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# The Biological Potential of Silver Nanoparticles Made from the *Sargassum tenerrimum* seaweed extract.

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**ABSTRACT**

Silver nanoparticles (AgNPs) have been extensively studied for their potential biological applications due to their unique physical and chemical properties. The Sargassum tenerrimum seaweed extract has been found to be a potential source for the synthesis of AgNPs. In this study, we have investigated the biological potential of AgNPs synthesized from Sargassum tenerrimum extract. Through dispersive X-ray analysis, Fourier Transformer Infrared Spectroscopy analysis, among other methods of research, these AgNPs have been shown to have strong antimicrobial activity against a variety of microorganisms, including bacteria, fungi, and viruses. Additionally, they have been shown to have antioxidant, anti-inflammatory, and wound-healing properties.

Research and observations thus showed the antibacterial activity of green synthesized AgNPs using Solanum torvum leaf extract. Observations have also indicated the presence of anti-cancer properties of silver nanoparticles.

## INTRODUCTION

Engineered nanomaterials have become increasingly important in recent years due to their unique properties and potential applications. Silver nanoparticles (AgNPs) are one of the most widely studied engineered nanomaterials due to their unique physical and chemical properties. They have been investigated for a wide range of commercial and biomedical uses, such as catalysts and optical receptors in cosmetics, electronics, and textile engineering, as well as a bactericidal agent in wound dressings, surgical instruments, and disinfectants.

As a result, Silver nanoparticles (AgNPs) have become a popular subject of research due to their unique chemical and physical properties, which have led to a wide range of applications, particularly in the field of medicine. However, the synthetic methods used to produce AgNPs often involve the use of hazardous chemicals, which can be both costly and environmentally damaging. These synthetic methods have been found to produce AgNPs that are highly toxic to living organisms and the environment. The toxicity of these AgNPs is due to their small size, which allows them to easily penetrate cells and disrupt normal cell function. Synthetic AgNPs can release silver ions, which can cause damage to DNA and other cellular structures. Furthermore, synthetic AgNPs have been found to accumulate in various organs and tissues, leading to toxicity over time. Due to these negative effects, many researchers have begun to explore natural alternatives for producing AgNPs, such as using plant extracts. This is to avoid the harmful effects of synthetic AgNPs on the environment and living organisms.

In recent years, researchers have begun to explore the use of natural sources, such as plant extracts, to synthesize AgNPs as a safer alternative. These natural silver nanoparticles are not only less toxic but also biocompatible, which makes them a more suitable option for biomedical applications. Moreover, naturally derived AgNPs are environmentally friendly, readily available, and relatively inexpensive compared to synthetic AgNPs. Additionally, natural AgNPs have been found to have similar or even better antimicrobial properties compared to synthetic AgNPs, making them a favorable alternative for use in medical applications. Furthermore, natural AgNPs have been found to be biocompatible with minimal toxicity to human cells, making them safe for use in biomedical and therapeutic applications. Overall, the use of natural sources to synthesize AgNPs offers a greener and safer alternative to synthetic methods.

**Materials and Methods**

**Rhizome extract preparation**

The process of preparing an extract from the rhizomes of sargassum tenerrimum seaweed extract involves several steps. First, the rhizomes are cleaned by placing them under running tap water for 30 minutes to remove any dirt or stones. They are then rinsed thoroughly with distilled water and left to dry completely in a shaded area for one week at room temperature. After the rhizomes have dried, they are then ground into a powder using a kitchen blender.

**Biological synthesis of silver nanoparticles**

Before beginning the synthesis process, 1 mM of AgNO3 was added to 50 mL of double distilled water in a beaker which was then placed in a boiling condition with a magnetic paddle mixer. After the water began to boil, 1 mL of a 5% (w/v) rhizome extract, prepared by boiling 5 g of rhizome powder in 100 mL of double distilled water for 30 minutes, was added to the AgNO3 solution. The mixture was kept boiling for approximately 20 minutes. The reduced silver colloidal solution was then removed from heat and placed in an ice bath to stop the reduction process. The silver colloidal solution was collected at different time points and analyzed using a UV-visible spectrophotometer with a double beam.

The methods that were used for the characterization of synthesized biogenic AgNPs are UV–vis spectroscopy, Fourier transform infrared spectroscopy (FTIR), transmission electron microscopy (TEM), and scanning electron microscopy (SEM) equipped with energy dispersive X-Ray, X-ray diffraction. The following characterization took place:

**UV-visible spectrophotometer**

A UV-visible spectrophotometer is a type of analytical instrument that is used to measure the absorption or transmission of light in the ultraviolet (UV) and visible regions of the electromagnetic spectrum. The deduction of silver ions into biogenic AgNPs was observed using UV–visible spectrophotometer.

**Fourier transform-infrared spectroscopy (FTIR)**

Fourier Transform Infrared Spectroscopy (FTIR) is a technique that uses infrared radiation to study the vibrations of chemical bonds in a sample. The sample is placed in an infrared spectrophotometer and exposed to a beam of infrared radiation. The radiation is absorbed by the sample, causing the bonds to vibrate at different frequencies. The absorption of radiation at these frequencies results in a unique spectrum, or "fingerprint," of the sample. The spectrum is then analyzed to identify the chemical compounds present in the sample and to study their structural properties.

**Transmission Electron Microscopy (TEM)**

TEM was used to determine the size and shape of synthesized AgNPs through structural analysis. Before looking at them under a microscope, the AgNPs were sonicated and a small amount was placed on a carbon-coated copper grid. The excess liquid was then evaporated. The study confirms the crystalline nature of the synthesized AgNPs.

**Scanning electron microscopy (SEM)**

In order to create SEM micrographs of the biosynthesized AgNPs, the particles were centrifuged and then redispersed in sterile distilled water. This process was repeated three times. A small amount of the aqueous solution of silver nanoparticles was then dropped onto a glass slide to create a thin film, which was dried under a hot air oven. The dried thin film was then examined under an SEM machine with EDS in order to determine the size and shape of the synthesized AgNPs at different magnifications.

**Research and Discussion.**

This study demonstrated the biological synthesis of AgNPs using the sargassum tenerrimum seaweed

extract. The formation of AgNPs through the reduction of AgNO3 ions was monitored by observing the change in color of the reaction mixture. The biosynthesis process began within minutes of adding the seaweed extract to a colorless silver nitrate solution. The change from colorless to dark brown indicated the reduction of silver ions, whereas no color change was observed in either the silver nitrate solution or aqueous rhizome extract alone. The intensity of the brown color increased over time and reached a maximum of 20-30 minutes. This is likely due to surface plasmon resonance (SPR) and the reduction of AgNO3. It has been previously reported that the size of the particles synthesized and their distribution percentage is inversely proportional to the temperature changes. This study revealed that the change from colorless to dark brown exhibited the synthesis of AgNPs from the sargassum tenerrimum seaweed extract.

A UV-visible spectrophotometer is an essential tool for confirming the formation of AgNPs in an aqueous medium. The UV-visible spectra of the synthesized AgNPs showed a strong and broad surface plasmon resonance, which confirms the formation of AgNPs. The stability of the AgNPs was also tested by storing the solution for 48 days at room temperature and observing for any precipitation or changes in the UV-visible absorption properties. No precipitation or changes in absorbance intensity or absorption maxima were observed, indicating that the particle size remained the same as originally recorded.

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The UV-visible spectrophotometer is a commonly used method to confirm the formation of silver nanoparticles (AgNPs) in aqueous solutions. The spectra of the synthesized AgNPs displayed a strong, broad surface plasmon resonance peak at 460 nm, indicating their formation. The aqueous extract of sargassum tenerrimum seaweed extract did not show this peak. The stability of the AgNPs was also tested by storing the solution for 48 days at room temperature, and observing no precipitation or changes in absorbance intensity or maxima, which confirms their stability. (Fig. 1)

**UV-vis spectroscopy analysis**

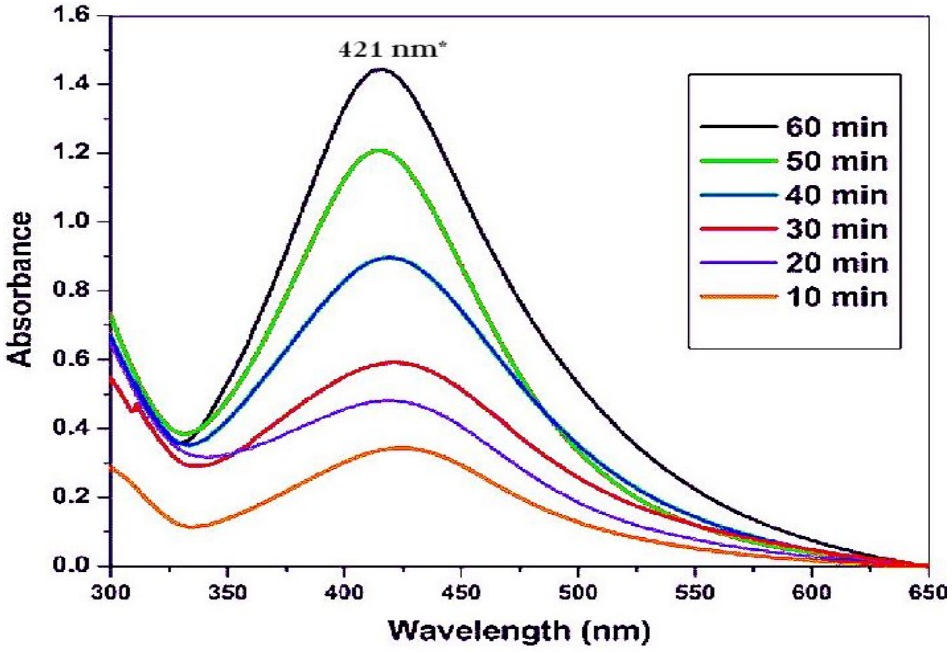


**Figure 1. UV-visible absorption spectra of biosynthesized Ag NPs *Sargassum tenerrimum* seaweed extract**

**Fourier Transformer Infrared Spectroscopy analysis**

Fourier transform infrared (FTIR) spectroscopy was introduced in 1991 as a technique to identify and classify microbes. Since then, it has gained growing interest and has undergone a remarkable evolution. FT-IR spectral analysis was performed to identify biomolecules in the aqueous extract of *Sargassum tenerrimum* seaweed extract that may have played a role in the formation of AgNPs. The analysis revealed intense peaks at 1072, 1413, 1546, 1652, and 2351cm$^{-1}$, indicating a strong interaction between the nanoparticles and the biomolecules in the FTIR spectrum of *Sargassum tenerrimum* seaweed extract. These peaks were shifted to 583, 1051, 1384, 1537, 1650, and 2351 cm$^{-1}$ after silver nanoparticle synthesis. (Fig. 2a).

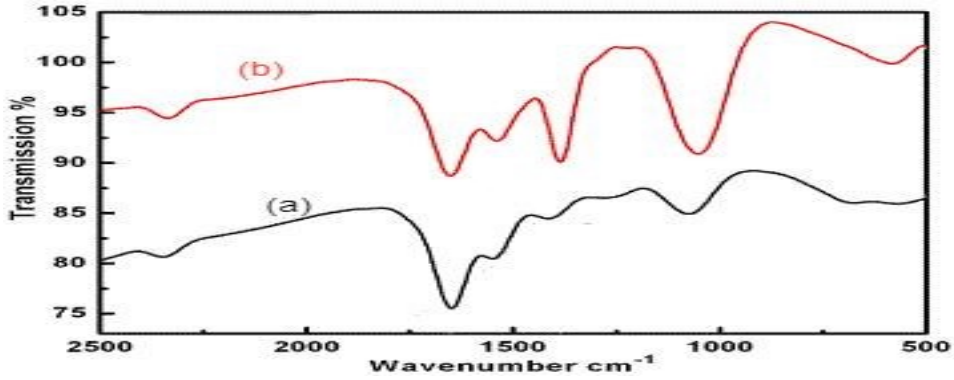


**Figure 2. a. FTIR-spectra of *Sargassum tenerrimum* seaweed extract and Figure 2. b. FTIR-spectra of Ag NP using *Sargassum tenerrimum* seaweed extract**

**TEM analysis**

The peak at 1072 cm-1 was identified as the C-C stretch skeletal vibration, the peak at 1453 cm-1 was identified as the methyl C-H asymmetric/symmetric bend, and the peak at 1646 cm-1 was identified as representing carbonyl groups (C=O). The peaks at 2832, 2917, and 2949 cm-1 represented tetrahedral carbon-hydrogen bonds. Previous research in our laboratory has shown that phytochemicals such as proteins, amino acids, lipids, and reducing sugars present in the rhizome extract of sargassum tenerrimum seaweed extract act as reducing and capping agents in the biogenic synthesis of AuNPs. The FT-IR results in the current study also showed the presence of amino acids, carbohydrates, and reducing sugars in the rhizome extract of sargassum tenerrimum seaweed extract, which may be acting as reducing agents for AgNP synthesis. This is in line with several other studies that have also reported the significant involvement of phytochemicals as reducing agents in AgNP synthesis.

The synthesized AgNPs were found to be spherical with an average size of 76 nm when analyzed using aqueous aliquots. TEM micrographs at different magnifications in Fig. 3 a and b showed that the AgNPs were biogenically synthesized. This is consistent with previous literature, where biosynthesized AgNPs were also found to be spherical with an average size ranging from 20 to 100 nm. The size distribution of the synthesized AgNPs is also shown.

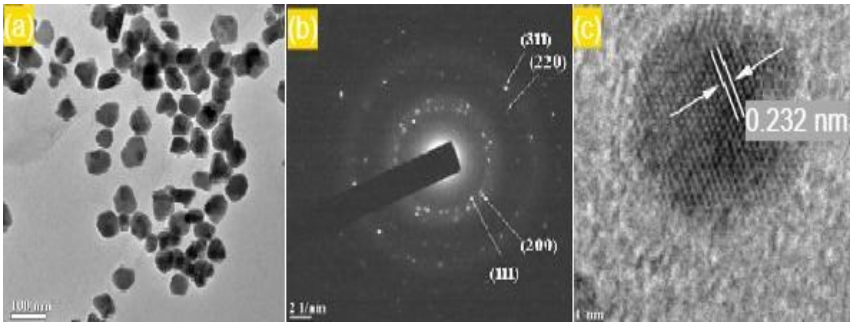


**Fig 3 (a) HR-TEM images of the synthesized AgNPs; (b) Selected area electron diffraction pattern of the face-centered cubic crystalline silver; (c) lattice fringes of the**

**synthesized AgNPs.**

**Energy-Dispersive X-Ray analysis**

The silver concentration of the nanoparticles was determined using a Scanning Electron Microscope (SEM) with an Energy Dispersive X-ray Spectrometer (EDX). AgNPs typically exhibit a characteristic absorption peak at around 3 keV due to the surface plasma resonance phenomenon (Prasad et al., 2012). As seen in Figure 2, the distinct peak detected at 3 keV confirms the presence of elemental silver in the nanoparticles. The EDX analysis revealed a silver concentration of 63.4± 1.6% in fresh algae and 55.4±0.9% in dried algae after incubation for 48 hours. This method of silver nanoparticles (AgNPs) synthesis does not involve the use of any toxic chemicals, making it a promising approach for use in various fields, especially in biomedical applications.

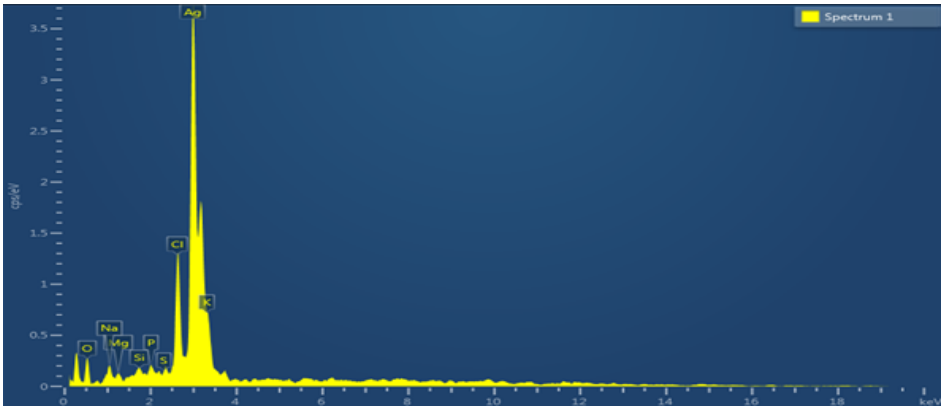


**Figure 4. EDX image of Ag NPs using *Sargassum tenerrimum* seaweed extract**

**DLS analysis**

The size distribution of the particles was determined through Dynamic Light Scattering analysis, with the highest intensity at 45 nanometers. Additionally, the zeta potential measurements revealed that the silver nanoparticles are highly stable, with a value of -27 millivolts-.

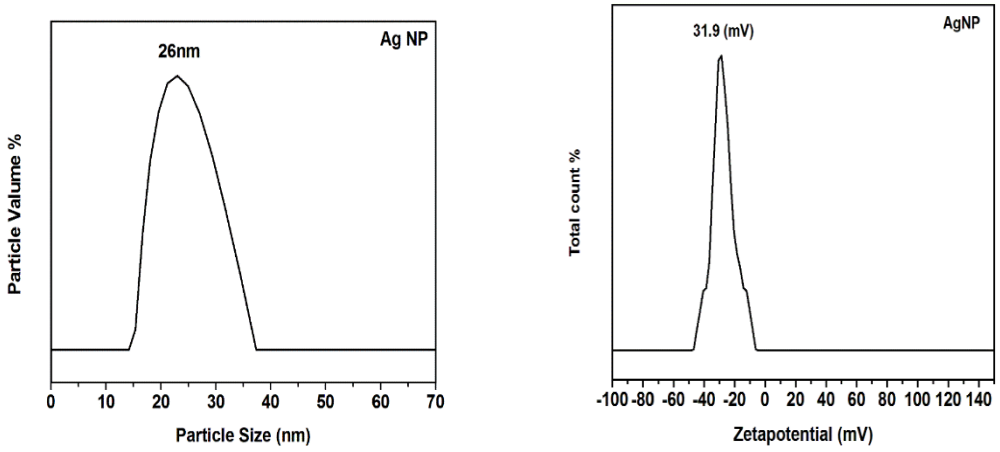


**Figure 5. (a) DLS analysis and (b) Zeta potential of AgNPs biosynthesized from a mixture of *Sargassum tenerrimum* seaweed extract.**

**X-ray diffraction analysis**

The X-ray diffraction pattern of silver nanoparticles produced after 120 hours of incubation, as shown in Figure 2, exhibits three prominent peaks that align with the (1 1 1), (2 0 0), and (2 2 0) planes of a standard cubic phase of silver, as identified by JCPDS card No 04-0783. These peaks indicate that the nanoparticles are of high purity and well-crystalline, as there are no reflections

from impurities such as nitrate ions present in the pattern. (Fig. 5)

Furthermore, the measurement of the lattice constant of the synthesized cubic phase of silver nanoparticles yielded a value of 4.083 Angstroms, which is consistent with the value from JCPDS (4.086 Angstroms). The size of the nanocrystals was calculated using the Debye-Scherrer formula, which takes into account the wavelength of the X-ray (1.5406 Angstroms), the Bragg angle, and the full width at half maximum (FWHM). Analysis of the X-ray line broadening revealed an estimated crystallite size of 17 nanometers, with a margin of error of 10%.

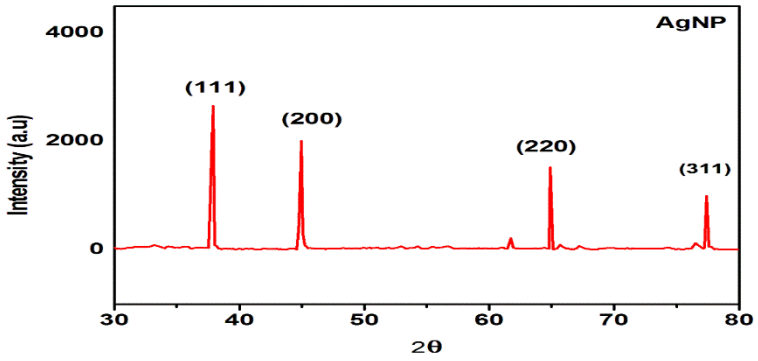


**Figure 5. XRD Pattern of Ag NPs using *Sargassum tenerrimum* seaweed extract**

**Table 1: XRD parameters of AgNPs using *Sargassum tenerrimum* seaweed extract**

| Peak position 2θ (˚) | FWHM B $_{size}$ (˚) | Crystallite size (nm) | (hkl) | Crystallite size Average (nm) |
|---|---|---|---|---|
| 37.89 | 0.752 | 11.67 | (111) | 13.92 |
| 44.86 | 0.857 | 10.47 | (222) | |
| 64.86 | 0.658 | 14.94 | (220) | |
| 77.33 | 0.571 | 18.61 | (311) | |

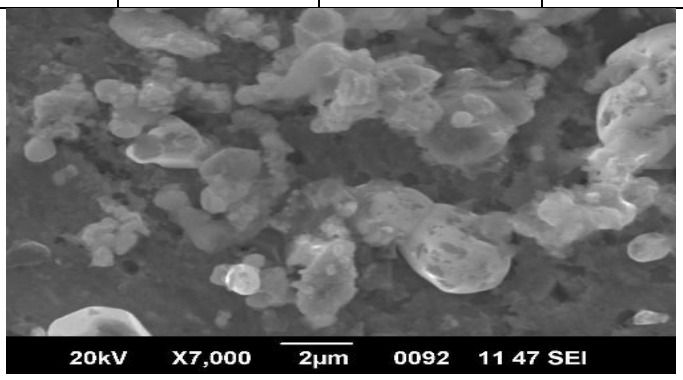


**Fig 6. SEM micrograph of green synthesized AgNPs using *Sargassum tenerrimum* extract**

To confirm the shape and size of the AgNPs, they were examined using SEM visualization. The SEM micrographs showed that the particles were spherical shaped and well distributed in solution. Analysis of energy dispersive spectroscopy (EDS) confirmed the presence of elemental silver, indicating that AgNPs were formed. The presence of copper may be due to the copper grid on which the AgNPs were deposited. (Fig. 6)

**Antibacterial activities of silver nanoparticles**

Silver nanoparticles were tested for antibacterial activity against S. aureus, E. coli, Candida albicans, P. aeruginosa, E. faecalis, B. subtilis, K. pneumoniae, and methicillin-resistant S. aureus. E. coli, P. aeruginosa, E. coli, P. aeruginosa, and K. pneumoniae are bacteria commonly found in the gut and can cause infections in humans. E. faecalis and B. subtilis are also bacteria. S. aureus and MRSA are a type of bacteria known as Staphylococcus aureus that can cause a range of infections in humans. MRSA is a strain of S. aureus that is resistant to certain antibiotics. C. Albicans is a type of fungus that can cause infections in humans, particularly in individuals with weakened immune systems. The results can be referred to in Table 2.

The effectiveness of the bio-synthesized silver nanoparticles as an antimicrobial agent against different human pathogens is demonstrated below. The results show that the silver nanoparticles inhibit the growth of almost all of the tested organisms. The highest zone of inhibition was observed against the gram-positive bacteria Staphylococcus aureus which is greater than the positive control, ampicillin, and the lowest zone of inhibition was against Streptococcus pneumoniae. Additionally, the gram-negative bacterium Salmonella typhi also showed a higher zone of inhibition compared to the positive control. One potential explanation for the antibacterial activity of silver nanoparticles is that they may attach to the cell membrane, disrupting its permeability and respiratory functions.

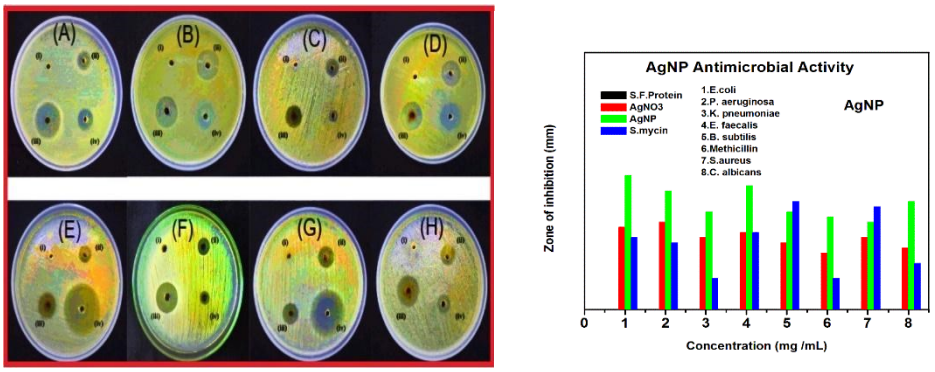


**Figure 7. Antibacterial activity of green synthesized AgNPs using *Solanum torvum* leaf extract against *E. coli*, *P. aeruginosa*, *K. pneumoniae*, *E. faecalis*, *B. subtilis*, *Methicillin-resistant S. aureus*, *S. aureus*, and *C. Albicans***

**Table 2: Zone of inhibition values of green synthesized AgNPs using *Sargassum tenerrimum* seaweed extract**

| Organism | *Sargassum tenerrimum* seaweed extract (25 µL) | Silver Nitrate (1 mM) | Silver nanoparticles (1 mg/mL) | Streptomycin (1 mg/mL) |
|---|---|---|---|---|
| *E. coli* | - | 17 | 32 | 16 |
| *P. aeruginosa* | - | 18 | 25 | 14 |
| *K. pneumoniae* | - | 16 | 22 | 7 |
| *E. faecalis* | - | 19 | 26 | 15 |
| *B. subtilis* | - | 15 | 23 | 17 |
| *Methicillin-resistant S. aureus* | - | 12 | 21 | 8 |
| *S. aureus* | - | 16 | 20 | 21 |
| *C. albicans* | - | 14 | 24 | 10 |

**Anticancer activities of silver nanoparticles**

In vitro cytotoxicity assays are used to evaluate the potentially toxic effects of materials on cells. The cytotoxicity of the nanoparticles was determined against the SK-MEL-28 cell line at different concentrations by MTT assay. Cell morphology and DNA fragmentation were also analyzed using AgNps In this study, the cytotoxicity of biosynthesized AgNPs was examined using the SK-MEL-28 cell line. The cells were treated with various concentrations of AgNPs, and the cytotoxicity was measured using a cell viability assay. The results showed that the AgNPs caused dose-dependent cytotoxicity in both cell lines, with the highest cell growth inhibition observed at a concentration of 100 µM/mL. Table 2 refers to the anticancer activity of AgNPs on the SKMEL 28 cell line using sargassum tenerrimum extract.

In contrast, the lower concentrations (1 to 25 µM/mL) of AgNPs did not have a significant inhibitory effect on the cell lines. Previous research has suggested that AgNPs can induce oxidative stress and DNA damage in triple-negative breast cancer cells compared to other types of breast cancer cells. Biogenic AgNPs synthesized using leaf extracts of Acalypha indica and Mentha arvensis have also been shown to exhibit significant cytotoxicity against the cancer cell line SK-MEL-28. The results of this study indicate that the biogenically synthesized AgNPs have potential cytotoxic effects against both triple-negative and non-triple-negative breast cancer cell lines.

**Table 3: Anticancer activity of AgNPs using *Sargassum tenerrimum* extract on SKMEL 28 cell line**

| Concentration µg/ml | Percentage viability | $IC_{50}$ µg/ml |
|---|---|---|
| 6.25 | 87.39 | 23.616 |
| 12.5 | 74.31 | |
| 25 | 56.95 | |
| 50 | 38.12 | |
| 100 | 27.48 | |

To evaluate the cytotoxicity of the synthesized AgNPs, the cancer cells were incubated with various concentrations of AgNPs. After 24 hours of exposure to the biogenically synthesized AgNPs at concentrations of 6.25, 12.5, 25, 50, and 100 µM/mL, the cell growth was significantly reduced to 87.39, 74.31, 56.95, 38.12, and 27.48% respectively, compared to the untreated cancer cells. The IC50 value of the photosynthesized AgNPs was23.616 µM/mL. This is similar to a previous report of cytotoxicity against AGS cancer cells using AgNPs photosynthesized by the leaf extract of Artemisia turcomanica by Mousavi et al., where a lower concentration (4.88 µM/mL) of AgNPs was required to inhibit cancer cells. Several studies have shown that AgNPs are capable of generating ROS and subsequent oxidative stress in cancer cells, leading to DNA damage and apoptotic cell death. To confirm the

induction of apoptosis in cancer cells, the morphological changes of the breast cancer cell line MDA-MB-231 on the addition of different concentrations of biogenically synthesized AgNPs were examined under a fluorescence microscope.

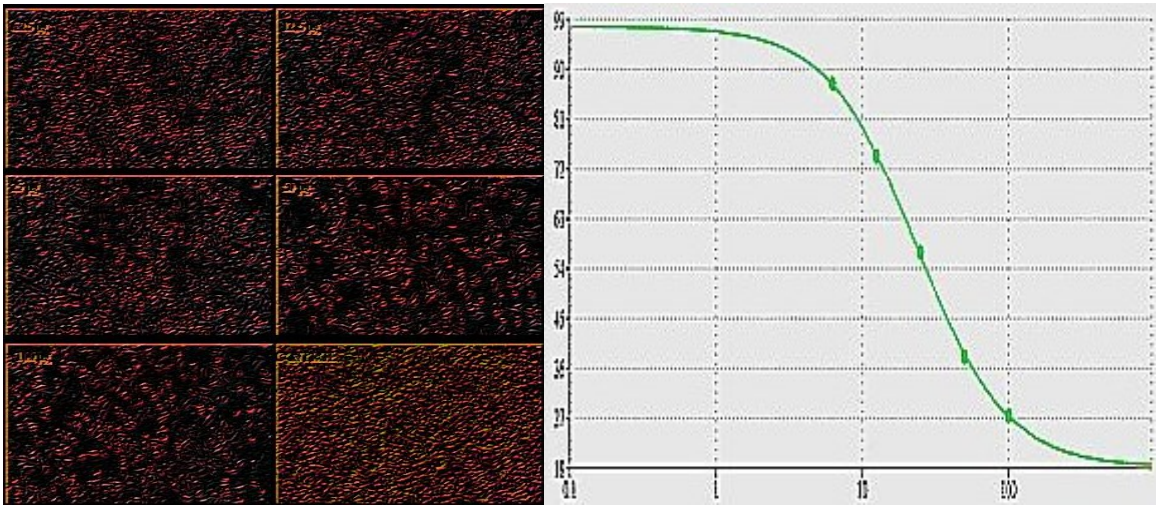

**Figure 8 a and b: Cell proliferation results of Fe2O3-NPs in SK-Mel 28 cell lines of AgNPs.**

The findings of this study support previous observations that the administration of different concentrations of AgNPs can trigger DNA fragmentation in human cervical cancer cells and Erlich Ascites murine carcinoma models, respectively. The results of this study suggest that the photosynthesized AgNPs have the potential to trigger nuclear condensation and fragmentation of DNA.

The cell cytotoxicity assays indicate that the photosynthesized AgNPs were cytotoxic to both breast and cancer cell lines. The higher concentrations of AgNPs were required to inhibit 50% of cell growth in MDA-MB-231 and MDA-MB-453 cell lines, compared to the AGS cancer cell line, which required a lower concentration of AgNPs. The biogenically synthesized AgNPs exhibited higher cytotoxicity towards both breast and cancer cells, which is possibly due to the higher uptake of AgNPs into cancer cells. This makes less use of anticancer agents, which is beneficial in protecting normal cells around the cancer cells. However, the activity of AgNPs depends on size, shape, cell type, media condition, and time and concentration dependence.

AgNPs were successfully produced using a rapid biological synthetic method from the seaweed extract of sargassum tenerrimum. The biogenically synthesized AgNPs were characterized and tested as a potent cytotoxic agent against breast and cancer cell lines for their effectiveness. The results showed that the AgNPs induced concentration-dependent inhibition of both breast and cancer cells. Their antiproliferative effects on both the cell lines of breast cancer and cancer were mediated by nuclear condensation or fragmentation of DNA, suggesting that biogenically synthesized AgNPs could be used as a novel anticancer agent for the treatment of breast and cancer cells. However, further research is needed to thoroughly study the mechanism of interaction of the photosynthesized AgNPs with cancer cells before further exploitation as a cancer drug and gene and drug delivery system.

In conclusion, the study demonstrates the potential of using Sargassum tenerrimum seaweed extract as a natural source for synthesizing silver nanoparticles (AgNPs). The bio-synthesized AgNPs exhibit good antibacterial and antimicrobial activity against different human pathogens and are found to be highly stable. The method of AgNP synthesis does not use any toxic reagents, making it a promising approach for use in the biomedical field. Additionally, SEM and TEM analysis showed that the AgNPs have a high density and spherical shape, with an average size of 20 nm. The high density of silver nanoparticles synthesized by the Sargassum tenerrimum extract confirms the development of silver nanostructures and their potential for successful use in various applications.

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