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Antibacterial Activity of Ecofriendly Biologically Synthesized Copper Oxide Nanoparticles

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Abstract

Copper oxide nanoparticles (CuONPs) were prepared using ecofriendly simple method that employs the extract of Syzygium Aromaticum (clove buds) as a reducing and stabilizing agent. The prepared CuONPs were characterized by FT-IR, SEM and EDS techniques to study their morphological and compositional properties. Fourier transform infrared spectroscopy (FTIR) spectrum indicates the presence of various functional groups at different positions. Morphological studies were carried out to study the shape of the synthesized nanoparticles. Energy dispersive X-ray spectroscopy (EDX) shows highly intense peaks of copper (Cu) and oxygen (O), while less intense peaks of carbon (C) and sulfur (S) elements due to the presence of biomolecules of bud extract used in CuONPs formation. The antibacterial activity of the synthesized CuONPs against gram –ve microorganisms viz. E-Coli, Salmonella and Enterobacter bacteria was studied. The produced CuONPs show resistance to the three types of bacteria with greatest resistance to E. coli followed by Salmonella and then Enterobacter.

Keywords: : Syzygium Aromaticum, Copper oxide nanoparticles (CuONPs), gram – ve microorganisms, antibacterial Activity.

1. Introduction

Metallic nanoparticles are gaining great attention worldwide due to their significant and wide applications in a variety of fields including medicine, agriculture, and chemistry [1]. Mainly, metal and metal oxide nanoparticles are synthesized either physically or chemically [2]. Physical means of metallic nanoparticles production include High-Energy Ball Milling and Ultrasonic Shot Peening (USSP) [3]. While chemical means include pulse electro-deposition [3], reduction of chemical salts of metals [4], and electrochemical synthesis [5].

Physical and chemical means are found to have great disadvantages as they are not environmentally friendly, expensive, time consuming, sometimes include toxic chemicals in the synthesis route [6, 7]. So, direction was shifted towards green methods for metallic nanoparticles synthesis which eliminated most of the drawbacks of the alternative conventional physical and chemical methods, green synthesis of metallic nanoparticles employs plants, bacteria or fungi in the process of synthesis as reducing and stabilizing agents [8-14]. It has been confirmed in previous work that plant extracts are the most effective among all for synthesis of metallic nanoparticles as they are much safer, easier to handle, more abundant, more cost effective, work at low temperatures and do not require harmful reagents during the reaction [15]. Another main advantage of using plant extracts is that they are very rich in valuable metabolites such as enzymes, carbohydrates, proteins, vitamins, alkaloids, terpenoids, tannins and saponins [16] that act as reducing and capping or stabilizing agents that help in modulation and controlling the size of produced nanoparticles by preventing possible aggregations that can enlarge the size of the particles [17]. In addition, the abundance of these metabolites in plant-based mechanisms extremely results in great antibacterial effects of the produced nanoparticles of metals and metal oxides.

Copper oxide nanoparticles (CuONPs) are among the most promising transition metal oxides that attract great attention in the last few years due to their emerging applications in pharmaceutical, chemical, industrial and medicinal fields [18-21]. For instance,

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in medicine and biochemical fields, CuONPs have been utilized as antibacterial, antioxidant, imaging and drug delivery agent [22]. CuO nanoparticles were prepared using various plant extracts including the extract of Aloe Vera leaves [23], Carica papaya leaves [24], tea leaves and coffee powder [25], Albizia lebbeck leaves [26], Abutilon indicum Leaf Extract [27]. Syzygium Aromaticum extract was used to produce CuNPs in previous research work [28]. But the problem with CuNPs is that they are easily and rapidly oxidized when subjected to air what makes their formation in natural environment relatively challenging [29].

Copper oxide nanoparticles synthesized using plant extracts efficiently showed antibacterial activities against several pathogens including gram positive and gram-negative bacteria such as E. coli, K. aerogenes and S. aureus [30]. An important feature of CuONPs is that they are more able to generate more reactive oxygen species which makes them more toxic to the bacterial cells [31].

However, the available information regarding the antimicrobial activity of CuO nanoparticles is yet limited and needs further research.

Based on the previous work and investigations, CuO nanoparticles will be synthesized using the extract of clove buds. The biologically synthesized CuONPs will be characterized by FT-IR, SEM and EDX techniques and their antibacterial activity against three types of gram-negative bacteria E. coli, Salmonella and Enterobacter, will be investigated.

2. Materials and methods

2.1. Materials

Clove buds (Syzygium aromaticum plant), used as a reducing agent for the synthesis of CuONPs were obtained from the local market. Copper sulfate, sodium hydroxide were purchased from Sigma Aldrich. All chemicals were utilized as obtained. All tested microorganisms were obtained from Biotechnology and Microbiology Center, Mansoura University, Egypt.

2.2. Preparations

2.2.1 Preparation of clove bud's extract

Clove buds' extract (Syzygium aromaticum plant) was prepared by crushing 5gm of clove buds into fine powder followed by filtration to remove unwanted debris. The fine powder was then mixed with 100 ml deionized water and boiled for 10 minutes. It was left to cool down at room temperature. The extract was then filtered using Whatman's No.1 filter paper. The filtered extract was collected in a clean and dried conical flask and was stored for further usage in CuONPs synthesis

2.2.2 Synthesis of CuO nanoparticles.

One ml of clove buds'extract was mixed with 10 ml of 0.1 mM CuSO4 with constant stirring at 60–65 ^{0}C then 0.1 M NaOH was introduced dropwise into the mixture until it reaches pH 11 and allowed to stir for 2 h. Formation of CuO was observed by the color change as shown in Figure 1. The mechanism of the reaction and related color changes are described in the mentioned study [32]. CuO nanoparticles were collected by centrifugation at 2000 rpm and repeated three times to confirm purification. The obtained precipitate was washed by distilled water and dried in oven at 150 ◦C.

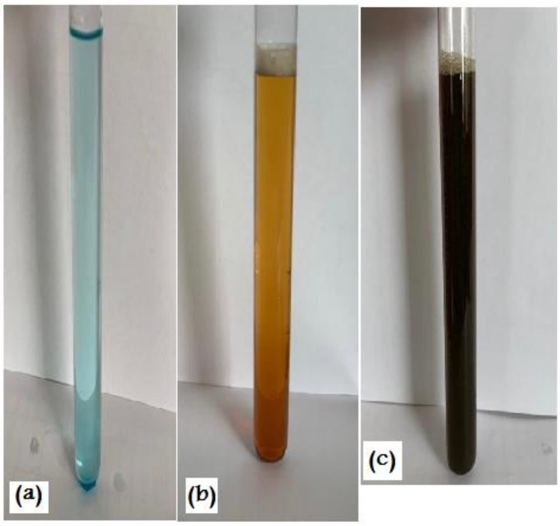


Fig. 1. Synthesis of CuONPs: (a) $CuSo_4$ solution, (b) clove buds' extract, (c) formed CuONPs.

2.3. Characterization

The synthesized CuONPs were characterized by FTIR, EDX, SEM techniques to get a closer look at the morphology and chemical composition.

2.3.1. Fourier-transform infrared spectroscopy FT-IR

This technique was used to identify the present functional groups associated with CuO synthesis process. The infrared spectrum of the sample was obtained in the wavelength range of 4000–400 cm^{-1} using Perkin Elmer FTIR spectrophotometer (Jasco 6100, Japan).

2.3.2. *Energy Dispersive X-ray (EDX)* was carried out to identify the elemental composition of the synthesized CuONPs. The interaction of the electrons transmitted through the specimen resulted in the formation of an image which is then magnified and focused onto an imaging device.

2.3.3. Scanning Electron Microscope (SEM) was used to gain information about the morphology of the produced CuONPs by scanning the surface at three different magnifications (5,000X, 15,000X and 27,000X).

2.4. Antimicrobial activities of the CuONPs

The three studied microorganisms E-coli, Salmonella and Enterobacter were cultured on nutrient agar plates following the streak-plating method. The synthesized CuO nanoparticles were pressed into 13 mm diameter pellets and located in agar plates with the tested species of bacteria and incubated for 24 hours at 37 ^{o}C. The mean diameter of each inhibition zone was measured in millimeters.

3. Results and discussion

3.1. Characterization of the synthesized CuONPs

3.1.1. FTIR spectroscopy

FTIR was carried out in order to identify the functional groups present. The infrared spectrum in Figure 2 shows two characteristic bands at 431 cm^{-1} and 481 cm^{-1} that can be assigned to Au modes of vibration of CuO, while the other important band at 605 cm^{-1} can be assigned to the Bu vibrational modes of CuO [33]. A broad band appeared in the range of 3200–3560 cm^{-1} that is ascribed to the stretching vibrations of hydroxyl groups that arise from adsorbed water molecules on the surface of CuONPs, this adsorption of moisture can be explained by high surface-to-volume ratio of the nanocrystalline materials [34]. Another sharp band appeared at 1107 cm^{-1} can be attributed to C–OH stretching and OH bending vibration, indicating the existence of a greater number of hydroxyl groups present in the chemical components of clove extract. The peak appeared at around 1413 cm^{-1} may indicate the presence of CO_2, which is usually adsorbed from the surrounding air.

3.1.2. ***Compositional studies:***

Energy Dispersive Analysis of X-ray (EDX) gives qualitative as well as quantitative analysis of chemical composition of compounds relying on the amount of energy emitted by the electrons of each element. Figure 3 shows the EDX results of CuONPs synthesized using clove buds' extract. The sharp peaks present correspond to Cu and O atoms that make up CuO. Weaker peaks for C atoms are also detected due to the organic nature of clove extract used during synthesis process.

3.1.3. Morphological studies

The SEM images of the CuO nanoparticles synthesized using clove buds extract was carried out in order to investigate the surface morphology. The SEM images that were obtained at three different magnifications namely (5000X, 15,000X and 27,000X) are shown in Figure 4. It can be straightly demonstrated from the images that the CuO nanoparticles possess a spherical morphology. The spherical shape of CuO nanoparticles is mainly due to the capping and stabilizing action of phytochemicals

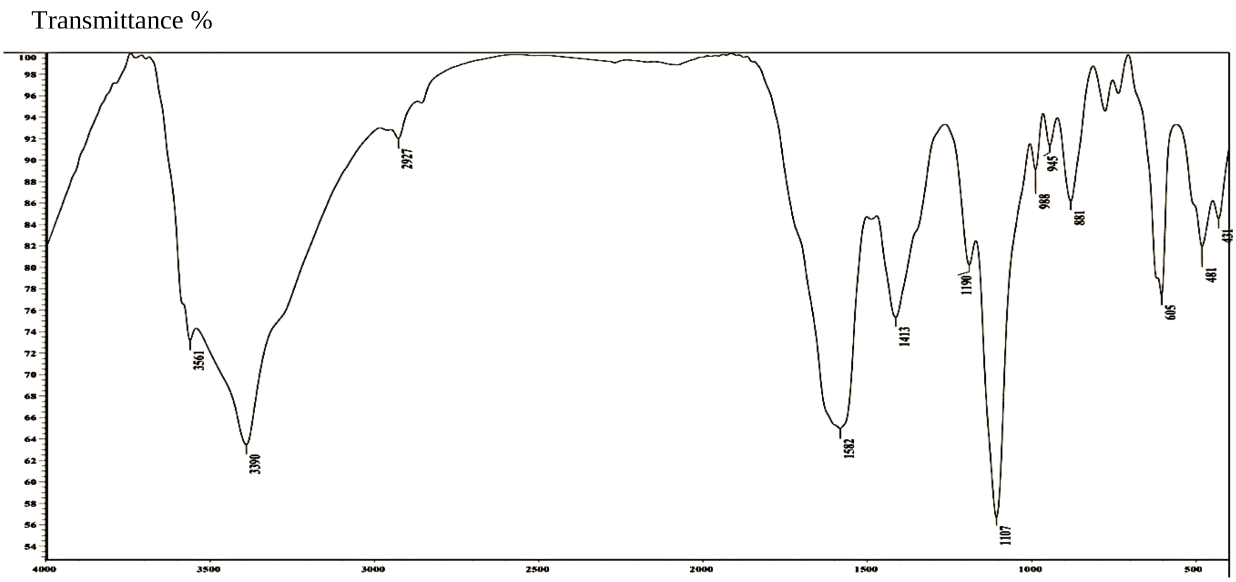


Fig.2. FT-IR spectrum of the synthesized CuO NPs Wavenumber (cm^{-1})

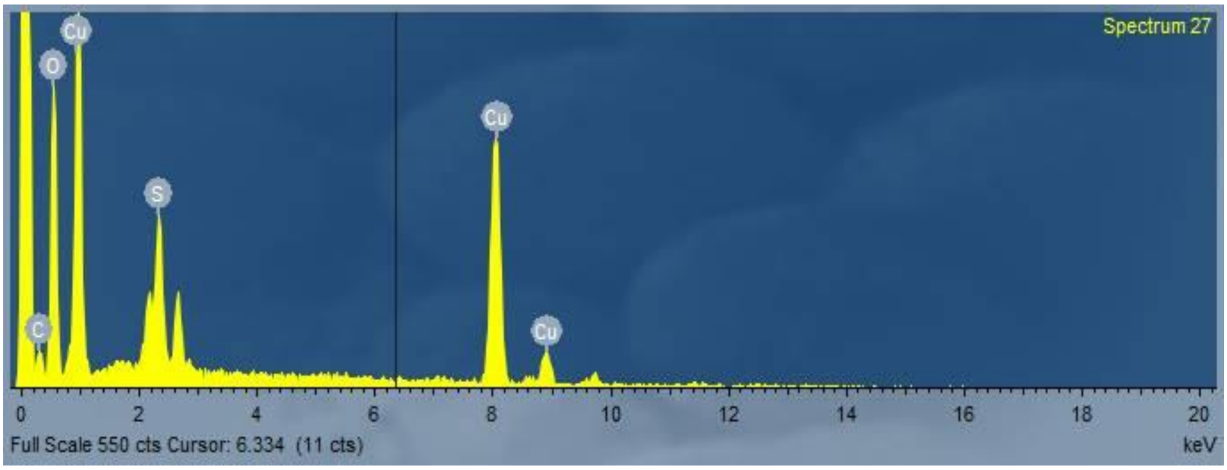


Fig.3. EDX spectrum of the synthesized CuO NPs

present in the plant extract that prevent agglomeration between the synthesized particles [35]. It can be also observed that the nanoparticles can be produced in large quantity as shown in the images using this simple and green method.

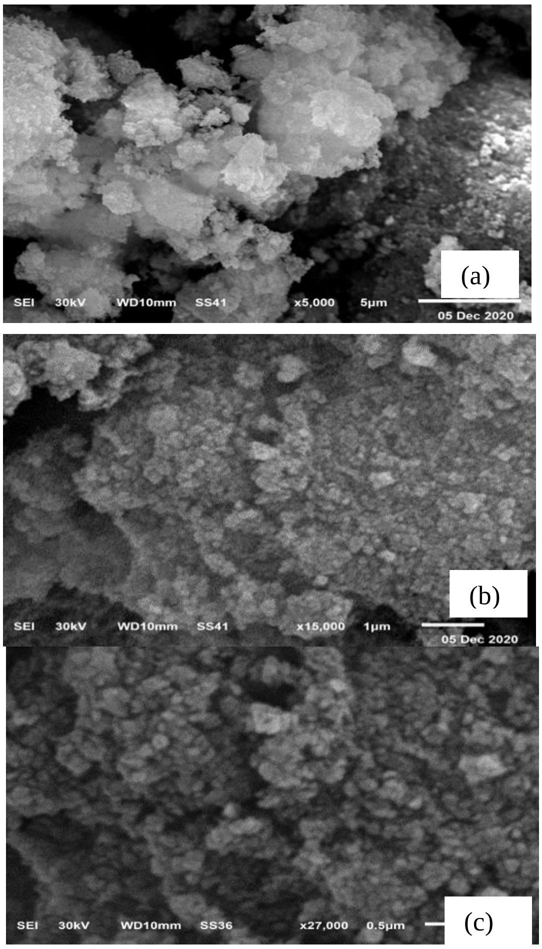
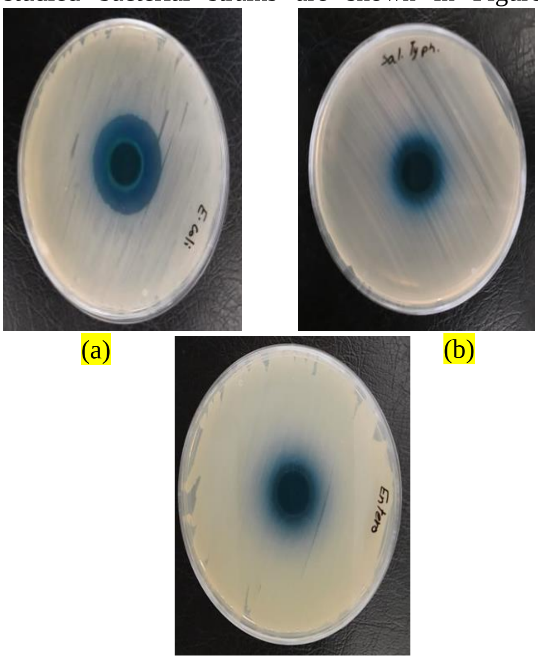


Fig.4. SEM images at magnifications of (a) 5000X, (b) 15000X, (c) 27000X

3.4. Antimicrobial activity

The antimicrobial activity of the synthesized CuONPs was evaluated against three pathogenic gram-negative bacteria namely E. coli, Salmonella, and Enteropacter bacteria.

The three types of bacteria were inhibited to different extents by CuONPs. The highest resistance activity of CuONPs was shown against E. coli bacteria with inhibition zone of diameter equals 29.6 mm. While the recorded zone of inhibition of Salmonella was of diameter 19 mm. CuONPs also show resistance against Enterobacter with an inhibition zone of 17.6 mm diameter. The images of clear zones that refer to the antibacterial behavior of CuONPs against the studied bacterial strains are shown in Figure 5.



(a) (b)

(c)

Fig.5. Images of antibacterial activity of CuONPs against (a) E. coli, (b) Salmonella, (c) Enterobacter bacteria

Although the exact antibacterial mechanism is still not completely understood, there are some proposed mechanisms that are believed to explain the antibacterial action of metal and metal oxide nanoparticles including release of metal ion species to the pathogen cell, generation of reactive oxygen species (ROS), penetration of cell wall, and cellular leakage [36].
Since CuO nanoparticles are smaller in size than bacterial pores, they are believed to have an exceptional ability to penetrate the membrane of bacterial cell causing damage and disturbance of cellular respiration processes which leads eventually to cell death [37]. Release of copper ions and generation of reactive oxygen species such as superoxide anions that cause toxicity and great damage to the bacterial cell are highly proposed mechanism for CuONPs antibacterial action [30, 36 38].

4. Conclusion

The CuO nanoparticles were biologically successfully synthesized utilizing the extract of clove buds. They were then characterized with several instrumental techniques. The antibacterial activity was investigated against E. coli, Salmonella and Enterobacter. The three types were sensitive to CuONPs, the highest resistance observed for E. coli and lowest to Enterobacter. Also, extra work is extremely required for further investigation of the antibacterial action of CuONPs.

5. Conflicts of interest

"There are no conflicts to declare".

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