nm with spherical, hexagonal in shape and poly-dispersed nature, and X-ray diffraction spectroscopy (XRD) was used to determine the crystalline structure. High positive Zeta potential (36.5 mV) of formed AgNPs indicates the stability and XRD pattern revealed the crystal structure of the AgNPs by showing the Bragg's peaks corresponding to (111), (200), (311) and (222) planes of face-centered cubic crystal phase of silver. The synthesized AgNPs exhibited effective anticancerous activity (at doses 25 and 50 μg/ml of AgNPs) against Breast cancer cell line (MCF7).

Keywords: *Sargassum muticum*; silver nanoparticles; antimicrobial activity; anti-cancerous efficacy

1. Introduction

The field of nanotechnology is one among the foremost important and active areas of research in modern science. Nanotechnology deals with the formulation of experimental processes for the synthesis of nanoparticles with different sizes and shapes (Mahasneh 2013). The application of nanoparticles, usually ranging from 1 to 100 nm, is a developing and interesting area of nanotechnology (Dahl *et al*. 2007). Nanoparticles synthesized using metals have received extensive attention in recent years because of their remarkable properties and wide range of applications in catalysis (Paul *et al*. 2014), plasmonics (Khlebtsov and Dykman 2010), optoelectronics (Muruganandam *et al*. 2014), biological sensor (Venkatesan and Santhanalakshmi 2014), water treatment (Con and Loan 2011) and pharmaceutical applications. To date, metallic nanoparticles are mostly prepared from noble metals, i.e., silver (Vankar and Shukla 2012), gold

*Corresponding author, Ph.D., Senior Scientist, E-mail: tnvkvprasad@gmail.com

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(Dash *et al*. 2014), copper, zinc and titanium (Schabes-Retchkiman *et al*. 2006) as well from cadmium (Suresh 2014), iron (Behera *et al*. 2013) and alginate (Asadi 2014). Among the noble metals, silver (Ag) is the metal of choice in the field of biological system, living organisms and medicine (Parashar *et al*. 2009). It is generally recognized that silver nanoparticles may adhere to the cell wall and damage the cell wall permeability. The cellular DNA and protein are destroyed by interaction of nanoparticles with the phosphorus of DNA and sulphur containing amino acids of protein (Elumalai *et al*. 2010). Different types of methods are available for the synthesis of silver nanoparticles for example, reduction in solutions (Goia and Matijevic 1998), chemical and photochemical reactions in reverse micelles (Taleb *et al*. 1997), thermal decomposition of silver compounds (Esumi *et al*. 1990), radiation assisted (Shahriari *et al*. 2011), electrochemical (Li *et al*. 2008), sonochemical (Moghimi-Rad *et al*. 2011), microwave assisted (Pal *et al*. 2014) and recently via green chemistry approach (Supraja *et al*. 2015).

Historically, seaweed is a readily available food source that has been consumed by coastal communities likely since the dawn of time. Seaweed is consumed habitually in many countries in South-East Asia. Marine algae refer to a wide variety of different species with different medicinal behavior, which are divided into two groups, namely microalgae and macroalgae. Marine macroalgae or seaweed, are plant-like organisms classified according to their pigmentation into green (chlorophytes), red (rhodophytes) and brown (phaeophytes). Seaweeds are well-known as functional food for their richness in lipids, minerals and certain vitamins, and also several bioactive substances like polysaccharides, proteins and polyphones, with potential medicinal uses against cancer (Namvar *et al*. 2012), oxidative stress (El Gamal 2010), inflammation (Khan *et al*. 2008), allergy (Zuercher *et al*. 2006), diabetes (Perez *et al*. 1998), thrombosis (Nishino *et al*. 1999), obesity (Miyashita 2009), lipidemia (Mohamed *et al*. 2012), hypertensive (Wada *et al*. 2011) and other degenerative diseases. Thus, their phytochemicals include hydroxyl, carboxyl, and amino functional groups, which can serve both as effective metal-reducing agents and as capping agents to provide a robust coating on the metal nanoparticles in a single step.

Green synthesis of nanoparticles is an emerging branch of nanotechnology (Supraja *et al*. 2016). The use of environmentally benign materials like plant extract (Prabha *et al*. 2014), bacteria (Seshadri *et al*. 2012), fungi (Muhsin and Hachim 2014) and marine algae (Supraja *et al*. 2016) for the synthesis of silver nanoparticles offers numerous benefits of eco-friendliness and compatibility for pharmaceutical and other biomedical applications as they do not use toxic chemicals for the synthesis protocol. Green synthesis are found to be superior over physical and chemical method as it is economically feasible, environmental friendly, scaled up for mass-scale production without any complexity.

The current work describes a green and rapid method using brown seaweed (*S. muticum* belongs to *Sargassaceae* family) aqueous extract solution for the biosynthesis of silver nanoparticles in ambient conditions. The current simple synthetic green method using rapid precursors of *S. muticum* aqueous extract provides high-yield nano-sized materials with good anti microbial and anti cancerous properties. In this work, the characterization and formation mechanisms of AgNPs are discussed.

2. Materials and methods

2.1 Methodology

Silver nitrate (> 99% pure) was purchased from Sigma Aldrich, India. Potato dextrose broth,

Potato dextrose agar, Nutrient broth, Nutrient agar plate, was supplied by Hi-media, India.

2.2 Sample collection and preparation (S. muticum, Algae)

The brown algae (*S. muticum*) were collected from the rameshwaram sea coast area near mandapam camp, Tamilnadu, India and were brought to the nanotechnology laboratory in new plastic bags containing sea water to prevent evaporation and washed with distilled water several times to remove the impurities. The clean algae were dried at room temperature in the shade for a week and powdered using a mortar and pestle.

2.3 Preparation of S. muticum (brown algae) extract

Dried powdered *S. muticum* (5 g) was mixed with 100 ml distilled water then the solution was kept for continues heating at 80°C for 1hour at room temperature with frequent shaking. After that the extract were filtered by using Whatmann No1 filter paper. The extract was collected and stored at 4°C for further use.

2.4 Synthesis of silver nanoparticles from S. muticum

10 ml of the aqueous extract of *S. muticum* was added into 90 ml of aqueous solution of 1 mM Silver nitrate. The mixture was exposed to a range of controlled temperatures for 24 h. Appearance of brown color in solution indicated the formation of AgNPs. The solution was then kept in dark for further analysis collected and stored at 4°C for further use.

2.5 Collection of microbes (Bacteria and fungi)

The microbes (Bacteria and fungi) samples were collected from Nanotechnology laboratory, Regional Agricultural Research Station, Tirupathi, (Chittoor District) Andhra Pradesh, India. These samples were stored in an ice box and transported to the laboratory for microbiological characterization. Through serial dilution pour plate technique, fungal sp. was isolated using potato dextrose agar (PDA) medium, and Gram negative and Gram-positive bacteria were isolated from nutrient agar medium. Further, it is maintained in potato dextrose agar slants (fungi) and nutrient agar slants (bacteria) for onward analysis.

2.6 Antibacterial activity of S. muticum produced AgNPs

The antibacterial activity of AgNPs was evaluated against the following pathogenic strains *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas fluorescence*, *Bacillus subtilis*, *Legionella pneumonia*, *Actinomyces israelii*, *Enterobacter cloacae* and *Helicobacter pylori*. These cultures were grown on appropriate medium at 37°C for overnight incubation and maintained at 4°C in a refrigerator (Vankar and Shukla 2012). Disc diffusion method disc of 5 Mm was made for nutrient agar medium and each disc was dipped at different concentration (170, 100, 50 ppm) efficiency of prepared AgNPs. The pure cultures of bacterial pathogens were sub-cultured on an appropriate medium. For comparison, plate of the same diameter with 5 Mm tetracycline (30 mcg) was used. After incubation at 37°C for 24 h the zones of bacterial inhibition were measured. The assays were performed triplicate.

2.7 Antifungal activity of S. muticum produced AgNPs

The antibacterial activity of AgNPs was evaluated against the following pathogenic strains fungal species *viz*., *Aspergillus flavus*, *Sclerotium rolfsii*, *Aspergillus niger*, *Rhizopus oligosporus*, *Aspergillus acidus*, *Athelia rolfsii*, *Aspergillus fumigates* and *Rhizopus oryzae* and these cultures were grown on appropriate medium at 25-28°C for overnight incubation and maintained at 4°C in a refrigerator (Elumalai *et al*. 2010). Disc diffusion method disc of 5 Mm was made on nutrient agar medium and each disc was dipped at different concentration (170, 100, 50 ppm) efficiency of prepared AgNPs. The pure cultures of fungal pathogens were sub-cultured on an appropriate medium. Discs of 5 mm diameter were made on potato dextrose agar medium. Each strain was swabbed uniformly onto the individual plate. For comparison, plate of the same diameter with 5 Mm ketoconazole (30 mcg) was used. After incubation at 37°C for 24 h the zones of fungal inhibition were measured. The assays were performed triplicate.

2.8 Anti cancer activity of S. muticum produced AgNPs

2.8.1 Cell line

The human breast adenocarcinoma cell line (MCF7) was obtained from National Centre for Cell Science (NCCS), Pune and grown in Eagles Minimum Essential Medium containing 10% fetal bovine serum (FBS). The cells were maintained at 37°C, 5% CO_2, 95% air and 100% relative humidity. Maintenance cultures were passaged weekly, and the culture medium was changed twice a week.

2.8.2 Cell treatment procedure

The monolayer cells were detached with trypsin-ethylenediaminetetraacetic acid (EDTA) to make single cell suspensions and viable cells were counted using a hemocytometer and diluted with medium containing 5% FBS to give final density of 1×10^5 cells/ml. One hundred microlitres per well of cell suspension were seeded into 96-well plates at plating density of 10,000 cells/well and incubated to allow for cell attachment at 37°C, 5% CO_2, 95% air and 100% relative humidity. After 24 h the cells were treated with serial concentrations of the test sample (Ag nanoparticles). They were initially dispersed in phosphate buffered saline by sonication and an aliquot of the sample solution was diluted to twice the desired final maximum test concentration with serum free medium. Additional four serial dilutions were made to provide a total of five sample concentrations. Aliquots of 100 μl of these different sample dilutions were added to the appropriate wells already containing 100 μl of medium, resulting in the required final sample concentrations. Following sample addition, the plates were incubated for an additional 48 h at 37°C, 5% CO_2, 95% air and 100% relative humidity. The medium containing without samples were served as control and triplicate was maintained for all concentrations.

2.8.3 MTT assay

3-[4,5-dimethylthiazol-2-yl] 2,5-diphenyltetrazolium bromide (MTT) is a yellow water soluble tetrazolium salt. A mitochondrial enzyme in living cells, succinate-dehydrogenase, cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan. Therefore, the amount of formazan produced is directly proportional to the number of viable cells (Supraja *et al*. 2016).

After 48 h of incubation, 15 μl of MTT (5 mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37°C for 4 h. The medium with MTT was then flicked off and

the formed formazan crystals were solubilized in 100 μl of DMSO and then measured the absorbance at 570 nm using micro plate reader. The percentage cell viability was then calculated with respect to control as follows

% Cell viability = [A] Test / [A]control × 100

2.9 Characterization of Ag nanoparticles

2.9.1 UV - Visible spectrum for synthesized nanoparticles

The nanoparticles were monitored by UV-visible spectrum at various time intervals. The UV - Visible spectra of this solution was recorded in spectra 50 ANALYTIKJENA Spectrophotometer, from 250 to 400 nm.

2.9.2 FT-IR analysis for synthesized nanoparticles

The nanoparticles were harvested and characterized by FT-IR. The FT-IR spectrum was taken in the mid IR region of 400-4000 cm^{-1}. The spectrum was recorded using ATR (attenuated total reflectance) technique. The sample was directly placed in the KBr crystal and the spectrum was recorded in the transmittance mode.

2.9.3 X-ray diffraction analysis for synthesized nanoparticles

The nanoparticles were harvested and characterized by XRD and TEM. The XRD pattern was recorded using computer controlled XRD-system, JEOL, and Model: JPX-8030 with CuK radiation (Ni filtered = 13418 A°) at the range of 40kV, 20A. The 'peak search' and 'search match' program built in software (syn master 7935) was used to identify the peak table and ultimately for the identification of XRD peak.

2.9.4 Dynamic light scattering (Particle size and zeta potential analyzer for synthesized nanoparticles)

The aqueous suspension of the synthesized nanoparticles was filtered through a 0.22 μm syringe driven filter unit and the size of the distributed nanoparticles were measured by using the principle of Dynamic Light Scattering (DLS) technique made in a Nanopartica (HORIBA, SZ-100) compact scattering spectrometer.

2.9.5 High resolution scanning electron microscope

The structural morphological characteristics of the bacterial sample were observed under scanning electron microscope (HR-SEM) Hitachi's SU6600 at magnification ranging from 10X to 600,000X operated at accelerating voltage of 30 kv.

3. Results and discussion

3.1 UV-Visible spectral analysis

From the (Fig. 1) *S. muticum* the AgNPs were synthesized, it is well- known that silver nanoparticles exhibit brown color, which arises due to excitation of surface Plasmon vibrations of the silver nanoparticles. After addition of 1mM silver nitrate solution to the aqueous extract of *S. muticum*, the colour of the composition has been changed colorless to dark brown colour. The

Fig. 1 *Sargassum muticum*

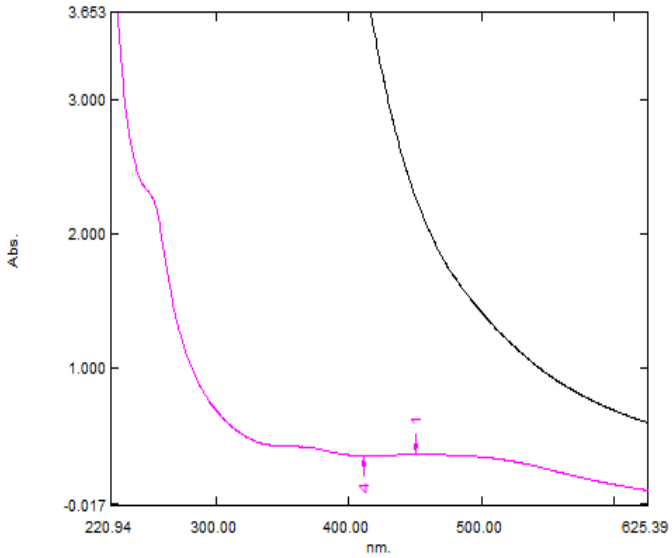


Fig. 2 UV/Visible absorption spectrum of synthesized silver nanoparticles from *S. muticum*

maximum absorbance peak is observed at 360 nm for *S. muticum* (Fig. 2). The overall observations suggest that the bio reduction of (silver ions) Ag^{+} to $Ag^{(0)}$ was confirmed by UV-Visible spectroscopy.

3.2 Fourier transform infrared spectrophotometry analysis

FT-IR spectrum of the biosynthesized silver nanoparticles using *S. muticum*. (Fig. 3) shows the absorption peaks at 3363, 2927, 2859, 2094, 1742, 1460 and 1165cm^{-1}. The peak at 3363 cm^{-1} reveals the presence of N-H stretching vibration, indicating the primary and secondary amines, 2927 cm^{-1} reveals the presence of C-H stretching vibration, indicating the presence of carboxylic/phenolic groups, 2859 cm^{-1} reveals the presence of C-H stretching vibration, indicating the presence of alkanes, 2094 cm^{-1} reveals the presence of –C ≡ C- stretching vibration, indicating

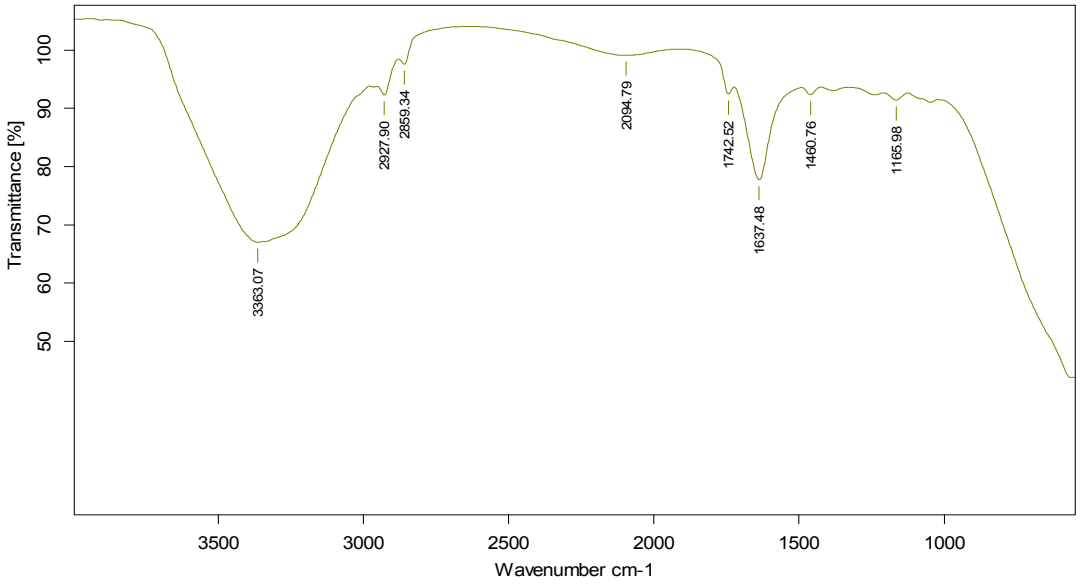


Fig. 3 FT-IR spectrum of synthesized silver nanoparticles from *S. muticum*

the presence of alkynes, 1742 cm^{-1} reveals indicating the presence of [N-H] C = O group that is characteristic of proteins shifted from after the synthesis of AgNPs, 1460 cm^{-1} reveals the presence of amide II and amide III of aromatic rings either may be poly phenols associated with synthesized silver nanoparticles which is segregated by *S. muticum* extract.

3.3 X-ray diffraction analysis

The sample of AgNPs could be also characterized by X-Ray Diffraction analysis of dry powder. The diffraction intensities were recorded from 10°-80° at 2θ angles (Fig. 4). Four different and important characteristic peaks were observed at the 2θ of 38.6°, 45.8°, 64.8° and 78.6° that correspond to (111), (200), (311) and (222) planes, respectively. All the peaks in XRD pattern can be readily indexed to a face centered cubic structure of silver as per available literature (JCPDS, File No. 4-0783). The XRD pattern of these peaks indicates the AgNPs is crystalline in nature and some of the unassigned peaks were observed it may be due to the fewer bio-molecules of stabilizing agents are enzymes or proteins in the *S. muticum* extract (Supraja *et al*. 2015).

3.4 Dynamic light scattering analysis

The particle size distribution spectra for the silver nanoparticles were recorded as diameter (nm) verses frequency (%/nm) spectra with diameter (nm) on *x*-axis and frequency (%/nm) on *y*-axis. The zeta potential spectra for the silver nanoparticles were recorded zeta potential verses intensity spectra with zeta potential (mV) on x-axis and intensity (a.u) on y-axis. Dynamic light scattering technique has been used to measure hydrodynamic diameter of the hydrosol (particle suspension). *S. muticum* AgNPs was found to be 65.9nm the recorded value of zeta potential of the silver nanoparticles was 36.5 mV (Fig. 5) which resulted in the agglomerated state of the formed AgNPs (Supraja *et al*. 2016).

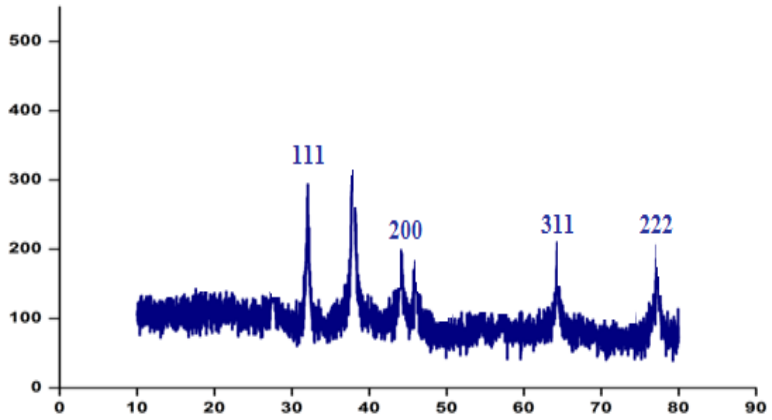


Fig. 4 XRD analyses of synthesized silver nanoparticles from *S. muticum*

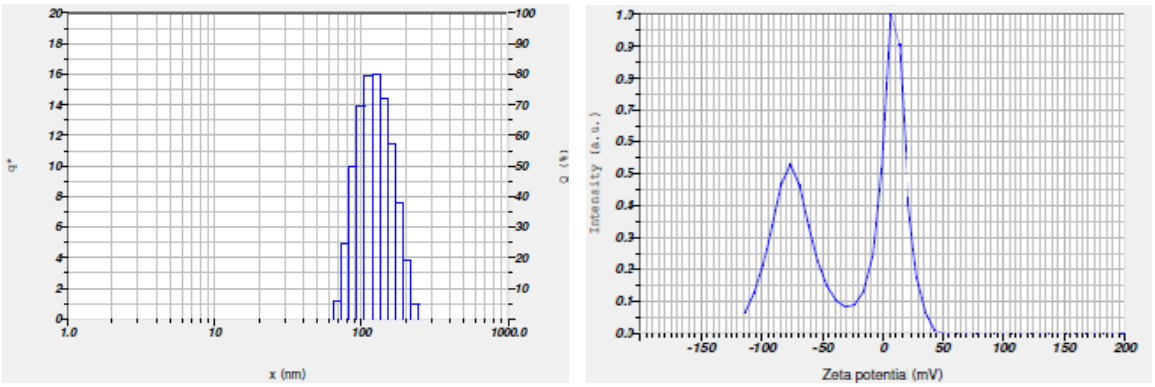


Fig. 5 DLS analysis of synthesized silver nanoparticles from *S. muticum*

3.5 Scanning electron microscopy analysis

The surface morphology, size and shape of *S. muticum* silver nanoparticles were characterized from the SEM micrograph, it is evident that AgNPs were spherical in shape and were poly-dispersed. The measured average size of AgNPs was 55 nm and the nanoparticles ranges from 40.4 nm to 57.6 nm occasional agglomeration of the AgNPs has been observed. The electrostatic interactions and hydrogen bond between the bioorganic capping molecules bond are responsible for the synthesis of silver nanoparticles using *S. muticum* extract (Supraja *et al*. 2015) (Fig. 6).

By comparing these FT-IR, XRD, DLS and SEM results with the chemical mediated

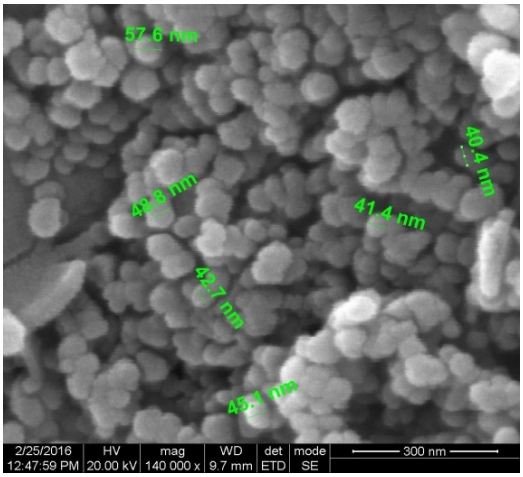
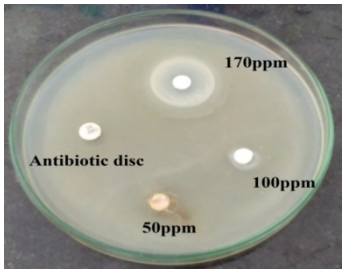


Fig. 6 SEM analyses of synthesized silver nanoparticles from *S. muticum*

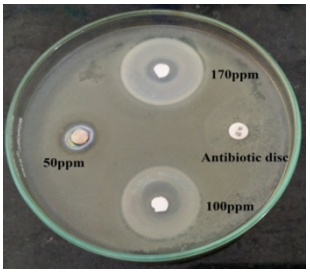
synthesized silver nanoparticles in FT-IR due to the presence of carbonyls, alkenes, alkynes and amines, and in XRD due to the presence of FCC with some disturbed picks indicates the presence of *S. muticum* compounds, In DLS the synthesized silver nanoparticles shown the high particle size less, and zeta potential is less it s due to the presence of *S. muticum* extract coated on AgNPs but when compared to commercially available AgNPs the particles size is low and zeta will be high due to presence of this will affect the cell wall damage to bacterial and fungal species in *Invitro* microbial activity analysis (Toxicity will be high even in low concentration) hence there is no extract is coated, coming to SEM AgNPs shown medium particle size with very little agglutination due to compounds present in *Sargassum muticum* where as in commercially available AgNPs the particle size is constant and there will be no agglomeration but coming to metallic nanoparticles the toxicity effect is less and they will show the highly effectible results due to presence of alkenes, alkynes, nitrogen and protein groups present in metallic nanoparticles.

3.6. Antimicrobial activity of S. muticum aqueous extract mediated silver nanoparticles

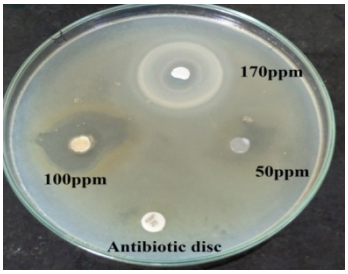
It is well- known that silver nanoparticles exhibit brown color, arising due to excitation of surface Plasmon vibrations in the silver nanoparticles. Silver nanoparticles obtained from *S. muticum* shown have very strong inhibitory action against fungal sp, Gram-positive and Gram-negative bacteria. These isolates were collected from nanotechnology laboratory, Acharya N G Ranga Agricultural University, Tirupathi. Three concentrations of NPs (170 ± 1.4, 100 ± 1.1, 50 ± 0.8 ppm) were prepared and were applied against an array of bacterial species *viz*., *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas fluorescence*, *Bacillus subtilis*, *Legionella pneumonia*, *Actinomyces israelii*, *Enterobacter cloacae* and *Helicobacter pylori*, fungal species *viz*., *Aspergillus flavus*, *Sclerotium rolfsii*, *Aspergillus niger*, *Rhizopus oligosporus*, *Aspergillus acidus*, *Athelia rolfsii*, *Aspergillus fumigates* and *Rhizopus oryzae*. The higher concentration (170 ± 1.4 ppm) of AgNPs showed significant antimicrobial effect compared with other concentrations (100 ± 1.1 and 50 ± 0.8 ppm). By comparing to (Nallamuthu *et al*. 2012, fight against Plasmodium falciparum and its vector Anopheles stephensi at different concentrations of ppm may be employed to develop newer and safer agents for malaria control. Azizi *et al*. (2013) tested antimicrobial



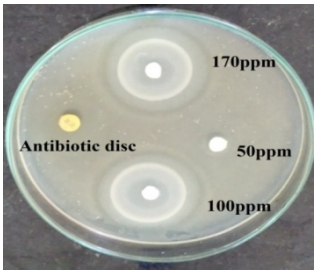
Escherichia coli



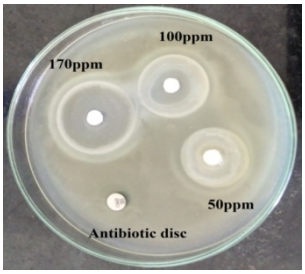
Staphylococcus aureus



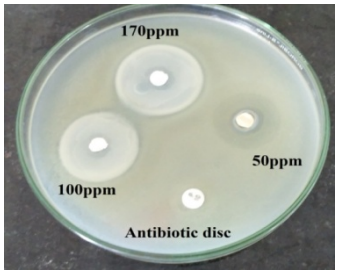
Pseudomonas fluorescence



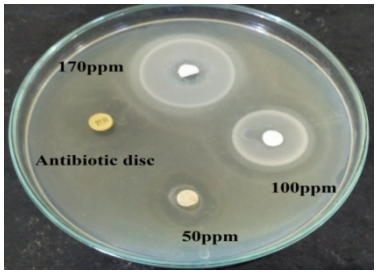
Bacillus subtilis



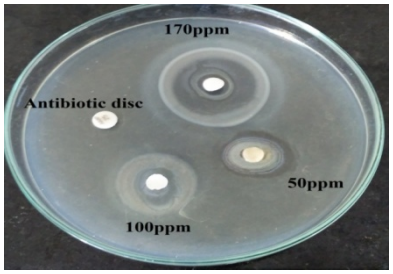
Legionella pneumonia



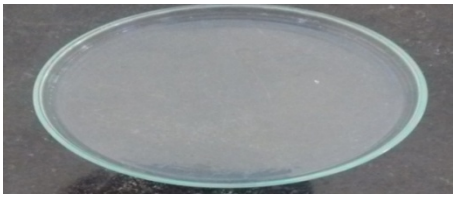
Actinomyces israelii



Enterobacter cloacae

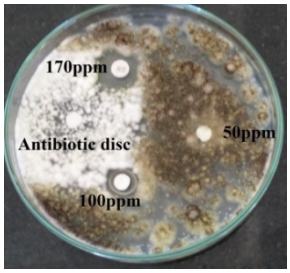


Helicobacter pylori

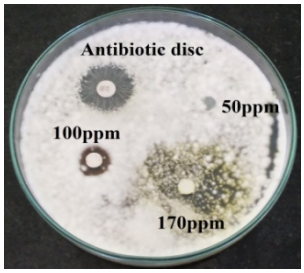


Control (Culture without AgNPs)

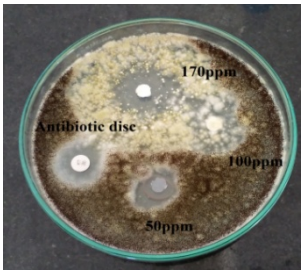
Fig. 7(a) Anti bacterial activity of synthesized silver nanoparticles from *S. muticum*



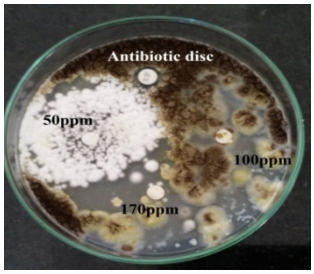
Aspergillus flavus



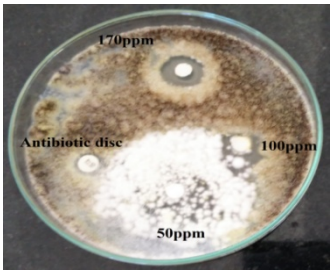
Sclerotium rolfsii



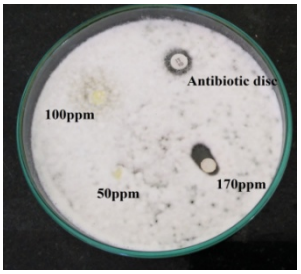
Aspergillus niger



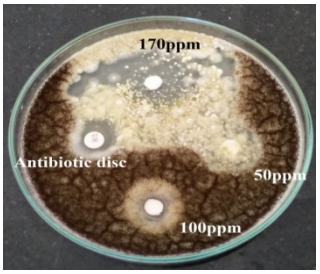
Rhizopus oligosporus



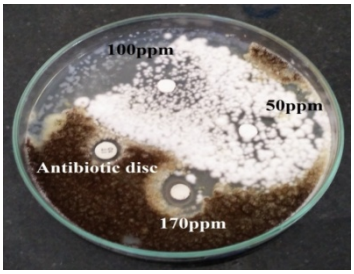
Aspergillus acidus



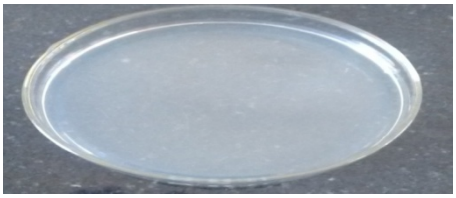
Athelia rolfsii



Aspergillus fumigates



Rhizopus oryzae



Control (Culture without AgNPs)

Fig. 7 (b) Anti fungal activity of synthesized silver nanoparticles from *S. muticum*

Table 1 *In vitro* antibacterial studies against bacteria using *S. muticum* extract mediated silver nanoparticles as inhibitors

S. no	Bacteria	*Sargassum muticum* (brown algae) aqueous extract mediated synthesis of silver nanoparticles			
		170ppm±1.4ppm	100ppm±1.1ppm	50ppm±0.8ppm	Tetracycline 30 mcg
1	Escherichia coli	3.7±0.6	2.7±0.3	2.3±0.5	0.3±0.02
2	Staphylococcus aureus	3.0±0.5	2.7±0.5	1.9±0.02	0.2±0.03
3	Pseudomonas fluorescence	3.4±0.4	3.0±0.4	1.2±0.08	0.2±0.04
4	Bacillus subtilis	3.7±0.6	2.4±0.3	1.8±0.05	0.3±0.02
5	Legionella pneumonia	2.6±0.7	2.0±0.5	1.0±0.04	0.4±0.03
6	Actinomyces israelii	2.6±0.5	1.6±0.2	1.1±0.02	0.1±0.04
7	Enterobacter cloacae	4.2±0.4	1.8±0.7	1.0±0.06	0.6±0.05
8	Helicobacter pylori	4.0±0.5	3.8±1.4	0.6±0.03	0.2±0.04

* The presented data are the mean (n = 3) ± standard error of three replicates

Table 2 *In vitro* antifungal studies against fungi using *S. muticum* extract mediated silver nanoparticles as inhibitors

S. no	Fungi	*Sargassum muticum* (brown algae) aqueous extract mediated synthesis of silver nanoparticles			
		170ppm±1.4ppm	100ppm±1.1ppm	50ppm±0.8ppm	Ketoconazole 30 mcg
1	Aspergillus flavus	1.3±0.05	0.9±0.02	0.6±0.01	0.8±0.02
2	Sclerotium rolfsii	1.3±0.03	0.8±0.04	0.5±0.02	1.6±0.06
3	Aspergillus niger	1.9±0.06	1.2±0.03	1.0±0.02	0.9±0.03
4	Rhizopus oligosporus	0.8±0.03	0.6±0.01	0.3±0.03	1.0±0.05
5	Aspergillus acidus	1.4±0.03	1.2±0.02	0.6±0.03	0.8±0.04
6	Athelia rolfsii	0.7±0.02	0.3±0.01	0.2±0.04	1.0±0.02
7	Aspergillus fumigates	1.6±0.05	1.0±0.03	0.5±0.02	1.2±0.04
8	Rhizopus oryzae	1.3±0.03	0.8±0.02	0.6±0.04	0.7±0.03

* The presented data are the mean (n = 3) ± standard error of three replicates

activities of ZnO nanoparticles/cellulose nanocrystals nanocomposites, Madhiyazhagan *et al*. (2015) showed antioxidant as well as anti-microbial activity, and therefore suggesting its therapeutic applications in various food samples but ours Silver nanoparticles obtained from *S. muticum* have shown both fungal and bacterial very effective antimicrobial activity when compared to other studies with less ppm concentrations.

The mechanism by which the nanoparticles are able to penetrate the bacteria is not understood completely, but studies suggest that when bacteria were treated with silver nanoparticles, changes took place in its membrane morphology that produced a significant increase in its permeability affecting proper transport through the plasma membrane (Auffan *et al*. 2009), leaving the bacterial cells incapable of properly regulating transport through the plasma membrane, resulting into cell death (Supraja *et al*. 2015). It is observed that silver nanoparticles have penetrated inside the

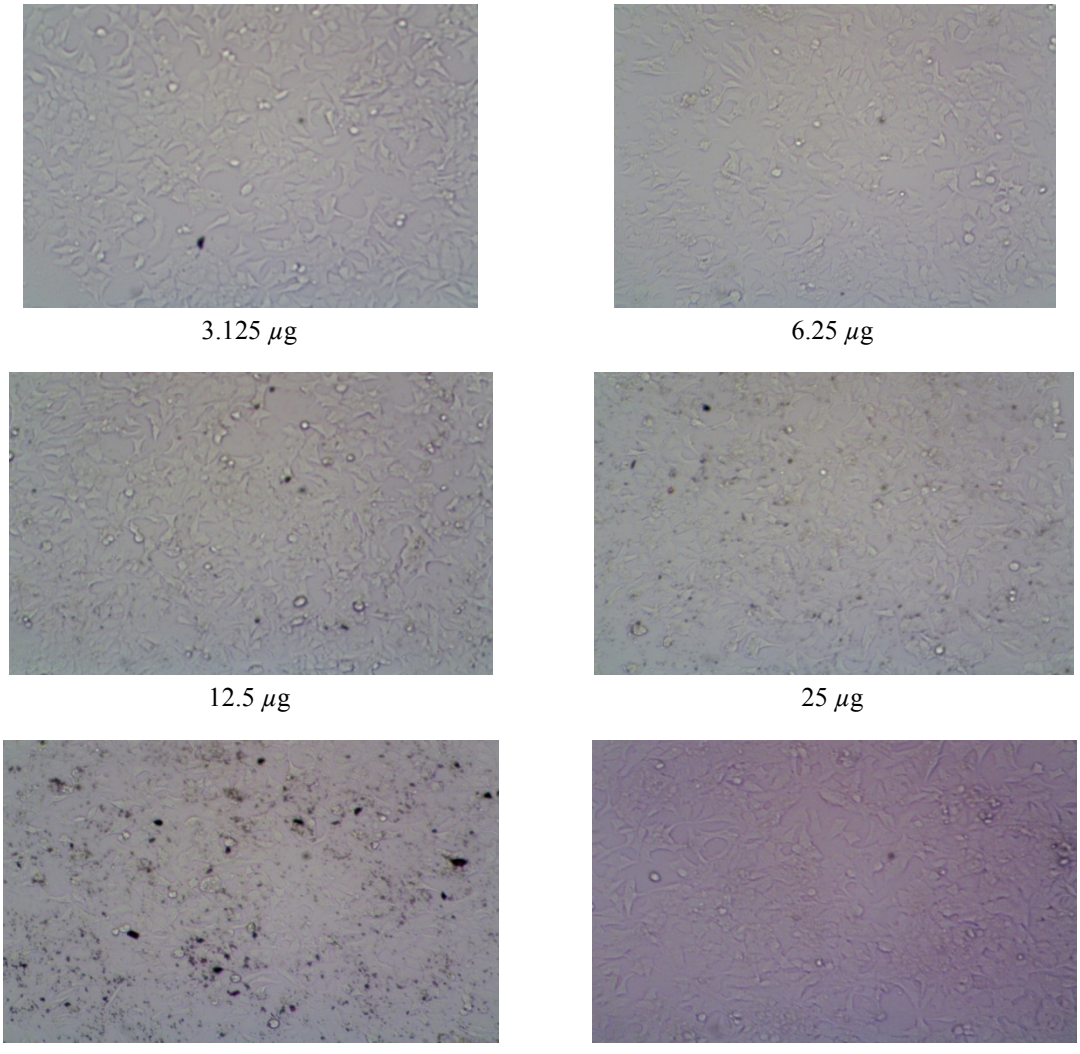


Fig. 8 Breast Cancer Cells (MCF 7) treated with 3.125-50 μg/ml of synthesized AgNPs using *S. muticum*

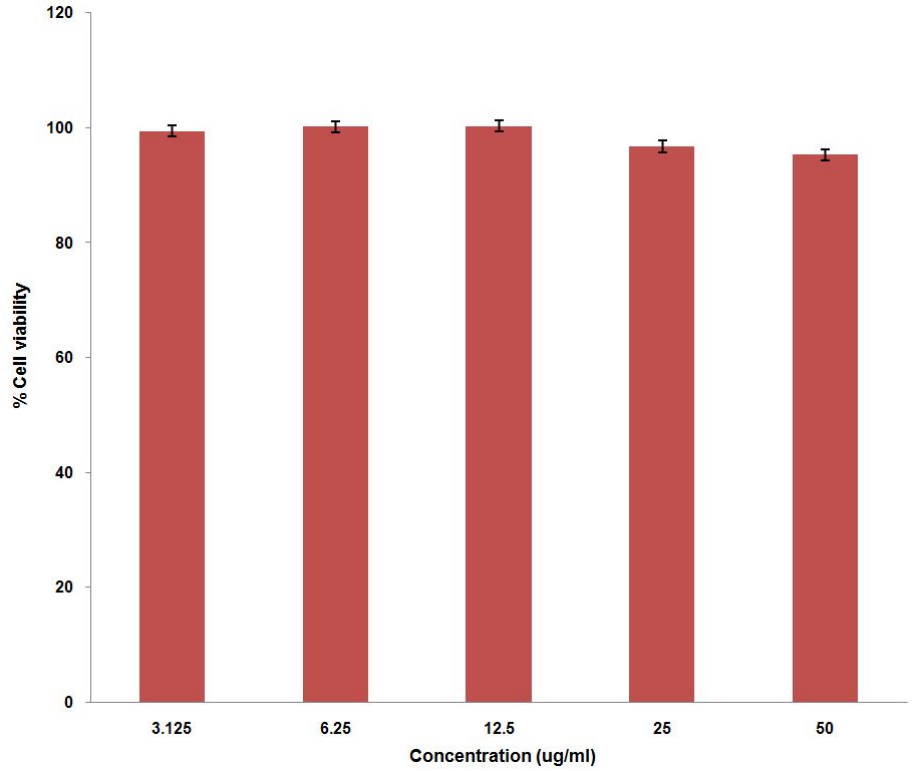


Fig. 9 Cytotoxic activity of *S. muticum* AgNPs against Human breast cancer cell line (MCF7)

bacteria and have caused damage by interacting with phosphorus- and sulfur-containing compounds such as DNA (He *et al*. 2008). Moreover, when compared to *S. muticum* AgNPs showed good antibacterial and antifungal activity, Further size and shape dependent uptake of silver nanoparticles into MCF 7 cells has been reported and points to the need of in depth study of size and shape dependent antimicrobial and cytotoxic effects of nanoparticles silver nanoparticles possess well-developed surface chemistry, chemical stability and appropriate smaller size, which make them easier to interact with the microorganisms (Supraja *et al*. 2015). Also, the particles interact with the building elements of the outer membrane and might cause structural changes, degradation and finally cell death (Figs. 7(a) and (b)) (Tables 1 and 2). The findings in this study may lead to the development of AgNPs-based new antimicrobial systems for medical applications.

3.7 MTT assay

The *in-vitro* cytotoxic effects of AgNPs were screened against MCF7 cell line and the percentage of cell inhibition was confirmed by MTT assay. The silver nanoparticles were not able to inhibit the MCF7 cells in a dose dependent manner. After 48 hours of treatment, the AgNPs at concentration of 3.125 μg/ml shown 99.49 viability, 6.25 μg/ml shown 100.22, 12.5 μg/ml shown 100.36, 25 μg/ml shown 96.795 and 50 μg/ml shown 95.339 of viability of MCF7 cells, and this was chosen as IC_{50} (Fig. 8). The cytotoxic effects of AgNPs are the active physico-chemical interaction of silver atoms with the functional groups of intracellular proteins, nitrogen bases and

phosphate groups in DNA. Although, further studies are needed to fully understanding the mechanism involved in the anticancer activity. From our results, it can be concluded that the *S. muticum* nanoparticles could have induced intracellular reactive oxygen species generation, which can be evaluated using intracellular peroxide-dependent oxidation and caused cell death slightly. The control cells were clustered, healthy and viable cells the *S. muticum* silver nanoparticles treated cells showed slightly increased apoptosis morphological changes also the clearly visible cell debris in (Fig. 9) is due to cell death by 12.5 μg/ml Silver nanoparticles treatment. Further studies have to be carried out to understand the nature of cytotoxicity and the death or proliferation of cells caused by *S. muticum* silver nanoparticles extract (Mosmann 1983, Monks *et al*. 1991, Rajeshkumar *et al*. 2016).

4. Conclusions

A critical need in the field of nanotechnology is the development of reliable and ecofriendly processes for synthesis of metal oxide nanoparticles.

- AgNPs with an average size of 50 nm and hexagonal shapes were synthesized by bioreduction of silver nitrate solution with a green method using brown seaweed (*S. muticum*) aqueous extract contain polysaccharides as the reducing agent and efficient stabilizer.
- The characteristics of the obtained AgNPs were studied using FT-IR, XRD, UV-Vis, DLS and FESEM techniques.
- The synthesized silver nanoparticles exhibited a strong antimicrobial activity against bacteria than fungi and shown excellent anticancerous efficacy.
- Biosynthesis of AgNPs using algal resources is a simple, environmentally friendly, pollutant-free and low-cost approach. This green method of synthesizing AgNPs could also be extended to Medical and industrial applications
- Further this *S. muticum* mediated synthesized AgNPs studies were carried out on Anti-diabetic and anti-oxidant purposes comparing with ZnO NPs.
- By using this brown algae *S. muticum* mediated synthesis of silver nanoparticles for Antimicrobial and anticancerous efficacy with different ppm concentrations, this studies carried out results is very Scant when compared to other related works carried out by using this *S. muticum*.

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