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Green Synthesis of Silver Nanoparticles (AgNPs) using dry Biomass of Green Alga Chlorella Ellipsoidea and their Photophysical, catalytic and Antibacterial Activity

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Abstract** - Silver nanoparticles (AgNPs) have a wide range of potential applications and can be produced in an environmentally sustainable and friendly manner using the dry biomass of the green alga Chlorella ellipsoidea. This paper aims to comprehensively investigate the photophysical properties, catalytic potential, and antibacterial activity of AgNPs synthesized through this method. In the realm of photophysics, the characteristics of AgNPs such as absorbance, fluorescence, and surface plasmon resonance are analyzed in detail to gain insights into their optical behavior. Understanding these properties is crucial for elucidating the interactions of AgNPs with light, which underpins their applications in various fields including sensors, imaging, and optoelectronics. Moreover, the catalytic activity of AgNPs is explored across different chemical reactions, highlighting their potential as catalysts. Due to their high surface area to volume ratio and unique surface properties, AgNPs exhibit catalytic capabilities in reactions such as reduction, oxidation, and hydrogenation, thus finding applications in green chemistry and industrial processes. Furthermore, the antibacterial efficacy of AgNPs against pathogenic microorganisms is evaluated, emphasizing their role as potent antibacterial agents. AgNPs possess inherent antimicrobial properties attributed to their small size and high surface area, which enable them to interact with bacterial cell membranes, disrupt cellular processes, and inhibit bacterial growth. This antimicrobial activity makes AgNPs promising candidates for various biomedical applications, including wound dressings, antimicrobial coatings, and drug delivery systems. Overall, this study provides valuable insights into the multifaceted applications of AgNPs synthesized using dry biomass of Chlorella ellipsoidea, showcasing their potential in photophysics, catalysis, and antibacterial activity. By leveraging the benefits of green synthesis and exploring the diverse properties of AgNPs, this research contributes to the advancement of green nanotechnology and the development of sustainable nanoparticle-based technologies.

***Keywords** - green synthesis, silver nanoparticles, antibacterial mechanisms.*

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1. INTRODUCTION

Nanotechnology is a rapidly growing field with several applications in science and technology, particularly the creation of novel nanomaterials and nanoparticles. These 1 nm to 100 nm-sized particles are used in biomedicine, agriculture, pharmaceuticals, textiles, food technology, catalysis, sensors, mechanics, electronics, and optics, among other scientific domains. Examples of nanoparticles include titanium dioxide, copper, iron, gold, zinc, cadmium sulphide, and silver. Each has a unique set of properties. Because of their many uses in biomedical science including drug delivery, wound dressings, biosensors, biocatalysis, and antibacterial, antifungal, antioxidant, and anti-cancer properties silver nanoparticles, or AgNPs, have drawn a lot of attention. The comprehensive exploration of AgNPs underscores their significance in scientific research over recent decades. Rajasekharreddy et al.(2020). Green synthesised AgNPs have demonstrated potent antibacterial, antioxidant, and anti-cancer properties in several recent investigations. AgNPs that were biosynthesized were also successfully utilised to break down a variety of harmful substances [9]. Green synthesised AgNPs can also be used for a wide range of other biotechnological applications, including food preservation, water filtering, sanitization, cosmetics manufacturing, and the synthesis of nanopesticides and nanoinsecticides Huq et al. (2022).

Silver nanoparticles (AgNPs) synthesized through environmentally friendly methods have demonstrated potent antibacterial properties against a spectrum of both Gram-positive and Gram-negative pathogenic bacteria. Salmonella epidermidis, Salmonella Typhimurium, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli, Bacillus subtilis, Vibrio parahaemolyticus, Streptococcus pneumoniae, Enterobacter hormaechei, Salmonella paratyphi, Klebsiella pneumoniae, Aeromonas hydrophila, Pseudomonas fluorescens, Flavobacter. The eco-friendly synthesis of AgNPs emerges as a promising avenue for combatting a diverse array of bacterial pathogens, highlighting its potential significance in addressing microbial challenges.

These nanoparticles are frequently synthesised using a variety of physical and chemical techniques, including chemical reduction, photochemical, electrochemical, physiochemical, and microwave irradiation. The primary disadvantages of these techniques are their high cost and potential for hazard due to the use of poisonous materials, expensive, work-intensive machinery, and the production of dangerous product or by-products Due to the severe limitations of physicochemical procedures, scientists are increasingly concentrating on biological methods for the simple, low-cost, non-toxic, and environmentally friendly synthesis of nanoparticles. Green synthesis is a cost-effective method that substitutes inexpensive, hazardous chemicals with natural molecules as reducing, capping, and stabilising agents. The green production of bioactive nanoparticles could make use of a variety of biological resources, including bacteria, fungi, algae, and plants and their various parts (fruit, leaves, and roots, for example) [20,21,22, 23]. The antibacterial activity of AgNPs synthesised environmentally using microorganisms or plant extracts has received a lot of attention lately.

Multidrug-resistant bacteria pose a major risk to global public health since they are the source of numerous infectious illnesses that can be fatal. The number of bacterial strains that are resistant to many treatments is constantly rising as a result of pollution, mutation, shifting environmental circumstances, and overuse of medications. Scientists are working to create novel medications to treat these kinds of microbial infections in an effort to solve this issue Huq et al. (2022). It has been discovered that green synthesised AgNPs work well in suppressing these bacterial strains that are resistant to many drugs. This paper presents a complete overview of the environmentally friendly production of silver nanoparticles (AgNPs) using a variety of biological resources. The article delves into the mechanisms underlying the biosynthesis of AgNPs, shedding light on their antibacterial applications, and presenting insights into the potential future developments and applications. Emphasizing the importance of stable, facile, and high-yield synthesis, the review considers various parameters crucial for achieving these objectives. This exploration not only contributes to the understanding of green synthesis processes but also anticipates promising avenues for the continued advancement and utilization of biosynthesized AgNPs.

Nanostructured metallic particles, characterized by a high surface-to-volume ratio and facile surface functionalization, exhibit fascinating opto-electronic, pharmacological, and catalytic effects. in addition to tuneable surface plasmon features.Applications of metal nanoparticles in chemical sensing, medicine, drug delivery, bioimaging, thermodynamics, water treatment, antibacterial, food packaging, nonlinear optical devices, catalysis, etc. have garnered a lot of interest recently. The potential applications of nanoparticles have sparked interest in new approaches to obtaining nanomaterials. Traditional physical and chemical synthesis techniques are frequently laborious, complex, and energy-intensive, with low conversion yield. In addition to being costly, these techniques are bad for the environment since they use organic solvents, dangerous reducing agents, and non-biodegradable stabilising chemicals Gupta et al. (2021). The newly generated nanoparticles become coated with the preparative chemicals, making them unsuitable for usage in clinical and biological settings. As a result, environmentally friendly methods for synthesising nanoparticles have been developed.

Alternative, environmentally benign methods for generating nanoparticles can be found in the synthesis process, which includes biological creatures like viruses, lichens, bacteria, fungi, and marine algae. It has now been established that these biological environments are ideal for the production of nanoparticles at room temperature with a pH that is naturally controlled. The phytochemicals act as stabilising and reducing agents to allow access to a variety of nanoparticle sizes, compositions, and forms. Extensive research has been conducted on environmentally sustainable approaches to produce nanoparticles using plant-based components. Numerous plant species, such as Prunus persica, Allium sativum (garlic), Curcuma longa (turmeric), Cinnamomum camphora, Jatropha curcas, Emblica officianalis, Aloe vera, Garcinia indica, Agrewia optiva, and Cymbopogan citratus (lemongrass) have all been utilised in these ways (tea leaves). Additionally, algae such as diatoms and cyanobacteria have been harnessed for the eco-friendly synthesis of green nanomaterials..

Silver nanoparticles, also known as AgNPs, are an inorganic nanomaterial that exhibit superior material properties and functional versatility. This versatility offers applications in a wide range of industries, including antimicrobials, biosensors, catalysts, composite fibres, cryogenic superconductors, cosmetics, electrical components, and nonlinear optics. For example, compared to other metallic nanoparticles, nanoscale silver particles exhibit a high level of anti-microbial activity against viruses, bacteria, and other eukaryotic microorganisms. As a result, these particles have gained significant relevance. In light of this, we report herein the environmentally benign and photo-catalytic production of silver nanoparticles mediated by green algae, as well as their antibacterial properties Gupta et al. (2021).

The rapid advancement of nanotechnology has ushered in a new era of scientific exploration and innovation, offering promising solutions to a myriad of challenges across various fields. Among the myriad applications of nanomaterials, silver nanoparticles (AgNPs) have garnered considerable attention due to their unique physicochemical properties and versatile applications. In particular, the synthesis of AgNPs via environmentally sustainable methods has emerged as a focal point of research, driven by the imperative to mitigate the ecological impact of conventional synthesis routes.

In this context, the eco-friendly synthesis of silver nanoparticles (AgNPs) using biological material has surfaced as a promising approach, leveraging the inherent reducing and stabilizing properties of natural compounds derived from plants, microorganisms, and algae. Among these biological resources, the green alga Chlorella ellipsoidea is a promising contender for the environmentally benign manufacture of AgNPs. The use of dry biomass from Chlorella ellipsoidea provides a sustainable alternative to standard synthesis methods, minimising the usage of harmful chemicals and reducing the environmental impact associated with nanoparticle manufacturing. Singh, et al. (2018). Green synthesis of AgNPs from dry Chlorella ellipsoidea biomass, with a focus on photophysical properties, catalytic potential, and antibacterial activity. We hope to explain the potential uses of AgNPs synthesised using this method by leveraging their distinctive features in a variety of domains, including catalysis and biomedicine. Additionally, this study aims to shed light on the underlying mechanisms governing the synthesis process and the antibacterial action of AgNPs against pathogenic microorganisms. through comprehensive analysis and experimentation, we endeavor to contribute to the growing body of knowledge surrounding green nanotechnology and its implications for sustainable development. By harnessing the power of nature to drive the synthesis of AgNPs, we aim to pave the way for the development of eco-friendly nanomaterials with diverse applications and minimal environmental impact. Ultimately, this research endeavors to propel the field of green synthesis towards a more sustainable and responsible future, where scientific innovation coexists harmoniously with environmental stewardship.

2. REVIEW OF LITERATURE

Huq et al. (2022) The ecologically sustainable synthesis of silver nanoparticles (AgNPs) through the utilisation of biological resources is a highly feasible, economical, and sustainable option that circumvents the limitations of chemical and physical approaches. Bioactive AgNPs can be produced quickly and non-toxically using a variety of biological sources, example as plants, bacteria, fungi, and algae. Plant extracts serve as capping and reducing agents throughout the synthesis method. They are abundant in a variety of biomolecules, including phenolic chemicals, alkaloids, terpenoids, and flavonoids. The production of AgNP also involves the contribution of a variety of metabolites from microorganisms that function as reducing and capping agents. AgNPs that have been biosynthesized have drawn a lot of interest due to their prospective uses in a variety of biomedical domains, namely in the treatment of bacterial infections, especially in light of the increasing prevalence of germs that are resistant to drugs. Due to their tiny size and large surface area, which allow for simple penetration of bacterial cell walls, several investigations have shown the bio-synthesized AgNPs' remarkable antibacterial activity against a wide range of human diseases. AgNPs cause bacterial cell death by rupturing cell membranes, producing reactive oxygen species, and impeding vital functions like protein synthesis and DNA replication after internalisation. In conclusion, this work offers a thorough review of the environmentally friendly and quick synthesis of AgNPs from biological resources, highlighting their antibacterial characteristics and clarifying the underlying mechanisms of action.

Gupta et al. (2021) This research outlines the significant antibacterial characteristics of silver nanoparticles (AgNPs) and discusses their environmentally friendly manufacturing. With an emphasis on ecologically acceptable techniques that use plant extracts, microbial agents, and other biological resources as both reducing and capping agents, it provides a thorough review of current advancements in AgNP synthesis. The document delves into the mechanisms by which biosynthesized AgNPs exhibit antibacterial effects, encompassing the disruption of bacterial cell membranes, generation of reactive oxygen species, and interference with cellular processes like DNA replication and protein synthesis. Additionally, the review explores the potential applications of antibacterial AgNPs across various sectors, including healthcare, food packaging, and water treatment, underscoring their pivotal role in combating infectious diseases and addressing challenges related to antibiotic resistance.

Rajasekharreddy et al. (2020) The goal of this work is to synthesise silver nanoparticles in an environmentally friendly manner by using plant extracts as capping and reducing agents. It offers a comprehensive overview of the synthesis methodologies employed, encompassing diverse extraction techniques for obtaining bioactive compounds from plants and their subsequent application in AgNP synthesis. The paper examines the crucial role played by phytochemicals present in plant extracts, including polyphenols, alkaloids, and terpenoids, in facilitating the reduction and stabilization of AgNPs. Furthermore, it underscores the potential applications of plant-mediated AgNPs in biomedical, agricultural, and environmental domains, with a particular emphasis on their antibacterial, antifungal, and antioxidant properties.

Kumar et al. (2019) The biogenic production of silver nanoparticles and its possible uses in the biomedical industry are thoroughly examined in this work. It provides a thorough elucidation of the mechanisms involved in the green synthesis of AgNPs utilizing biological resources, with a particular emphasis on the contributions of biomolecules derived from plants, microbes, and other organisms in the formation of nanoparticles. The review delves into the physicochemical properties of biosynthesized AgNPs, including their size, shape, and surface characteristics, and discusses their impact on various biomedical applications such as drug delivery, imaging, and wound healing. Additionally, the paper highlights the antibacterial activity of AgNPs, emphasizing their effectiveness against multidrug-resistant pathogens and positioning them as potential alternatives to conventional antibiotics.

Singh et al. (2018) This paper provides a thorough description of the biological synthesis of nanoparticles using microbes and plant extracts. It delves into various green synthesis methods, encompassing extracellular and intracellular routes facilitated by diverse biomolecules sourced from biological entities. The review elucidates the fundamental principles governing nanoparticle synthesis, employing plant extracts abundant in phytochemicals and microbial cultures capable of generating metabolites with reducing and capping properties. Moreover, it underscores recent advancements in the field, including the utilization of genetically engineered microorganisms to enhance nanoparticle synthesis, and discusses the potential applications of biosynthesized nanoparticles across diverse fields such as medicine, agriculture, and environmental remediation.

3. MATERIAL AND METHOD

3.1 Reagents and chemicals

Sigma Aldrich, USA, provided the methyl orange, methylene blue, and silver nitrate that were utilised. Rankem grade water, whereas TCI, Japan supplied sodium borohydride.

3.2 The process of making algae powder

The algae Chlorella ellipsoidea was isolated, identified, and cultured at an algal germplasm unit using water taken from residential sewage systems. Following a thorough cleaning with distilled water, the wet algae was subjected to drying in the shade. Subsequently, the freshwater algae was finely ground into a powder using a pestle and mortar once it had completed the drying process. The synthesis of nanoparticles was directly conducted using the powdered algae.

3.3 Synthesis of silver nanoparticle

A 100 ml aqueous solution containing 10^{-3}M silver nitrate was mixed with around 1 g of dry algal powder and let to stand for 12 hours at room temperature. By keeping an eye out for a colour shift and taking UV-vis spectra at regular intervals, the course of the reaction was tracked. The creation of silver nanoparticles was indicated by the gradual browning of the initially colourless silver nitrate solution. When the solution became dark brown and showed a clear surface plasmon resonance (SPR) peak after 12 hours, the procedure was complete. Centrifugation was used to gather the silver nanoparticle-containing supernatant, and the residual algal biomass was discarded. The bio-matrix loaded AgNPs were then extracted by evaporating the supernatant, rinsing the residue several times with distilled water, and letting it air dry.

3.4 Characterization AgNPs'

Several microscopic and spectroscopic methods were employed to analyse and describe the synthesised AgNPs. A Fourier transform infrared (FT-IR) spectrum spanning wavelengths from 400 to 4000 cm^{-1} was obtained using a Shimadzu Prestige-21 FT-IR spectrophotometer equipped with a KBrdisk. A UV–vis spectrophotometer (Jasco V-700) was used to measure the bioreduction of Ag^+ ions to Ag^0 (AgNPs) at different time intervals. Transmission electron microscopy (TEM) images of the as-synthesised AgNPs were taken with a Jeol JEM2100 equipment. The presence of elemental silver in the synthesised nanoparticles was confirmed by energy-dispersive X-ray spectroscopy (EDX) analysis, which was performed with an acceleration voltage of 20 kV and an acquisition length ranging from 60 to 100 s.

3.5 Photocatalytic Function

Methyl orange and methylene blue, two dangerous dyes, were broken down by the produced AgNPs' photocatalytic activity when exposed to UV light. The higher energy of UV light was expected to enhance the efficiency of the reduction process. A common protocol involved mixing 1 millilitre of freshly made NaBH4 solution with 25 millilitres of each dye's 10−4M stock solution. To the resultant solution, colloidal AgNPs (1 mg AgNPs dissolved in 1 ml water) were added. Under UV light, the AgNP-containing sample progressively changed colour, shifting from orange to colourless for the methyl orange (MO) dye and from deep blue to light blue to colourless for the methylene blue (MB) dye. A UV-vis spectrophotometer was used to regularly evaluate the decrease in dye concentration. Normal temperature was used for the experiment.

3.6 Antimicrobials Study

The agar well plate diffusion method was used for the antibacterial activity assay against Klebsiella pneumoniae, Escherichia coli, and Pseudomonas aeruginosa, as well as Gram-positive bacteria such as Staphylococcus aureus. Each bacterial culture was injected into Muller Hinton agar following a 24-hour incubation period on nutrient agar. Paper discs containing 5, 10, 15, and 20 μg/ml nanoparticles were prepared and filled using an aseptic micropipette. After that, the plates were kept in the dark at 37°C for a whole day. AgNP-free blank paper discs were used as a negative control in this experiment. Millimetres were used to express the zone of inhibition's diameter.

3,7 Data interpretation

The OriginPro 9.0 programme was utilised to conduct the data analysis for this research. All tests were carried out in triplicate to guarantee the results' dependability and reproducibility, and the data are shown as means ± standard deviation. Testing in triplicate improves the dependability of the data obtained and reduces experimental mistakes. A central measure of the data is provided by the mean value, and the degree of variation or dispersion around the mean is shown by the standard deviation. In addition to helping to depict the results' central tendency, this statistical presentation sheds light on the accuracy and consistency of the experimental findings. The correctness and validity of the study's conclusions are increased when the experimental data are examined thoroughly and rigorously using OriginPro 9.0.

4. RESULT AND DISCUSSION

4.1 AgNP synthesis using the Chlorella ellipsoidea platform

The algal species, with dimensions of 9 μm for length and 7 μm for width, was extracted from the polythene surface of wastewater collected from residential areas. They possessed a dry cell weight of 1 g L−1 and generated 3.4 g of fresh biomass daily. UV-visible spectrophotometry was used to quantitatively assess a wide range of phytochemicals, including chlorophyll-a, carotenoids, carbohydrates, protein, lipids, exopolysaccharides, vitamin C, phenol, flavonoids, polyphenols, and tannins. The purposeful combination of Ag+ ions and dry algae powder in an aqueous solution produced silver nanoparticles (AgNPs). At ambient temperature, this process converted the silver ions to metallic silver (Ag). Phenolic-OH groups from hydrolyzable phytochemicals were identified as being essential to the reduction pathway as they bound Ag+ ions, oxidised them to quinone, and finally reduced Ag+ to Ag0. Algal phytochemicals played a dual role in stabilising the silver nanoparticles by coating their exterior and actively participating in the chemical reduction process. By taking advantage of the natural phytochemical composition of algae, this platform-based method to nanomaterial manufacturing offers a unique and heuristic means of effectively reducing and stabilising AgNPs.

Table 1. lists the phytochemicals that Chlorella ellipsoidea contains.

Phytochemicals	Concentration (μgml=1)
Chlorophyll a	1.4 ± 0.20
Carotenoids	2.9 ± 0.30
Carbohydrates	209 ± 0.67
Protein	93 ± 0.11
Lipid	2.8 ± 0.18
Exopolysaccharides	18.2 ± 0.33
Vitamin C	0.93 ± 0.01
Phenol	22.7 ± 0.17
Flavonoid	8.9 ± 0.03
Polyphenol	2.1 ± 0.02
Tannin	6.8 ± 0.18

- **Study of the UV–VIS spectrum**

The Ag+ ions in the AgNO3 solution were assimilated by C. ellipsoidea, which used enzymes or other biochemicals produced by cellular activity to reduce them to their elemental state, resulting in nanoparticles. The observation that the solution's colour changed from colourless (the start of biosynthesis) to brown over the course of a 12-hour period during the reduction process (Ag+ to Ag0) was corroborated by the emergence of a large peak at 436 nm in the UV-vis spectrum. The generation of elemental nanosilver was suggested by the development of a surface plasmon at 436 nm, which was brought on by the activation of free conduction electrons in AgNPs' outermost orbitals. The AgNP solution's consistent brown hue, which developed within 12 hours and remained unchanged over the course of the 15-day investigation, proved the stability of the nanoparticles. The polydispersity and size distribution of the nanoparticles were confirmed by the wide shape of the SPR peak, which featured a tail in the longer wavelength region.

- **Antimicrobial efficaciousness**

The antibacterial effectiveness of the synthesised silver nanoparticles (AgNPs) against three pathogenic Gram-negative bacteria (E. coli, K. pneumoniae, and P. aeruginosa) and one Gram-positive bacterium (S. aureus) was evaluated using the disk-diffusion method. Figure 8a demonstrates how AgNPs greatly inhibited the growth of bacteria. Despite the strong peptidoglycan-containing cell wall of Gram-positive S. aureus being generally impervious to nanoparticles, the as-synthesised AgNPs effectively prevented the bacteria's growth, exhibiting 15 mm zones of inhibition in 10, 15, and 20 µg/mL solutions. Notably, different zones of suppression were detected in Gram-negative bacteria after AgNP treatment, owing to their thin lipid coats, which allow for easy nanoparticle entrance and disruption. P. aeruginosa showed distinct zones of inhibition (16 mm) at all dosages, indicating increased sensitivity to the synthesised AgNPs. K. pneumoniae showed the narrowest zone of inhibition (8 mm) at 5 µg/ml concentration, regardless of the AgNP concentration utilised. E. coli's zone of inhibition remained stable at 9 mm regardless of AgNP concentration. Overall, the AgNPs coated with as-synthesized phytochemicals displayed considerable activity against all four strains at low doses. The antibacterial properties were attributed to the free radicals generated on the particle surface, causing membrane damage. This study aligns with recent research, indicating a strong correlation between the antibacterial activity of silver nanoparticles and various investigations.

5. CONCLUSIONS

Using the green alga C. ellipsoidea, a fast-growing, renewable bioresource, a simple, inexpensive green technique was used to successfully synthesise biocompatible silver nanoparticles in an aqueous media. Nontoxic, moderate phytochemicals are used in the preparation procedure, and no external stabiliser or reductant is needed. The AgNPs as synthesised are primarily spherical and have a crystalline structure. It has been shown that these nanoparticles are effective photocatalysts for the destruction of methyl orange and methylene blue, two water-soluble pollutants. Application potential for the dye degradation process in the removal of toxic dyes from industrial effluents is evident. Furthermore, the phytosynthesized silver nanoparticles (AgNPs) showed outstanding efficiency against four harmful bacterial strains, indicating their potential as antibacterial agents. This suggests that the current method of synthesising AgNPs using algae has the potential for large-scale production and could be expanded to investigate additional metallic nanoparticles, aligning with the pursuit of cleaner, nontoxic, and environmentally friendly approaches to nanomaterial synthesis. The demonstrated antibacterial efficacy of phytosynthesized AgNPs emphasises their potential uses in a variety of domains, emphasising the need of ecologically friendly nanoparticle synthesis methods.

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