Spectroscopic and Antibacterial Properties of CuONPs from Orange, Lemon and Tangerine Peel Extracts: Potential for Combating Bacterial Resistance

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ABSTRACT

Green synthesis of nanoparticles using citrus peel extracts is known to be environmentally friendly and non-toxic when compared to chemical methods. In this study, different citrus peel extracts obtained with the solvents acetone and distilled water were used to synthesize copper oxide nanoparticles (CuONPs). The nanoparticles were characterized using cyclic voltammetry, ultraviolet-visible spectroscopy, energy-dispersive X-ray spectroscopy (EDS), transmission electron microscopy (TEM), scanning electron microscopy (SEM) and Fourier-transform infrared spectroscopy (FTIR). The absorption spectrum of CuONPs prepared with acetone exhibited characteristic peaks at the wavelengths between 280–293 nm, while those with distilled water had peaks at 290 nm. The acetone-synthesized CuONPs were spherical while those produced using distilled water were rodshaped. Based on EDS, the analysis revealed a trace spectrum of CuO nanoparticles with different weight compositions that varied with the type of citrus peel and solvent used. FTIR measurements were carried out in the range of 500–4000 cm−1 for citrus peel extract mediated CuONPs. The spectra had five vibrations occurring at approximately 473, 477, 482, 607 and 616 cm–1 for all samples, which can be attributed to the vibrations of CuO, validating the formation of highly pure CuONPs.

Keywords: green synthesis; citrus peels; copper oxide nanoparticles; antibacterial activity

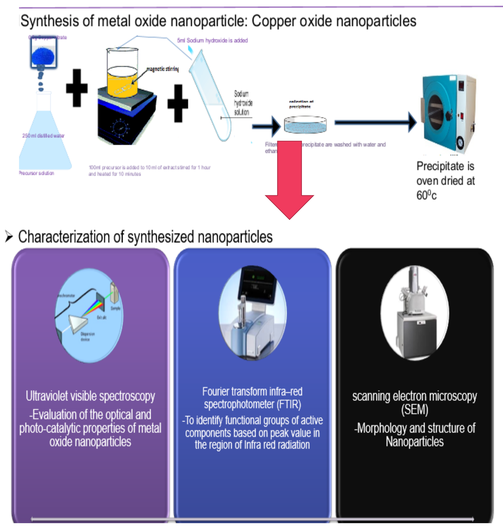
INTRODUCTION

According to the National Institute of Communicable Diseases (NICD), there are constant outbreaks and re-emergence of infections caused by pathogenic bacteria such as *Escherichia coli*, *Salmonella typhi* and *Klebsiella pneumonia* [1]. Infectious diseases cause suffering and death to humans and severe economic implications, which are not always immediately appreciated. Thus, the increase in the number of contagious diseases poses significant constraints to public health and healthcare facilities. Moreover, these microbes' pathogenicity is also enhanced by the possession of antibiotic resistance determinants [2,3]. This is aggravated by the fact that antimicrobial resistance among bacterial pathogens is rising, making this an issue of severe public health concern [4]. Furthermore, due to the very little progress in the development of new antimicrobial agents and given that the goal of all health-related measures mostly involves an effective treatment control strategy, sustainable investment in countering antimicrobial resistance needs to be ensured [5]. To achieve this, highly coordinated strategies that may facilitate the discovery of alternative antibacterial agents is imperative. Recently, nanoparticles have proven to be very effective against clinically relevant antibiotic-resistant pathogenic bacterial strains [6]. Much attention has been directed towards exploring the antimicrobial activity of various plant extracts and their components, and some have produced significant inhibitory effects against pathogenic bacteria, yeasts, fungi and viruses [7–10]. Among the potential candidates, citrus fruit extracts generate considerable interest due to the wide range of phytochemical components they possess [11,12]. Green synthesis is a set of principles that eliminates the use of hazardous substances in manufacturing and applications of chemical products [13].

MATERIALS AND METHODS

All chemicals used were of analytical-reagent grade. Field emission scanning electron microscopy (FESEM) images were obtained from high resolution scanning electron microscope (Zeiss Ultra Plus 55 HRSEM, Germany). Infrared spectroscopy data were obtained with fourier transformed infrared spectrometer (Agilent Technology, Cary 600 series FTIR spectrometer, USA). UV-Vis experiments were performed with a UV-1901 UV-vis spectrophotometer (Agilent Technology, Cary series) using quartz cell with path length of 1.0 cm. The antibacterial potency of the synthesized metal oxide nanoparticles was evaluated using cells of the ten pathogenic bacterial strains. An aliquot of 100 µL of the overnight culture of each bacterial strain.

EXPERIMENT



RESULTS

Spectroscopic characterization

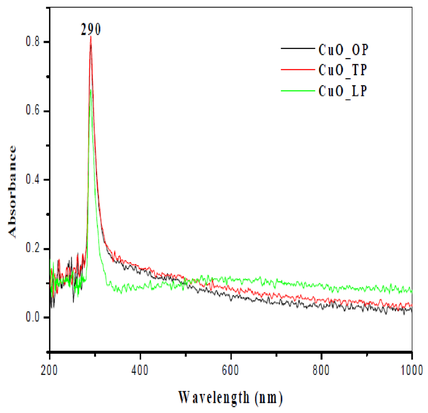


Fig. 1: UV-Vis spectra of CuONPs from citrus peels extracted with distilled water solvent.

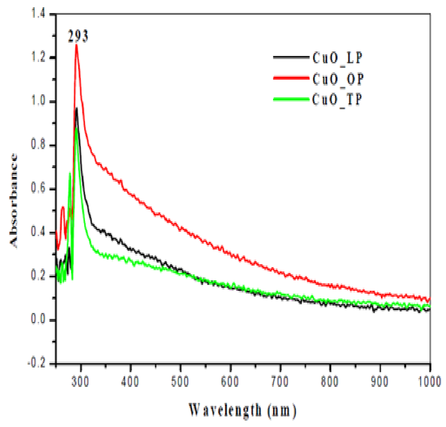


Fig. 2: UV-Vis spectra of CuONPs from citrus peels extracted with acetone solvent.

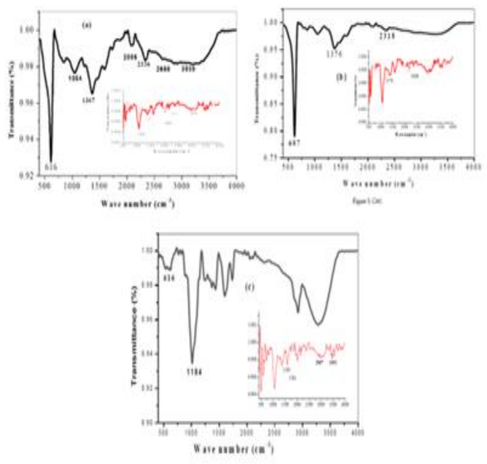


Fig. 3: The FTIR spectra of CuONPs from (a) orange (b) tangerine and (c) lemon peel extracts in distilled water; insert is the spectra for the respective citrus extracts

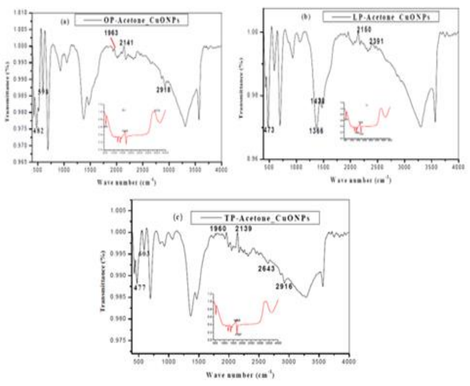


Fig. 4: The FTIR spectra of CuONPs from (a) orange (b) lemon and (c) tangerine peel extracts in acetone; insert is the spectra for the respective citrus extracts

RESULTS - CON'T

Morphological characterization

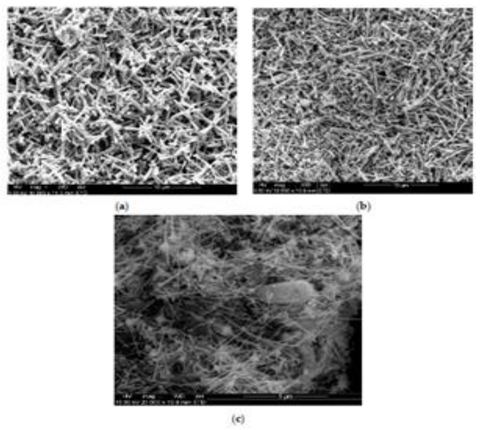


Fig. 5: SEM images of CuONPs from citrus peel extracts using distilled water as a solvent. (a) Lemon, (b) tangerine and (c) orange.

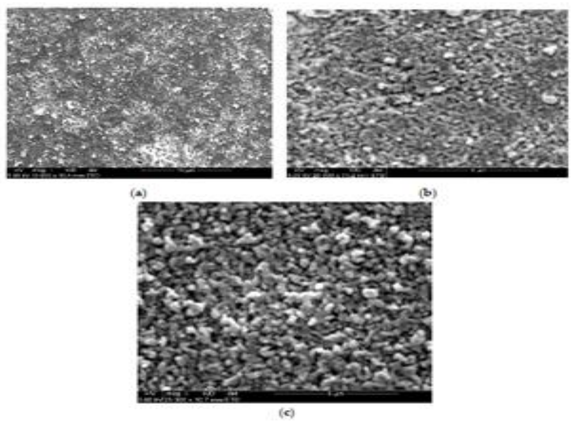


Fig4: SEM images of CuONPs from citrus peel extracts using acetone as a solvent. (a) Lemon, (b) tangerine and (c) Orange.

Antimicrobial sensitivity test

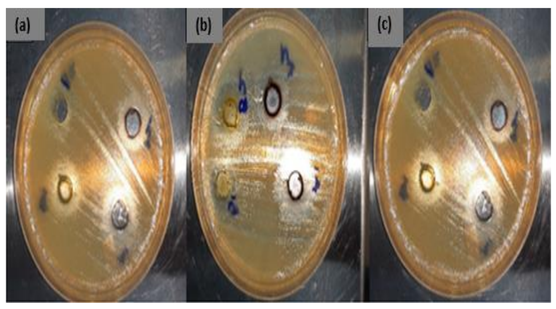


Fig.5: Antimicrobial sensitivity test of copper oxide nanoparticles. (a) *Escherichia coli*, (b) *Campylobacter coli* and (c) *Staphylococcus aureus*.

Pathogenic Bacterial Strains	Influence of Different Concentrations of CuONPs (Tangerine Extract) on Zone of Inhibition			
	(25 µg/mL) (Acetone)	(25 µg/mL) (Deionized Water)	(50 µg/mL) (Acetone)	(50 µg/mL) (Deionized Water)
C. perfringens	0 mm	0 mm	0 mm	0 mm
C. coli	0 mm	0 mm	0 mm	0 mm
E. coli	0 mm	0 mm	0 mm	0 mm
S. aureus	0 mm	0 mm	0 mm	0 mm
L. monocytogenes	0 mm	0 mm	0 mm	0 mm
S. pneumonia	0 mm	8 mm	0 mm	14 mm
P. aeroginosa	0 mm	0 mm	0 mm	0 mm
M. catarrhalis	0 mm	0 mm	0 mm	0 mm
S. diarizonae	0 mm	0 mm	0 mm	0 mm
E. faecalis	0 mm	0 mm	0 mm	0 mm

Table 1. Antibacterial activity of CuO-NPs-T on pathogenic bacterial strain.

Pathogenic Bacterial Strains	Influence of Different Concentrations of CuONPs (Lemon Extract) on Zone of Inhibition			
	(25 µg/mL) (Acetone)	(25 µg/mL) (Deionized Water)	(50 µg/mL) (Acetone)	(50 µg/mL) (Deionized Water)
C. perfringens	0 mm	0 mm	0 mm	0 mm
C. coli	0 mm	16 mm	0 mm	25 mm
E. coli	0 mm	0 mm	0 mm	0 mm
S. aureus	0 mm	17 mm	0 mm	23 mm
L. monocytogenes	0 mm	9 mm	0 mm	13 mm
S. pneumonia	0 mm	0 mm	0 mm	0 mm
P. aeruginosa	0 mm	6 mm	0 mm	10 mm
M. catarrhalis	0 mm	0 mm	0 mm	0 mm
S. diarizonae	0 mm	0 mm	0 mm	0 mm
E. faecalis	0 mm	0 mm	0 mm	0 mm

Table 2. Antibacterial activity of CuO-NPs-L on pathogenic bacterial strain.

DISCUSSION

This study aimed to determine the spectroscopic, morphological and antimicrobial properties of synthesized green mediated copper oxide nanoparticles from citrus peels extracts. This was motivated by the constant detection of multidrug-resistant bacterial strains that pose severe public health challenges to both humans and animals worldwide. In addition, the emergence and dissemination of multidrug-resistant strains is associated with their misuse which exerts selective pressure in the environment. There is the need to search for alternative antibacterial agents that are not only effective but are environmentally friendly. The present study presents findings of the successful green synthesis of CuONPs prepared through a biosynthetic technique using citrus extracts. The spectroscopic, morphological, and antimicrobial properties of the nanoparticles were examined. The UV–visible absorption spectrum designates that it was copper oxide nanoparticles synthesized from selected peel extracts exhibiting characteristic peaks at a wavelength between 280 and 320 nm as shown in fig.1 and 2. The FTIR characteristic peaks ranged from 600-3413 cm-1 and were more visible in the synthesized nano metal oxide respectively as shown in fig. 3 and 4. The vibration at 616 cm-1, is conveyed to be the vibration of Cu-O, confirming the formation of pure copper oxide nanoparticles. The images from the SEM revealed that the particles appear to be almost rod-like for the distilled water solvent, and sphere-shaped for the acetone solvent. Furthermore, the EDS produced trace spectra of CuONPs that had different weight compositions spectra of copper and oxygen respectively for both solvents. A close observation of the images from the TEM showed globular shapes and the frequency distribution ranged between 48-76 nm; the acetone solvent had bigger globular shapes compared to the distilled water solvent. Copper oxide has shown mild inhibitory zone effects against *E. faecalis*, *Salmonella*, *E. coli* and *S. aureus* [14]. The CuO nanoparticles exhibited significant antibacterial activity against *C. perfringens* ATCC 13124, *C. coli* ATCC 33559, *E. coli* ATCC 25922, *L. monocytogenes* ATCC 19115, *S. pneumonia* ATCC 13883, *P. aeruginosa* ATCC 27853 and *M. catarrhalis* ATCC 25240. Amongst them, *S. aureus* ATCC 25923 and *E. coli* ATCC 25922 showed significant sensitivity and zones of inhibition to CuONPs compared to the positive control (Tetracycline). *C. perfringens* ATCC 13124, *L.monocytogenes* ATCC 19115, *S.pneumonia* ATCC 13883, *P.aeroginosa* ATCC 27853 and *M.catarrhalis* ATCC 25240 strains showed the moderate zones of inhibition compared to the positive control Tetracyline. The study successfully validates the use of copper oxide nanoparticles with citrus extracts to have antimicrobial properties on various microorganisms that are resistant to antibiotics.

Conclusions

This study reports on the green synthesis of CuONPs prepared by the biosynthesis technique using citrus extracts. The UV-Vis absorption spectra show that copper oxide nanoparticles synthesized from selected peel extracts exhibit characteristic peaks at the wavelengths 280–320 nm. SEM images reveal that the particles appear to be almost rod-like and sphere-shaped. TEM images showed spherical shapes and the size estimation, and the subsequent presentation in different histograms revealed an average size ranging between 48–76 nm. The CuO nanoparticles exhibited significant antibacterial activity against *C.perfringens* ATCC 13124, *C. coli* ATCC 33559, *E. coli* ATCC 25922, *L. monocytogenes* ATCC 19115, *S. pneumonia* ATCC 13883, *P. aeroginosa* ATCC 27853 and *M. catarrhalis* ATCC 25240.
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