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Research Article

Antibacterial and anticancer activity of green synthesised silver nanoparticles using polysaccharides extracted from the marine alga *Portieria hornemannii*

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Abstract
The increasing incidence of cancer cases and multi-drug-resistant bacteria, which are major threats to humankind, forces the research world to innovate new molecules to deal with them. The main aim of the present work is to prepare silver nanoparticles using macroalgal polysaccharides and to study biological activities. The silver nanoparticles (NPs) were prepared using polysaccharides extracted from the marine macro alga *Portieria hornemannii* by stirring them with 1 mM silver nitrate after 24 h at 90 ºC. The formed silver nanoparticles were characterized using UV-visible spectrophotometry, Fourier transform infrared spectroscopy (FTIR) analysis, Transmission Electron Microscopy (TEM) analysis, selected-area electron diffraction (SAED), and Energy Dispersive X-ray (EDX) analysis. UV-visible spectrum analysis revealed a surface plasmon peak at 380 nm, showing the development of silver nanoparticles. The nanoparticle size varied between 40 and 50 nm and the functional group was analyzed using FT-IR spectrum. The broadband was observed at 3304 cm^{-1} (hydroxyl and amino group) and the narrow band was observed at 2907 cm^{-1} (C–H stretching vibration), 1657 cm^{-1} (stretching of carbonyl groups), and 1001 cm^{-1} (C–O stretching vibration). The crystalline nature of silver NPs was confirmed by SAED. EDX analysis reveals the purity and the chemical composition of silver NPs. Nanoparticles were highly effective against *Proteus mirabilis* (24 mm zone of inhibition) and *Bacillus substilis* (24 mm zone of inhibition). The anticancer activity of the silver nanoparticles tested against colorectal adenocarcinoma cell lines increased at increasing concentrations of nanoparticles.

Keywords: Anticancer, Antibacterial, Green synthesis, Marine alga, Polysaccharides, Silver nanoparticles

INTRODUCTION

Marine algae are widely distributed in the sea and are plant-like organisms in coastal waters (Bhuyar*et al*., 2020). They are generally attached to sand, rocks, dead plants and algae from underwater and are available on the sea surface (Bhuyar*et al*., 2019a). The size of macroalgae or seagrass varieswidely;however, they are grouped under multicellular algae (Bhuyar*et al*., 2019b). Seaweed contains several micro and macronutrients, carbohydrates, proteins, aminoacids, and vitamins. Seaweeds are widely used to extract varioususeful industrial products such as alginates, carrageenan, phycocolloids and agar (Chandraprabha *et al*., 2012; Al-Dhabi *et al*., 2019). Among seaweeds, Sargassum is one of the important groups and has antimicrobial activity with various pathogenic bacteria such as *Pseudomonas aeruginosa*, *Escherichia coli*, *Shewanella* sp., *Salmonella* sp., *Klebsiella pneumoniae*, *Escherichia coli*, and *Staphylococcus aureus*(Ayesha *et al*., 2010). Nanotechnology is an emerging field and the green synthesis of nanoparticles has attracted much more attention in recent years because of its excellent antibacterial, antifungal and anticancer properties

(Elango *et al*., 2016). These nanoparticles show superior, unique and excellent properties and have attracted much more attention for their unique properties that are not observed in macroscopic forms. They achieved these characteristics because of their increased surface –to–volume ratio (Sahayaraj*et al*., 2012; Surendra *et al*., 2016).

Recently, nanostructured or nanophasic substances exhibited maximum potential to achieve selectivity and specific processes, mainly in pharmaceutical and biological applications, showing various health benefits. Silver has been widely used to treat various infections. It is generally non-toxic and exhibits bacteriostatic activity against several bacterial floras. Silver nanoparticles have a wide range of biological applications such as antifungal, antibacterial against several drug-resistant bacteria, wound healing, preventing bacterial and fungal infections, and anti-inflammatory properties. Silver ions are also used in the formation of bone cement, dental resin composite materials, ion coating and ion exchange fibers for medical devices because of its antibacterial activities. These silver nanoparticles show strong activity against Gram-positive and Gram-negative bacteria, fungi, viruses and other pathogenic organisms (Bhuyar*et al*., 2020). Silver nanoparticles can be green synthesized using algae extract by an environmentally friendly approach as they do not require highly toxic chemicals. Marine algae are available throughout the season and naturally have biological and reducing power. Hence, researchers are interested in marine macro algae-mediated green synthesis of silver nanoparticles (Vijayaraghavan *et al*., 2022). The green synthesized nanoparticles were characterized using UV-Visspectroscopy, Dcanning electron microscopy (SEM), Dynamic light scattering (DLS) and Absorbance spectroscopy (Arasu *et al*., 2019). The study's main objective was to synthesize silver nanoparticles using marine macro alga (*Portieria hornemannii*) extract, characterize the nanoparticles using analytical methods, and study the antibacterial and anticancer properties of the nanoparticles.

MATERIALS AND METHODS

Study area

The marine macro alga, *P. hornemannii* was collected from the South Coast of India. It was collected in the rocky coastal region of the Kanniyakumari coast (8° 5' 17.9016'' N and 77° 32' 18.4272'' E). The location of the sampling station is depicted in Fig. 1a. The collected marine macro alga was brown and is illustrated in Fig. 1b.

Extraction of phytochemicals from the marine alga

The marine alga was collected from the Southern tip of India (Kanniyakumari) and was washed with tap water to remove all impurities and epiphytes. It was dried, and the dried alga was used to extract polysaccharidesas described previously (El-Rafie et al., 2013). 50 g of dried algal powder was soaked in 500 mL ethyl acetate and stirred for 72 h. It was stirred frequently, and the mixture was filtered using a Buchner funnel. It was evaporated using a rotary evaporator and the sample was concentrated by evaporating solvent at 80 rpm under vacuum conditions (Helan *et al*., 2016).

Gas-Chromatography-Mass Spectrophotometry (GC-MS) analysis of algal extract

The ethyl acetate extract was used for GC-MS analysis. GC-MS chromatograph was combined with the mass selective detector, which consists of an HP-5 MS low bleed capillary column and helium gas was used as a carrier. The flow rate was maintained at1.25 mL/min. The temperature was maintained at 200 °C and programmed to 250°C in the injector line. About 10 *µ*l sample was loaded and the separated phytochemicals were detected. The mass spectrum was matched with the National Institute of Standards and Technology (NIST) library (*Yaazh Xenomics, Coimbatore, India*) and the compounds were identified.

Extraction of alga polysaccharide

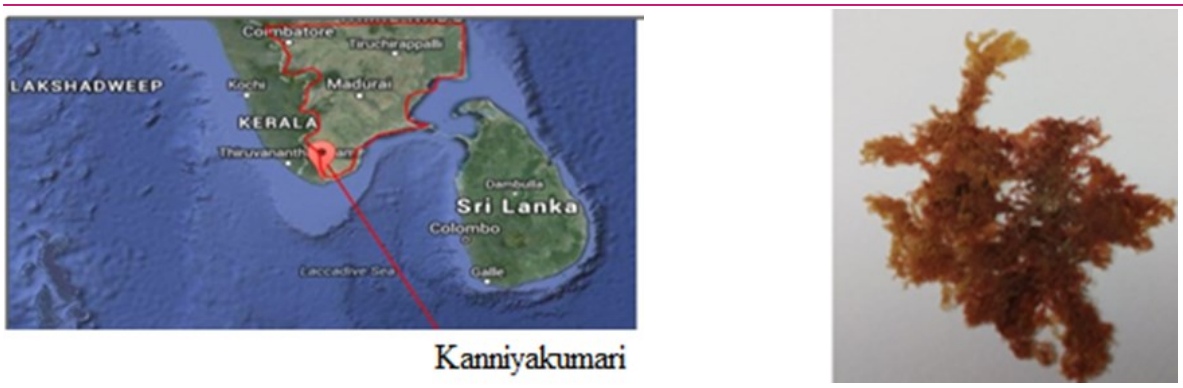
The collected alga was sun-dried for 2 – 3 days, and the dried seaweed was used to extract polysaccharides. Briefly, 10 g of dried seaweed was stirred with 500 mL double distilled water for 4 h. It was further filtered using a funnel and the pH of the solution was adjusted to 7.0 using 0.1 N NaOH. To this solution (50 mL) 150 mL ethanol was added. It was stirred continuously for 30 min , filtered and used to reduce silver nanoparticles (Álvarez-Viñas *et al*., 2022).

Green synthesis of silver nanoparticles

Silver nanoparticles were prepared using algal polysaccharides, as described previously. Briefly, 25 mL of the algal extract (polysaccharide) was mixed with 25 mL of aqueous silver nitrate (1 mM) solution with continuous stirring. The temperature of the solution was increased to 90 ºC. It was centrifuged at 5000 rpm for 10 min. and the clear supernatant was removed. The developed silver nanoparticles were collected and characterized (Bindhu *et al*., 2020).

Characterization of silver nanoparticles

The development of silver nanoparticles was analyzed using a UV-Visible spectrophotometer. Fourier-transform infrared spectroscopy (FT-IR) analysis was performed and the functional group was determined. The nanoparticles were dried and prepared in KBr pellets, and the functional group was analyzed using a Shimadzu FT-IR model 8300 machine (400 – 4000 cm). The morphology of the green synthesized nano-



a b

Fig. 1. *Map showing the sampling location of Kanniyakumaricoast (a) and characterized P. hornemannii*

particles was determined using a Jeol JEM-1010 electron microscope equipped with photomicrographedintegrated with Energy Dispersive X-ray (EDX) analyser (Femi-Adepoju *et al*., 2019; Arokiyaraj*et al*., 2014).The crystalline nature of silver nanoparticles was tested by a Selected Area Electron Diffraction (SAED) pattern as described previously (Wan Mat Khalir *et al*., 2020).

Antimicrobial assay

The antimicrobial activity of silver nanoparticles was analyzed against bacteria (*Staphylococcus aureus* (*MTCC* 10787), *Streptococcus mutans*(*MTCC*890), *Bacillus subtilis* (MTCC736), *Klebsiella pneumoniae* (MTCC109), *Pseudomonas aeruginosa*(MTCC1688) and *Proteus mirabilis* (MTCC425)) and fungi (*Aspergillus flavus*(MTCC13062) and*Candida albicans* (MTCC3017)). All bacterial and fungal strains were obtained from Microbial Type Culture Collection and Gene Bank, India. All bacterial strains were cultivated in a nutrient broth medium for 24 h, whereas fungi were cultured for 74 h at 37 ºC. 10 µg silver nanoparticles were dissolved in dimethyl sulfoxide and antimicrobial activity was evaluated using the disc diffusion method (Zhang *et al*., 2020). Mueller-Hinton agar plates were prepared and 0.1 mL bacterial or fungal suspension was spread using a cotton swab. The sample or standard was loaded and incubated at 37 ºC and the zone of inhibition (mm) was determined. The antimicrobial activity was assessed based on the zone of inhibition around the disc on the agar surface (Atif *et al*., 2019).

Anticancer activity

Anticancer activity of the silver nanoparticles was tested using colorectal adenocarcinoma cell lines (DLD1) and procured from National Centre for Cell Sciences (NCCS), Pune, India. Briefly, the cancer cell lines were maintained in RPM1 culture medium and incubated for 24 h. 100 µg sample was suspended in dissolved in DMSO and added separately at five different concentrations (6.25 µL, 12.5 µL, 25 µL, 50 µL and 100 µL). DMSO was used as the negative control. The cells were incubated for 2 days at 30 ± 1 ℃ and the effect of nanoparticles on cancer cell lines was assessed (Malar *et al*., 2020).

RESULTS AND DISCUSSION

GC-MS analysis of ethyl acetate fraction of *P. hornemannii*

GC-MS analysis revealed that the marine algae exhibited the presence of significant bioactive compounds. The prominent components were Cyclohexanepropanoic acid (RT – 6.667 min), hexadecanoic acid (RT - 14.01 min), 2,3-Dihydro-benzofuran (RT – 4.858 min). The other minor compounds such as beta-Sitosterol (RT - 35.783 min)and Campesterol (RT – 33.75 min). The GC-MS spectrum of the ethyl acetate fraction of *P. hornemannii* is depicted in Fig. 2.

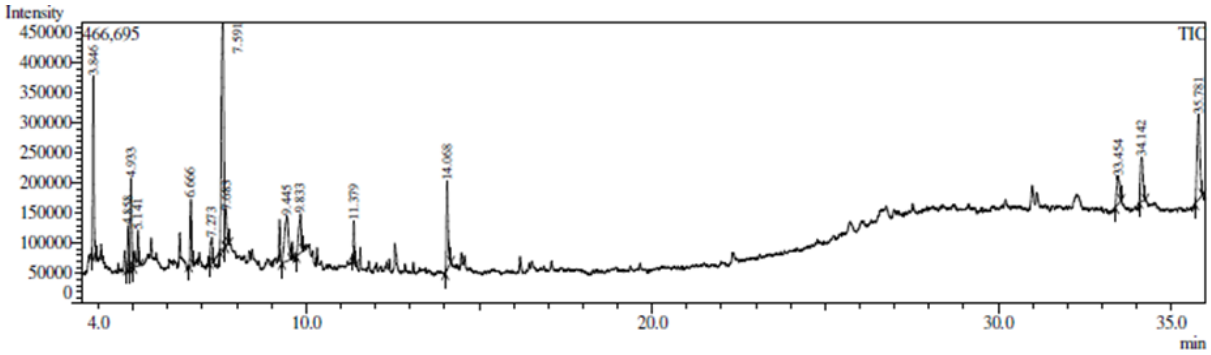


Fig. 2.*GC-MS spectrum of ethyl acetate fraction of marine alga collected at Kanniyakumari coast*

Characterization of silver nanoparticles

The algal extract was mixed with 1 mM silver nitrate, and the silver ions' development was monitored using a UV-visible spectrophotometer. In the present study, an algal polysaccharide was used to prepare silver nanoparticles and the capping property of an algal polysaccharide as reported previously by Surendra *et al*. (2016). It was revealed that such amino bond capping agents are involved in reducing silver ions to silver nanoparticles. A characterized peak observed between 350 nm and 450 nm revealed the formation of silver nanoparticles (Fig. 3a). The colour of the silver nitrate solution changed to dark brown, indicating nanoparticle synthesis. Green synthesis of silver nanoparticles exhibited higher yield at higher temperatures (>60 ºC) than at room temperature (30 ºC). The improved nanoparticle synthesis at higher temperatures may be due to the excitation of surface plasmon vibrations at increased temperatures (Valsalam*et al*., 2019). Rapid accumulation of the reactant substances further induced the development of small nanoparticles (20 – 40 nm). The temperature was increased to 90 ºC to improve the yield and obtain small-sized nanoparticles. The development of silver nanoparticles was confirmed using UV-visible spectrophotometry analysis. It has been reported that metal nanoparticles, such as iron silver nanoparticles, develop the SPR absorption band. In the present study, silver nanoparticles showed a maximum absorbance peak between 350 nm and 450 nm, revealing the formation of silver nanoparticles. The characterized peak within this range has been reported previously in silver nanoparticles derived from the cyanobacterium *Oscillatoria limnetica*(Hamouda*et al*., 2019).

The Infrared spectrum was analyzed and the functional groups were identified. The broadband was observed at 3304 cm^{-1} (hydroxyl and amino group) and the narrow band was observed at 2907 cm^{-1} (C–H- stretching vibration), 1657 cm^{-1} (stretching of carbonyl groups), and 1001 cm^{-1} (C–O stretching vibration) (Fig. 3b). In this study, a broad peak was observed at 370-380 indicating the silver nanoparticles in the medium. The functional groups observed in FT-IR analysis were similar tothose in theearlier study (Mohanta *et al.,* 2017). Vibration bands in the IR spectrum were similar to silver nanoparticles prepared using aqueous rhizome extract of *Zingiber officinale* and *Curcuma longa* (Venkatadri *et al*., 2020). The particle size of the silver nanoparticles was analyzed using TEM. The particle size was approximately 40 – 50 nm (Fig. 3c). The crystalline nature of silver nanoparticles was determined by the SAED analysis (Fig. 3d). EDX spectra was analyzed from the nanoparticles synthesized using algal extractconfirming the crystalline nature of silver nanoparticles (Fig. 3e) and exhibited various peaks at 39.02, 43.08, and 66.04 at 2θ. In present study, the highly intense EDX peaks showed crystalline nature. The EDX pattern observed in this study was similar to nanoparticles prepared using Actinobacteria (Vimala *et al*., 2022).

Antimicrobial properties of silver nanoparticles

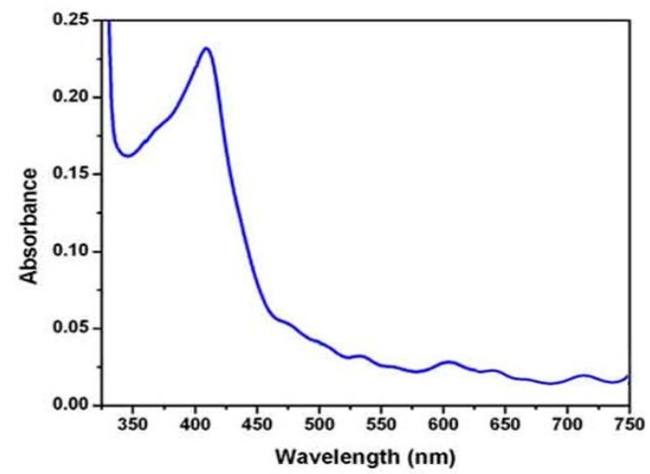
The green synthesized nanoparticles were used to test antimicrobial activity against Gram-negative and Gram-positive pathogenic bacteria (*S. aureus*, *S. mutans*, *B. subtilis*, *K. pneumoniae*, *P. aeruginosa* and *P. mirabilis*). Nanoparticles were highly effective against *P. mirabilis* (24 mm zone of inhibition) and *B. substilis* (24 mm zone of inhibition). Moreover,particles were also highly effective against other pathogens, viz. *P. aeruginosa*, *K. pneumoniae*, *S. aureus* and *S. mutans* (Fig. 4). The disc diffusion test revealed that the green synthesized NPs were highly active against *C. albicans* and *A. flavus* (Fig. 5). Nanoparticles directly or indirectly act on pathogenic Gram-positive and Gram-negative bacteria (Al-Dhabi *et al*., 2018). Bacteria exposure to silver nanoparticles leads to particles binding on the cell surface. Further, silver nanoparticles interact with proteins and weakencell wall structure. Nanoparticles disrupt bacterial cell walls, damaging microbial cell factories (George *et al*., 2020). In this study, the green synthesized nanoparticles showed significant antibacterial and antifungal activity against Gram-positive and Gram-negative bacteria.

Anticancer activity

The anticancer activity of the silver nanoparticles was tested against colorectal adenocarcinoma cell lines. The cytotoxicity activity was observed after 48 h of treatment with 6.25 µL - 100 µL silver nanoparticles.Cytotoxic activity was observed at 6.25 µL nanoparticle concentrations, 12.3% inhibition, and 64.3% inhibition at 100 µL concentrations. This revealed that anticancer activity or cytotoxic effect against adenocarcinoma cell lines increased at increasing concentrations of nanoparticles (Fig. 6).The anticancer activity of silver nanoparticles was reported previously. In a study, Alduraihem*et al*. (2023) reported the anticancer activity of silver nanoparticles prepared using *Acacia nilotica* pod extract. Rajawat *et al*. (2016) reported the anticancer properties of green silver nanoparticles prepared using black tea leaf extract and showed activity against MCF-7 breast cancer cell lines.

Conclusion

Algal polysaccharide was used for the green synthesis of silver nanoparticles. The developed nanoparticles exhibited antibacterial and antifungal activities. The formed silver nanoparticles showed potent activity against various Gram-positive and Gram-negative bacteria; and pathogenic fungi. Anticancer activity was tested against cancer cell lines and exhibited anticancer



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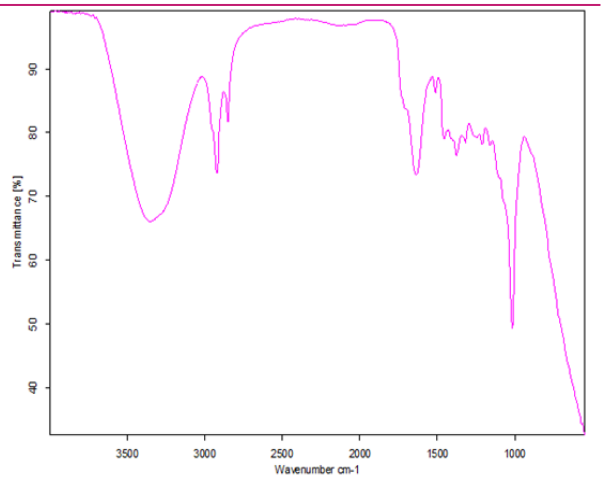

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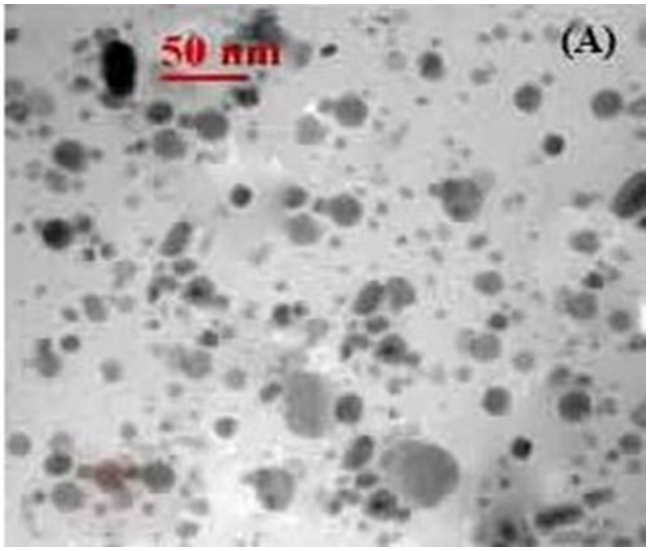

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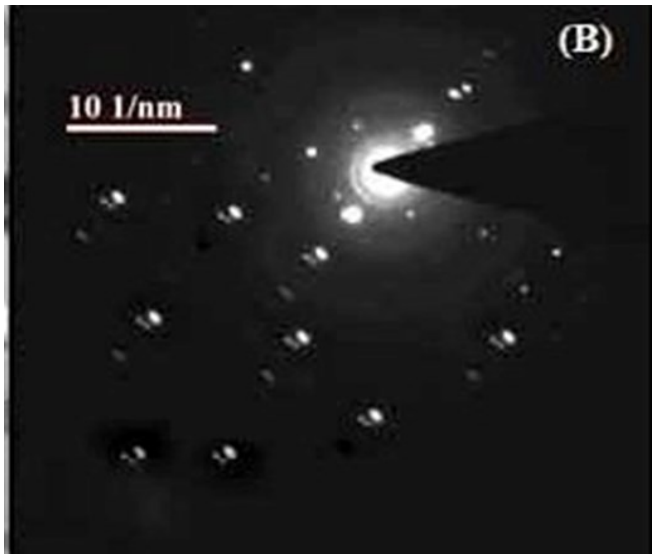

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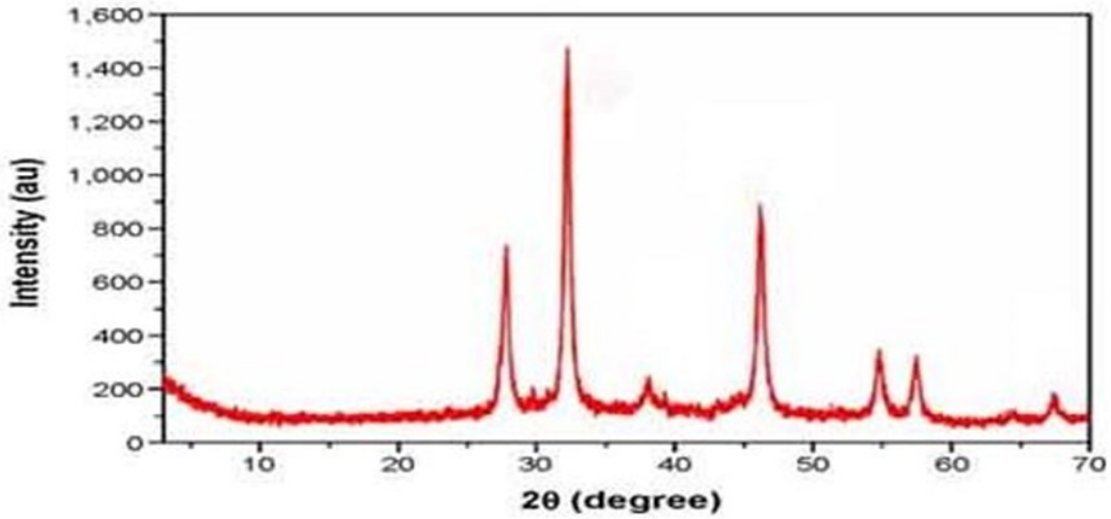


e

Fig. 3.*Characterization of green synthesized silver nanoparticles using marine algal extract. (a) UV-Visible spectrum of algal polysaccharide-reduced silver nanoparticles, (b) FT-IR spectrum of the nanoparticles, (c) TEM analysis of nanoparticles, (d) SAED analysis, and (e) EDX analysis of silver nanoparticles.*

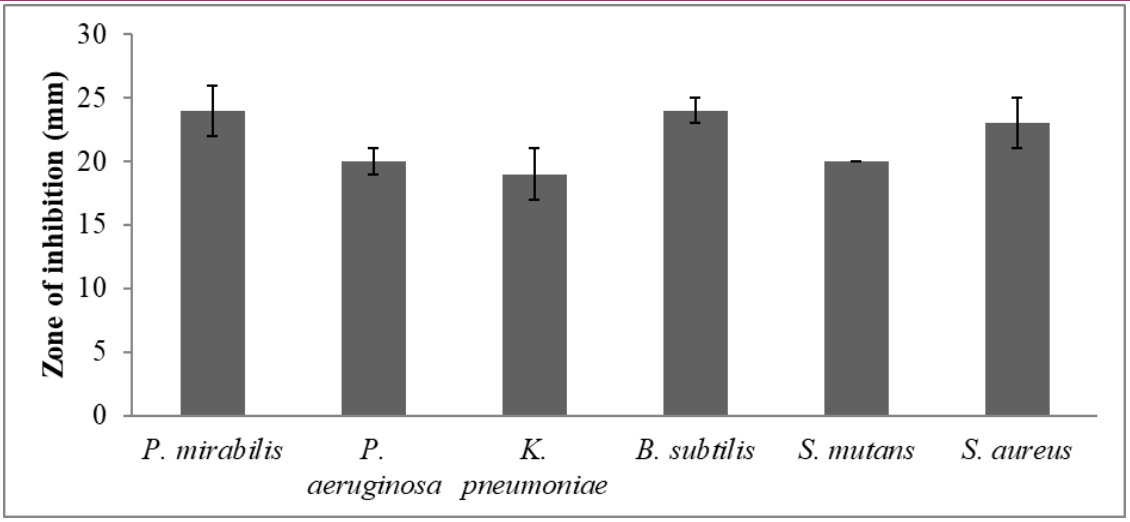
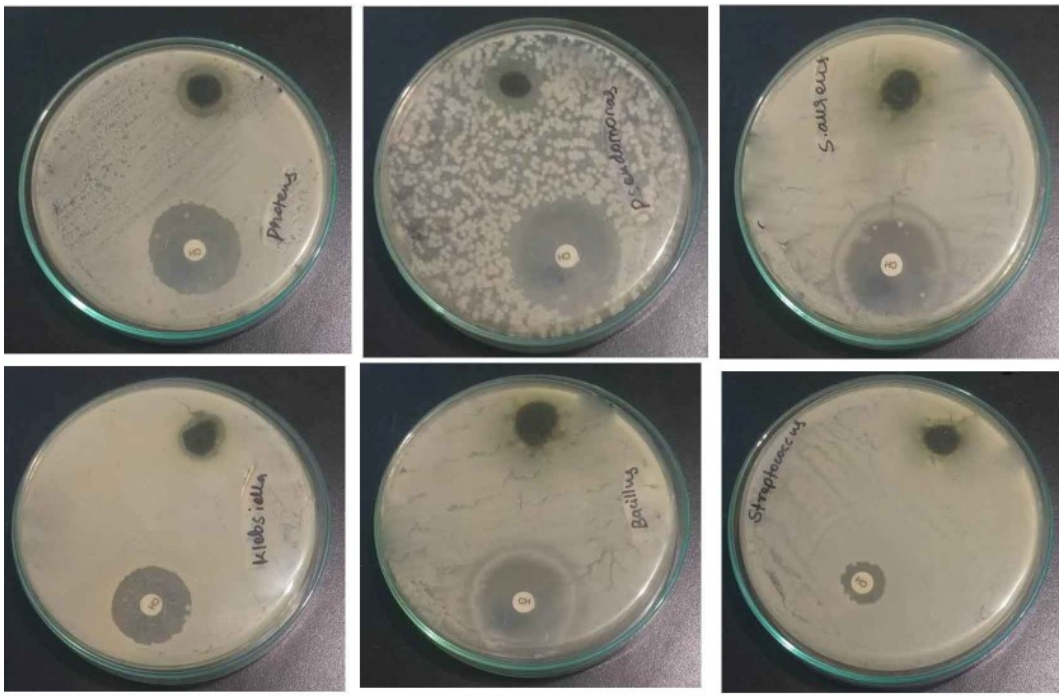


Fig. 4. *Antibacterial activity of silver nanoparticles against Gram-positive and Gram-negative bacteria. Error bar represents standard deviation.*



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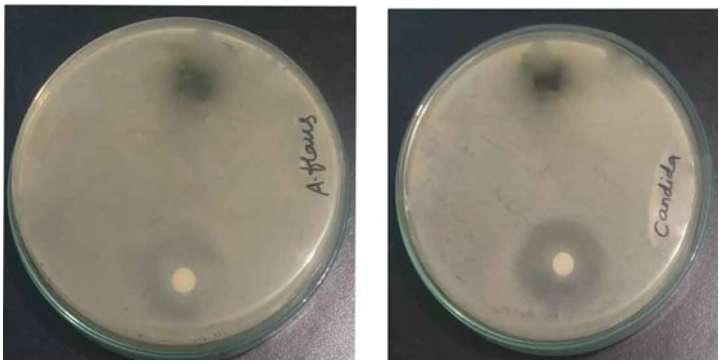

b

Fig. 5. *Antimicrobial activity of silver nanoparticles against bacteria (a) and fungi (b). Green synthesized nanoparticles were loaded on the disc and the zone of inhibition was assayed after 24 h (for bacteria) and after 72 h (for fungi); Zone of inhibition expressed as mm.*

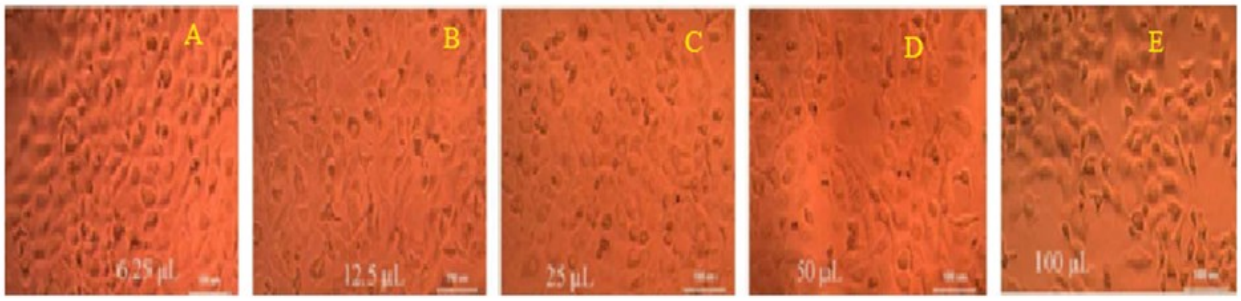

Fig. 6. *Anticancer activity of silver nanoparticles against adenocarcinoma cell lines; Green synthesized silvernanoparticles treated with cell lines at various concentrations (A-6.25 µL sample, 12.5 µL sample, 25 µL sample, 50 µL sample, and 100 µL sample) and incubated for 48 h. Nanoparticles induced morphological changes among cell lines.*

activity in a dose-dependent manner. Hence, the present findings seem to be an alternative approach since using silver nanoparticles is largely considered an alternative to various sectors, including anticancer therapy.

Conflict of interest

The authors declare that they have no conflict of interest.

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