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Algal Mediated Green Synthesis Of Silver Nanoparticles Using Turbinaria Ornata.

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ABSTRACT

Background Information: Nanoparticles exhibit improved performance due to their large surface to volume ratio. *T. Ornata* is a brown alga, which is widely distributed in the tropical and subtropical seas were selected for the synthesis of silver nanoparticles. This brown alga is composed of many bioactive compounds. These secondary metabolites contain functional groups which can reduce the silver ions to silver and act as stabilizing agent for nanoparticles.

Materials and methods: Brown seaweed *Turbinaria Ornata* was collected at the Gulf of Mannar region (latitude 78° 8' East and longitude 9° 17' North) along the eastern coast of Tamil Nadu, India. The collected seaweeds were cleaned thoroughly to avoid salts, sand, shells, debris with associated epifauna and epiphytes. Cleaning was done by tap water and rinsing with double distilled water. Furthermore, the seaweeds were allowed to dry in the shade for over a week. Then, the dried seaweeds were crushed or grounded and aqueous extract preparations Synthesis of silver nanoparticles and estimation of anti-oxidant and anti-cancer activity were performed.

Results: The SEM results depicts that the obtained silver nanoparticles were cubical in shape. The UV-Vis spectra confirms the biosynthesis of silver nanoparticles at 438 nm. The presence of AgO nanoparticles is confirmed by the spectrum vibration at 815 cm^{-1}. The EDX confirms the presence of silver nanoparticles. The significant anti-oxidant potential of AgO nanoparticles was evaluated by DPPH radical scavenging assay having concentration of 50 µg. The ascorbic acid was used as a standard. The cytotoxic activity on breast cancer cell lines shown decreasing the cell viability by increasing the concentration of silver nanoparticles.

Conclusion: The synthesized AgNPs is found to have a potential anti-oxidant activity and has cytotoxic effect against breast cancer cell line. Further, it has to be analyzed for other biomedical applications so as to present it as an effective agent in future

Key Words: Nanoparticles; silver; anti-oxidant; anti-cancer.

INTRODUCTION

Despite all the efforts and advancements in detection and treatment, cancer remains one of the top causes of death globally (1). It has often been reported that many pathways are linked to the development of cancer, among other things, these include the growth and death of cancer cells (2). Furthermore, it is well known that the development of colorectal carcinomas is accompanied by a progressive inhibition of apoptosis, which may confer resistance to cytotoxic cancer agents and promote the growth and progression of tumor (3,4). The creation of medicines that encourage apoptosis and prevent cell proliferation while having few to no side effects and toxicities is therefore crucial. Moreover, none of the present therapies, including the triage of surgery for tumor removal, chemotherapy, and radiation, are free from significant problems that have not yet been resolved. Nano medicine appears in this context as a valuable alternative in the treatment of cancer (5,6),(7). Due to their unique physicochemical characteristics and inventive potential, silver nanoparticles are among the many options offered by

nanoscience and make excellent candidates for cancer therapy and detection (6,8),(9,10),(11). While practically all chemical procedures call for the employment of toxic and expensive chemicals, modern chemical and physical approaches enable the synthesis of silver nanoparticles of various sizes and shapes (12). Because of this, there is a lot of interest in and room for expansion in the development of environmentally friendly processes for the synthesis of silver nanoparticles

Utilizing extracts from natural goods such as plant leaves(13)(14), fruits(15,16)(17) , and microorganisms(18) are a few examples of green biosynthesis of AgNPs. These processes offer advantages over the traditional chemical and physical approaches since they are economical and environmentally benign. We concentrated on marine seaweeds in this regard. A number of bioactive substances found in seaweeds, including terpenoids, steroids, polysaccharide fucoidans (19) , laminarians , and others, have been shown to have pharmacological effects, including anti-cancer, anti-inflammatory, and anti-bacterial characteristics (20). Additionally, algae have recently been referred to as "bionano factories" because of the various ways in which they can be used to synthesize nanoparticles.

Turbinaria ornata has been a brown algae present in tropical and subtropical regions which has been used to synthesize these nanoparticles. This way of green synthesis could possibly reduce the toxic levels of the production of silver nanoparticles. The brown sea algae *Turbinaria ornata* was selected as part of our project focused on the green synthesis of novel nanomaterials with biomedical applications. The species that should be targeted in order to create silver nanoparticles with anti-tumor activity. The North East Atlantic, Baltic Sea, and Mediterranean all contain large populations of *Turbinaria ornata* which live at exposed rocky coasts at low intertidal and subtidal levels. The utilization of *Turbinaria ornata* in the biosynthesis and characterization of silver nanoparticles with a biological application is being tested for the first time in the scientific literature in this study. The aim of the study is to synthesize the silver nanoparticles using *Turbinaria ornata* brown algae extract and to characterize and analyze for anti-oxidant and anti-cancer properties.

2. MATERIALS AND METHODS

2.1 Sample collection and extract preparation

Brown seaweed *Turbinaria Ornata* was collected at the Gulf of Mannar region (latitude 78° 8’ East and longitude 9° 17’ North) along the eastern coast of Tamil Nadu, India. The collected seaweeds were cleaned thoroughly to avoid salts, sand, shells, debris with associated epifauna and epiphytes. Cleaning was done by tap water and rinsing with double distilled water (DDW). Furthermore, the seaweeds were allowed to dry in the shade for over a week. Then the dried seaweeds were crushed or grounded and aqueous extract preparations were performed based on our previous experiment.

Synthesis of nanoparticles

10 ml of seaweed filtrate was added in 90 ml of 103 M aqueous AgNO3 solutions at room temperature for the biogenesis of Ag nanoparticles. Visual inspection can prove that silver nitrate was bio-reduced into silver nanoparticles.(21)

Physical characterization

The spectral vibration at 815 cm^{-1} confirms the presence of AgO nanoparticles. FTIR spectra show the appearance of five prominent absorption peaks of seaweeds at ~3100, 2690, 1893, 1494, and 1004 cm^{-1}. The strong absorption was found at ~ 3100 cm^{-1} indicating the presence of polyphenols due to the binding of silver ions with hydroxyl group which referred to the stretching of OH group or free hydroxyl group. Furthermore, the presence of −C = C− stretched at around 1494 cm^{-1} confirms the presence of a broad range of alkene groups in the synthesized nanoparticles. The sharp band at ~1494 cm^{-1} possible due to N-O asymmetric stretching indicates the active involvement of nitro compounds. Another medium peak at ~1028 cm^{-1} indicates the presence of aliphatic amines due to C-N stretching.

The SEM results shown that the synthesized materials were in cubical shape. EDX results confirmed that synthesized nanoparticles are AgO.

On a Jasco Spectrometer V-670, room temperature UV-Vis spectra were captured. To determine the precise reaction time, time course measurements were taken at the wavelength with the highest absorbance. The amount of silver was measured. By employing an internal standard of indium and a Perkin Elmer Optima-4300 DV ICP-OES.

Cell viability

The cytotoxic activity of both NPs-EPS was determined using MTT assay (Promega, Madison, WI, USA) as previously described [69, 72]. Briefly, cells were plated at 5×10^3 cells/well in 96-well plates. NPs-EPS, diluted to the desiderated concentrations in culture medium, were added to the wells with respective vehicle control (H_2O). Doxorubicin hydrochloride (Sigma, st. Louis, MO) was used as a reference drug. After 24 h, of incubation 20 µL of the Cell titer 96®AQueous reagent was added to each well after three washes with phosphate buffer saline (PBS) and incubated for 1-4 h at 37 °C in a CO_2 incubator. The absorbance was recorded at 490 nm using a 96-well plate reader (Spark® 20M, Tecan Trading AG, Switzerland). The percentage of cell viability was calculated with respect to untreated control cells for each treatment after subtraction of the blank. The concentration necessary for 50% of growth inhibition (IC50) was calculated using a dose–response model, which was obtained from sigmoidal fitting of response curves of percent inhibition versus

logarithmic concentration of DOSs using Graph Pad Prism software. Each result was the mean value of three different experiments performed in triplicate.

Anti-oxidant assay

The significant antioxidant potential of AgO nanoparticles was evaluated by DPPH radical scavenging assay having concentration of 50 µg. The ascorbic acid was used as a standard.

3. RESULTS AND DISCUSSION

3.1. SEM analysis of AgNPs

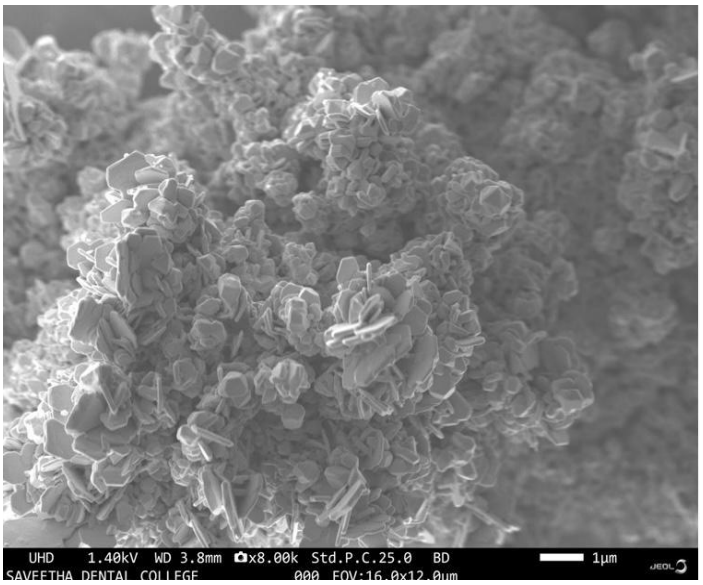

Fig 1. The picture represents scanning electron microscopic pictures of AgNPs.

The SEM results depicts that the obtained silver nanoparticles are cubical in shape. They are uniform in their shape, there are no agglutination which could be a big advantage for the biomedical applications, the drugs prepared using these nanoparticles will be more effective due to their uniform shape and they will more volume of distribution.

3.2. UV-Vis spectra of AgNPs

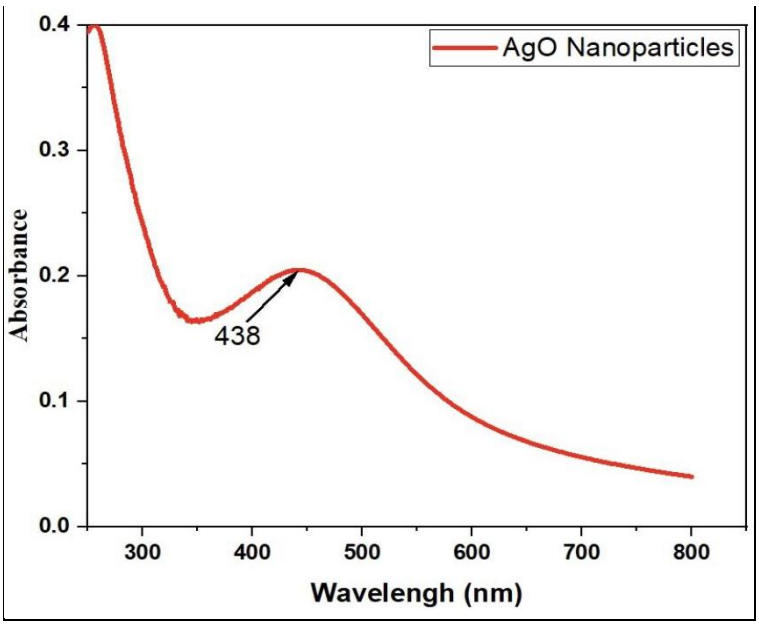

Fig 2. This graph represents UV-Vis spectra of AgNPs.

The bio-reduction of silver ions (Ag+) into silver nanoparticles (AgO) was monitored in aqueous solution by a UV-Vis spectrophotometer (Jasco V730, Japan) at regular intervals in wavelength ranges between 200 and 1000 nm.

Mohanta et al., 2016; and Nayak et al., 2016 observed the absorption peak at 420–450 nm for AgNPs. In our study, the observed absorption peak at 438 nm further confirms the biosynthesis of Ag nanoparticles (22).

The UV-Vis spectra confirms the biosynthesis of silver nanoparticles at 438 nm.

3.3. FTIR SPECTRA OF AgNPs

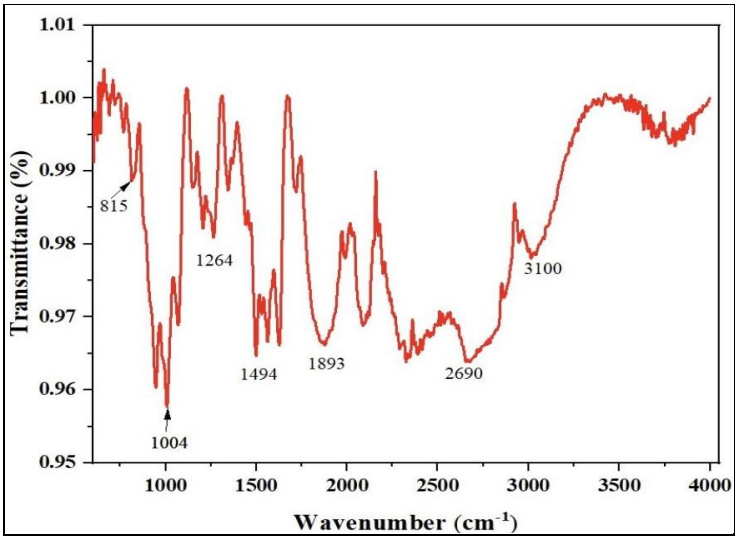

Fig 3. The graph represents FTIR spectra of AgNPs

The presence of AgO nanoparticles is confirmed by the spectrum vibration at 815 cm^{-1}. Five distinct seaweed absorption peaks may be seen in FTIR spectra, located at 3100, 2690, 1893, 1494, and 1004 cm^{-1}. Due to the interaction of silver ions with the hydroxyl group, which related to the stretching of the OH group or free hydroxyl group, a high absorption at 3100 cm^{-1} was discovered, confirming the existence of polyphenols. Additionally, the existence of "C = C" extended at about 1494 cm^{-1} demonstrates that the produced nanoparticles contain a variety of alkene groups. The sharp band at 1494 cm^{-1} caused by N-O asymmetric stretching suggests that nitro compounds are actively involved. The presence of aliphatic amines is indicated by another medium peak at 1028 cm^{-1}, which is caused by C-N stretching. The FTIR graph confirms the synthesis and the elements present in the synthesized nanoparticles at a spectral vibration.

3.4. EDX OF AgNPs

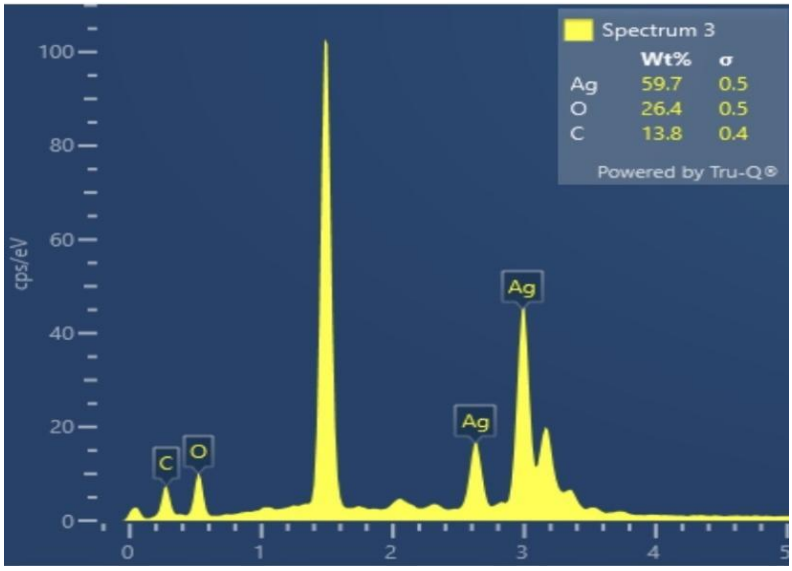

Fig 4. This graph represents EDX of AgNPs.

The EDX confirms the Synthesis of nanoparticles and the elemental analysis of AgNPs, there is more silver and oxygen which confirms the synthesized nanoparticle is AgO nanoparticle.

3.5. Cytotoxic activity against Breast cancer cells

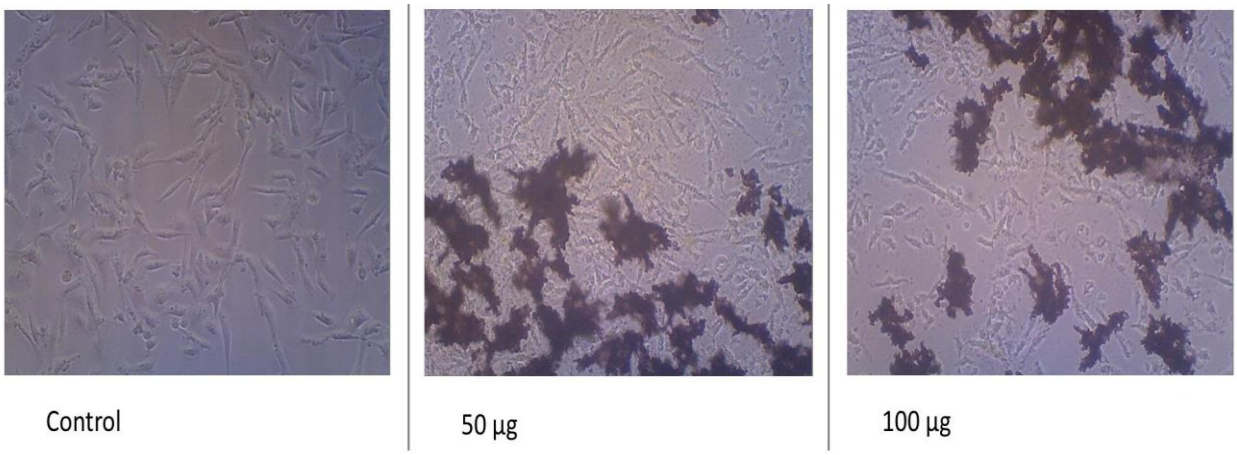

Figure 5. The picture represents cytotoxic activity against breast cancer cells MDA-MB-231.

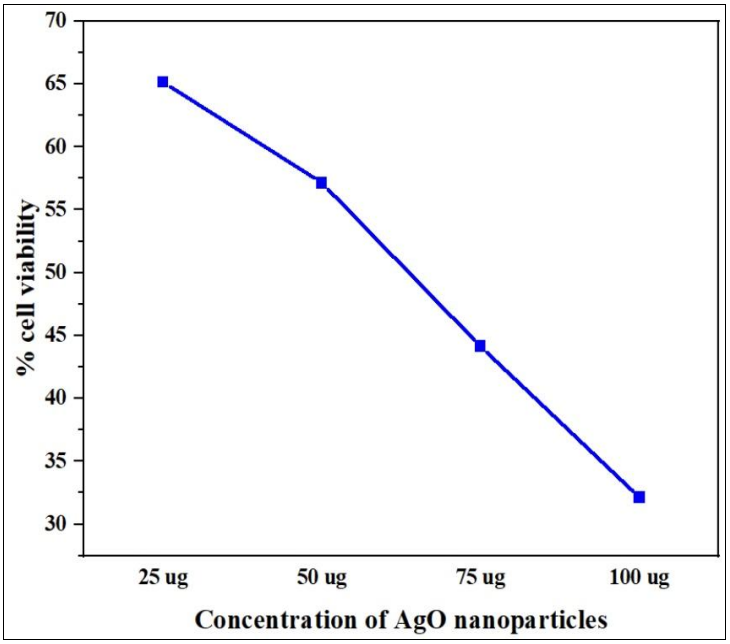

Figure 6. The picture represents cell viability of Ago nanoparticles.

The cytotoxic activity of both NPs-EPS was determined using MTT assay (Promega, Madison, WI, USA), as previously described [69, 72]. Briefly, cells were plated at 5 × 103 cells/well in 96-well plates. NPs-EPS, diluted to the desiderated concentrations in culture medium, were added to the wells with respective vehicle control (H_2O). Doxorubicin hydrochloride (Sigma, st. Louis, MO) was used as a reference drug. After 24 h of incubation 20 µL of the Cell titer 96®AQueous reagent was added to each well after three washes with phosphate buffer saline (PBS) and incubated for 1-4 h at 37 °C in a CO_2 incubator. The absorbance was recorded at 490 nm using a 96-well plate reader (Spark® 20M, Tecan Trading AG, Switzerland). The percentage of cell viability was calculated with respect to untreated control cells for each treatment after subtraction of the blank. The concentration necessary for 50% of growth inhibition (IC50) was calculated using a dose–response model, which was obtained from sigmoidal fitting of response curves of percent inhibition versus logarithmic concentration of DOSs using Graph Pad Prism software. Each result was the mean value of three different experiments performed in triplicate.

In this graph we could observe that as the concentration is increased the cell viability is decreased which shows that the AgNPs has anti-cancer property and it can inhibit cell proliferation.

3.6. Anti-oxidant activity of AgNPs

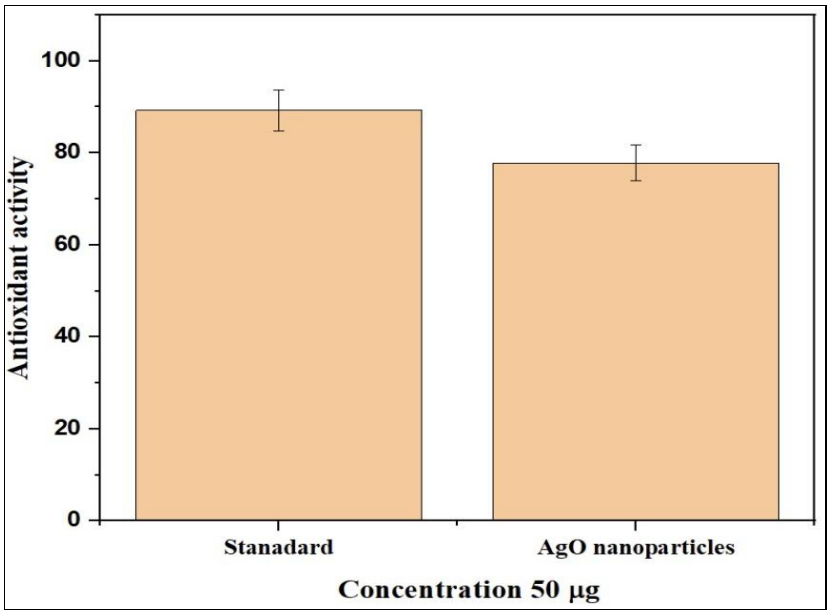

Figure 7. The graph represents antioxidant activity of AgNPs

The significant antioxidant potential of AgO nanoparticles was evaluated by DPPH radical scavenging assay having concentration of 50 µg. The ascorbic acid was used as a standard. We could observe that in case of AgNPs there is a decrease in antioxidant activity when compared to standard. Maybe the concentration of AgNPs must be increased to observe a better result.

Physicochemical production involves potentially harmful ingredients and toxic leftovers that disrupt the environment and because they are expensive, physicochemical production procedures are commonly abandoned in the preparation of nanoparticles because they are unsuited for the clinical area. The green production of metallic nanoparticles has sparked interest in nanotechnology studies because of its superior optoelectronic, catalytic, magnetic, and non-toxic properties as well as their eco-sustainable attitude and adaptability in biomedicine (23) (24,25). In light of all of this, the current study described a green synthesis of silver nanoparticles mediated by plant extracts. Nanoparticle synthesis using biological methods *Turbinaria ornata* secretes functional biomolecules like alkaloids, flavonoids, sterols, and others that actively reduce metal ions, making it more biocompatible than other plants that make nanoparticles with the help of different biomaterials like polymers, which are less effective and more expensive. (26) Confirmatory results may be found in (27), which show that *Turbunaria ornata* reacts with silver nitrate solution to shift from a yellow color to a brown suspension by reducing Ag+ ions to Ago. BP-AgNPs may exhibit a surface plasmon resonance spectrum between 440 and 558 nm (28). Silver nanoparticles show absorbance maxima at 458 and 446 nm in a prior research by Das et al.(29) .Due to the surface plasmon resonance property, the greatest absorbance peak in the current experiment occurred at 438 nm. The results imply that the extract's phytoconstituents function as reducing and capping agents. Additionally, Scanning Electron Microscopy (SEM), which indicated a cubical structure as in Ibrahim et al. (30), was used to forecast the morphology and size of silver nanoparticles. The average particle size determined by Image J software in the current investigation was 12 nm, with the histogram showing a particle size distribution between 10 and 50 nm. The histogram shows an average particle size distribution of approximately 10-80 nm, according to Aslam et al. (31). The elemental composition of silver nanoparticles anticipated by the energy dispersive X-ray (EDX) examination showed the presence of chlorine, oxygen, and silver. The presence of silver nanoparticles is indicated by higher levels of Ag (32). FTIR measurements predicted the practical stability of silver nanoparticles by phytoconstituents on particle surfaces. The nanoparticles were in agreement with those previously described by (33) and were conjugated with alkane (O-H), alcohol (C-H), fluoro (C-F), nitro (C-N), carboxyl, amine, alkenes (C=C), phenol (O-H), sulfone (S-O), and halo compound (C-Cl).

4. CONCLUSION

Using *Turbinaria ornata* extract, we have reported a productive process for the production of gold nanoparticles. The outcomes show that nanoparticle formation occurs within the extract within the first 100s of processing. After that, only nanoparticles with mostly polycrystalline structures are still present in the extract. This proposes a nucleation step into the extract as part of the formation mechanism, which encourages polycrystallinity, separation, and stability of the resulting silver nanoparticles. The discovery of various functional groups in proteins, polyphenols, and polysaccharides that may be

involved in the reduction of silver and the capping of Ag was made possible by FTIR spectral analysis. Breast cancer cells MDA-MB-231 cell lines were evaluated, and AgNps had considerable cytotoxic action, particularly against breast cancer cells. Surprisingly, AgNPs demonstrates remarkable biocompatibility on the human body. AgNPs, apoptotic activity was measured, and the activation mechanism was investigated. Results demonstrated that AgNPs can activate apoptosis by the mitochondrial and extrinsic pathways. Overall, the outcomes point to a significant potential for the treatment of breast cancer. Therefore, more research should be done to better understand the impacts of AgNPs in an *in vivo* model in order to confirm the capabilities of green approaches to provide powerful instruments for cancer therapy.

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