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## Biosynthesized silver nanoparticles from *Colpomenia peregrina*: A novel approach to combat bacterial infections

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### Abstract

The rapid emergence of antibiotic-resistant bacteria necessitates innovative approaches to combat bacterial infections. This study explores the biosynthesis of silver nanoparticles (AgNPs) using the marine brown alga *Colpomenia peregrina* as a cost-effective and eco-friendly method. The aqueous extract of *C. peregrina* was employed as a reducing and stabilizing agent for synthesizing AgNPs. Characterization of the nanoparticles was performed using UV-Vis spectroscopy, Fourier-transform infrared (FTIR) spectroscopy, transmission electron microscopy (TEM), and X-ray diffraction (XRD) to confirm their formation, size, shape, and crystalline nature.

The synthesized AgNPs exhibited potent antibacterial activity against Gram-positive and Gram-negative bacterial strains, as determined by the Kirby Bauer disc diffusion method. The antibacterial mechanism was attributed to the disruption of bacterial cell walls, reactive oxygen species (ROS) generation, and interference with intracellular processes.

This study highlights the potential of *C. peregrina*-mediated AgNPs as a novel antibacterial agent, paving the way for sustainable nanotechnology-based solutions in the fight against bacterial infections. These findings provide a promising foundation for developing alternative antimicrobial therapies and applications in healthcare.

**Keywords:** Nanomedicine, silver nanoparticles, green synthesis, antibacterial efficacy

### Introduction

The increasing prevalence of multidrug-resistant bacterial infections poses a significant challenge to global healthcare systems. Conventional antibiotics are losing their efficacy due to the adaptive mechanisms of bacteria, necessitating the exploration of alternative antimicrobial strategies [5]. In this context, nanotechnology has emerged as a promising field, with silver nanoparticles (AgNPs) gaining particular attention for their broad-spectrum antimicrobial properties [11].

Silver nanoparticles exhibit unique physicochemical characteristics, such as high surface-to-volume ratio and enhanced reactivity, which enable their interaction with bacterial membranes and intracellular components. These interactions disrupt vital processes, leading to bacterial cell death [3]. While physical and chemical methods for AgNP synthesis are well-established, they are often associated with high costs, toxic by-products, and environmental concerns [4]. To address these challenges, biosynthesis using natural sources offers a sustainable and eco-friendly alternative.

Marine algae, particularly brown algae, are rich in bioactive compounds such as polysaccharides, phenolics, and proteins, making them excellent candidates for nanoparticle biosynthesis [8]. *Colpomenia peregrina*, a marine brown alga, is known for its abundance of bioactive metabolites, including phlorotannins and sulfated polysaccharides, which can serve as natural reducing and capping agents [10]. Despite its potential, the utilization of *C. peregrina* in nanoparticle synthesis remains largely unexplored.

This study focuses on the biosynthesis of AgNPs using aqueous extracts of *C. peregrina* and evaluates their antibacterial efficacy against clinically relevant bacterial pathogens. The objectives include characterizing the biosynthesized AgNPs and investigating their antibacterial mechanism. By leveraging the bioactive potential of *C. peregrina*, this research aims to provide a sustainable and effective solution for combating bacterial infections, addressing both environmental and healthcare challenges.

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## Review of Literature

The biosynthesis of nanoparticles, particularly silver nanoparticles (AgNPs), has gained significant attention due to their potential applications in medicine, agriculture, and environmental remediation. The use of biological systems such as plants, fungi, and algae for synthesizing nanoparticles offers an eco-friendly and sustainable alternative to conventional physical and chemical methods [4].

Silver nanoparticles are well-known for their potent antibacterial properties against a broad spectrum of pathogens, including multidrug-resistant bacteria. Studies have shown that AgNPs exert their antimicrobial effect by disrupting the bacterial membrane, generating reactive oxygen species (ROS), and interfering with intracellular components like DNA and proteins [3]. Additionally, their nanoscale size enhances their penetration and interaction with bacterial cells, making them effective even at low concentrations [11].

Marine algae are a rich source of bioactive compounds such as polysaccharides, polyphenols, and proteins, which can act as natural reducing and stabilizing agents in nanoparticle synthesis [8]. Brown algae, in particular, are abundant in phlorotannins and sulfated polysaccharides, which have been effectively utilized for the green synthesis of AgNPs. Studies have reported that algal-mediated nanoparticles exhibit enhanced stability and significant antimicrobial properties due to the bioactive compounds capping their surface [10, 13, 14].

*Colpomenia peregrina*, a marine brown alga, is rich in secondary metabolites with strong reducing and antioxidant properties [5, 6, 16]. Although limited studies have explored its application in nanoparticle biosynthesis, related species have shown promising results. For example, *Sargassum* and *Padina* species have been extensively studied for AgNP synthesis, demonstrating potent antibacterial, antifungal, antidiabetic, and anti-inflammatory activities [15, 19, 20, 21]. This highlights the untapped potential of *C. peregrina* for synthesizing biofunctional nanoparticles.

AgNPs synthesized using algal extracts have been shown to disrupt bacterial membranes through electrostatic interactions, leading to leakage of intracellular components. Furthermore, the generation of ROS induces oxidative stress, damaging vital cellular components. Studies have also suggested that the bioactive compounds from algae, capping the nanoparticles, enhance their antibacterial efficacy by synergistically interacting with the silver ions [12].

While biosynthesized AgNPs present a promising solution, challenges such as scalability, reproducibility, and stability need to be addressed. Future research should focus on optimizing synthesis parameters and exploring the synergistic effects of bioactive compounds for enhanced antibacterial properties. Additionally, studying the biocompatibility and toxicity of these nanoparticles is essential for their safe application in clinical and environmental settings.

## Materials and Methods

### Biosynthesis of silver nanoparticles

For the biosynthesis of silver nanoparticles (AgNPs), a solution was prepared in a 500 mL Erlenmeyer flask by suspending *Colpomenia peregrina* powder (1.0 g) in 50 mL of  $10^{-3}$  M aqueous silver nitrate ( $\text{AgNO}_3$ ). The solution was

incubated at  $37^\circ\text{C}$  for 30 minutes and then air-dried over three days. A noticeable color change from colorless to dark brownish-red was observed, indicating the formation of AgNPs. During this process, the algal extract served as a capping agent, while  $\text{AgNO}_3$  acted as the metal precursor [17, 18]. Within 24 hours of continuous stirring, approximately 95% of the  $\text{AgNO}_3$  ions were reduced.

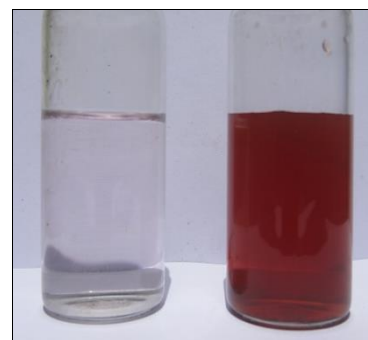
### Characterization of silver nanoparticles

The characterization of silver nanoparticles (AgNPs) synthesized using *Colpomenia peregrina* was performed using advanced analytical techniques. UV-Vis spectroscopy (SHIMADZU UV-3150PC) was utilized to monitor the reduction of silver ions within a wavelength range of 200-600 nm, with water as a reference. TEM analysis (JEOL 2000 EX) provided insights into the morphology and structural features of AgNPs, employing a carbon-coated copper grid for sample preparation and operating at 80 keV [9]. X-ray diffraction (XRD) analysis, conducted using a Rich Seifert P3000 with  $\text{Cu-K}\alpha 1$  radiation, was applied to determine crystal structure, phase purity, and nanoparticle size using Bragg's law and the Debye-Scherrer equation [1, 2]. Thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) assessed the thermal stability and purity of lyophilized AgNPs (Hitachi STA7200, TA Instruments Q200). FT-IR spectroscopy (Thermo-Nicolet 6700) was employed to identify functional groups and chemical bonds, analyzing the samples in the infrared range of  $4000\text{-}400\text{ cm}^{-1}$  [22, 23]. These techniques provided comprehensive data on the physical, chemical, and thermal properties of AgNPs, confirming their successful biosynthesis and potential applications.

## Results

### Biosynthesis of silver nanoparticles

In the green synthesis, *Colpomenia peregrina* powder was mixed with  $10^{-3}$  M  $\text{AgNO}_3$  solution and reacted for 20 minutes at  $121^\circ\text{C}$ . The reaction mixture turned dark brown, indicating the formation of AgNPs, while the control solution without algal powder showed no color change (Fig 1). This color change was attributed to the excitation of surface plasmon vibrations, facilitated by bio-metabolites in the algal extract acting as capping agents. Persistent heating further intensified the color modulation, confirming the successful synthesis of AgNPs.



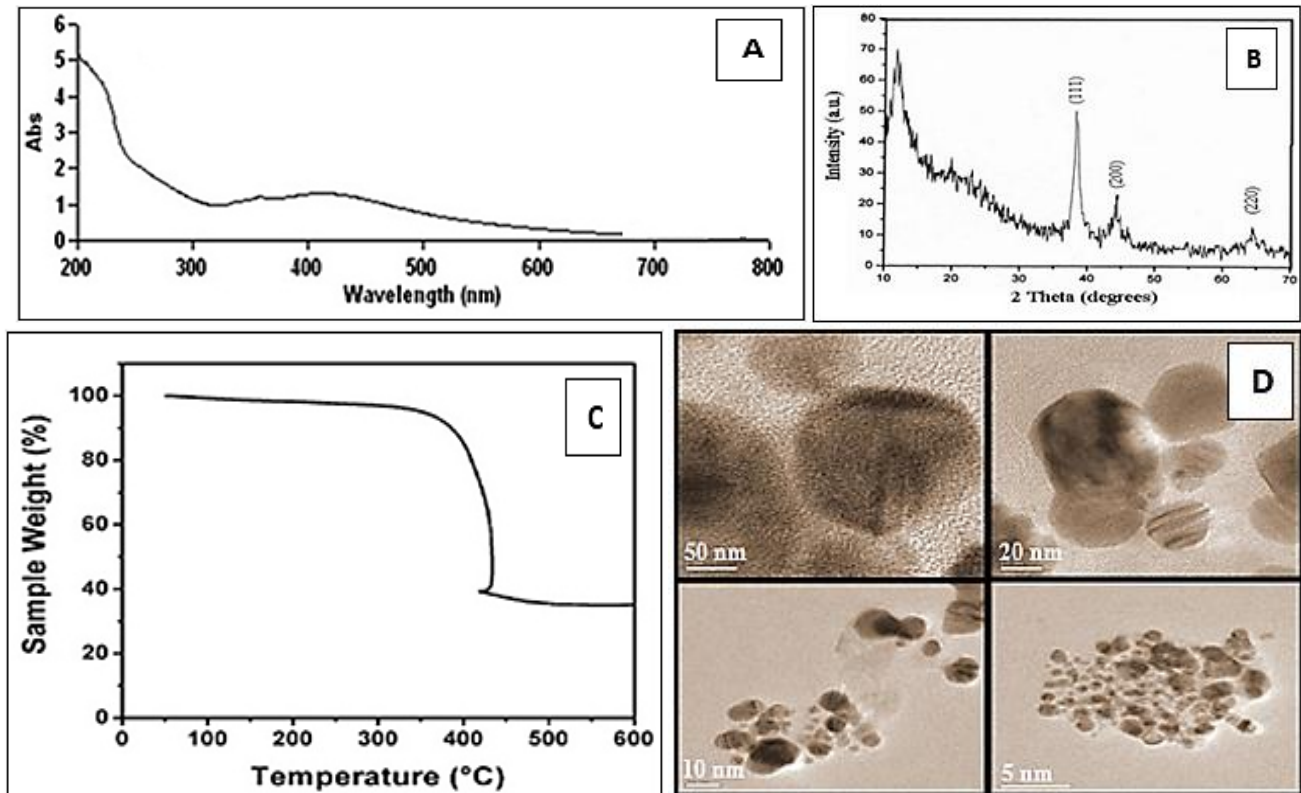
**Fig 1:** Biogenic silver nanoparticles from *Colpomenia peregrina*

### Characterization of silver nanoparticles

The silver nanoparticles (AgNPs) synthesized from the marine alga *Colpomenia peregrina* were analyzed using various characterization techniques (Figure 2). UV-Vis spectrophotometry revealed a distinct absorption peak at

420 nm, a characteristic feature of AgNPs synthesized by the alga. The peak broadening indicated polydispersity, with intensity increasing over reaction time, suggesting an interaction between biosynthesized AgNPs and biomolecules in the algal extract. Morphological analysis using TEM confirmed that the AgNPs were predominantly spherical, cubical, or hexagonal, with some irregular shapes, and had an average size of approximately 34 nm, aligning with DLS histogram calculations. The crystalline nature of the AgNPs was established using XRD, which displayed Bragg reflections corresponding to a face-centered cubic (FCC) silver structure with particle sizes ranging from 10 to 50 nm. The diffraction peaks, primarily at 39.87°, 45.13°,

and 65.87°, matched the planes {111}, {200}, and {220}, respectively, with background noise attributed to nanosized particles and biological macromolecules in the extract. TGA revealed the thermal stability of AgNPs, showing no significant weight loss up to 250°C, but a 16% weight loss occurred between 250–400°C. FT-IR spectroscopy identified functional groups associated with AgNPs, with absorption peaks at 3419.52 cm<sup>-1</sup> (N-H stretching), 2920.28 cm<sup>-1</sup> (C-H stretching), 1640.28 cm<sup>-1</sup> (N-H bending), and other characteristic peaks confirming the presence of biomolecules acting as capping agents. These results collectively confirmed the successful biosynthesis and stability of AgNPs from *C. peregrina*.



**Fig 2:** Characterization of biogenic silver nanoparticles from *Colpomenia peregrina* [A] UV Vis Spectroscopy, [B] XRD analysis, [C] TGA analysis, [D] TEM analysis

### Antibacterial activity of silver nanoparticles synthesized from marine brown alga

#### *C. peregrina*

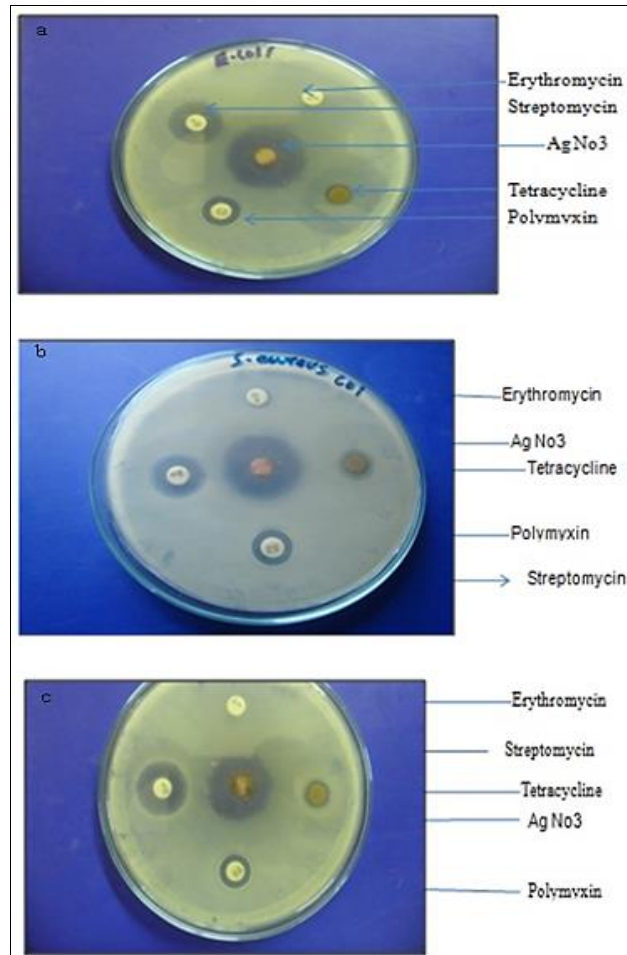
The antibacterial potential of silver nanoparticles (AgNPs) synthesized from the marine brown alga *Colpomenia peregrina* was evaluated and compared with various standard antibiotics, including erythromycin, polymyxin, streptomycin, and tetracycline. The study focused on bacterial cultures of *Escherichia coli*, *Staphylococcus aureus*, and *Proteus vulgaris*. The Kirby-Bauer disc diffusion method was employed to measure the zone of inhibition, which indicates antimicrobial susceptibility.

The biosynthesized AgNPs demonstrated significant efficacy in inhibiting the growth of the tested organisms. The bacterial pathogens showed susceptibility to the AgNPs within a zone of inhibition range of 14.5 to 16.5 mm in diameter. These results, detailed in Table 1 and illustrated in

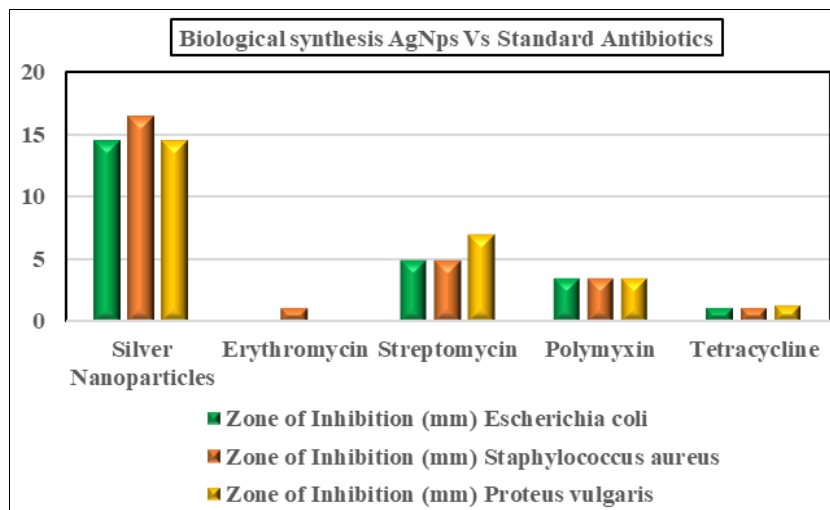
Figures 3 and 4, were compared to the zones of inhibition exhibited by standard antibiotics.

Among the antibiotics tested, streptomycin exhibited the maximum zone of inhibition against both Gram-positive and Gram-negative bacteria. The green-synthesized AgNPs from *C. peregrina* displayed superior antibacterial activity against the tested pathogens compared to erythromycin, polymyxin, and tetracycline.

Notably, the AgNPs showed good antibacterial activity against *E. coli* and *P. vulgaris*, and the highest activity against *Staphylococcus aureus* underscoring their potential as a robust alternative to conventional antibiotics. These findings highlight the consistent and effective antibacterial properties of AgNPs synthesized via green methods using *C. peregrina*, making them a promising candidate for combating human pathogenic bacteria.



**Fig 3:** Antimicrobial susceptibility test (Kirby -Bauer method) of silver nanoparticles biosynthesized from *Colpomenia peregrina*, (a) *Escherichia coli* (b) *Staphylococcus aureus* (c) *Proteus vulgaris*



**Fig 4:** Antimicrobial susceptibility test (Kirby -Bauer method) of silver nanoparticles biosynthesized from *Colpomenia peregrina*, (a) *Escherichia coli* (b) *Staphylococcus aureus* (c) *Proteus vulgaris*

**Table 1:** Antimicrobial susceptibility test (Kirby -Bauer method) of silver nanoparticles biosynthesized from *Colpomenia peregrina*, (a) *Escherichia coli* (b) *Staphylococcus aureus* (c) *Proteus vulgaris*

S. No	Biosynthesized AgNPs from <i>C. peregrina</i> standard antibiotics	Zone of Inhibition (mm)		
		<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Proteus vulgaris</i>
1	Silver nanoparticles	14.5	16.5	14.5
2	Erythromycin	0	1.1	0
3	Streptomycin	5	5	7
4	Polymyxin	3.5	3.5	3.5
5	Tetracycline	1.1	1.1	1.3



## Discussion

The antibacterial activity of silver nanoparticles (AgNPs) synthesized from the marine brown alga *Colpomenia peregrina* was evaluated and compared with standard antibiotics such as erythromycin, polymyxin, streptomycin, and tetracycline. The bacterial cultures of *Escherichia coli*, *Staphylococcus aureus*, and *Proteus vulgaris* were tested using the disc diffusion method. The biosynthesized AgNPs effectively inhibited the growth of these organisms, with zones of inhibition ranging between 14.5 mm and 16.5 mm. While streptomycin exhibited the largest zone of inhibition against all Gram-positive and Gram-negative bacteria, the green-synthesized nanoparticles from *C. peregrina* displayed significant antibacterial activity, against *E. coli* and *S. aureus*, *P. vulgaris*, outperforming erythromycin, polymyxin, and tetracycline. The findings underscore the potential of *C. peregrina*-mediated AgNPs as a consistent and eco-friendly alternative for combating human pathogenic bacteria.

The potent antibacterial efficacy of AgNPs is attributed to their large surface area, which facilitates effective interaction with bacterial cell walls. These nanoparticles disrupt the bacterial cell by interacting with sulfur-containing proteins in the membrane and phosphorus-containing compounds such as DNA. Upon entering the bacterial cell, AgNPs induce the formation of a low molecular weight region, which helps protect DNA from silver ions while simultaneously attacking the respiratory chain and affecting cell division, ultimately leading to cell death. This mechanism aligns with observations made by researchers like Vishnu Kiran Manam 2020<sup>[18]</sup> and Vishnu Kitan Manam 2014<sup>[19]</sup>, who documented the antibacterial effects of green-synthesized AgNPs from various sources. The eco-friendly synthesis process of *C. peregrina* ensures the stability of AgNPs due to protein capping from the algal extract. This study highlights the potential applications of biosynthesized AgNPs in medical biology, nanotechnology, and nanomedicine, offering an efficient and sustainable alternative for antimicrobial interventions.

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