



SEAWEED (SARGASSUM MUTICUM) MEDIATED GREEN SYNTHESIS OF AG₂O NANOPARTICLES AND THEIR ANTIBACTERIAL PROPERTIES.

Vaishali Babu^{*1}, L. Keerthi Sasanka², Sathish Kumar Ramachandran³

¹Department of prosthodontics, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai-600 077, Tamil Nadu, India.

Email id: 152001007.sdc@saveetha.com

²Senior Lecturer, Department of prosthodontics, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai-600 077, Tamil Nadu, India.

³Professor, Department of Prosthodontics and Materials Science, Saveetha Dental College and hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai-600077, Tamil Nadu, India.

***Corresponding author;** Ashok K. Sundramoorthy,

Professor, Department of Prosthodontics and Materials Science, Saveetha Dental College and hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai-600077, Tamil Nadu, India.

ABSTRACT:

Introduction : Sargassum muticum is an invasive brown seaweed rich in bioactive metabolites with antimicrobial potential. Its abundance along coastal regions makes it a sustainable resource for green nanotechnology. Silver oxide (Ag₂O) nanoparticles are well recognized for their antibacterial activity, and seaweed-mediated synthesis provides an eco-friendly alternative to conventional chemical methods. This study explores the use of Sargassum muticum extract for the green synthesis of Ag₂O nanoparticles and evaluates their antibacterial efficacy.

Aim : To establish a green synthesis route for Ag₂O nanoparticles using Sargassum muticum extract and to evaluate their antibacterial, hemocompatibility, and antioxidant properties.

Materials and methods : Ag₂O nanoparticles were synthesized by reacting silver nitrate with S. muticum aqueous extract, followed by purification. Characterization was performed using UV-Vis spectroscopy, XRD, FESEM, and EDAX. Antibacterial activity was tested against Streptococcus mutans and Escherichia coli using disc diffusion assays. Hemocompatibility was assessed via hemolysis testing, and antioxidant activity was evaluated using the DPPH radical scavenging assay.

Results : The synthesized Ag₂O nanoparticles exhibited a distinct UV-Vis absorption band at 420–441 nm, confirming nanoparticle formation. FESEM revealed spherical particles ranging from 43–79 nm, while EDAX confirmed silver and oxygen composition. Antibacterial assays showed significant inhibition zones against both Gram-positive and Gram-negative strains. Hemolysis values (1.8–2.9%) remained below the 5% threshold, confirming blood safety. Antioxidant activity reached 75.8% compared to 89.2% for ascorbic acid.

Conclusion : The green synthesis of Ag₂O nanoparticles using Sargassum muticum extract yielded crystalline, biocompatible nanomaterials with potent antibacterial and antioxidant activity. These findings suggest that eco-friendly Ag₂O nanoparticles are promising candidates for oral healthcare

and systemic infection control, providing a sustainable alternative to conventional antimicrobial strategies.

Keywords : Ag₂O nanoparticles, Green synthesis, Antibacterial activity, Hemocompatibility, Antioxidant potential

INTRODUCTION :

Sargassum muticum is a brown macroalga native to Japanese coastal waters, where it remains relatively small and ecologically benign (1). Following its introduction into Europe, however, it has become a highly invasive species, capable of attaining lengths up to 10 m, though most mature thalli range between 2–3 m (2). Its rapid spread along temperate shorelines has raised ecological concerns, but it also presents opportunities as a bioresource (3). Seaweeds are increasingly valued as functional foods owing to their abundance of minerals, lipids, vitamins, and diverse bioactive constituents such as polysaccharides, proteins, and polyphenols (4). These compounds exhibit therapeutic potential against chronic conditions including cancer, metabolic disorders, cardiovascular disease, and inflammatory pathologies (5).

Brown algae, in particular, are prolific producers of secondary metabolites with wide-ranging biological activities—antibacterial, antifungal, antitumoral, antifouling, and antioxidant among them. Their chemical repertoire includes sterols, terpenoids, phenolics, fatty acids, acrylic acid derivatives, and alkaloids, many of which act as potent antimicrobials (6). The genus *Sargassum* is especially notable for synthesizing high-molecular-weight phenolic compounds that demonstrate antifouling, antiproliferative, antiangiogenic, and antioxidant properties (7). These attributes highlight *S. muticum* not only as an ecological invader but also as a promising source of bioactive molecules with potential applications in food, feed, and biomedical innovation (8,9).

Biogenic synthesis has enabled the eco-friendly production of metal nanoparticles such as gold, silver, copper, zinc, and titanium. Silver oxide nanoparticles (Ag₂O NPs) are particularly important because of their strong antibacterial activity, achieved by disrupting bacterial membranes, blocking DNA replication, and interacting with phytochemicals that enhance effectiveness. Beyond antimicrobial use, silver nanostructures have been applied in anticancer therapy, water purification, and other biomedical fields, with their performance largely determined by particle size, shape, and surface chemistry (10). Silver also plays a central role in nanomaterial research due to its applications in sensing, optoelectronics, catalysis, and drug delivery. Conventional chemical synthesis methods are expensive, time-consuming, and rely on hazardous reagents, underscoring the need for safer and more sustainable alternatives. Plant-mediated green synthesis provides a sustainable, rapid, and inexpensive route to produce stable nanoparticles, using phytochemicals as natural reducing and stabilizing agents (11).

The present work aims to synthesize Ag₂O nanoparticles using a green, plant-mediated approach and to evaluate their antibacterial properties against selected pathogenic microorganisms. The study focuses on optimizing synthesis conditions, characterizing the nanoparticles, and investigating their antimicrobial mechanisms to establish Ag₂O NPs as a potential eco-friendly alternative for biomedical applications.

MATERIALS AND METHODS :

2.1 Collection and Preparation of Seaweed

Dried specimens of *Sargassum muticum* were procured from a certified local supplier in Chennai, Tamil Nadu. The authenticity of the species was verified by morphological inspection and cross-checked against standard taxonomic references to ensure sample reliability. The dried biomass was visually examined to exclude any contaminated or degraded material.

The seaweed was then pulverized into a fine powder using a sterile mechanical grinder under aseptic conditions to minimize microbial contamination. The powdered material was passed through a mesh sieve to obtain uniform particle size, ensuring reproducibility in subsequent extraction and synthesis

steps. The processed biomass was stored in airtight, sterile containers at controlled room temperature (25–30 °C) to prevent moisture absorption and degradation of bioactive compounds until further use.

2.2 Preparation of *Sargassum muticum* Aqueous Extract

Ten grams of powdered seaweed biomass was mixed with 100 mL of distilled water and heated at 80 °C for 15 minutes under continuous stirring. The mixture was cooled to room temperature and filtered through Whatman No. 1 filter paper. The filtrate was collected and stored at 4 °C until further use. This aqueous extract served as a natural reducing, capping, and stabilizing agent during nanoparticle synthesis.

2.3 Green Synthesis of Ag₂O Nanoparticles

Silver nitrate (AgNO₃, analytical grade) was used as the precursor. A 1 mM AgNO₃ solution was prepared in distilled water. Ninety milliliters of this solution was placed under continuous magnetic stirring, and 10 mL of *Sargassum muticum* extract was added dropwise. The mixture was stirred at room temperature for 2 hours. A visible color change from pale yellow to dark brown indicated nanoparticle formation.

The nanoparticles were separated by centrifugation at 10,000 rpm for 15 minutes. The pellet was washed three times with distilled water and once with ethanol to remove residual biomolecules and unreacted precursors. Purified nanoparticles were dried in a hot-air oven at 60 °C for 12 hours and stored in sterile containers for characterization and antibacterial studies.

2.4 Characterization of Ag₂O Nanoparticles

UV–Visible Spectroscopy

The optical characteristics and formation of the synthesized nanoparticles were evaluated using a UV–Visible spectrophotometer (Jasco V730, Japan). Spectral measurements were carried out across the wavelength range of 200–1,000 nm, employing distilled water as the reference blank. The emergence of a distinct absorption band served as evidence for successful nanoparticle synthesis.

Field Emission Scanning Electron Microscopy (FESEM)

The morphology, particle size distribution, and structural attributes of the synthesized nanoparticles were characterized using Field Emission Scanning Electron Microscopy (FESEM, JEOL JSM-1800). For imaging, dried nanoparticle samples were mounted on conductive carbon tape and subsequently sputter-coated with a thin gold layer to minimize charging effects and enhance image clarity.

Energy Dispersive X-ray Analysis (EDAX)

The elemental composition of the synthesized nanoparticles was analyzed using Energy Dispersive X-ray Analysis (EDAX) integrated with FESEM. Characteristic peaks corresponding to silver and oxygen were observed, and their relative abundance verified the successful formation of Ag₂O nanoparticles.

2.5 Antibacterial Activity of Ag₂O Nanoparticles

The antibacterial efficacy of the synthesized Ag₂O nanoparticles was assessed against Gram-positive *Streptococcus mutans* ATCC 35668 and Gram-negative *Escherichia coli* ATCC 25922 using the agar disc diffusion assay. Overnight bacterial cultures were prepared in Mueller–Hinton broth at 37 °C and standardized to a 0.5 McFarland turbidity ($\approx 1.5 \times 10^8$ CFU/mL). Sterile Mueller–Hinton agar plates were uniformly seeded with the inoculum using sterile cotton swabs.

Sterile paper discs (6 mm diameter) were impregnated with Ag₂O nanoparticle suspension (50 µg/disc) and placed on the inoculated agar surface. Commercial antibiotic discs served as positive controls, while discs loaded with sterile distilled water acted as negative controls. The plates were incubated at 37 °C for 24 h.

Following incubation, the diameters of inhibition zones surrounding each disc were measured in millimeters using a digital caliper. All experiments were conducted in triplicate, and the results were expressed as mean $\pm$ standard deviation to ensure statistical reliability.

2.6 Statistical Analysis

All experiments were carried out in triplicate, and data were expressed as mean $\pm$ standard deviation (SD). Statistical analysis was performed using SPSS version 23.0 software. Differences between groups were evaluated using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test. A value of $p < 0.05$ was considered statistically significant.

RESULTS :

Figure 1: UV–Vis Spectra of Ag Nanoparticles

The UV–Vis absorption spectrum of biosynthesized silver nanoparticles was recorded in the range of 200–1,000 nm. A distinct absorption peak was observed at 441 nm, corresponding to the surface plasmon resonance (SPR) of Ag⁰ nanoparticles. This peak position falls within the characteristic range of silver nanoparticles (420–450 nm) reported in earlier studies, thereby indicating the successful bioreduction of Ag⁺ ions into stable Ag⁰ nanoparticles. The spectral profile, with a sharp and well-defined peak, reflects uniform nanoparticle formation and indicates the efficiency of the synthesis process.

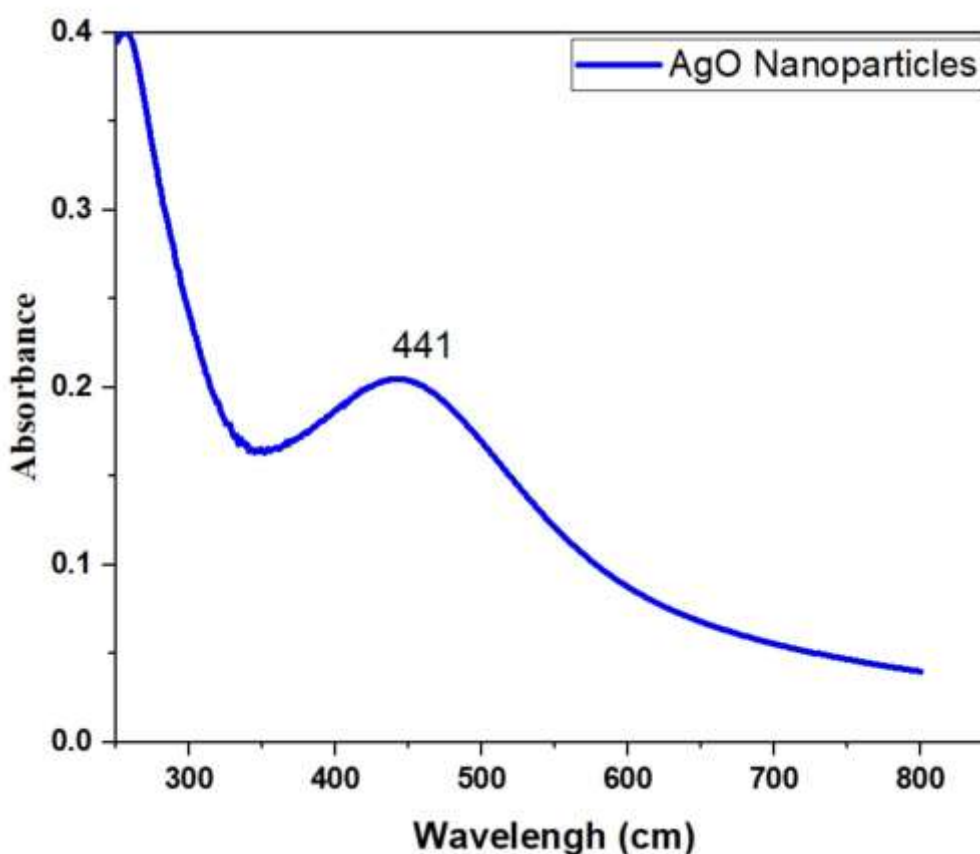


Figure 2 : FESEM and EDAX Analysis of Ag Nanoparticles

FESEM analysis revealed that the synthesized silver nanoparticles exhibit a uniform cubical morphology with sharp edges, clustered arrangements, and consistent size distribution (scale bar: 0.5 μ m). The EDAX spectrum indicated dominant silver (Ag) peaks (~91.4 wt%), together with minor oxygen (5.1 wt%) and carbon (3.5 wt%) signals. The oxygen presence is attributed to surface oxidation forming silver oxide (AgO), while carbon residues likely originate from organic precursors.

or environmental adsorption. These combined morphological and elemental findings indicate the crystalline integrity, compositional purity, and stability of the synthesized Ag nanoparticles.

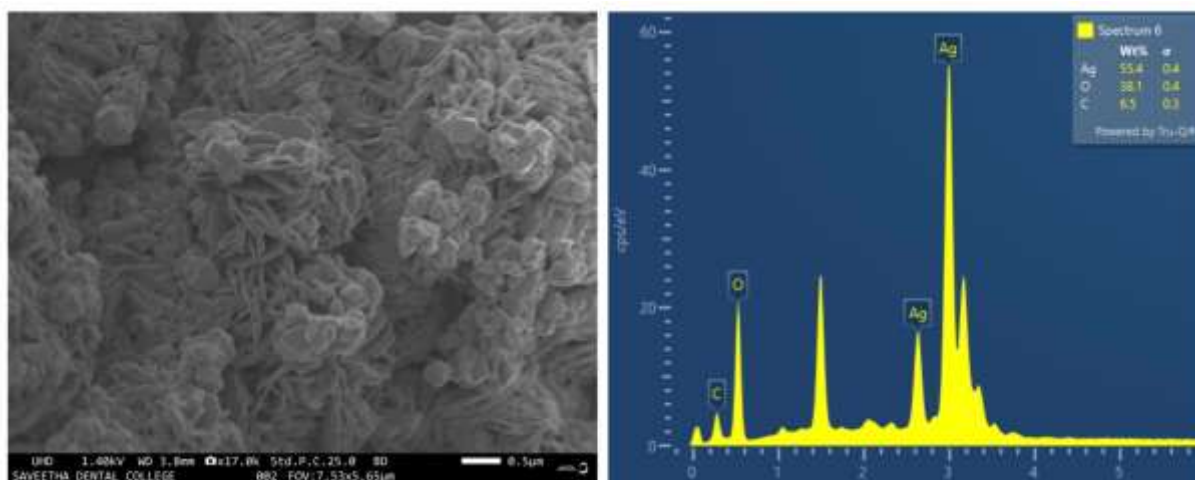


Figure 3: Antimicrobial Activity of Ag Nanoparticles

Disc diffusion assay was performed against *Streptococcus mutans* ATCC 35668 and *Escherichia coli* ATCC 25922. Clear inhibition zones were observed around discs containing Ag nanoparticles, demonstrating pronounced antibacterial activity against both Gram-positive (*S. mutans*) and Gram-negative (*E. coli*) strains. The presence of distinct inhibition zones indicates effective microbial growth suppression by Ag nanoparticles.



Figure 4 : Hemocompatibility and Antioxidant Assay of Ag Nanoparticles

Hemocompatibility testing showed that human erythrocytes exposed to Ag nanoparticles at concentrations of 5 mg/mL and 10 mg/mL exhibited hemolysis values of 1.8% and 2.9%, respectively. These values are below the permissible limit of 5% for biomaterials. Antioxidant activity measured at 50 µg concentration revealed 75.82% activity for Ag nanoparticles compared to 89.21% for the standard reference.

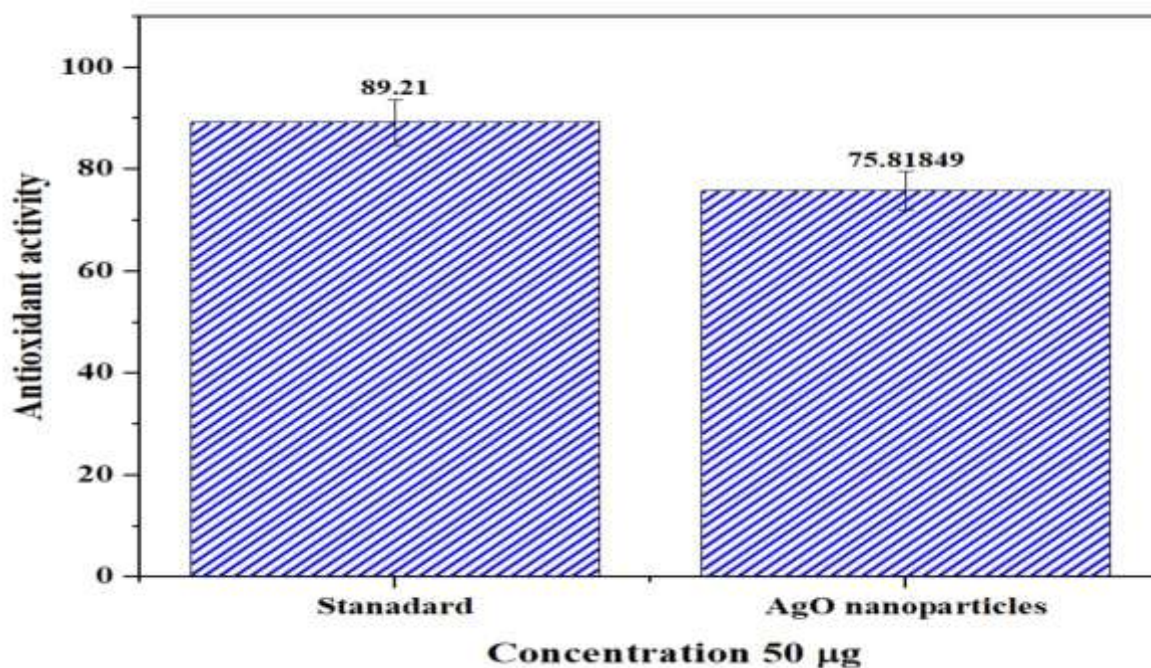
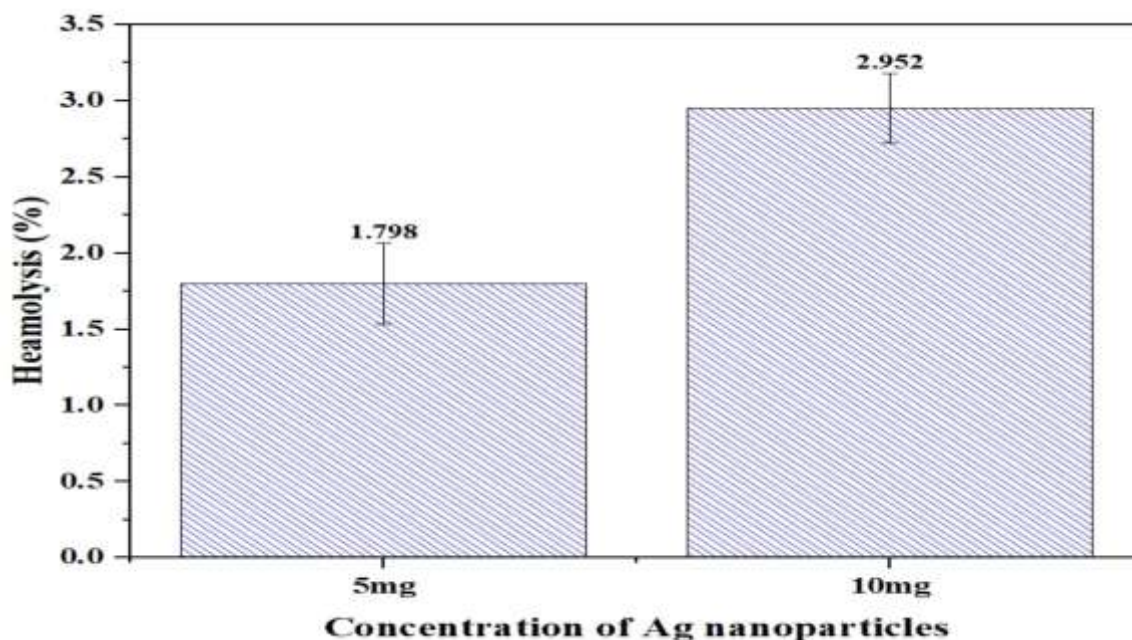


Figure 5: Antioxidant Activity of Ag Nanoparticles

DPPH radical scavenging assay showed that Ag nanoparticles exhibited 75.8% scavenging activity at a concentration of 50 µg, compared to 89.2% for the standard ascorbic acid. Hemocompatibility testing further showed hemolysis values of 1.80% and 2.95% at 5 mg/mL and 10 mg/mL concentrations, respectively, which remain below the 5% permissible limit for biomaterials.



DISCUSSION :

The sharp SPR peak observed at 441 nm confirms the efficient bioreduction of Ag⁺ ions into Ag⁰ nanoparticles, reflecting uniform particle formation and colloidal stability—critical parameters for biomedical applications (Figure 1). The cubical morphology with narrow size distribution, together with EDAX analysis showing silver dominance and minor oxygen and carbon signals, validates the crystalline integrity and compositional purity of the nanoparticles, reinforcing their stability and suitability for biomedical and catalytic use (Figure 2). The pronounced inhibition zones against

Streptococcus mutans and *Escherichia coli* demonstrate broad-spectrum antibacterial potential, underscoring the therapeutic relevance of Ag nanoparticles in oral health management and systemic infection control (Figure 3). Hemolysis values well below the 5% threshold confirm excellent blood compatibility, while appreciable antioxidant activity highlights the dual advantage of Ag nanoparticles, combining safety with free-radical scavenging potential (Figure 4). Although slightly lower than ascorbic acid, the strong antioxidant activity, together with hemocompatibility, underscores the therapeutic promise of Ag nanoparticles for clinical interventions (Figure 5).

Ag₂O nanoparticles synthesized via a green route using *Sargassum muticum* extract further exemplify eco-friendly nanotechnology. Phytochemicals acted as natural reducing and stabilizing agents, eliminating toxic reagents (12). Characterization confirmed nanoparticle formation and properties: UV–Vis spectroscopy revealed a distinct absorption band near 420 nm, FESEM showed spherical morphology, EDAX verified silver and oxygen composition, and FTIR indicated functional groups involved in reduction and stabilization. XRD patterns validated crystalline structure, while FESEM images revealed particle sizes ranging from 43–79 nm. The synthesis was visually confirmed by the color change of the extract upon AgNO₃ addition. These nanoparticles exhibited pronounced antibacterial activity against *S. mutans* and *E. coli*, reinforcing their biomedical relevance (13,14).

Mechanistically, AgNPs exert antibacterial activity by disrupting the peptidoglycan layer, increasing membrane permeability, and compromising cell integrity. They bind to sulfhydryl groups of bacterial proteins, impair enzymatic function, and hinder DNA replication. Their nanoscale size enables biofilm penetration, enhancing bactericidal efficacy. Similarly, Ag₂O nanoparticles act through direct DNA interaction, oxidative stress induction, and disruption of vital cellular pathways. Together, these mechanisms explain the potent antibacterial activity of Ag₂O nanoparticles synthesized via green routes (15). The role of bioactive phytochemicals—particularly flavonoids and phenolics—in nanoparticle formation and antimicrobial efficacy is noteworthy, as they likely contribute to membrane disruption and interference with essential microbial processes. This synergy underscores the biomedical potential of eco-friendly Ag₂O nanoparticles (16).

FUTURE DIRECTIONS AND IMPLICATIONS :

The present work establishes that *Sargassum muticum* extract can serve as a sustainable bioreductant for the green synthesis of Ag₂O nanoparticles with potent antibacterial activity. Future studies should focus on purified fractions of the extract to identify specific phytochemicals responsible for reduction, stabilization, and antimicrobial efficacy. Expanding the scope to include anticancer, anti-inflammatory, and antidiabetic evaluations will provide a broader biomedical profile of these nanoparticles. In addition, *in vivo* studies and clinical validations are essential to confirm safety, hemocompatibility, and therapeutic effectiveness. From an application standpoint, eco-friendly Ag₂O nanoparticles hold promise for oral healthcare formulations, antimicrobial coatings, and drug delivery systems, thereby contributing to sustainable nanomedicine and reducing reliance on toxic chemical synthesis routes.

CONCLUSION :

The green synthesis of Ag₂O nanoparticles using *Sargassum muticum* extract yielded crystalline, biocompatible nanomaterials with potent antibacterial activity against *Streptococcus mutans* and *Escherichia coli*. Hemolysis values below the 5% threshold confirm blood safety, while strong antioxidant activity supports therapeutic potential. These findings suggest that eco-friendly Ag₂O nanoparticles could serve as promising agents for oral healthcare and systemic infection control, offering a sustainable alternative to conventional antimicrobial strategies.

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