Review Article

A Systematic Review of the Anti-proliferative Activity of Algae-synthesized Silver Nanoparticles against Cancer Cell Lines

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Submission Date: 19-06-2023; Revision Date: 14-08-2023; Accepted Date: 14-08-2023.

ABSTRACT

Context: Chemotherapeutic agents and radiation therapy have been acknowledged as the sole cancer treatment, even though they cause significant adverse reactions. Recent research has demonstrated that treatment-based nanoparticles can specifically target cancer cells, thereby overcoming these detrimental effects. Nanotechnology is a promising new anti-cancer treatment since it focuses primarily on the cytotoxic processes that induce apoptosis in cancerous cells. Algae, which can be subdivided into microalgae and macroalgae, appear to be the most suitable substrate for nanoparticle synthesis among the various plant divisions utilized thus, for the bioreduction of metal nanoparticles. **Objectives:** This review focuses on the essential elements and techniques involved in the algal-mediated synthesis of AgNPs, as well as the cytotoxicity of these biosynthesized silver nanoparticles generated by microalgae and macroalgae against various forms of human cancer. **Data Sources:** Data were acquired utilizing a variety of online databases, including ScienceDirect, PubMed Central, Springer Link, National Center for Biotechnology Information, and ResearchGate. The accumulating literature highlights the unique properties of AgNPs produced by various algae, their method of anti-proliferative action, and their cytotoxic evaluation of several cancer cell lines. **Results:** This review found that the antiproliferative effect of macroalga and microalga biosynthesized silver nanoparticles is dependent on particle size, concentration, and cancer cell lines tested. Since their high growth rate and short harvesting technique without causing damage to normal cells, macroalgae are more typically used to synthesize AgNPs than microalgae in this research.

Keywords: Silver nanoparticles, Cancer cell lines, Algae, Apoptosis, Biosynthesis.

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INTRODUCTION

Nearly every branch of science, including biology, computer science, chemistry, physics, materials science, and even engineering, utilizes nanotechnologies. Specifically, nanotechnology is an emerging technology in cancer treatment with many different *in vitro* studies producing favorable results.

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DOI: 10.5530/ajbls.2023.12.31

In the field of medicine, synthesized metallic nanoparticles like silver, gold, zinc, and magnesium have been broadly used for targeting and drug delivery due to the low cost of manufacturing, its physicochemical properties, simplicity of synthesis, and ease of characterization.[1] Silver nanoparticles are usually synthesized using different methods, such as physical and chemical synthesis. In the chemical reduction method, glucose is used as a reducing agent, silver nitrate as the source of nitrate, and polyvinyl alcohol as the stabilizing agent while using ultraviolet irradiation. Even though these methods are efficient at synthesizing nanoparticles, most are costly and require a capping reagent such as thiophenol and mercapto acetate to

prevent nanoparticle aggregation in which the majority of these capping agents are hazardous. Due to its cost-effectiveness, eco-friendly, and less time-consuming procedures, the biological synthesis of nanoparticles is the critical cause of this growing interest in using algae to synthesize nanoparticles.[2]

Green synthesis is more advantageous due to its low-cost manufacturing and less exposure to hazardous chemicals, such as thiourea and thiophenol, which are created in the laboratory through chemical methods. Therefore, green biosynthesizing methods are employed to create stable AgNPs incorporating different algae extracts as reducing and capping agents due to the natural component of algae such as phenols, enzymes, carboxylic compounds, and polysaccharides.[3]

Algae are polyphyletic and autotrophic, thus their ability to produce food-utilizing light, water, and carbon dioxide groups of photosynthetic eukaryotic creatures. They can be found in the vast majority of sea and freshwater environments and can be further classified as unicellular microalgae or multicellular and macroalgae, such as seaweeds, mostly based on their morphological traits.[4]

With cancer being one of the most life-threatening conditions today which causes approximately 10 million deaths it is known that cancer is the leading cause of mortality of 1 out of 6 deaths worldwide. Typically, chemotherapy and radiation therapy are used as a way to treat cancer by killing cancerous cells, however, these treatments are non-selective in which it can destroy normal human cells as well.

Due to these toxic treatments, there is a dire need for the medical field to continuously search for an alternative treatment that can effectively kill cancer cells while being a non-toxic substance in the human body. Nanoparticle treatments are appealing avenues for clinically relevant drug development.[5] These particles are known as cancer-specific targeting particles that induce fewer side effects and are capable of lysing cancer cells.[6]

In humans, silver nanoparticles have the mechanism to enter the cell through endocytosis, macropinocytosis, and phagocytosis in intracellular and paracellular pathways.[7] With their relatively small size and potent ability to destroy cancer cells, silver nanoparticles are a better option than other nanomaterials in this field.

This systematic review focuses on the important elements and procedures involved in the synthesis of AgNPs by algal-mediated processes and the cytotoxicity of silver nanoparticles synthesized by micro- and macroalgae against various types of human cancer. This paper discussed various studies but only had algal syntheses as its focus.

MATERIALS AND METHODS

Literature Search

In order to gather information for this review, the authors retrieved information through a variety of search engines. Such as ScienceDirect, The Institution of Engineering and Technology (IET), PubMed, Nature, Hindawi, International Journal of Pharmaceutical Sciences and Research, Longdom Publishing SL, Springer Nature, Multidisciplinary Digital Publishing Institute, Springer Link, National Center for Biotechnology Information (NCBI), and ResearchGate. Research journals and articles retrieved from these internet databases were consulted in March 2023 and assessed in April 2023 to verify the information gathered.

Eligibility Criteria

Articles fulfilling the following requirements were included in the systematic review: (1) studies that used MTT assay and half maximal inhibitory concentration (IC_{50}) to measure the cytotoxic activity of the silver nanoparticles; (2) published studies from reputable search engine sites like ResearchGate, Springer Link, National Center for Biotechnology (NCBI), PubMed, and ScienceDirect; (3) studies that are published in full-text. However, published research that is excluded from this systematic review meets one or more of the following criteria: (1) publications from before 2013; (2) publications from questionable/unreliable search engine websites; (3) research written in a language other than English; (4) studies that are not focused on the antiproliferative activity of the aforementioned silver nanoparticle; (5) articles and studies that did not utilize algae-synthesized silver nanoparticles against cancer cell lines.

Selection Strategy

The publication dates were also considered in filtering the used sources, considering the requirement to only access articles and journals from 2013 to 2023. Related works of literature were retrieved by inputting the combination of the following terms on reliable search engines, including "Algae-synthesized", Silver," "Nanoparticles," "Anti-proliferative," "Cancer," "Cancer cells," and "Cancer cell lines." Additionally, only English was employed in the study because it is the most accessible language to understand the published studies. The authors also carefully reviewed and evaluated the abstracts per the specified criteria, as they might be particularly significant and valuable to the study.

Data Extraction

The authors evaluated the inclusion-eligibility and irregularities of the selected publications. Before selecting each acceptable research article, exclusion and inclusion criteria were devised and taken into consideration. The following study characteristics were used to further extract data from the eligible articles: (1) the study or research article's title; (2) the year of publication; (3) the size and shape of the algae-synthesized silver nanoparticles; (4) a list of cancer cell lines and its corresponding organ of origin; (5) studies and articles that used the MTT assay; (6) the IC_{50} values of the cytotoxicity evaluation at 24 and 48 hr exposure.

RESULTS

A total of eighty-four (84) research papers were retrieved for the initial screening from the four search engines selected: NCBI, ScienceDirect, Springer Link, PubMed, and ResearchGate. A total of four (4) duplicate studies were manually removed, leaving eighty (80) pieces of study that were sought for retrieval once duplicate studies were found. Furthermore, seventy-four (74) studies remained to be evaluated after six (6) studies that were not available in full-text versions were eliminated. The authors screened each piece of literature's title, abstract, and year of publication based on the established inclusion and exclusion criteria, discarding twenty-four (24) research and leaving fifty (50) articles to be evaluated for eligibility. The relevant study characteristics, which were explicitly based on the type of algae (microalgae or macroalgae) that was used in biosynthesized silver nanoparticles, cancer cell lines, the most antiproliferative concentration used against the cancer cell lines, and IC_{50} values of the cytotoxicity evaluation, were used to evaluate each prospective eligible research paper. A total of forty-six (46) studies were included in this review following a full-text evaluation for eligibility.

Type of Algae used in Biosynthesis of Silver Nanoparticles

Based on the systematic review, Figure 1 presents the two kinds of algae used for biosynthesis from the forty-six (46) studies. The types of algae used are separated into two categories (2): microalgae and macroalgae. As presented in Figure 2, more studies used macroalgal species rather than microalgal species in the biosynthesis of silver nanoparticles. Of the forty-six (46) journal studies, thirty-two (32) made use of macroalgae (68%), and fourteen (14) used microalgae (32%).

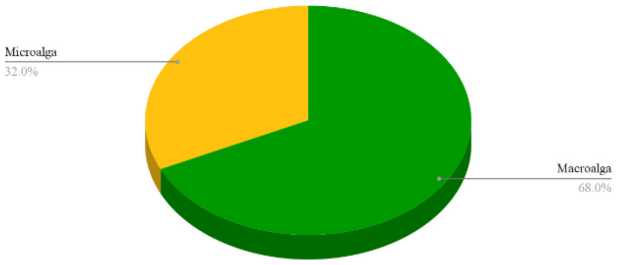

Figure 1: Types of Algae Used in Biosynthesis.

Algae against Different Cancer Cell Lines

Numerous cancer cell lines were utilized in this study to assess the anti-proliferative activity of algae-synthesized silver nanoparticles (AgNPs) against various known cancer cell lines. In this systematic review, a total of eighteen (18) established cancer cell lines were thoroughly sought and recorded, particularly carcinomas of the breast, colon, liver, and cervix. As seen in Figure 2, this review documented the following cancer cell lines: MCF-7 and MDA-MB-231 which were derived from breast cancer; HCT-116, HT-29, and Caco-2 which were derived from colon carcinoma; Hep-G2 was derived from hepatocellular carcinoma; HeLa was initially acquired from cervical cancer; PC-3 from prostatic adenocarcinoma; A549 and NSCLC from lung carcinoma; and AGS from gastric adenocarcinoma. However, the figure below did not display the percentages of the other cancer cell lines, such as Vero, EAC, Colo 205, HL60, T47D, 786-O, and HFB-4.

Figure 2 depicts the percentage of the cancer cell lines that were predominantly centralized by the studies included in this review. Most studies utilized the breast cancer cell line, MCF-7 (27.3%), the liver cancer cell line, Hep-G2 (12.1%), and the cervical cancer cell line, HeLa (10.6%). The least utilized cancer cell lines from the forty-six (46) accumulated studies included in this review were NSCLC, Vero, EAC, Colo 205, HL60, T47D, 786-O, and HFB-4, all of which accounted for 1.5%.

The human breast cancer cell line, MCF-7, is evidently the most frequently utilized in cytotoxicity studies. Comsa *et al.* (2015) stated that the aforementioned cell line has been proven to be a suitable model for cancer investigations, including cancer treatment drugs. This may be accounted for by its characteristics, such as its poorly-aggressive nature and non-invasiveness, that can be applied as a model of easy-stage cancer disease which may be advantageous in cancer-related investigations and studies.

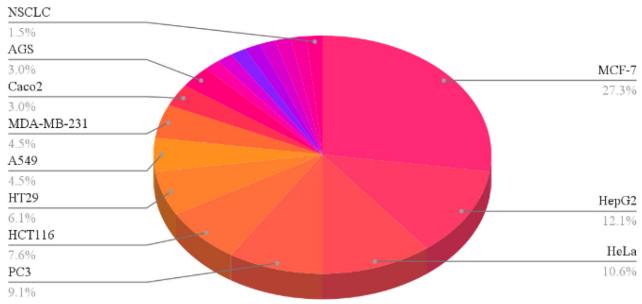

Figure 2: Algae against Different Cancer Cell Lines.

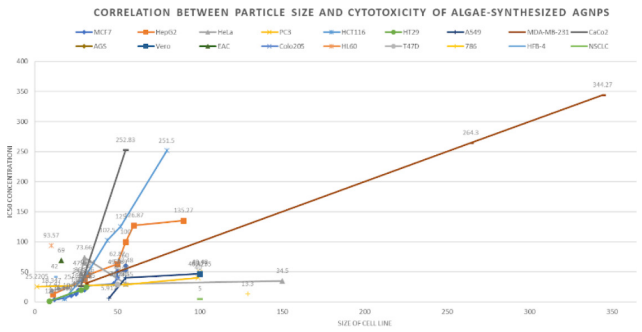

Figure 3: Correlation Between Particle Size and Cytotoxicity Value of Algae-synthesized AgNPs.

Size and Anti-Proliferative Activity of Algae-synthesized Silver Nanoparticles

The antiproliferative activity data of nanoparticles synthesized by algae demonstrated that AgNPs elicit an anti-metabolic activity and diminish cell viability against various NP sizes. Fifteen (15) cancer cell lines were reduced with algae-synthesized AgNPs with a size range of 10–30 nm which defines a more substantial anti-proliferative effect against cancer cell lines in terms of cell viability inhibition among the algae-synthesized AgNPs depicted in Figure 3. Moreover, fifteen (15) cancer cell lines were also reduced by AgNPs that have the size range of 31-60 nm, nine (9) cancer cell lines were reduced by AgNPs that have the size range of 61-100 nm, three (3) cancer cell lines were reduced by AgNPs that have the size range of >100 nm. Lastly, four (4) cancer cell lines were reduced by AgNPs that have a size range of <10 nm.

The IC_{50} values of algae-synthesized AgNPs were plotted against the corresponding particle size to correlate their anti-proliferative activity (Figure 3). According to Figure 3, there is a direct correlation between the particle size and the IC_{50} values of AgNPs produced by algae. This means smaller nanoparticles require a lower concentration to reduce cell viability by 50%, indicating a higher cytotoxicity potency. This anti-proliferative activity is observed in all 18 types of cancer cell lines.

However, a minor discrepancy was observed in the study of El-Sheekh *et al.* (2021), wherein smaller-sized (28 nm) algae-synthesized NPs induced a higher IC_{50} value (286.74 ug/mL) when compared to the larger-sized algae-synthesized AgNPs (30 nm) that requires a lower IC_{50} value (73.66 µg/mL) against HeLa cells (a cervix cancer cell line).

DISCUSSION

Types of Algae against Cancer Cell Lines

Due to its abundance in the environment, algae have grown interest in the field of nanotechnology. In this systematic review, macroalgae which are commonly known as seaweeds have been the most common source of biosynthesized silver nanoparticles which cover 68% of all the accumulated literature, while microalgae which are known as phytoplanktons comprised the 32% minority of the group. Since macroalgae are more economically significant, and a much easier sample to acquire due to seaweed forests, most researchers employ the use of them instead of microalgae which requires coagulation and flocculation to harvest. There is no concrete evidence that macroalgae induce a more cytotoxic effect than microalgae in this systematic review, however, in a pertinent study by Monteiro *et al.* (2020), macroalgae were observed to have a more antioxidant capacity than microalgae since phenolic compounds of microalgae do not appear to be the main contributors in its capability to induce antioxidant capacity.

Size in cytotoxicity of various cancer cell lines

The findings of this review demonstrated that the cytotoxic activity of silver nanoparticles varies depending on the types of cell lines they are exposed to when used against various cancer cell lines. Based on the journals presented, the particle size, which ranges from 10 to 30 nm, is directly proportional to the IC_{50} values of AgNPs. This means that the smaller the synthesized silver nanoparticles from macroalgae and microalgae, the lesser concentration of silver nanoparticles needed to induce cytotoxicity in different cancer cell lines.

Compared to larger nanoparticles, smaller nanoparticles have a larger surface area-to-volume ratio, which can increase their interaction with cancer cells. Moreover, cytotoxicity is increased when nanoparticles are smaller, which can penetrate through cell membranes. AgNPs can take on many shapes, but spherical AgNPs are the most commonly produced in cytotoxic studies. According to Helmlinger *et al.* (2016), spherical silver nanoparticles are less prone to oxidation and are more stable than other shapes like rods or triangles.

Subsequently, the elongated nanoparticles require a more protracted process of membrane wrapping than the spherical nanoparticles of similar size, which explains why spherical nanoparticles of the same size can be internalized more readily and much faster.[8] In the endocytosis process, the shape and size of an object have a direct relationship with its internalization within the cell.

Most Anti-Proliferative Concentration against Cancer Cell Lines

In relation to the illustrated results showing the different anti-proliferative concentrations of related literature, it has shown that biosynthesized silver nanoparticles inhibit proliferation at the peak concentration of >200 µg/ml after 24 hr of exposure to the cancer cell lines. This supports the idea that algal biosynthesized silver nanoparticles have a dose-dependent relationship with their ability to inhibit the proliferation of cancer cells. This follows the same trend as the positive control used by most of the studies which is cisplatin wherein viable cancer cells decrease as the dose of the cisplatin increases. This presents that the dose of the biosynthesized AgNPs has an inversely proportional relationship with cancer cell viability because the higher the dose, the lower cell viability is presented.

In addition, the most common anti-proliferative concentration at both 24 and 48 hr exposure is at >200 ug/mL. However, it is not well established whether the time intervals of the inoculation of silver nanoparticles increase the anti-proliferative activity of AgNPs against cancer cell lines. Subsequently, in a study by Acharya *et al.* (2020), they found that there is a 75% reduction of the anti-proliferative activity of silver nanoparticles after 48 hr of exposure in the cancer cell lines. This can also be supported by a study conducted by Jayaprakash *et al.* (2017) wherein there was a drop in anti-proliferative activity of the AgNPs solution from 49% inhibition to 8% inhibition after 48 hr. Due to this, it can be stated that the potential to inhibit the proliferation of cancer cells varies from each other depending on the cell type, particle size, and exposure time.[9] However, for most of the cases, the higher the concentration of the silver nanoparticles, the higher the inhibition of cell proliferation that happens.

CONCLUSION

Conclusively, the data gathered from different literature reveals eminent cytotoxic activities of macroalga and microalga biosynthesized silver nanoparticles against different cell lines. The systematic review revealed that the anti-proliferative activity of macroalga and microalga biosynthesized silver nanoparticles depends on the particle size, concentration, and cancer cell lines used. Between the two types of algae used in this study, macroalgae are more commonly used to synthesize AgNPs because of their rapid growth rate, low cost, and brief harvesting procedure without causing any cytotoxicity to normal, healthy cells.

Furthermore, besides the morphological characteristics of the silver nanoparticles (AgNPs), the potency of the AgNps depends on the cancer cell lines in which the following predominates in this study are MCF-7 (Breast), HepG2 (Liver), and HeLa (Cervix). The potency of the algae-synthesized AgNPs depends on the size and IC_{50}; a half-maximal inhibitory concentration that can induce antiproliferative activity in each cancer cell line. Because of this, the IC_{50} values of AgNPs are directly correlated with the particle size, which ranges from 10 to 30 nm. This implies that the smaller the synthesized silver nanoparticles from macroalgae and microalgae, the lower the concentration of silver nanoparticles required to induce cytotoxicity in various cancer cell lines.

ACKNOWLEDGEMENT

This systematic review is the result of the dedication and perseverance of all the authors who participated in the study; however, it would not have been possible without the guidance of several people.

We would like to convey our deepest gratitude to our research professors, Ma'am Ariane Laranang and Mr. Francis Lirag, for their helpful advice and preparations for this review study. We thank you for your guiding comments and suggestions that assisted us in polishing and completing this study. We also like to extend a sincere thank you to Ma'am Pamela Rose Bremner, our research adviser, for allowing us to work on this project and for her unwavering support and guidance as we endeavor to achieve our goals and objectives for this systematic study. We were able to write this review paper as plainly as we could, all thanks to your encouragement, perseverance, sincerity, and drive.

CONFLICT OF INTEREST

The authors can attest that no conflict of interests exist.

ABBREVIATIONS

AgNP: Silver Nanoparticles; **IC_{50}:** Half-maximal Inhibitory Concentration; **NP:** Nanoparticles; **MTT:** 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide.

SUMMARY

Recent research has revealed that a nanoparticle-based treatment can specifically target malignant cells, overcoming the disadvantages of traditional medicines such as radiation treatment and chemotherapy. These nanoparticles are a promising anti-cancer therapeutic since they primarily target the cytotoxic mechanism leading to cancer cell apoptosis. Silver nanoparticles (AgNPs) have various biomedical applications; their large surface area improves their ability to interact with biological cells, and this work describes their permeability and retention against malignant cells. A thorough screening procedure was utilized to determine whether papers and journals gathered from various databases satisfied the stated eligibility standards. The studies chosen for this review concentrated on the morphological influence of AgNPs in size and concentration-induced cytotoxicity. Because of differences in synthesis, purification, and characterization, nanoparticles can exist in various morphologies.

The findings demonstrated that AgNPs inhibited cancer cell proliferation by a variety of apoptosis-induced cytotoxic mechanisms, including DNA fragmentation, Reactive Oxygen Species (ROS)-induced cytotoxicity, mitochondrial-related apoptosis, and scavenging action against diverse cancer cell lines. According to the findings of this review, smaller nanoparticles are more efficient than larger particles because they can aggregate and penetrate tissues more effectively while requiring less silver nanoparticle concentration to elicit cytotoxicity in diverse cancer cell types.

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Cite this article: Millano, Jan Gabrielle, Moril, Katherine, Muro, Ricardo J, Narvaez, Audrey J, Nieves, Joy Ma. L, Nino, Jane E, Palo, Kenn R, Prias, Jhennifer Ma. CA, and Bremner, Rose P. A Systematic Review of the Anti-proliferative Activity of Algae-synthesized Silver Nanoparticles against Cancer Cell Lines. Asian J Biol Life Sci. 2023;12(2):224-9.