



Original article

Biosynthesized silver nanoparticles using *Caulerpa taxifolia* against A549 lung cancer cell line through cytotoxicity effect/morphological damage



Danjie Zhang^a, Govindan Ramachandran^b, Ramzi A. Mothana^c, Nasir A. Siddiqui^c, Riaz Ullah^c, Omer M. Almarfadi^c, Govindan Rajivgandhi^{b,*}, Natesan Manoharan^b

^a Department of Thoracic Surgery, Second Affiliated Hospital of Xi'an Jiaotong University, Xi'an 710061, China

^b Department of Marine Science, Bharathidasan University, Tiruchirappalli 620024, Tamil Nadu, India

^c Department of Pharmacognosy, College of Pharmacy, King Saud University, P.O. Box 2457, Riyadh 11451, Saudi Arabia

ARTICLE INFO

Article history:

Received 19 July 2020

Revised 2 September 2020

Accepted 7 September 2020

Available online 14 September 2020

Keywords:

Caulerpa taxifolia

Ag NPs

Physiochemical characterization

In-vitro studies

Morphological damage

Increased death cells

ABSTRACT

The *Caulerpa taxifolia* is excellent marine green algae, which produced enormous bioactive compounds with more biological activities. Also, it is an excellent source for synthesis of Ag NPs with increased bioactivity against various infections. In our study, the marine algae *Caulerpa taxifolia* mediated Ag NPs was synthesized effectively. The synthesized Ag NPs was characterized well using UV-spectrometer and X-ray powder diffraction (XRD) and confirmed as synthesized particle was Ag NPs. The available structure of the Ag NPs was morphologically identified by scanning electron microscope (SEM), and exact minimum size, polydispersive spherical shape of the entire Ag NPs structure was confirmed by Transmission electron microscope (TEM). Further, the anti-cancer efficiency of biosynthesized Ag NPs against A549 lung cancer cells was found at 40 µg/mL concentration by cytotoxicity experiment. In addition, the phase contrast images of the result were supported the Ag NPs, which damaged the A549 morphologically clearly. Finally, florescence microscopic images were effectively proved the anti-cancerous effect against A549 lung cancer cells due to the condensed morphology of increased death cells. All the confirmed *in-vitro* results were clearly stated that the *Caulerpa taxifolia* mediated Ag NPs has superior anti-cancer agent against A549 lung cancer cells.

© 2020 The Authors. Published by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Recent decades, syntheses of nanoparticles are increased attention in the entire field including chemical, physical, biological, pharmaceutical, environmental and biomedical with greater properties (Batool et al., 2019). The nanomaterial opened very big window in material science research of physical, chemical and engineering. It concentrated on synthesis of nanoparticles, having at least one dimension range of ≤ 100 nm. It contained with various physiochemical components and most of them undefined materials (Ramesh et al., 2018). Nanomaterials provide more

advantages to environment and more compatibility with excellent biomedical applications including anti-microbial, anti-cancer, larvicidal, immunosuppressive, biosensor, catalysis and drug delivery (Vijaya anand et al., 2019). Nanomaterials are divided in different types such as nanoparticles, nano rods, nano films, fullerenes and etc. Among these types, nanoparticles have the ability to exhibit the zero dimensions with significant biochemical properties. Last 10 years, the uniform sized nanoparticle synthesis is increased worldwide, and the size based morphology of the nanoparticles is frequently used to deal with advanced technology and environmental challenges (Ebrahimzadeh et al., 2020). The smaller sized nanoparticles are synthesized in ordinary laboratory conditions and low cost, high surface area. Various routes are available in nanoparticles synthesis; they are physical, chemical, biological. Among these, biological route is considered more efficient than other routes due to the low toxicity, low cost, increased surface nature and volume zero (Al-Brahim and Mohammed, 2020). Sometimes, the chemical mediated nanoparticles seriously affected the environment and others directly or indirectly due to the toxic nature (Lakhan et al., 2020).

* Corresponding author at: Department of Marine Science, Bharathidasan University, tiruchirappalli, Tamil Nadu, india.

E-mail address: rajivgandhimicro@yahoo.com (G. Rajivgandhi).

Peer review under responsibility of King Saud University.



Production and hosting by Elsevier

Among these nanoparticles, biological syntheses of nanoparticles are heightened in the research field due to the biocompatibility, greater efficient, cost efficient and decreased environmental toxicity (Kup et al., 2020).

In particular Ag NPs was gained notable attention worldwide in the entire field, and it's owing to its beneficial role of increased potentials including anti-bacterial, anti-viral, anti-malarial, anti-oxidant, anti-cancer, larvicidal, wound healing properties. It has enormous activity with naturally renewable, cost-effective (Sahoo et al., 2020; Batool et al., 2019; Lakhan et al., 2020; Vijaya Anand et al., 2019; Ebrahimzadeh et al., 2020; Ramesh et al., 2018). Recent years, considerable studies are going on Ag NPs synthesis through biological synthesis using plant extract, microbes, sponge associated extract, marine organism mediated Ag NPs and some other marine and terrestrial microorganisms (Shah et al., 2020) Among these, the plant associated Ag NPs was very high due to the increased biological properties (Li et al., 2020). It has the absorption capacity and utilizes the some important chemical substituents from plants. Previously, the some plants have excess nutrients and produced the increased biological properties. Also, they have high phenolic and flavonoid contents with rich anti-oxidant activity (Vijaya Anand et al., 2019). The synthesized Ag NPs from these kinds of plants were given more advantages due to the utilization of plant chemical components (Ahila et al., 2016). The plant mediated Ag NPs has low toxicity compared with chemical and physical synthesis.

In biological state, Ag NPs has the ability to permeable into the bacterial DNA and arrest the replication of bacterial cell cycle (Das et al., 2020) Due to this factor, the bacterial was lost their developmental genes and did not produce the cell wall in bacteria. Finally, the bacteria fallen on the decline phase and could not produce their role to develop multi drug resistant effect. The plant synthesis Ag NPs has improved anti-cancer activity against various cell line and it destroy the cells completely (Ravichandran et al., 2018). The complete mechanism of Ag NPs and bacterial inhibition was still unknown. But, it can enter into the metabolic pathway of cancer cells and inhibit the nucleus of the cells completely (Khorrami et al., 2020). Recent reports are clearly stated that the plant mediated Ag NPs synthesis was excellent method and inhibit the cancer cells at very lowest concentration. Based on the statement, we have concluded that the non-hazardous, biocompatible, low cost and eco- friendly method of plant mediated Ag NPs synthesis is very effective method than any other. In addition, seaweed synthesized Ag NPs has considerable attention in biological route worldwide due to the rich carbohydrate nature and different environmental conditions (Hashemi et al., 2020). It has rich phytochemical constituents, rich carbohydrates and more anti-oxidant properties. Previous researchers are also reported that the seaweed has effective anti-cancer properties against various cancer cells. Among the various seaweed, the marine green algae *Caulerpa* is an edible algae and used as salad in Asian countries, especially India and China (Sasikala and Geetha Ramani, 2017). Among the *Caulerpa*, *Caulerpa taxifolia* is the important species and more secondary metabolites producer such as sesquiterpenoid, diterpenoids which protects plant from herbivores. In addition, it produces di-indole pigments and various biological activities including anti-bacterial, anti-viral, immunomodulatory, anti-viral and anti-biofilm activities (Etcherla, 2014). Particularly, more oxidative stress responses produced *Caulerpa taxifolia* against breast and lung cancer cells (Mehra et al. 2019). Therefore, our research is focused on biosynthesized Ag NPs using marine seaweed of *Caulerpa taxifolia* for inhibition of various cancer cells.

2. Materials and methods

2.1. Needed chemicals and materials

Silver nitrate (Ag NO₃), sodium hydroxide (NaOH), D.DH₂O and other nanoparticles are purchased from Suresh Scientific@Co, Tamil nadu, India. A549 human non-small lung cancer cells were obtained from King Institute of Prevention and Medicine, Guindy, Chennai, Tamil nadu, India. The complete medium of Dulbecco's Modified Eagle medium (DMEM), AO/EB fluorescence dye was purchased from Merk, India. The cell culture was continuously maintained at 37 °C with 5% CO₂ incubator at (T-25) tissue culture plate.

2.2. Preparation of seaweed extract

The 30 days dried green seaweed *Caulerpa taxifolia* extracts was grinded for fine powder by using mortar and pestle. The obtained powder was dissolved in 1000 mL of Millipore water and boiled at 80 °C for 20 min and filtered by whatman NO.1 filter paper. The filtrate of the crude plant material was extracted with methanol by soxhlet experiment and maintained at 37 °C maceration. After removal of methanol, the extract was maintained at 45 °C reduced pressure using rotary evaporator for 1 d. After evaporation, almost 50% w/w yield of unclear dark green color compound was recovered. Based on the previous report of Rajivgandhi et al., 2020a, the crude compound was added into the preparative HPLC column (Bruker serious 2500 pump with Gilson FC203B fraction collector and Bruker serious UV/VIS detector set at 240 nm, Merck, India) for purified the active secondary metabolites. After 6 h, the biomedical components of HPLC purified extract was recovered for various biomedical applications.

2.3. Synthesis and characterization of Ag NPs

The 10 mL of HPLC purified *Caulerpa taxifolia* extract (5 W/V) was taken together with 100 mL Ag NO₃ (4 Mm) aqueous solution at 250 mL sterile conical flask. The mixed samples were stirred and maintained at 90 °C water bath for 1 h. The 1 N NaOH and 1 N H₃PO₄ were used to maintain the adjustment of pH. After incubation, the reaction mixture of the sample was gradually changed their color from yellow to reddish brown. This color change was indicating the Ag NPs synthesis in the reaction mixture (Venugopal et al., 2017). Further, the synthesized Ag NPs was initially confirmed by using UV-spectrometer (Merck, India). Then, the powder sample of Ag NPs was thoroughly diluted with KBr and makes a thin pellet, and consecutively analyzed by XRD (X pert PRO, analytical, China, Cu Ka radiation, λ = 1.541870 Å) to identify the intensity differentiation between the 10 ° to 80° of 2 θ range (Lateef et al., 2016). In addition, the morphological observation of synthesized Ag NPs was observed by scanning electron microscope analysis (SEM model JEOL JSM-7600F) at 0.1–30kv of accelerating voltage. The particular morphological structure and size of the biosynthesized Ag NPs was analyzed by transmission electron microscope at 120–200 kV voltage accelerated (Nilavukkarasi et al., 2020).

2.4. Anti-cancer experiments

2.4.1. Cytotoxicity assessment of A549 lung cancer cells

The anti-cancer effect of biosynthesized Ag NPs was initially detected by cytotoxicity assay using the solvent of dimethylthiazol-diphenyltetrazolium bromide (MTT) with the previous report of Kumar et al. (2019). Briefly, overnight incubated A549 culture ratio of ~2 × 10⁴ cells was taken in new complete medium of DMEM containing 96-well plate and incubated at

37 °C for 24 h under 5% CO₂ atmosphere and 95% humidity. After 24 h, DMSO diluted 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 µg/mL of Ag NPs concentrations were added separately into the respective 96-well plate. Whereas, without addition of Ag NPs concentrations with A549 cells, and A549 cells containing DMSO samples were acted as a positive and negative control respectively. All the samples containing plate was incubated till overnight at room temperature. After overnight incubation, the cells were taken and gradually added the MTT solution diluted by PBS with 25 µl of 5 mg/mL and covered by aluminum foil followed by 4 h incubation with room temperature. After room temperature, the plate was taken to atmospheric temperature 5 min and formation of crystal formazan production appeared in the treated cells well or not was noted on the naked eye. The color intensity of treated and untreated wells was monitored by UV-spectrometer at O.D wavelength of 540 nm. The replicated values of the result was noted and changed to inhibition percentage using following equation,

$$\text{Percentage of IC}_{50} [\text{Mean O.D}_{\text{Control}} - \text{Mean O.D}_{\text{Test}}] \times 100,$$

The control well of the plate percentage was considered as 100%, and tested result was compared with this control. Finally, the inhibition percentage (IC₅₀) was detected based on the equation result.

2.4.2. Detection of morphological changes by phase contrast microscope

The effect of Ag NPs against human lung cancer A549 cells was initially identified using morphological observation. In this initial morphological observation, the IC₅₀ concentration of Ag NPs treated and untreated cells were morphologically viewed by phase contrast microscope (Rajivgandhi et al., 2018). Shortly, the mature A549 cell culture was diluted with freshly prepared DMEM medium containing 96-well plate and treated with IC₅₀ concentration of biosynthesized Ag NPs and exposed to room temperature at overnight incubation. After incubation, the cells were taken and fixed by formaldehyde (4%) for 15 min. After 15 min, the damaged or undamaged morphologies of treated or untreated A549 cells were observed by phase contrast microscopy using 40 × magnifications.

2.4.3. Live/dead cell variation (AO/EB) assay

The survival and unsurvival A549 cells in the treated and untreated 96-well plate wells were morphologically viewed by fluorescence microscope using acridine orange and ethidium bromide (AO/EB) dyes. This method is followed by previous method of Rajivgandhi et al., 2019a, with some alteration. The 24 h staled cultures was allowed to grow on 6-well plate containing cover slip, and treated with IC₅₀ dose of biosynthesized Ag NPs. Whereas, the cover slip containing A549 cell culture without treatment of Ag NPs was performed as a control. Both the samples were allowed to maintain in room temperature for overnight. After overnight incubation, the cover slips were taken separately and rinsed with 1x PBS followed by drying the PBS. After, the 15 10 µg/mL AO/EB dye was used on the treated and untreated cells and allowed to maintain in dark room 30 min. Finally, the treated and untreated cells were viewed the live/dead cell modification by fluorescence microscope (Carl Zeiss, Japan) at 40× magnification.

3. Result

3.1. Preparation of seaweed extract

After recovered the HPLC purification, the marine seaweed *Caulerpa taxifolia* was analyzed the HPLC-MS to identify the complete phytochemical constitution and anti-cancer derivatives. It is a green algae, *Ulvophyceae* class and *Caulerpaceae* family. After care-

ful interpretation, the previously reported anti-cancer compounds were identified in the third fraction of the HPLC purified extract (Rajivgandhi et al., 2020b). Based on the retention time and occupied area, the compounds 2,4-Di-*tert*-butyl-phenol, Tetracosenoic acid, 9-H-Pyrido[3,4-b]indole, Bis(2-ethylhexyl) phthalate, Neophytadiene, 1-Deacetylasperulosidic acid and Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro were present in the purified HPLC extract of *Sargassum weightii* (Fig. 1). In our result was suggested that the active anti-cancer compounds are acted as a reducing substances in Ag Nps synthesis. They may influence the enhanced anti-cancer activity against tested cancer cells. Previously, Nourozi et al., 2019, reported that the seaweed extract of *Caulerpa taxifolia* has excellent anti-cancer property against cancer cells (Adyani and Soleimani, 2019). Recently, the reported evidence of Sumbal et al., (2019), also supported to our result and indicated that the marine *Caulerpa taxifolia* could played enhanced biological properties in synthesized Ag NPs. This enhanced effect may be occurred by different environmental conditions of sea including different temperature, different pH, different salt nature and different NaCl (Kumara Swamy et al., 2017; Sharma et al., 2020). Therefore, our result was suggested that the purified extract of seaweed *Caulerpa taxifolia* was excellent source for synthesis of Ag NPs and improved anti-cancer effect.

3.2. Synthesis and characterization of Ag NPs

3.2.1. UV-Vis spectroscopy analysis

After careful identification, the color intensity was changed from yellow to reddish brown color in the reaction mixture of purified seaweed extract and Ag NO₃ solution. It was indicated that the Ag NPs was formed from the reaction mixture. In addition, the phytochemical constituents of seaweed may influence the Ag Nps synthesis as a substance (Jha et al., 2017). Further, the synthesized Ag Nps was pure or not was confirmed by various range result of UV-spectrometer analysis. The surface plasmon resonance of the Ag NPs was initially exhibited in the UV absorption peak of 420 nm for purified *Caulerpa taxifolia* extract, indicating the formation of Ag NPs, and confirmed primarily (Fig. 2a). In the reaction mixture, the peak was shifted when the nanoparticle was completely linked with surface plasmon resonance phenomena and the phytochemical derivatives of crude extract acted as a precursor for synthesis of silver nanoparticles (Vemuri et al., 2019). In our result, the Ag NPs was synthesized between the peaks of 310–490. The formed Ag Nps indicated that the various biomolecules of *Caulerpa taxifolia* participated in the synthesis process.

3.2.2. XRD pattern analysis

The crystalline condition of biosynthesized Ag NPs was effectively confirmed by powder XRD analysis. The XRD result of biosynthesized Ag NPs was indicated in Fig. 2b. Based on the findings, the available indexed peaks of Ag Nps was exhibited at (1 1 3), (1 1 1), (2 0 3), (2 2 6), (3 1 9) and (2 1 9) and their reflection patterns were exhibited at 2θ value of 31.66°, 40.11°, 47.12°, 70.14°, 19.21° respectively. The measurement of lattice plane peaks patterns were corresponded with the JCPDS, File No. 04–0783 (Rajivgandhi et al., 2020b; Shahid et al., 2020). The mentioned result of XRD was indicated that the biosynthesized Ag NPs was crystalline condition and it derived from seaweed extract.

3.2.3. Morphological images of Ag NPs by SEM

The surface morphology of biosynthesized Ag NPs was exhibited with uniform sized particles with dispersed spherical like agglomerated morphology. In addition, it shown with average size of the molecules. The exhibited result was suggested that the Ag NPs was converted from Ag NO₃ and seaweed extract. The chemical composition and morphological evidences of SEM images were

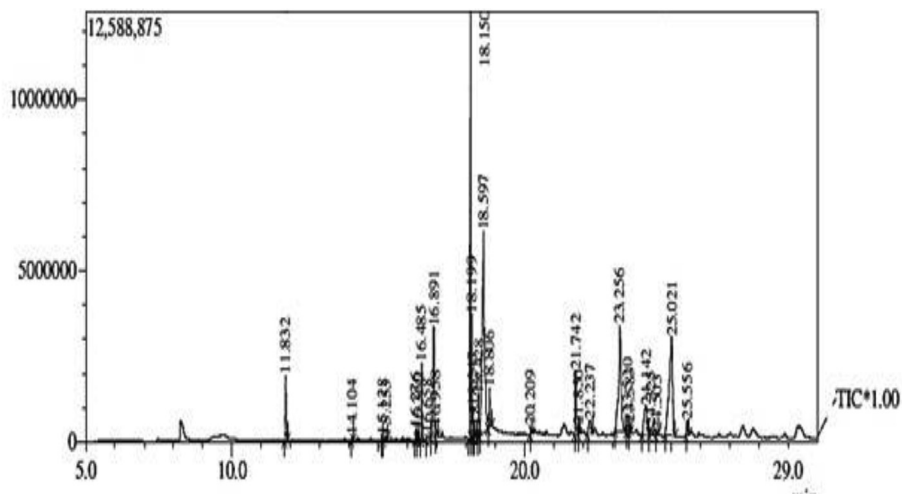


Fig. 1. Detection of available available anti-cancer compound form marine algae *Caulerpa taxifolia* by GC–MS analysis.

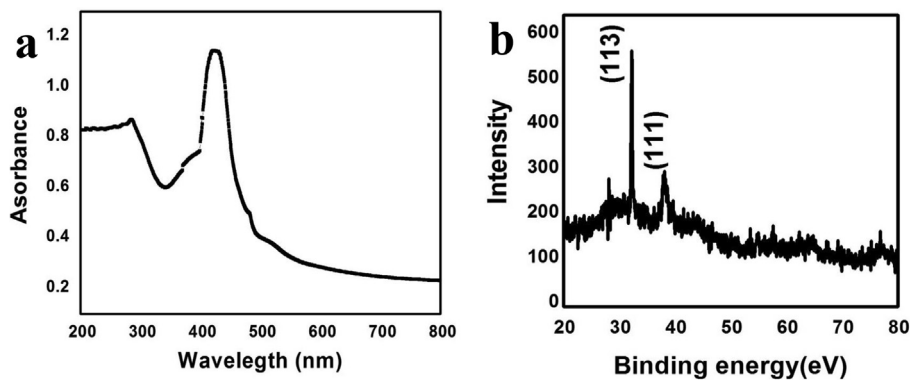


Fig. 2. Spectroscopic characterization of biosynthesized Ag NPs in UV-spectrometer (a) and XRD spectrum analysis (b).

indicated that the seaweed was essential source for Ag NPs synthesis (Saber et al., 2018). The absorption peak of 3KeV in the surface plasmon resonance was characterized successfully with stronger structures (Rajivgandhi et al., 2019b; Jalilian et al., 2020). The SEM images of biosynthesized Ag NPs was depicted in Fig. 3a, b.

3.2.4. Confirmation of Ag NPs images by TEM

To detection of Ag NPs morphology, TEM is an important instrument (Zayed et al., 2020). It is used to detect the morphology, size and exact shape of the Ag NPs in different angle. It is helped to

detect the individual size and shape of the biosynthesized Ag NPs. Also, it suggest, the synthesized Ag NPs result was accurate and it shown in 10 and 100 nm along with polydispersed spherical morphology. It exhibited with small sized Ag NPs with larger surface area. The size and shape of the resulted Ag NPs was indicated that the seaweed allows only small sized Ag Nps. Basically, seaweed mediated Ag NPs damage the cancer cells through ROS generation, mitochondrial damage and cell cycle arrest (Khaleghi et al., 2019). Previously, Kumaresan et al., (2018) was agreed our result and seaweed mediated Ag NPs was exhibited with smaller sized

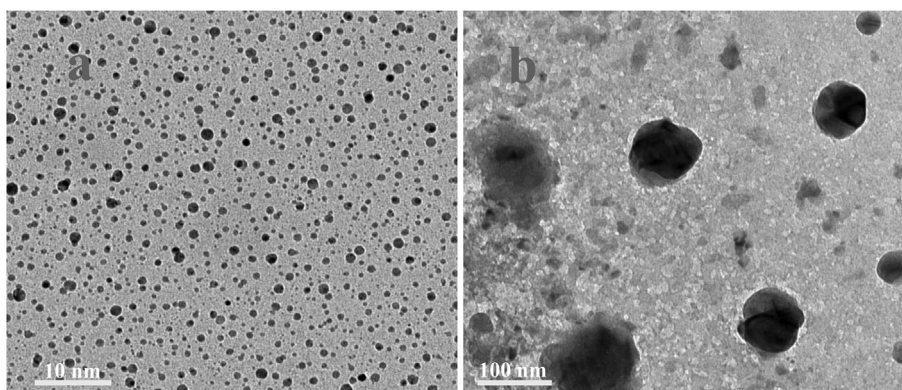


Fig. 3. Morphological observation of biosynthesized Ag NPs by scanning electron microscope.

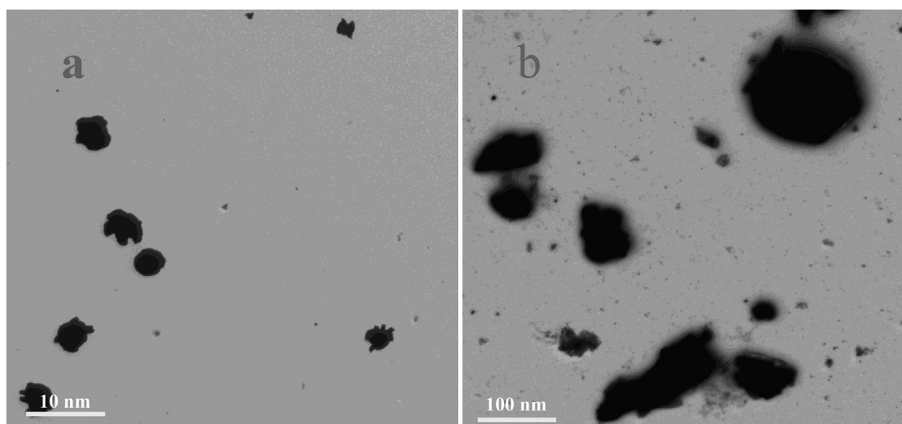


Fig. 4. Size and shape of the biosynthesized Ag NPs by transmission electron microscope.

particle. The polycrystalline nature of the biosynthesized Ag NPs was synthesized from seaweed extract and this result was very good agreement with previous report of Manjunath Hulikere et al., 2017. The purified seaweed extract mediated Ag NPs was depicted in Fig. 4a, b.

4. Anti-cancer studies

4.1. Cytotoxicity measurement of Ag NPs

Recently, Venugopal et al., 2017 was indicated that the biosynthesized Ag NPs is an important nanomaterial that has been effectively used in cancer cells study. The present result was exhibited also clearly exhibited that the biosynthesized Ag NPs was excellent nanomaterial for A549 lung cancer cell and it very effective at 70 $\mu\text{g}/\text{mL}$ concentration. The gradual decrease of the growth in the increased concentration of O.D was shown with 90% of inhibition at same concentration. Interestingly, the decreased half growth was shown at 40 $\mu\text{g}/\text{mL}$ concentration and it shown increased turbidity in 96-well plate. After addition with MTT in the plate of A549, the formazan was formed rapidly and continuously. This formation was changed the color of the culture in the treated wells. When we found the control wells, it was not shown any turbidity and color formation. Therefore, IC_{50} concentration of 40 $\mu\text{g}/\text{mL}$ was fixed for future study due to the exhibited value of $\sim 52\%$ inhibition against A549 cells (Fig. 5). Our result was deliver, the formation of necrosis and condensation morphology of A549 cells were observed under the effect of Ag NPs. Recently, Jha et al., 2017; Vijaya Anand et al., 2019 documented that the necrosis formation was influenced by biosynthesized Ag NPs. Previously, Rajivgandhi et al. (2018) was agreed our result and received statement of increased concentration of biosynthesized Ag NPs. Previously, the anti-cancer properties of *Caulerpa taxifolia* were reported with excellent result against breast and lung cancer (Mehra et al., 2019).

4.2. Morphological damage of Ag NPs

At 40 $\mu\text{g}/\text{mL}$ concentration, the A549 cells were damaged in the influence compound of Ag NPs. This evident was viewed by phase contrast microscope and images were shown in Fig. 6. In phase contrast microscope result, the wrinkled cells and Ag NPs retaliated forces of the A549 surface morphology was shown. The retaliated Ag NPs surface was shown in Fig. 6b, whereas normal, original and clear A549 cell morphology was observed in the untreated control A549 cells (Fig. 6a, b). Initially, the phase contrast images were taken for unclear, more turbidity of IC_{50} concen-

tration treated wells as well as untreated control wells were used for control and test respectively. Phase contrast microscope images were used for differentiate the control and treated cells morphology initially and agreed by Venugopal et al. (2017); Rajivgandhi et al., 2018. Also, their Ag NPs was synthesized from biological sources and effective role against A549 lung cancer cells at IC_{50} concentration with increased damages.

4.3. Combined fluorescence staining method

Based on the fluorescence color intensity, the control and treated A549 cells were clearly shown in the Fig. 7. The color emission confirmation was followed by previous statement of Saber et al., 2018 by AO/EB fluorescence dyes. In our result, the treated cells were emitted with red color after IC_{50} concentration of Ag NPs. More necrotic and condensation of the cells with intact morphology was shown in the exhibited red color. The result of AO/EB support was evidenced in the control and treated places correctly. The AO stain was undergone and bind in the untreated cells, whereas the EB undergone the treated cells and apoptosis places. So, the Ag NPs was influenced the A549 cells intracellular level. In our result, the more condensation of Ag NPs retaliated cells was shown in Fig. 7a. It was shown with clumped apoptosis and decreased cell growth. More apoptosis was also shown in treated cells with more condensation. In our result, the fluorescence images were also supported to treated and untreated cells in morphology and also intracellular level. Compared with control and treatment, the green/red colors for AO/EB perfectly suited with clear differentiation. The

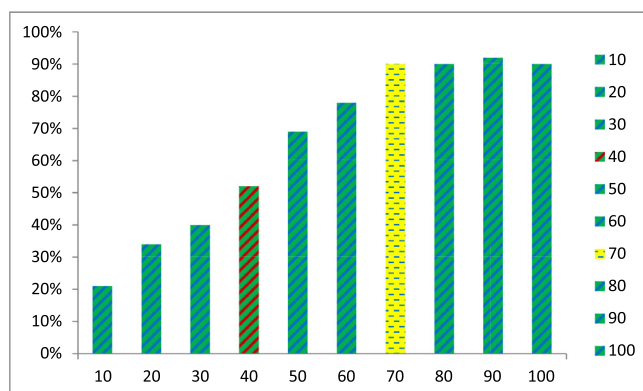


Fig. 5. Cytotoxicity effect of biosynthesized Ag NPs against A549 lung cancer cells at different concentration.

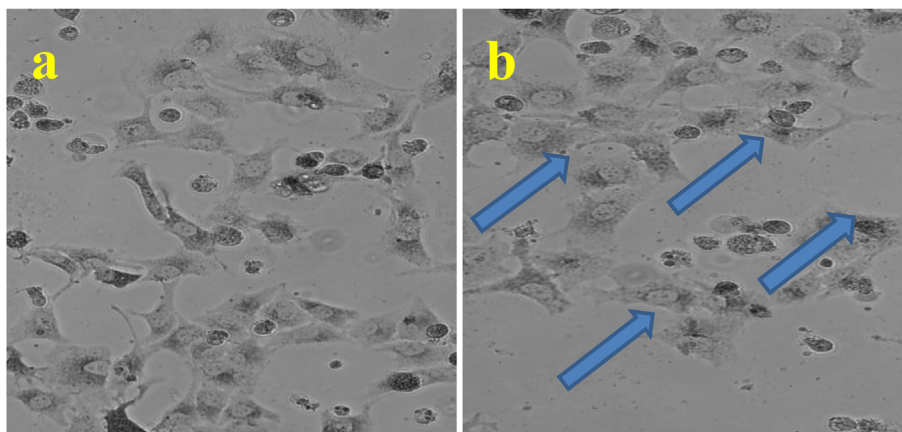


Fig. 6. Detection of morphological changes of biosynthesized Ag NPs treated A549 lung cancer cells by phase contrast microscope.

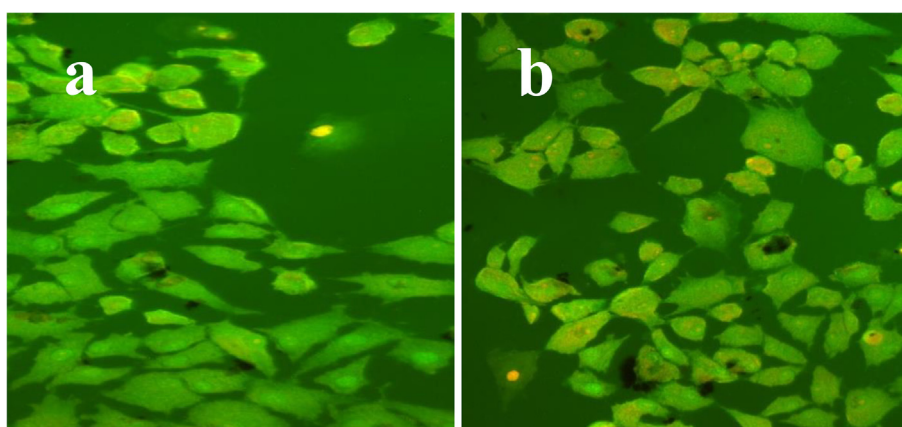


Fig. 7. Differentiation of live/dead cells (a, b) of Ag NPs treated A549 lung cancer cells by fluorescence microscope analysis using AO/EB as a fluorescent dye.

control cells of undamaged, original, normal morphology of A549 lung cancer cells were shown in Fig. 7a. Whereas, unattached and non-adherence ability of treated cells with red color emission of A549 cells also observed in the treated cells. The apoptosis variation using AO/EB stains was agreed by Hashemi et al., 2020. Supported morphological evidences with increased treated cells of red color emission in the A549 of Ag NPs treated cells were indicated by Jha et al., 2017. Recently, Vemuri et al., 2019; Saber et al., 2018 also supported to our evidence and using AO/EB dyes for control/treated A549 cells in the excellent influence of Ag NPs. Hence, our result was suggested that the IC₅₀ dose of *Caulerpa taxifolia* based synthesized Ag NPs was more effective against A549 lung cancer cells.

5. Conclusion

Initially, the anti-cancer compounds present in the marine seaweed were confirmed by GC–MS analysis. After GC–MS result, the six major anti-cancer compounds were identified based on the NIST library of Bharathidasan University, Tiruchirappalli, Tamil nadu, India. *Caulerpa taxifolia* The Ag NPs was synthesized from marine seaweed of *Caulerpa taxifolia* and it was confirmed by various spectroscopic analysis including UV-spectrometer and XRD. The morphological observation of biosynthesized Ag NPs was clearly observed by SEM analysis at 40x magnification. In addition, the polydispersed spherical morphology of the biosynthesized Ag NPs was confirmed by transmission electron microscope. Further, the anti-cancer effect of biosynthesized Ag NPs was exhibited excellent cytotoxicity against A549 lung cancer cells at 70 µg/mL

concentration. Furthermore, the phase contrast microscope result was initially confirmed that the IC₅₀ concentration of Ag NPs damaged the A549 cells morphology. Finally, the fluorescence microscopic result was proved that the IC₅₀ concentration of Ag NPs treated A549 cells were showed with more death cells. Hence, our result was confirmed that the biosynthesized Ag NPs has more anti-cancer property against A549 lung cancer cells.

Acknowledgement

The authors extend their appreciation to the Deputyship for Research & Innovation, “Ministry of Education” in Saudi Arabia for funding this research work through the project number IFKSURG-073.

References

- Batool, S., Hussain, Z., Niazi, M.B.K., Liaqat, U., Afzal, M., 2019. Biogenic synthesis of silver nanoparticles and evaluation of physical and antimicrobial properties of Ag/PVA/starch nanocomposites hydrogel membranes for wound dressing application. *J. Drug Deliv. Sci. Technol.* 52, 403–414.
- Ramesh, A.V., Rama Devi, D., Battu, G.R., Basavaiah, K., 2018. A Facile plant mediated synthesis of silver nanoparticles using an aqueous leaf extract of *Ficus hispida* Linn. f. for catalytic, antioxidant and antibacterial applications, *South African. J. Chem. Eng.* 26, 25–34.
- M.A. Vijaya Anand, R. Vinayagam, S. Vijayakumara, A. Balupillaia, F.J. Herbert, S. Kumar, A.Y. Ghidan, T.M. Al-Antaryc, E. David, Green synthesis, characterization and antibacterial activity of silver nanoparticles by *Malus domestica* and its cytotoxic effect on (MCF-7) cell line, *Microb. Pathogen.* 135, 2019, 103609.
- Ebrahimzadeh, M.A., Naghizadeh, A., Amiri, O., Shirzadi-Ahodshti, M., Mortazavi-Derazkol, S., 2020. Green and facile synthesis of Ag nanoparticles using

- Crataegus pentagyna* fruit extract (CP-AgNPs) for organic pollution dyes degradation and antibacterial application. *Bioorg. Chem.* 94, 103425.
- Al-Brahim, J.S., Mohammed, A.E., 2020. Antioxidant, cytotoxic and antibacterial potentials of biosynthesized silver nanoparticles using bee's honey from two different floral sources in Saudi Arabia. *Saudi J. Biol. Sci.* 27, 363–373.
- M.N. Lakhan, R. Chen, A. H. Shar, K. Chand, A. H. Shah, M. Ahmed, I. Ali, R. Ahmed, J. Liu, K. Takahashi, J. Wang, Eco-friendly green synthesis of clove buds extract functionalized silver nanoparticles and evaluation of antibacterial and anti-diarrhoeal activity, *J. Microbiol. Methods*, 173, 2020, 105934.
- Kup, F.O., Coşkunçay, S., Duman, F., 2020. Biosynthesis of silver nanoparticles using leaf extract of *Aesculus hippocastanum* (horse chestnut): Evaluation of their antibacterial, antioxidant and drug release system activities. *Mat. Sci. Engin. C* 107, 110207.
- Sahoo, C.R., Maharana, S., Mandhata, C.P., Bishoyi, A.K., Paidsetty, S.K., Padhy, R.N., 2020. Biogenic silver nanoparticle synthesis with cyanobacterium *Chroococcus minutus* isolated from Bahiharachandi sea-mouth, Odisha, and *in vitro* antibacterial activity. *S. J. Biol. Sci.* 27 (6), 1580–1586.
- Z. Shah, S. Hassan, K. Shaheen, S.A. Khan, T. Gul, Y. Anwar, M.A. Al-shaeri, M. Khan, R. Khan, M.A. Haleem, H. Suo, Synthesis of AgNPs coated with secondary metabolites of *Acacia nilotica*: An efficient antimicrobial and detoxification agent for environmental toxic organic pollutants, *Mat. Sci. Eng. C*, 111, 2020, 110829.
- Li, R., Pan, Y., Li, N., Wang, Q., Chen, Y., Phisalaphong, M., Chen, H., 2020. Antibacterial and cytotoxic activities of a green synthesized silver nanoparticles using corn silk aqueous extract, *Colloids and Surfaces A: Physicochem. Eng. Asp.* 598, 124827.
- Kumar, V., Singh, S., Srivastava, B., Bhadoria, R., Singh, R., 2019. Green synthesis of silver nanoparticles using leaf extract of *Holoptelea integrifolia* and preliminary investigation of its antioxidant, anti-inflammatory, antidiabetic and antibacterial activities. *J. Environm. Chem. Eng.* 7, 103094.
- Ahila, N.K., Sri Ramkumar, V., Prakash, S., Manikandan, B., Ravindran, J., Dhanalakshmi, P.K., Kannapiran, E., 2016. Synthesis of stable nanosilver particles (AgNPs) by the proteins of seagrass *Syringodium isoetifolium* and its biomedical properties. *Biomed. Pharmacot.* 84, 60–70.
- Das, G., Patra, J.K., Shin, H.S., 2020. Biosynthesis, and potential effect of fern mediated biocompatible silver nanoparticles by cytotoxicity, antidiabetic, antioxidant and antibacterial, studies. *Mat. Sci. Eng. C* 114, 111011.
- Ravichandran, A., Subramanian, P., Manoharan, V., Muthu, T., Periyannan, R., Thangapandi, M., Ponnuchamy, K., Pandi, B., Marimuthu, P.N., 2018. Phyto-mediated synthesis of silver nanoparticles using fucoidan isolated from *Spatoglossum asperum* and assessment of antibacterial activities. *J. Photochem. Photob. B: Biology* 185, 117–125.
- Khorrani, S., Kamali, F., Zarrabi, A., 2020. Bacteriostatic activity of aquatic extract of black peel pomegranate and silver nanoparticles biosynthesized by using the extract. *Biocat. Agricult. Biotech.* 25, 101620.
- Hashemi, S.F., Tasharofi, N., Mahmoudi Saber, M., 2020. Green synthesis of silver nanoparticles using *Teucrium polium* leaf extract and assessment of their antitumor effects against MNK45 human gastric cancer cell line. *J. Molecul. Struct.* 1208, 127889.
- C. Sasikala, D. Geetha Ramani, Comparative study on antimicrobial activity of seaweeds, *Asi. J. Phar. Clin. Res.* 10, (2017), 1–3.
- Etcherla, M., Narasimha, G.M., 2014. Rao *in vitro* study of antimicrobial activity in marine algae *Caulerpa taxifolia* and *Caulerpa racemosa* (C. agardh). *Int. Appl. Biol. Pharm. Tech.* 5, 1–6.
- Mehra, R., Bhushan, S., Prasad Yadav, U., Bast, F., Singh, S., 2019. *Caulerpa taxifolia* inhibits cell proliferation and induces oxidative stress in breast cancer cells. *Biologia* 74, 187–193.
- Rajivgandhi, G.N., Maruthupandy, M., Li, J.L., Dong, L., Alharbi, N.S., Kadaikunnan, S., Khaled, J.M., Alanzi, K.F., Li, W.J., 2020. Photocatalytic reduction and antibacterial activity of biosynthesized silver nanoparticles against multi drug resistant *Staphylococcus saprophyticus* BDUMS 5 (MN310601). *Mat. Sci. Engin. C* 114, 111024.
- Venugopal, K., Ahmad, H., Manikandan, E., Thanigai Arul, K., Kavitha, K., Moodley, M. K., Rajagopal, K., Balabhaskar, R., Bhaskar, M., 2017. The impact of anticancer activity upon *Beta vulgaris* extract mediated biosynthesized silver nanoparticles (ag-NPs) against human breast (MCF-7), lung (A549) and pharynx (Hep-2) cancer cell lines. *J. Photochem. Photob. B: Biol.* 173, 99–107.
- Lateef, A., Ojo, S.A., Oladejo, S.M., 2016. Anti-candida, anti-coagulant and thrombolytic activities of biosynthesized silver nanoparticles using cell-free extract of *Bacillus safensis* LAU 13. *Process Biochem.* 51, 1406–1412.
- Nilavukkarasi, M., Vijayakumar, S., Prathip Kumar, S., 2020. Biological synthesis and characterization of silver nanoparticles with *Capparis zeylanica* L. leaf extract for potent antimicrobial and anti proliferation efficiency. *Mat. Sci. for Energy Technol.* 3, 371–376.
- Naveen kumar, S., Rajivgandhi, G., Ramachandran, G., Manoharan, N., 2018. A marine sponge *Fascaplysinopsis* sp. derived alkaloid fascaplysin inhibits the HepG2 hepatocellular carcinoma cell. *Front. Laborat. Med.* 2, 41–48.
- Rajivgandhi, G., Muneeswaran, T., Maruthupandy, M., Ramakritinan, C.M., Saravanan, K., Ravikumar, V., Manoharan, N., 2018. Antibacterial and anticancer potential of marine endophytic actinomycetes *Streptomyces coeruleorubidus* GRG 4 (KY457708) compound against colistin resistant uropathogens and A549 lung cancer cells. *Microb. Pathog.* 125, 325–335.
- Rajivgandhi, G., Maruthupandy, M., Quero, F., Li, W.J., 2019a. Graphene/nickel oxide nanocomposites against isolated ESBL producing bacteria and A549 cancer cells. *Mat. Sci. Engin. C* 102, 829–843.
- Nourozi, E., Hosseini, B., Maleki, R., Mandoulakani, B.A., 2019. Pharmaceutical important phenolic compounds overproduction and gene expression analysis in *Dracocephalum kotschyi* hairy roots elicited by SiO₂ nanoparticles. *Indust. Crop. Product.* 133, 435–446.
- Adyani, S.H., Soleimani, E., 2019. Green synthesis of Ag/Fe₃O₄/RGO nanocomposites by *Punica Granatum* peel extract: Catalytic activity for reduction of organic pollutants. *Int. J. Hyd. Energy.* 44, 2711–2730.
- Sumbal, A., Nadeem, S., Naz, J.S., Ali, A., Mannan, M., 2019. Zi, Synthesis, characterization and biological activities of monometallic and bimetallic nanoparticles using *Mirabilis jalapa* leaf extract. *Biotech. Rep.* 22, 00338.
- Kumara Swamy, M., Arumugam, G., Kaur, R., Ghazemzadeh, A., Yusoff, M.M., Sinniah, U.R., 2017. GC-MS based metabolite profiling, antioxidant and antimicrobial properties of different solvent extracts of Malaysian *Plectranthus amboinicus* Leaves. *Evid. Base. Complement. Alternat. Med.* 1517683, 1–10.
- Sharma, C., Ansari, S., Ansari, M.S., 2020. Single-step green route synthesis of Au/Ag bimetallic nanoparticles using clove buds extract: Enhancement in antioxidant bio-efficacy and catalytic activity. *Mat. Sci. Engin. C* <https://doi.org/10.1016/j.msec.2020.111153>.
- Jha, D., Thiruveedula, P.K., Pathak, R., Kumar, B., Gautam, H.K., Agnihotri, S., Sharma, A.K., Kumar, P., 2017. Multifunctional biosynthesized silver nanoparticles exhibiting excellent antimicrobial potential against multi-drug resistant microbes along with remarkable anticancerous properties. *Mat. Sci. Eng. C* 80, 659–669.
- Vemuri, S.K. et al., 2019. Novel biosynthesized gold nanoparticles as anti-cancer agents against breast cancer: Synthesis, biological evaluation, molecular modelling studies. *Mat. Sci. Engin. C* 99, 417–429.
- G.N. Rajivgandhi, G. Ramachandran, M. Maruthupandy, N. Manoharan, N. S. Alharbi, S. Kadaikunnan, J.M. Khaled, T.N. Almana, W.J. Li, Anti-oxidant, anti-bacterial and anti-biofilm activity of biosynthesized silver nanoparticles using *Gracilaria corticata* against biofilm producing *K. pneumoniae*, *Colloid. Surf. A: Physicochem. Engin. Asp.*, 600, 2020b, 124830.
- M. Shahid, R. Rajoka, H.M. Mehwish, H. Zhang, M. Ashraf, H. Fang, X. Zeng, Y. Wu, M. Khurshid, L. Zhao Z. He, Antibacterial and antioxidant activity of exopolysaccharide mediated silver nanoparticle synthesized by *Lactobacillus brevis* isolated from Chinese koumiss, *Colloid. Surf. B: Biointerf.* 186, 2020, 110734.
- Saber, M.M., Mirtajani, S.B., Karimzadeh, K., 2018. Green synthesis of silver nanoparticles using *Trapa natans* extract and their anticancer activity against A431 human skin cancer cells. *J. Drug Deliv. Sci. Technol.* 47, 375–379.
- Rajivgandhi, G., Maruthupandy, M., Muneeswaran, T., Anand, M., Quero, F., Manoharan, N., Li, W.J., 2019b. Biosynthesized silver nanoparticles for inhibition of antibacterial resistance and biofilm formation of methicillin-resistant coagulase negative *Staphylococci*. *Bioorg. Chem.* 89, 103008.
- Jalilian, F., Chahardoli, A., Sadrjavadi, K., Fattahi, A., Shokoohinia, Y., 2020. Green synthesized silver nanoparticle from *Allium ampeloprasum* aqueous extract: Characterization, antioxidant activities, antibacterial and cytotoxicity effects. *Advan. Powd. Technol.* 31 (3), 1323–1332.
- Zayed, M.F., Mahfoze, R.A., El-kousy, S.M., Al-Ashkar, E.A., 2020. *In-vitro* antioxidant and antimicrobial activities of metal nanoparticles biosynthesized using optimized *Pimpinella anisum* extract. *Colloid Surf. A: Physicochem. Engin. Aspects* 585, 124167.
- Khaleghi, M., Khorrani, S., Ravan, H., 2019. Identification of *Bacillus thuringiensis* bacterial strain isolated from the mine soil as a robust agent in the biosynthesis of silver nanoparticles with strong antibacterial and anti-biofilm activities. *Biocatal. Agricult. Biotech.* 18, 101047.
- Kumaresan, M., Vijai Anand, K., Govindaraju, K., Tamilselvan, S., Ganesh Kumar, V., 2018. Seaweed *Caulerpa taxifolia* mediated preparation of zirconia (ZrO₂) nanoparticles and their antibacterial activity against gram positive and gram negative bacteria. *Microb. Pathog.* 124, 311–315.
- Manjunath Huliker, M., Joshi, C.G., Danagoudar, A., Poyya, J., Kudva, A.K., Dhananjaya, B.L., 2017. Biogenic synthesis of gold nanoparticles by marine endophytic fungus *Cladosporium cladosporioides* isolated from seaweed and evaluation of their antioxidant and antimicrobial properties. *Process Biochem.* 63, 137–144.