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Green Synthesis, Characterization of Copper Sulfide Nanoparticles and Antibacterial Activity Evaluation

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Abstract
This study focused on the eco-friendly and economical synthesis of copper sulfide nanoparticles at certain temperatures using myrtle leaf extract. Green synthesis methods are mostly considered to be safe and efficient in the nanoparticle synthesis field. The nanoparticles were characterized using FT-IR, AFM, UV-visible spectrophotometer, FESEM with EDX, and XRD. where the crystal size in XRD was 21.4 nm. The antibacterial properties of CuS nanoparticles were evaluated against two bacterial strains, namely, *Staphylococcus aureus* and *Escherichia coli*, using various concentrations. The Minimum Inhibitory Concentration (MIC) was determined for both bacteria at a range of CuS NPs conc. from 0.5229×10^{-1} M to 0.3268×10^{-2} M.

Keywords: Antimicrobial activity, Green synthesis, Myrtle leaf plant, Nanoparticles.

التوليف الأخضر وتوصيف جسيمات كبريتيد النحاس النانوية وتقييم النشاط المضاد للبكتيريا

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الخلاصة
ركزت هذه الدراسة على توليف صديق للبيئة واقتصادي لجسيمات كبريتيد النحاس النانوية عند درجات حرارة معينة باستخدام مستخلص أوراق نبات الآس. تعتبر طرق التوليف الأخضر في الغالب آمنة وفعالة في مجال تخليق الجسيمات النانوية. تم توصيف الجسيمات النانوية المصنوعة باستخدام مطيافية الأشعة تحت الحمراء ومجهر القوة الذرية ومطيافية الأشعة المرئية ومجهر الإلكترون الماسح المعدني مزود بجهاز تحليل الطاقة المنبعثة بالأشعة السينية وتحليل البلورات بواسطة الأشعة السينية. حيث كان حجم البلورة في حيود الاشعة السينية يساوي 21.4 نانوميتر. تم دراسة تأثير تراكيز مختلفة من جسيمات كبريتيد النحاس النانوية على نشاط البكتيريا لنوعين من البكتيريا، وهما المكورة العنقودية والإشريكية القولونية. تم تحديد التركيز المثبط الأدنى (MIC) اللازم لتثبيط نمو البكتيريا عند مدى معين من تراكيز كبريتيد النحاس النانوي من 0.5229×10^{-1} الى 0.3268×10^{-2} مولاري.

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1. Introduction

Nanotechnology has been an active and dynamic field of research in the past decade. The size, distribution, and shape of nanoparticles all affect their unique properties and characteristics. The synthesis of nanoparticles with particular morphologies and controlled sizes has witnessed important improvements in the field of nanotechnology through recent years. The practical utilization of nanoparticles, defined as particles with sizes below 100 nm, is attributed to their unique and fascinating properties that surpass those of their bulk counterparts. The present study focuses on developing efficient methods for producing noble nanoparticles with precise composition, uniform dispersion, and well-defined shape and size. These characteristics significantly influence the chemical, physical, catalytic, magnetic, electrical, optical, and electronic properties of nanoparticles, making them excellent candidates for various environmentally friendly applications. One notable area of application is in biomedical research and biotechnology [1]. Copper nanoparticles have distinctive characteristics that are absent in conventional copper, such as exceptional catalytic efficacy and significant antifungal and antibacterial properties. One of the key factors contributing to their exceptional catalytic performance is their significantly increased surface area, which enhances their catalytic activity [2]. The antimicrobial activity of copper nanoparticles arises from their close interaction with microbial membranes and the release of metal ions into solutions. These characteristics are inherent to copper nanoparticles, which possess a small size and a high surface-to-volume ratio. These unique properties contribute to the effective antimicrobial action displayed by copper nanoparticles [3]. As copper nanoparticles gradually oxidize in liquid environments, cupric ions are released. In the presence of nearby lipid membranes, these cupric ions can generate hydroxyl free radicals, which might be hazardous. The oxidation of lipids inside cellular membranes by free radicals results in the breakdown of these membranes. Consequently, the damaged membranes lose their functionality, causing the leakage of cellular contents and impairing essential metabolic functions. Eventually, cell death occurs because of these internal modifications induced by the action of free radicals [4]. Living plant secretions are utilized in the synthesis of nanoparticles. Various parts of plants, such as leaves, roots, latex, seeds, and stems, are commonly employed in the synthesis of metal- and metal oxide-based nanoparticles. The synthesis process involves the utilization of specific chemicals found in plants, including flavones, terpenoids, amides, aldehydes, ketones, and other compounds, which play a crucial role in nanoparticle synthesis [5]. During the nanoparticle synthesis process, combinations of chemicals derived from plant extracts can serve as both stabilizing (capping) and reducing agents. These plant-derived compounds play a dual role by stabilizing the nanoparticles and facilitating the reduction of precursor materials during the synthesis process [6]. Previous research has demonstrated the environmentally friendly synthesis of copper sulfide nanoparticles using various plant sources. Among these studies is the work conducted by a group of researchers, which focused on synthesizing copper sulfide nanoparticles using leaf extracts of Urtica dioica. The study yielded promising results, showing a noticeable enhancement in crystallinity following heat treatment [7]. This indicates the effectiveness of the plant-based approach for synthesizing copper sulfide nanoparticles. The biomedical research community has shown a significant interest in copper sulfide (CuS) nanoparticles, as they have gained widespread applications in laboratories, particularly for the detection of biomolecules, pathogens, and chemicals. Furthermore, preclinical studies have explored the potential *in vivo* applications of CuS nanoparticles. These applications include drug delivery and therapeutics, leveraging the photothermal properties of CuS for cancer therapy, as well as molecular imaging using various techniques. The versatile properties of CuS nanoparticles have made them a valuable tool in biomedical research, with promising potential for diverse applications [8]. Additionally, nanoparticles (NPs) are extensively employed as a potential alternative to antibiotics for targeting bacteria [9]. Previous studies have also examined the inhibition of these gram-negative and gram-positive bacteria using different types of nanoparticles. For instance, in a 2021 study conducted by Mar and Tag, environmentally friendly silver nanoparticles demonstrated significant antibacterial activity against both gram-positive and gram-negative bacteria, including *Staphylococcus aureus* and *Escherichia coli*. Similarly, in another study by the same researchers, gold nanoparticles exhibited inhibitory effects on these two types of bacteria [10,11]. The effect of silver nanoparticles synthesized from plant latex was studied for these two types of bacteria, as the results showed an inhibitory effect at 500µg/ml of synthesized AgNPs [12]. Also, in a previous study, the antibacterial activity of MgO nanoparticles synthesized by a green method using the peels of Persimmon extract was evaluated on *Staphylococcus aureus and Escherichia coli*, where MgO NPs showed activity at the minimum inhibitory concentration (MIC) of 500 µg/mL [13]. In this study, copper sulfide nanoparticles from myrtle plants will be used to specifically target the bacteria *Staphylococcus aureus and Escherichia coli*. The natural habitat of *Staphylococcus aureus* in humans is the skin and also the nasopharynx. One of the dangers of this bacteria is that it can cause a wide range of infections, including soft tissues, skin, internal organs, and vascular sites. It is an important pathogen in hospitals and the community, where it can spread from a superficial site through the bloodstream to internal organs. Surgical wounds and static medical devices are the main sites of infection in hospital patients [14]. *Escherichia coli* are a type of vertebrate gut bacteria that is involved in both intra- and extra-intestinal infections. *Escherichia coli* are an opportunistic pathogen [15]. This bacterium is associated with infections as well; including urinary tract infections, diarrhea, and meningitis [16], *Escherichia coli* is well suited for the study of antimicrobial resistance. This is because it easily gains resistance and is commonly found in many different species [17].

2. Materials and methods

Copper (II) sulfate pentahydrate ($CuSO_4.5H_2O$), and sodium sulfide (Na_2S), myrtle leaves, all the chemicals were used without further purification. All the solutions were prepared with distilled water.

2.1. Instrumentation

The ultraviolet spectrum was recorded at room temperature using the Shimadzu UV-1800 spectrophotometer, while the FTIR spectra were obtained using the Shimadzu FT-IR 84005 spectrometer at room temperature. For FT-IR analysis, the plant extract containing CuS NPs was dried for one hour at 60 °C and then mixed with an appropriate amount of KBr. The biosynthesis of CuS NPs was confirmed through X-ray diffraction (XRD) analysis using the Shimadzu XRD-6000 diffractometer. The morphology and contact surface of the copper sulfide nanoparticles were analyzed using AA300 Angstrom AFM Atomic Force microscopy. Additionally, the FESEM S-4160 electron microscope was used to evaluate aliquots of plant extract filters containing CuS NPs.

2.2. Green synthesis of CuS NPs

Fresh myrtle leaves were collected from the home garden and properly washed and rinsed with distilled water. The leaves were air-dried at room temperature and subsequently cut into small pieces. For the extraction solution, leaves (10 g) were boiled with distilled water (200 mL) for 20 minutes. The resulting solution was filtered using filter paper to obtain a purified extract that was suitable for nanoparticle synthesis, as depicted in Figure 1.

Figure 1: The myrtle leaf extract

For the preparation of copper sulfide nanoparticles [7], $CuSO_4.5H_2O$ solution (200 mL, 0.02 M) was combined with myrtle leaf extracts (200 mL). A solution of Na_2S (0.02 M) was gradually added after 30 minutes, drop by drop, and the resulting solution was allowed to proceed through reduction for 20 hours. The nanoparticles were separated from the solution through centrifugation at 3000 rpm and subsequently dried in an oven. Following drying, thorough grinding was performed, as depicted in Figure 2. To enhance crystallinity and eliminate any organic components derived from the plant material, a calcination stage was conducted at 500 °C.

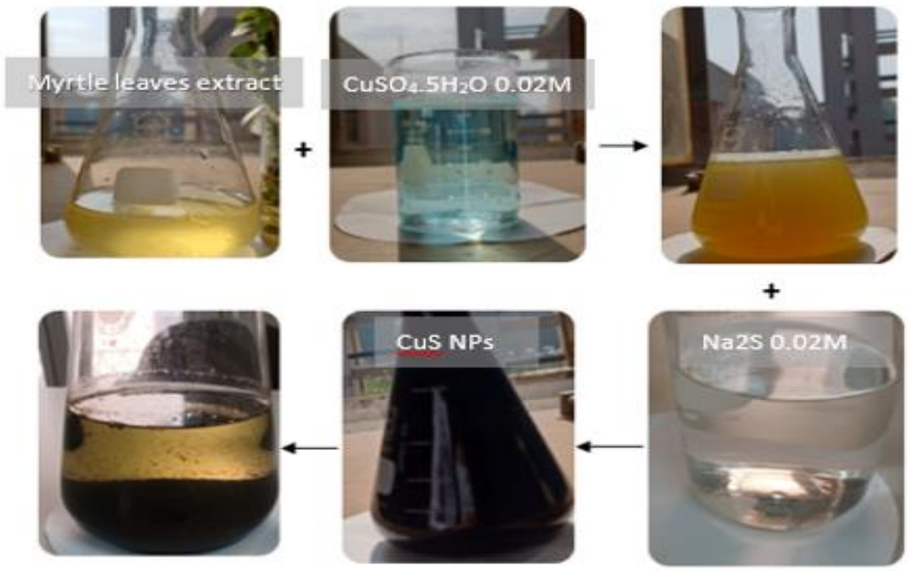

Figure 2: Preparation of copper sulfide NPs

2.3. Antibacterial activity and minimum inhibitory concentration (MIC)

The antibacterial effects of copper nanoparticles were examined using two types of bacteria: *Staphylococcus aureus*, a gram-positive bacterium, and *Escherichia coli*, a gram-negative bacterium. The bacterial strains were activated and exposed to room temperature. After that, a nutrient broth (volume of 100 μL), distilled water, and bacteria (10 μL) were combined in a microliter plate. The produced solution was gradually diluted by half to test different CuS nanoparticle concentrations, as shown in Table 1.

Table 1: Concentration of CuS NPs on *Staphylococcus aureus* and *Escherichia coli*

Sample of CuS NPs	Concentration (M)
A	0.5229×10^{-1}
B	0.2614×10^{-1}
C	0.1307×10^{-1}
D	0.6536×10^{-2}
H	$0. 3268 \times 10^{-2}$

Figure 3 illustrates the impact of five different concentrations of nanoparticles on two types of bacteria: *Staphylococcus aureus*, a gram-positive bacterium (represented as E and F), and *Escherichia coli*, a gram-negative bacterium (represented as G and H). The concentrations of nanoparticles (labelled as A to H) were applied to the bacteria from left to right, with the rightmost column serving as the control group, where no nanoparticles were added. The bacteria were subsequently incubated in the microliter plate for 24 hours to observe any observable changes.

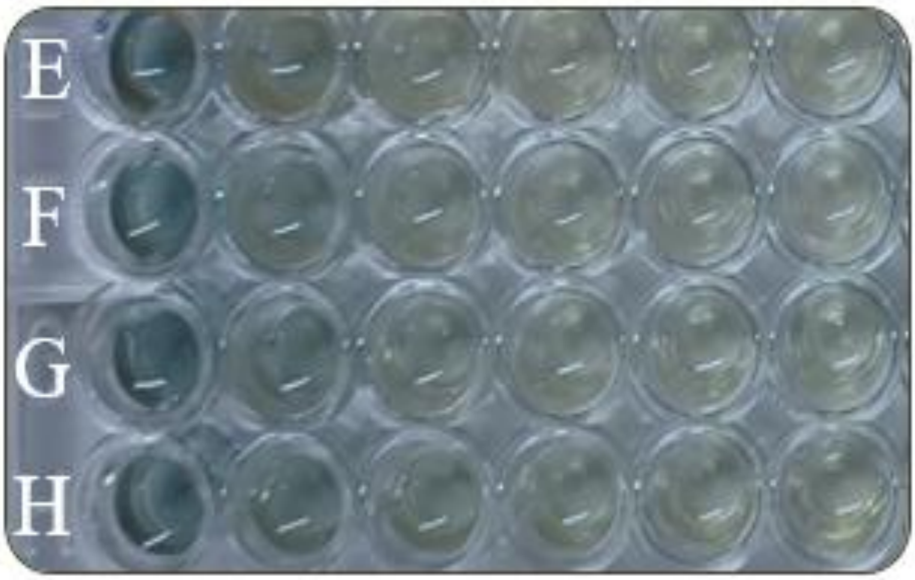

Figure 3: Effect of CuSNPs concentration on *Staphylococcus aureus* and *Escherichia coli*

3. Results and discussion

3.1. Characterization techniques

The nanocrystals size, composition, morphology, and other vital characteristics were thoroughly analysed and investigated using a variety of analytical techniques, including UV-*via* spectroscopy, FT-IR, FE-SEM with EDX, AFM, and XRD. These techniques provided valuable insights into the properties and features of the nanocrystals.

3.1.1. Green synthesis of CuS NPs and UV-Vis spectroscopic analysis

The UV-visible spectrum of CuS nanoparticles typically exhibits a characteristic peak in the range of 290-310 nm, corresponding to the absorption of CuS nanoparticles. The intensity and shape of this peak may change depending on factors such as nanoparticle size, shape, and concentration. Other peaks or shifts in the spectrum may indicate the presence of impurities or structural modifications in the nanoparticles. UV-Visible spectroscopy is a valuable technique for investigating the optical properties of CuS nanoparticles. The obtained results demonstrated that copper sulfide nanoparticles exhibit optical absorption at approximately 295 nanometers, as depicted in Figure 4, which illustrates the absorption spectrum of copper sulfide particles. The result of the measurement was close to the results of UV-Visible in the researchers' work "Rawat, Pooja, Anju Nigam, and Shubhra Kala" in the preparation of CuS NPs from plants [7].

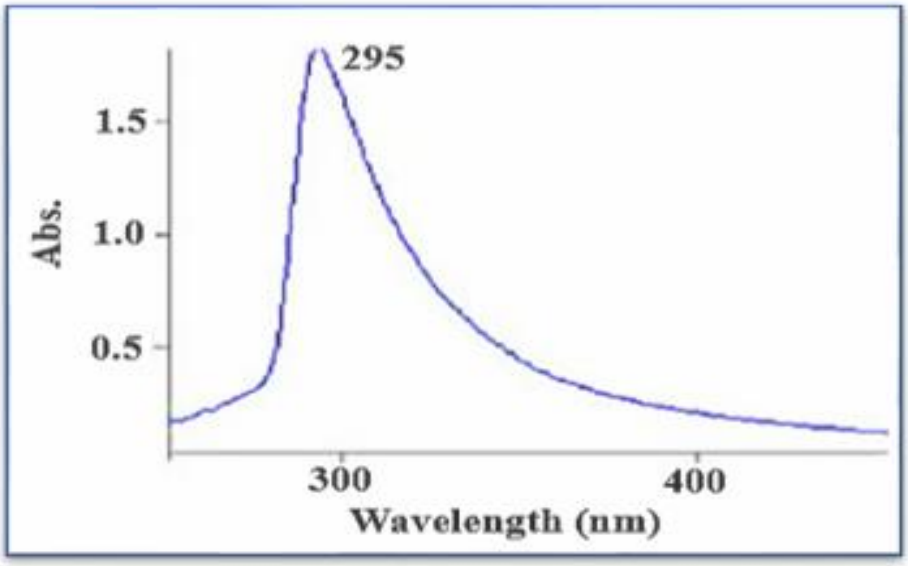

Figure 4 - The UV-visible spectra of CuS NPs

3.1.2. Atomic force microscopy (AFM)

The primary objective of the AFM study is to examine surface characteristics and analyse topography. By generating three-dimensional images of nanoparticle surfaces at a microscopic scale, AFM enables the achievement of this objective. The two- and three-dimensional images obtained (as depicted in Figures 5) showcase the synthesis of copper sulfide nanoparticles utilizing myrtle leaf extract, with an average particle diameter of 24.64 nm at nanoscale.

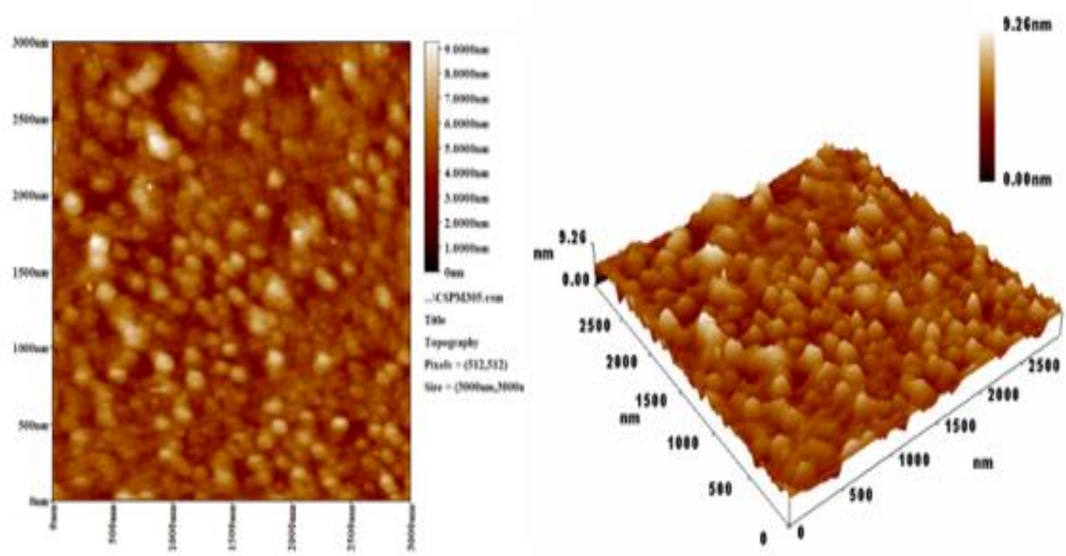

Figure 5: Two- and three-dimensional AFM images of CuSNPs

In Figure 6, the percentage of granularity cumulation distribution of copper sulfide nanoparticles is displayed, indicating the highest distribution at a diameter of 18. The results of the AFM measurements are summarized in Table 2.

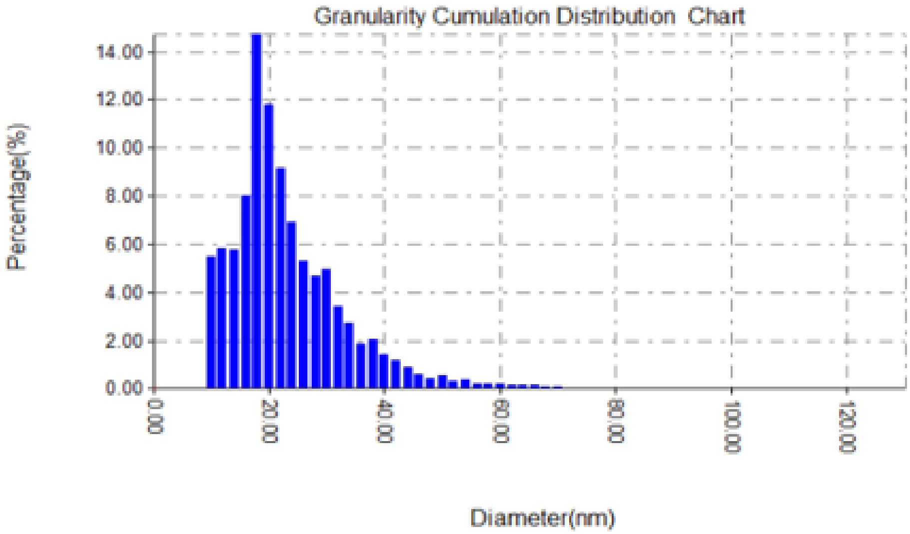

Figure 6: Granularity cumulation distribution of copper sulfide NPs

Table 2 - Grain size analysis

Total Grain	16525
Avg. Size	477.01 nm^2
Avg. Height	5.17 nm
Avg. Diameter	24.64 nm
Max Size	12977.60 nm^2
Min Size	68.66 nm^2

3.1.3. FESEM/EDX analysis of CuS NPs

FESEM analysis is a powerful technique that allows for the examination of surface topography and element identification at different zoom levels, ranging from 10x to 300,000x. As depicted in Figure 7a-c, the shape of the copper sulfide nanoparticles is shown clearly, with noticeable aggregation. The size, shape, and distribution of the CuS nanoparticles synthesized using myrtle leaf extract were also determined; it showed that the lowest dimensions were equal to 73.72 nm. Previous studies have shown that there are different forms of morphology in nanocrystalline copper sulfide due to several factors that determine the shape, including heating time, temperature, reaction time, and sulfur source [18].

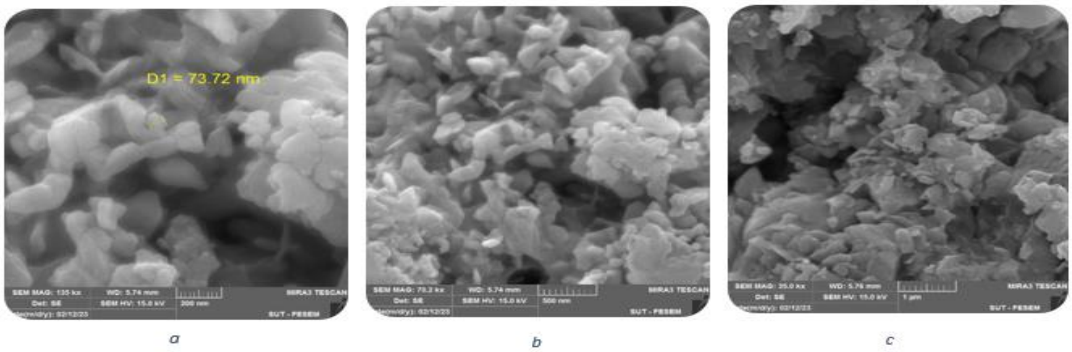

Figure 7: FESEM pattern of synthesized CuS NPs

EDX analysis is a valuable technique implemented to examine the elemental composition and chemical properties of a sample. The EDX spectrum in Figure 8 confirms the presence of copper (Cu), sulfur (S), carbon (C), and oxygen (O) in the analyzed sample. The detection of carbon might be attributed to the combustion of organic matter derived from the plant extract during the calcination process. The results showed a great similarity to the published work [19].

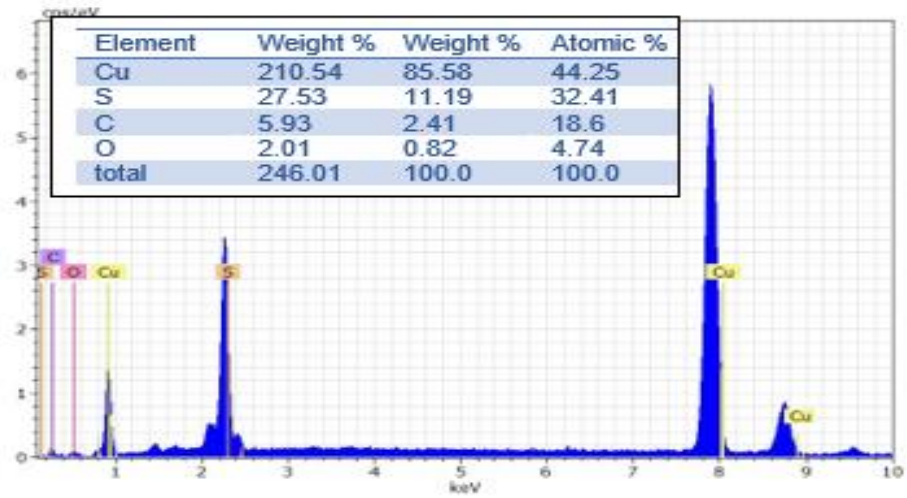

Figure 8: EDX analysis of CuS NPs

3.1.4. X-Ray diffraction (XRD) analysis of CuS NPs

The XRD pattern in Figure 9 shows that the prepared copper sulfide nanoparticles have formed an orthorhombic crystal system of CuS. The pattern exhibits five distinct peaks located at 2θ values of 21.942, 32.097, 33.174, 39.221, 44.745, 48.237, 58.589, 61.369, 68.041, and 74.821 degrees, corresponding to (hkl) values of 004, 013, 006, 015, 008, 017, 019, 024, 118, and 028, respectively. The peaks are in good agreement with the database (ICSD) (98-003-2107). The crystallite size of the sample was determined using Debye-Scherrer's approximation as described in equation 1, indicating a crystal size of 21.4 nm.

$$D = \frac{K\lambda}{\beta cos\theta} \quad (1)$$

Where, D is the crystallite size of copper sulfide nanoparticles, λ represents the wavelength of the X-ray (source 0.1541 nm) used in XRD, β is the full width at half maximum of the diffraction peak, K is the Scherrer constant with a value from 0.9 to 1, and θ is the Bragg angle.

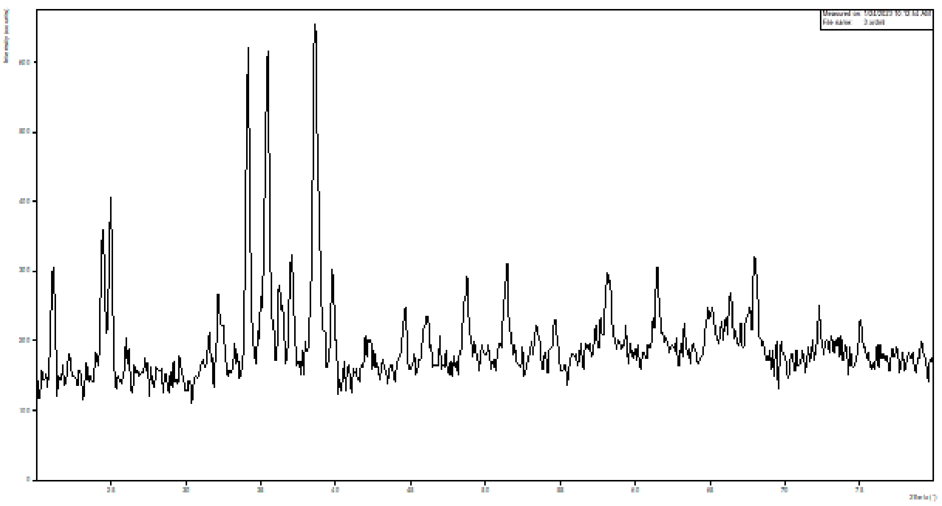

Figure 9: XRD pattern of synthesized copper sulfide nanoparticles (CuS NPs)

3.1.5. Fourier transforms infrared analysis

FT-IR analysis of copper sulfide nanoparticles typically exhibits distinct peaks that correspond to the chemical bonds present in the sample. These peaks provide valuable insights into the functional groups and chemical composition of the nanoparticles. However, the specific position and intensity of these peaks can vary depending on factors such as the synthesis method, nanoparticle size and shape, and surface chemistry. In Figure 10, the main peaks observed for the prepared CuS nanoparticles are as follows: a sharp peak in the range of 600-700 cm^{-1}, which corresponds to the stretching vibrations of the Cu-S bond, and a peak around 1000–1100 cm^{-1}, associated with the bending vibrations of the S-Cu-S bond.

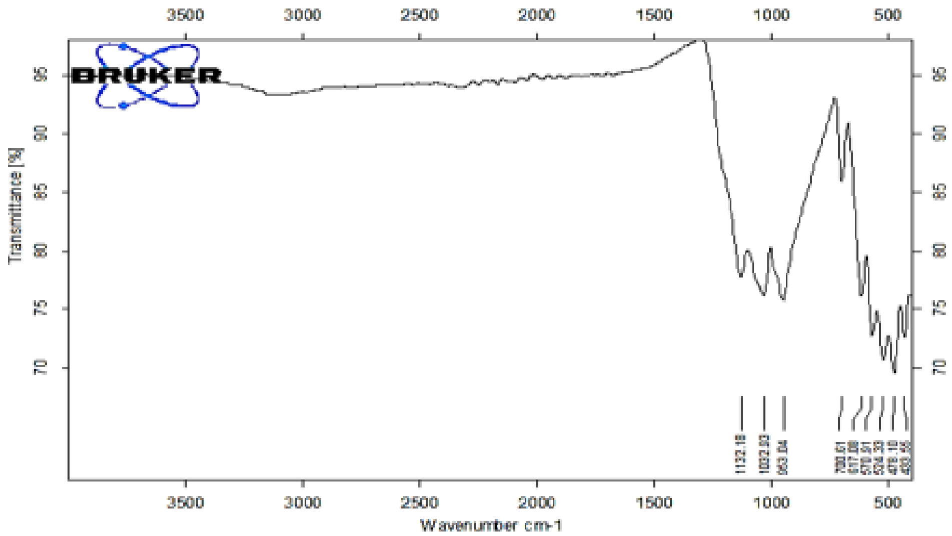

Figure 10: FT-IR spectrum of CuS nanoparticles

3.2. Antimicrobial analysis of CuS NPs

Copper sulfide nanoparticles made from myrtle leaves were tested for their ability to kill gram-positive *Staphylococcus aureus* and gram-*negative Escherichia coli* bacteria. The results, presented in Figure 11, demonstrate that the nanoparticles effectively inhibit the growth of both bacterial strains. The minimum inhibitory concentration (MIC) determines whether a particular bacterial strain is susceptible to an antibiotic *in vitro* or resistant to it. The minimum inhibitory concentration of an antibacterial agent is measured in mg/L, and it is the lowest concentration at which the test strain of an organism cannot grow in any way that can be seen. Many factors, such as the appropriate method selection, adherence to labelling regulations, and competent interpretation of the data, must be considered in order to produce credible MICs. [20]. The MIC of copper sulfide nanoparticles against *Staphylococcus aureus* bacteria was determined to be 0.1307×10^{-1} M, indicating the minimum concentration required to inhibit bacterial growth. Similarly, the MIC of copper sulfide nanoparticles against *Escherichia coli* bacteria was found to be 0.2614×10^{-1} M, as shown in Table 3. These findings indicate the potential of copper sulfide nanoparticles synthesized from myrtle leaves as antimicrobial agents against both gram-positive and gram-negative bacteria.

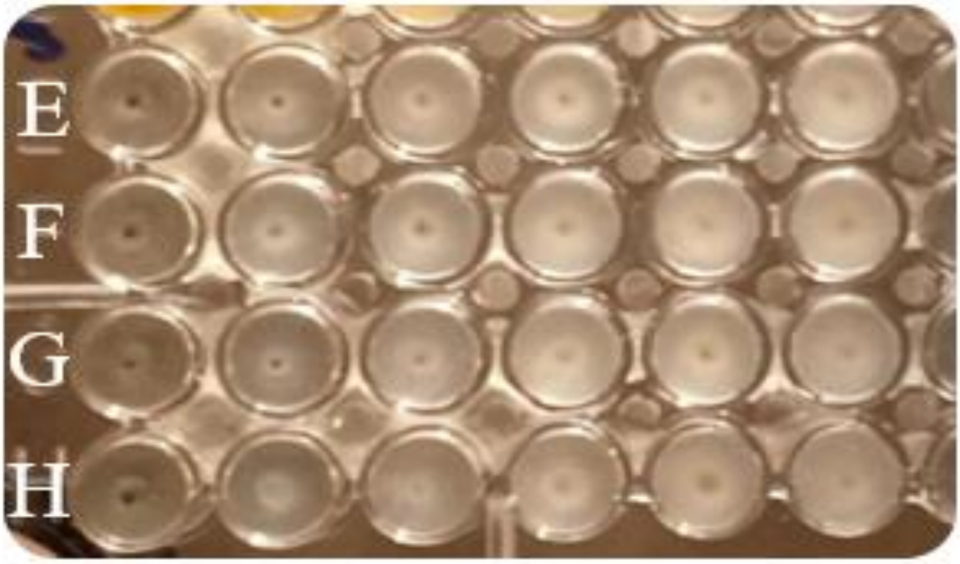

Figure 11: Antibacterial activity of different concentrations of synthesized CuSNPs

Table 3: Antibacterial activity of copper sulfide nanoparticles

Concentration (M)	Staphylococcus	E.Coli
0.5229×10^{-1}	Kill	Kill
0.2614×10^{-1}	Kill	Kill
0.1307×10^{-1}	Kill	No effect
0.6536×10^{-2}	No effect	No effect
$0. 3268 \times 10^{-2}$	No effect	No effect

4. Conclusion

The green synthesis method was employed to synthesize copper sulfide nanoparticles using the extraction of myrtle leaves as a reducing agent. The nanoparticles were subjected to heat treatment to enhance their crystallinity, resulting in a well-crystallized form of copper sulfide nanoparticles at a temperature of 500 °C. This treatment also led to a significant decrease in the carbon content of the nanoparticles. Various analytical techniques, including X-ray diffraction, FESEM, EDX, UV-Visible, and AFM, were utilized to characterize the synthesized copper sulfide nanoparticles. Furthermore, the antibacterial activity of the prepared nanoparticles was assessed against two types of bacteria, namely gram-negative *Escherichia coli* and gram-positive *Staphylococcus aureus*, using different concentrations of copper sulfide nanoparticles. The findings supported the effectiveness of the nanoparticles in inhibiting the growth of bacteria.

Conflicts of interest

The author declares that they have no conflicts of interest.

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