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Green Synthesis of Copper Nanoparticles from Artemisia Maritima: Characterization and Evaluation of Antibacterial Properties

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ABSTRACT

Copper nanoparticles (Cu NPs) attracted many researchers due to their potential biomedical and pharmacological activities that depend on the shape and size of the nanoparticles. **Objective:** To extract and characterize nanoparticles from the aqueous extract of the *Artemisia maritima* plant. **Methods:** UV-V spectroscopy indicates the presence of Cu NPs with unique optical characteristics. FTIR analysis identified functional groups and chemical bonds in the Cu NPs. XRD analysis revealed a hexagonal crystal structure for the Cu NPs. Antibacterial activity of the synthesized Cu NPs was evaluated against *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Bacillus subtilis,* and *Staphylococcus aureus*. **Results:** The Cu NPs exhibited varying zones of inhibition (ZOI) against different bacterial strains, with the largest ZOI observed against *Staphylococcus aureus* (20 mm), followed by *Bacillus subtilis* (19 mm), *Pseudomonas aeruginosa* (17 mm), *Klebsiella pneumoniae* (16 mm), and *Escherichia coli* (11 mm). These results highlight the potential of *Artemisia maritima*-synthesized Cu NPs as antimicrobial agents, particularly against Gram-positive bacteria. **Conclusions:** Artemisia maritima-mediated Cu NPs offer a promising, green alternative for antimicrobial development, warranting further research for clinical and environmental applications.

INTRODUCTION

Medicinal plants describe a broad group of natural herbs and botanicals which have been traditionally employed in herbal medicine. Research teams investigate the healing potential of these plants, which medical communities value for medicinal purposes. The natural compounds in medicinal plants serve as the principal factors for their health benefits, which enable the treatment of various medical conditions, including infectious diseases [1]. The rising interest in herbal medicines stems from their reputation for being safe while remaining affordable as well as environmentally friendly. The natural remedies have grown in popularity because they reportedly produce fewer serious adverse effects than conventional pharmaceutical medicines [2]. Plants, including Artemisia maritima, contain medicinal phytochemicals that enable their application in herbal medicine manufacturing and in producing natural dyes and beverages and flavorings, as well as herbal teas and cosmetics. The scientific community found anti-malarial properties in Artemisia japonica alongside Artemisia maritima and Artemisia nilegarica through laboratory testing [3]. Scientific research has demonstrated that extracts derived from Artemisia japonica and Artemisia maritima, along with Artemisia nilegarica, possess promising anti-malarial properties [3]. The culinary herb tarragon exists among numerous Artemisia genus species, with Artemisia

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dracunculus as its widely recognized variety. Many chefs use tarragon in various dishes, including meat preparations and cheese blending, as well as pickled foods and both vinegar-based condiments and mustards and decorative use for meats and fruits. Tarragon serves nutritional purposes for the kitchen, yet its phytochemical compounds could support cardiovascular health and provide pain treatment to teeth and open wounds [4]. Science has identified the plant Artemisia capillaris under its alternative name capillaristhunb, which contains multiple active compounds, including capillarisin and apigenin and hesperidin and coumaric acid. Experimental studies have proven that these substances exhibit anticancer effects and antimicrobial properties [5, 6]. The Asteraceae family includes Artemisia maritima, which serves essential purposes in both traditional medicine and contemporary pharmaceutical industries. Many known names exist for this plant, including wormseed and Santonica, along with Drooping Sea wormwood, See mugwort, and Old Woman. Artemisia maritima plant expands throughout multiple global areas, which include France, the UK, Italy, Belgium, Germany, Denmark, Pakistan, China, India, Sweden, Bulgaria, Russia, Afghanistan and the Himalayan range [7, 8]. The development of new therapeutic agents depends on antimicrobial property exploration, and silver nanoparticles demonstrate strong antibacterial and antifungal, and antiviral actions [9]. Research demonstrates that Artemisia maritima exhibits vital anticoagulant capabilities [10]. Research shows that A. maritima extracts can block enzymatic activities involved in blood clotting processes [11]. Research evidence supports A. maritimia's effectiveness in cancer treatment [12]. Research demonstrates that A. maritima extract-derived nanoparticles demonstrate dual efficacy by fighting bacterial infections and cancer cells [13]. Nanoparticles engineered with Artemisia ciniformis have shown their ability to activate apoptosis-programmed cell death as an essential mechanism to destroy cancerous cells, according to research [14]. Studies reveal that Artemisia-based nanoparticles disrupt parasite life cycles because they block the developmental progress of the parasites [15]. Scientists have discovered that silver nanoparticles excel at killing bacteria yet maintain low toxicity levels toward human cells [16]. For centuries, people have utilized Artemisia annua to treat malaria, while its active component artemisinin, remains fundamental to World Health Organization-approved combination therapies for this disease [17]. Researchers have identified multiple physical and chemical methods for creating nanoparticles. Bio-based methods receive increased attention because of their negligible effect on the environment [18]. Medicinal plant extracts proved efficient for noble metal nanoparticle synthesis, especially for silver, gold, platinum and palladium nanoparticles [19]. The well diffusion method demonstrated that synthesized nanoparticles exhibited strong antimicrobial effects against multiple disease-causing pathogens [20]. The exploitation of Artemisia maritima for biological nanoparticle synthesis represents an innovative approach because there is no documented literature regarding its utilization in producing copper nanoparticles. The current work utilizes this plant in green synthesis to generate valuable knowledge about sustainable nanotechnology while developing eco-friendly substitutes for conventional chemical-intensive syntheses. We chose Artemisia maritima because it contains rich phytochemicals alongside abundant endogenous copper that could help make efficient Cu NPs and boost their activity levels. A unique study emerges because no previous research has fully examined Artemisia maritima as a method for environmentally friendly copper nanoparticle manufacturing.

This study aims to synthesize and characterize Cu NPs using A. maritima leaf extract and evaluate their antimicrobial efficacy against selected bacterial strains. We hypothesise that A. maritima-derived phytochemicals will enable the eco-friendly synthesis of Cu NPs and confer significant antimicrobial activity.

METHODS

For extract preparation, 20g of fine powder of dried plant was mixed in 200 ml of distilled water in a flask and homogenized on a magnetic stirrer at 70°C for 1 hour. Later, the mixture with filtered with a muslin cloth to remove large particles to obtain a liquid solution. The obtained mixture was centrifuged at 3000 rpm for 15 minutes. After centrifugation, the supernatant was filtered by Whatman filter paper No. 1 three times to obtain a clear *Artemisia maritima* plant extract. The extract was stored at -4°C for further use as a reducing and stabilizing agent in the synthesis of Cu NPs. Sterility was maintained throughout the experiment. An aqueous solution of 1 mM Copper sulfate ($CuSO_4$) was prepared in a 250 ml Erlenmeyer flask and used for the synthesis of Cu NPs. *Artemisia maritima* extract was added drop-wise into 50 ml of 0.4M copper nitrate solution at lab temperature for 1 hour with continuous stirring. The colour of the nanoparticle solution changed to a dark brown precipitate, indicating the formation of copper nanoparticles. The entire reaction took place in the dark. The complete reduction of $CuSO_4$ to Cu^{+2} ions was confirmed by the change in color from colorless to colloidal brownish yellow. The colloidal mixture

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was then sealed and stored properly for future use. To isolate the nanoparticles, either unloaded or Ampicillin loaded, the solution was centrifuged (15 minutes; 15,000 rpm) and the pellet was kept for lyophilization. Copper ions and plant extract residue were removed from the Cu-NPs-containing pellet by washing it three to four times with deionized water. After drying in the hot air oven, the obtained powder was stored in Eppendorf tubes for future analysis. The nanoparticles were further confirmed spectroscopically. To check the antibacterial activity of Cu NPs synthesized by *Artemisia maritima* against bacterial strains (such as *Escherichia coli, Staphylococcus aureus, Klebsiella pneumonia, Pseudomonas aeruginosa*, and *Bacillus subtilis*), the serial dilutions of Cu NPs (4 mg, 2 mg, 1 mg, and 0.5 mg) were prepared in 1ml of DMSO (dimethyl sulfoxide). For bacterial growth, nutrient broth and nutrient Cuar media were prepared. The nutrient broth is a liquid medium that is used for the expansion of bacterial growth. For the preparation of the nutrient broth, 3g of the nutrient broth powder was taken and added to 100 ml of distilled water in a conical flask. After mixing and dissolving, the media were sterilized in an autoclave at 121°C for 15 minutes. For nutrient Agar preparation, 28g of nutrient Agar media was dissolved in 1000 ml of distilled water and autoclaved at 121.5°C and approximately >15 psi. Media was poured into plates and then left the solidify. When the gel was completely solidified then poured 200 microliters of the bacterial strains mentioned above were poured into each plate. After, pouring bacterial strains were streaked over the plates with sterile cotton swabs. For the preparation of antibiotic dilution, 4 mg of powder of Ampicillin was taken, and 1ml of distilled water in an Eppendorf tube. The solution was sonicated in an ultrasonic bath for 3 minutes to mix it properly. The dilutions were stored in the refrigerator at 4°C for further use. Cu NPs made from *Artemisia maritima* aqueous extract were prepared for dilution by adding 1 milliliter of dimethyl sulfoxide to an Eppendorf tube. To thoroughly mix the solution, it was sonicated for three minutes in an ultrasonic bath. Twenty microliters of the stock's bacterial strains were added to culture tubes with one milliliter of nutrient broth medium to create inoculate. For a whole day, the culture tubes holding the different bacterial strains were kept in an incubator. The bacterial inoculum was ready after a day. Once turbidity was detected, the inoculum was applied to nutrient agar plates. After being autoclaved, the nutrient agar medium was allowed to cool to room temperature. After being transferred onto Petri dishes, the liquid agar medium was allowed to set. Using a micro-pipette, the 200 µl inoculum was transferred into agar plates. With the use of a sterile cup propagator, the inoculum was dispersed throughout agar medium until it was entirely digested under laminar flow. The Well Diffusion assay is primarily used to determine bacterial strain susceptibility to antibiotics, with a clear zone around the well reflecting bacterial antibiotic sensitivity. We used nutritional Agar plates spread with bacterial strains instead of discs. Then, 10mm diameter wells were prepared in the gel and named A, B, C, D, +ve, and -ve. A has the highest concentration (4 mg/ml), and D has the lowest concentration (0.5 mg/ml). DMSO was used as a negative (-ve) control, and Ampicillin as a positive control (+ve). Cu NPs synthesized by Artemisia maritima were injected into the wells and incubated for 24 hours at 37°C to determine the zone of inhibition (ZOI). Both gram-positive and gram-negative bacterial strains were used to examine the ZOI. After an incubation period of 24-48 hours, the plates were examined for the efficacy of antibacterial samples by measuring the area of the barrier (i.e., ZOI) in millimeters (mm).

RESULTS

In the present UV characterization, the broad peak suggests that the Cu NPs synthesized by *Artemisia maritima* have a distribution of sizes, which could be due to various factors such as the synthesis method, reaction conditions, and stabilizing agents used during the synthesis process (Figure 1).

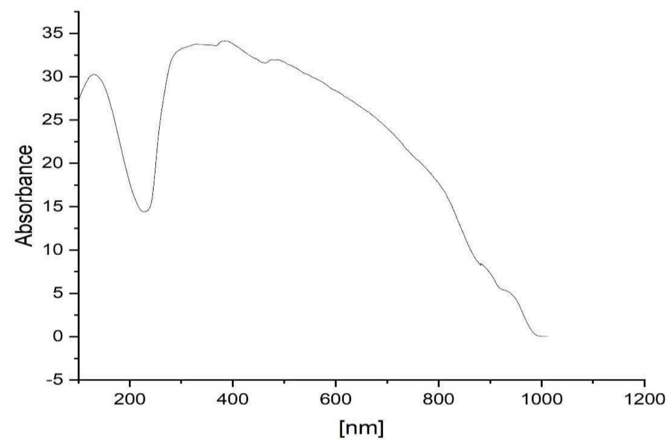

Figure 1: UV-Vis Representing the Absorbance Peaks of Artemisia Maritima-Mediated Cu NPs

The FTIR spectrum of *Artemisia maritima*-mediated CuNPs exhibited characteristic bands at 3593.38 cm-1, 2877.79 cm-1, 2349.29 cm-1, 1680 cm-1, 1502.5 cm-1, and 430.13 cm-1. FTIR analysis shows a strong broad stretching of the free–OH functional group at 3593.38 cm-1. The sharp peak at 2877.79 cm-1 is attributed to C-H asymmetric stretching. Strong C=O stretching is involved in the absorption band at 1680 cm-1. The C-O bond of carbonate ions, assigned to the oxy-carbonate structure (Figure 2).

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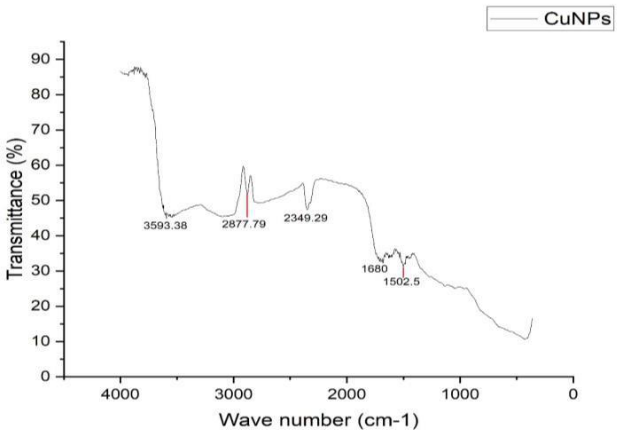

Figure 2: FTIR Spectrum of Cu NPs Synthesized from Artemisia Maritima

The XRD diffraction pattern shows the XRD pattern of Cu NPs of Artemisia maritima, which manifests various angles at 2θ, several sharp diffraction peaks at positions (2θ) are 20.7647°, 38.2520°, 44.4898°, 44.5963°, 64.8751°, 65.0540°, 78.0168°, and 78.2430°. The peak at 20.7647° could correspond to a copper phase with a particular crystal structure. The peaks at 44.4898° and 44.5963° might indicate a separate copper phase or a possible impurity(Figure 3).

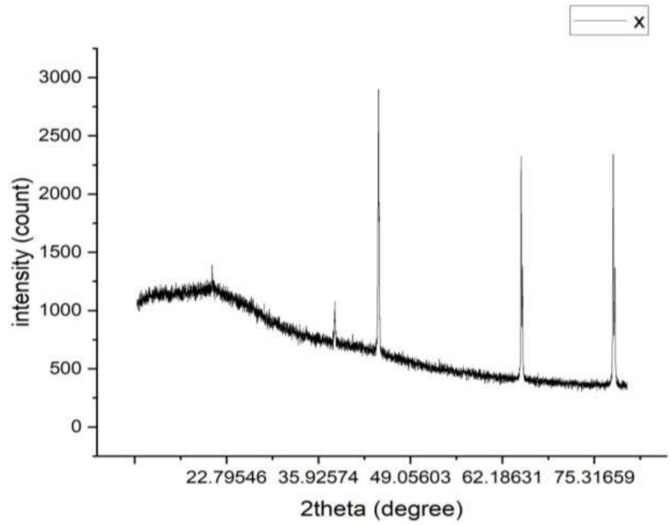

Figure 3: XRD Pattern of Cu NPs Synthesized from Extract of Artemisia Maritima

At a concentration of 40 µg/ml, Cu NPs demonstrated notable antimicrobial activity against all tested bacterial strains. The largest inhibition zone was observed for *Staphylococcus aureus* (20 mm), followed by *Bacillus subtilis* (19 mm), *Pseudomonas aeruginosa* (17 mm), *Klebsiella pneumoniae* (16 mm), and *Escherichia coli* (11 mm). These findings suggest that Cu NPs are more effective against Gram-positive bacteria than Gram-negative bacteria at this concentration. Conversely, at the lowest tested concentration (5 µg/ml), the antimicrobial activity of Cu NPs was significantly reduced. No inhibition zones were observed for the Gram-negative Bacteria E. coli and K. pneumoniae, indicating a lack of activity at this dosage. However, *P. aeruginosa* showed a moderate inhibition zone of 12.5 mm, while B. Subtilis and S. aureus exhibited zones of 12mm and 13mm, respectively. Despite reduced efficacy, these results confirm that Cu NPs retained some degree of antimicrobial activity even at low concentrations (Table 1).

Table 1: Zone of Inhibitions (mm) Demonstrating the Antibacterial Activity of Artemisia Maritima-Synthesized Cu NPs Against Selected Bacterial Species

Concentrations of Cu-NPs	Zone of Inhibition (mm) Against Bacterial Species				
	Gram Negative			Gram Positive	
	Escherichia coli	*Klebsiella pneumonia*	*Pseudomonas aeruginosa*	*Bacillus subtilis*	*Staphylococcus aureus*
40 µg/ml	11	16	17	19	20
20 µg/ml	10	12	16	16	17
10 µg/ml	-	11	13.5	15	14
5 µg/ml	-	-	12.5	12	13
Positive Control	44	45	40	45	39
Negative Control	0	0	0	0	0

As expected, the negative control did not show any zone of inhibition for any of the tested bacterial strains, confirming the validity of the results and excluding the possibility of external contamination. Overall, the results affirm that Cu NPs possess concentration-dependent antimicrobial activity, with greater effectiveness observed against Gram-positive bacteria (Figure 4).

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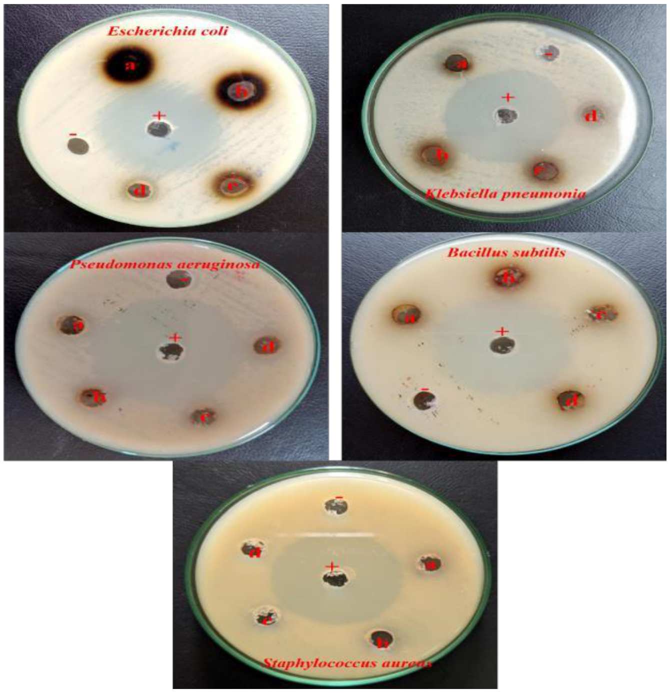

Figure 4: Zone of inhibition (mm) Demonstrating the Antibacterial Activity of Artemisia Maritime-Synthesized Cu NPs Against Selected Bacterial Species

DISCUSSION

This study highlights the promising potential of Artemisia maritima as a sustainable source of the green synthesis of copper nanoparticles (Cu NPs), an area still underexplored. The plant's phytochemical richness and inherent copper content make it a viable candidate for eco-friendly nanoparticle production. The successful synthesis and characterization of Cu NPs from A. maritiima mark a new contribution, as limited studies to date have evaluated their application in nanoparticle fabrication. MIC values indicated a higher inhibitory effect against Bacillus subtilis, Klebsiella pneumonia, and Staphylococcus aureus. The enhanced antibacterial performance may be attributed to the ability of Cu NPs to disrupt bacterial membranes, generate reactive oxygen species (ROS), and release copper ions that interfere with intracellular functions [21, 22]. These multitarget mechanisms enhance their antimicrobial efficacy while reducing the chances of resistance development. At 5 µg/mL, Cu NPs still showed moderate inhibition against *P. aeruginosa*, *B. subtilis*, and S. aureus, strengthening their potency at even low concentrations. The UV-Vis spectroscopy confirmed nanoparticle synthesis, with a surface plasmon resonance (SPR) peak observed between 370-419 nm, typical for copper nanoparticles. FTIR analysis revealed functional groups such as hydroxyl, carbonyls, and aromatic rings-suggesting the involvement of plant phytochemicals in reduction and stabilization. Though flavonoids are known to contribute to these processes, their broader biological relevance is well established and need not be elaborated further here. The XRD analysis provides information about the diffraction pattern of Cu NPs synthesized from the extract of *Artemisia maritima*. The XRD pattern exhibits several sharp diffraction peaks at different angles [2θ]. The specific peaks mentioned in the results are at 20.7647°, 38.2520°, 44.4898°, 44.5963°, 64.8751°, 65.0540°, 78.0168°, and 78.2430°. The peak at 20.7647° suggests the presence of a copper phase with a particular crystal structure. The peaks at 44.4898° and 44.5963° indicate the existence of either a separate copper phase or a potential impurity. These results indicate that Cu NPs at this concentration effectively inhibited the growth of Gram-positive bacteria to a greater extent compared to Gram-negative bacteria. The antimicrobial activity reduced substantially when the Cu NPs concentration reached the lowest tested level of 5 µg/ml. Antibiotic susceptibility testing demonstrated that Pseudomonas aeruginosa developed a 12.5 mm zone of inhibition. The data shows that Cu NPs at 5 µg/ml displayed reduced antimicrobial activity when compared to concentrations above it. The negative control without antimicrobial agents showed no inhibitory effect which proves that the observed zone of inhibition was directly caused by the presence of Cu NPs and not affected by external elements. The results from this study show solid evidence to support the antimicrobial properties of Cu NPs. This study demonstrates that Cu NPs synthesized from Artemisia maritima show promise as an antimicrobial agent. The antimicrobial activity of Cu NPs showed greater inhibition against Gram-positive bacteria while also producing strong effects against both bacterial strains tested. Results show that increased concentrations of Cu NPs lead to more potent bacterial growth inhibition. Future research must study how Cu NPs work and investigate their possible uses in fighting bacterial diseases. Future studies need to broaden their investigation of bacterial strains to determine the full antimicrobial spectrum of Cu Nps.

CONCLUSIONS

It was concluded that artemisia maritima-mediated Cu NPs offer a promising, green alternative for antimicrobial development, warranting further research for clinical and environmental applications.

Authors Contribution

Conceptualization: SA
Methodology: SA, MA, MNA, UUR
Formal analysis: SA
Writing review and editing: HAA, MNA

All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

All the authors declare no conflict of interest.

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