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Copper oxide nanoparticles impregnated antibacterial surgical gloves for potential application in prevention of nosocomial transmission infections during nursing

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Abstract
This work showed the preparation of Copper oxide nanoparticles (CuO NPs) from leaf extract of Cinnamomum camphora (C.camphora) by a green synthetic method. To prepare CuO NPs, about 10 ml of 0.01 M copper sulphate and 30 ml of C.camphora extract were mixed by heating for 60 min at a temperature of 80 °C. Various techniques such as x-ray diffraction analysis (XRD), Energy-dispersive spectroscopic analysis (EDS), Fourier-transform infrared spectroscopy (FTIR), dynamic light scattering (DLS), UV–vis spectroscopy (UV–vis) and Transmission electron microscopy (TEM) were used for the characterization of biosynthesized CuO NPs. The formation of CuO NPs was indicated by gradual color change of brownish yellow solution into dark brown. Poly-dispersive and spherically shaped NPs were seen from TEM images with an average particle size of about 23 nm. FTIR results confirmed that polyphenols were capped onto the surface of the formed CuO NPs. On the other hand, the Gloves coated with CuO NPs were extremely successful in suppressing contamination of the outside glove surface with nosocomial-resistant microorganisms and hence beneficial of their use in the food sector or clinical context. CuO NPs-coated latex gloves significantly reduced all experimental bacteria within 30 s, including Methicillin-resistant Staphylococcus aureus, Vancomycin resistant enterococci, Escherichia coli, Acinetobacter, and Candida albicans (P < .05). In addition, research must be undertaken to assess the effectiveness of CuO NPs coated gloves in health care setting to know their effectiveness in protection from contaminated fluids that may infiltrate gloves.

Introduction
A massive attention grew in the field of Nanotechnology is mainly because of its wide range of applications present in various human endeavored areas like energy, industry, communication technology, healthcare and agriculture [1]. Research in nanotoxicity has also gained interest as a result of its unavoidable exposure to humans; therefore, it has been a part in our day to day life [1]. The effect of nanoparticles (NPs) on human health when used in applications of biomedicine stands as a specific critical issue. Latest challenges for management of probable adverse effects on health are involved after exposure to NPs, due to their biological properties and physicochemical characteristics [2]. The fluorescence property of NPs has an advantage over usual organic dyes in the field of biomedicine which is useful in applications of clinical and diagnosis [3].

CuO NPs have drawn major importance due to their diverse uses in solar energy devices, superconductors with higher Tc, catalysts, materials of huge magnetic resistance, gas sensors and in fabrication of organic-inorganic...
CuO NPs can be used as antibiotic, antibacterial and antifungal agent when applied in textiles and coatings [9]. Additionally, CuO NPs have shown some prominent properties of biocide similar to those found in formulations of pesticides [10]. For preparation of CuO NPs, various methods have been already reported such as chemical reduction [11], sonochemical [12], microwave irradiation [13], and electrochemical [14] methods. The NPs produced using chemicals suffer by surface decoration of chemical reagents on their own surface, thus making it hazardous in various applications of biomedicine. Therefore, cost efficient and eco-friendly approach to synthesize CuO NPs is necessary. In recent times, various nanomaterials have been biosynthesized with the help of plants and green reducing agents [15, 16]. Biosynthesis of nanomaterials using extracts of Aloe vera [17], Albizia lebbeck [18], algae [19] and gum karaya [20] have been reported in earlier research.

Many bacteria, including methicillin-resistant Staphylococcus aureus (MSA) and vancomycin-resistant enterococci (VE), are considered to transmit across healthcare institutions mostly through direct contact between patients and healthcare workers [21, 22]. Inappropriate gloving and poor hand sanitation may raise the risk of pathogen transmission, which frequently result in hospital acquired nosocomial infections [21, 23, 24]. On the other hand, harmful bacteria regularly contaminate the gloves of healthcare workers both through close interaction with patients and environment surroundings [22, 25].

On the other hand, Campylobacter, Salmonella and Escherichia coli (E. coli), are responsible for over 30% of infections induced by contaminated food [26]. Common sources of contamination include tainted raw resources and cross-infection by kitchen staff [27]. Gloves have been prominent in the food sector as a protection against cross-contamination; nevertheless, gloves only reduce the passage of pathogens from hands to food and other surroundings [28]. Many research studies indicated that forced glove wear frequently reduces washing hands and sanitation, leading in a rise in cross-contamination due to excess use or misuse of gloves [29, 30].

Following the appropriate hygiene practices and gloving techniques, antibacterial gloves are required to reduce infections caused by the transmission of pathogenic microbes by hospital care workers. This study evaluated the efficacy of gloves coated with biosynthesized CuO NPs an antiseptic dye against harmful pathogens. The extract of dried clove obtained from leaves of C. camphora was used to synthesize CuO NPs. The characterization of obtained CuO NPs was done using TEM, DLS technique, XRD and XPS analysis, FTIR and ultraviolet-visible spectrum. The prepared CuO NPs were coated on gloves which were studied for their effective antimicrobial efficacy against pathogenic drug resistant bacteria.

### Materials and methods

#### Materials

Sigma Aldrich Chemical Ltd., Shanghai have provided necessary chemicals such as Copper sulfate, potassium bromide (KBr), dimethyl sulfoxide (DMSO) along with other solvents and chemicals.

#### Plant extract preparation

Leaves of *C. camphora* plant were obtained from the surroundings of Qingdao Hiser Hospital in July 2019 (temperature of 28 °C with 52% humidity). Later, 2 g of dried leaves of *C. camphora* were added to 30 ml of double distilled water and heated for around 2 h on water bath at 90 °C temperature. The filtration of obtained mixture was performed to get a clear extract of *C. camphora*.

#### Preparation of copper oxide NPs

To prepare CuO NPs, 10 ml solution of 0.01 M copper sulphate (CuSO₄) and 30 ml prepared extract of *C. camphora* were mixed by continuous shaking. The subsequent solution was heated for 60 min at temperature of 80 °C. The CuO NPs formation was visually known by gradual colour change of brownish yellow copper sulfate solution into dark brown, which was identified from UV–vis spectroscopy and XRD (discussed in results). Later, the subsequent reaction mixture was allowed for washing with double distilled water through centrifugation and the obtained pellet was dried in oven at 60 °C to obtain the powder of CuO NPs.

#### Fabrication of CuO NPs coated gloves

Initially, CuO NPs (3 mg ml⁻¹) aqueous dispersion was prepared by resuspending the purified nanoparticles in double distilled water under ultrasonication conditions for about 5 min. By following dip-coating method, CuO NPs were accumulated onto the surface of glove. First, the latex gloves (2.5 cm × 2.5 cm size) were dipped into the prepared CuO NP’s dispersion and allowed to stay for about 10 min. The CuO NPs-coated gloves were then taken out and permitted to stand at room temperature for twenty-four hours for drying. The gloves were then used for future studies.
Antimicrobial assay
The effectiveness of uncoated and coated gloves against Methicillin-resistant Staphylococcus aureus (MSA), Multi drug resistant-Escherichia coli (MDR-E. coli), Multi drug resistant-Acinetobacter baumannii (MDR-A. baumannii), Vancomycin resistant enterococci (VE), and Candida albicans (C. albicans) was evaluated. Bacteria analyzed include a wide range of gram-ve (E. coli, A baumannii), gram+ve (MRSA, VRE), and yeast (C albicans), that also reflect several of the pathogenic strains that may attribute to health care-associated infections. All bacteria are MDR strains taken from Qingdao Hospital of Traditional Chinese Medicine.

The outer surfaces of uncoated and coated gloves were swabbed with $1.5 \times 10^9$ CFU ml$^{-1}$ bacterial inoculum followed by drying at 27 °C for different time intervals (30 s, 10 min, 30 min, or 1 h). Facedown segments were then smeared all over Muller Hinton with 5% sheep blood agar. To quantify this technique, control segments of uncoated gloves were inoculated with microbes at specified quantities. Microbial isolates were undergone for serial dilution from $1 \times 10^8$ CFU ml$^{-1}$ to $1 \times 10^1$ CFU ml$^{-1}$ and swabbed over uncoated gloves. The segments were then put face down on Muller Hinton 5% sheep blood agar and streaked. Each plate was flipped and incubated at 37 °C, overnight. The amount of decrease due to antiseptic NPs coating was determined by comparing the growth of uncoated and coated glove segments at various time intervals to segments with specific microbial concentrations.

Characterization
XRD pattern for the prepared CuO NPs was obtained by using a Bruker D8 Advance diffractometer which was scanned at a rate of 4° min$^{-1}$ with Cu Kα radiation of $\lambda = 1.54\AA$ and 0.02° of step size, ranging from 10° to 80°. To know the capping of biomolecules on the surface of prepared CuO NPs, FTIR analysis was carried out using Jasco system. For measurements of FTIR, pellets were prepared using a purified and dried sample mixed with potassium bromide powder. JEOL JEM 2100, a high-resolution TEM was used to analyze the size and morphology of the produced CuO NPs. The diluted nanocolloid of CuO was placed onto copper grid surface and later vacuum dried for the preparation of sample to undergo TEM analysis. Furthermore, average size of the particle along with charge on surface of produced CuO NPs was analysed by Nanoparticle Analyzer, Horiba Scientific Nanopartici, SZ-100. Double distilled water was used to dilute the CuO NPs colloid to prepare the samples for the analysis of zeta potential.

Statistical analysis
All the obtained results of cytotoxicity studies were determined as mean ± S.D. One-way ANOVA or Student’s t-test were used to conduct the analytical and statistical comparisons of the mean. Statistical significance was indicated by considering p value less than 0.05.

Results and discussion
The dark brown coloured precipitate present at the bottom of flask indicated the successful formation of CuO NPs. The filtrate from the extract of C.camphora that contains 0.5 M Copper sulphate solution changed its color after reacting for 30 min and later turned totally into dark brown after 60 min. An experiment for control, without adding the plant extract was performed which neither showed change in color nor evidence for formation of CuO NPs.

UV–Vis spectrum of dispersed aqueous CuO has exhibited an intense absorption band of SPR (surface plasmon resonance) at wavelength of 250 nm, indicating the successful formation of CuO NPs. The spectrum of UV–vis analysis of the plant extract showed no absorption peak (figure 1). The surface plasmon resonance of CuO NPs was attributed to free surface conductive electrons having collective vibrations which were excited by the incident electromagnetic radiation [31].

CuO NPs synthesized using extract of C.camphora revealed characteristic peaks of XRD (Shown in figure 2). Strong peaks of diffraction at 35.56°, 48.74°, 32.54°, 38.77°, 66.29°, 58.37°, 68.17°, 53.53° and 61.56° in relation to (002), (202), (110), (111), (311), (202), (113), (020) and (113) planes, correspondingly indicated the formation of a typical CuO NPs monoclinic structure with no impurities. The extremely crystalline structure of produced CuO NPs was confirmed by the definite and sharp reflections of CuO in X-Ray Diffraction pattern (JCPDS card no. 801268). The crystal size was calculated using Scherrer’s formula which is found to be 22 nm.

HRTEM microscopic images of produced CuO NPs as shown in figure 3 represented branched and agglomerated CuO NPs formation with size existed between 20–30 nm. TEM images revealed that the mean size of particle was about 30 nm which agrees with the results of DLS. The EDS spectrum of the formed CuO NPs revealed that only peaks of Cu and O were present and no peaks of impurity were evident (figure 4). Furthermore, the presence of elemental oxygen and copper was confirmed by their corresponding peaks in EDS spectrum indicated CuO NPs formation. Additionally, the bio-constituents of the extract adsorbed on the
surface of NPs were attributed by the smaller peaks and strong carbon peak. These studies are in accordance with the previous results in which the biosynthesized copper oxide nanoparticles were fabricated by sublimated precursors [32, 33].

Zeta potential analysis showed the negative surface charge of NPs was observed to be $-7 \text{ mV}$ (figure 4). The characteristic zeta potential and size are well known to play major roles in the physiological efficacy of synthesized nanoparticles. Moreover, the surface zeta potential of fabricated CuO NPs was observed to be negatively charged because of the capping of \textit{C.camphora} extract bio-constituents onto the surface of fabricated nanoparticles after the process of reduction [34]. This negative charge on the surface of the fabricated CuO NPs may possibly generate the intense repulsive electrostatic forces in between the nanoparticles, which resulting the stability of copper oxide nanoparticles by preventing the aggregation of NPs. Remarkably, the negative charge on produced copper oxide NPs surface using extracts of plants was also reported in previous studies [35]. On the other hand, the dispersion of CuO NPs was found to be stable even after 3 months without any change in agglomeration which was also supported by negligible change in UV–vis absorption peak, further indicating the good stability of NPs.

From figure 5, FTIR spectra of produced CuO NPs showed the bands present at wavelengths 1632 cm$^{-1}$, 1103 cm$^{-1}$ and 3427 cm$^{-1}$ corresponding to C = O stretch, O-C-O stretching and hydroxyl group (-OH) stretching correspondingly [34]. Moreover, the bands present at 1632 cm$^{-1}$ and 2923 cm$^{-1}$ corresponds with vibrations of hydrogen bonding and C-H stretching. The absorption of Cu-O showed bands at 668 cm$^{-1}$ [36]. The presence of hydroxyl and C-O functional groups on the surface of the nanoparticles was shown by the CuO NP’s spectrum. The Fourier-transform IR band at 1632 cm$^{-1}$ was attributed to the intermolecular hydrogen bonding with oxidized polyphenols present in the extract of \textit{C.camphora}, indicating the capping of \textit{C.camphora} extract by polyphenolic constituents onto the NPs surface [37]. Furthermore, the presence of stretching bands at 668 cm$^{-1}$ corresponding to the absorption of Cu-O, suggesting the CuO NPs formation. All these results show that the bio-constituents of the plant extract stabilize the produced CuO NPs.
Figure 3. (A, B) High-resolution TEM images (B) EDS spectrum of produced CuO NPs induced by extract of *C. camphora*.

Figure 4. Zeta potential analysis of produced CuO NPs mediated by extract of *C. camphora*. 
The analysis of XPS is performed to characterize the synthesized CuO NPs in order to study the chemical composition of the formed particles (figure 6). Furthermore, figure 6(a) showed the peaks that are corresponding to O 1s (31.2%), Cu2p3 (8.9%) and C 1s (59.9%) obtained from the survey scan. In addition, the corresponding region of O 1s is represented in figure 6(b), and the fitting of the O1s XPS spectra showed two binding energies at 530.88 and 532.23 eV. The peak at 530.88 eV corresponding to the binding energy for oxygen defects/vacancies \((O_{\text{v}})^2\) within the matrix of CuO \([38]\). The other peak appeared at 532.21 eV ascribed to the binding energy related to carbon adsorbed or other surface oxygen species, which certainly react with the CuO surface \([39]\).

On the other hand, the strong C1s spectrum with high resolution is represented in figure 6(c), which showed an unexpected carbon peak at 284.8 representing C = O, C–O, and C–C functional groups which is because of the low amounts of amorphous carbon that is present on the surface of NPs and carbon impurities from the surroundings \([40, 41]\). The strong Cu 2p Gaussian fitting peak in 934—954 eV range is represented in figure 6(d). The presence of two peaks at about 963 and 943 eV were the corresponding satellite peaks, that are representing the of open shell 3d9 corresponding to small amount of Cu2O \([42, 43]\). The presence of 934 eV peak with a minimum binding energy is attributed to CuO, which corresponds to the Cu 2p3/2 state, also the Cu 2p1/2 state is attributed to the peak formed at 954 eV and these results are noticed to be in accordance with the earlier reported study \([44–46]\).
Antimicrobial assay of CuO NPs coated gloves

In tests involving yeast, MDR gram +ve pathogens and MDR gram -ve bacteria, CuO NPs-coated gloves performed better than uncoated gloves with respect to antibacterial activity. CuO NPs coated gloves were very effective in vitro against all of the experimental pathogens (Table 1). CuO NPs-coated latex gloves significantly reduced all experimental bacteria within 30 s, including Acinetobacter, MSA, VE, E. coli, and C. albicans (P < .05). A decrease in C. albicans and Acinetobacter was also observed in CuO NPs-coated gloves within 30 s, although it was not considerable. In addition, both species exposed to CuO NPs-coated gloves decreased significantly within 10 min time, correspondingly. CuO NPs-coated gloves totally eliminated all bacteria within one hour. It is reported from literature that about 25 phytochemicals existed in aqueous leaf extract of C. camphora, which majorly constitutes ketones, alcohols, esters and terpenoids. However, the major constituents includes 6-epi-shyobunol, camphor and linalool [47]. On the other hand, Wang et al, showed that the antibacterial activity of aqueous C. camphora extract may be because of the bioconstituents present in extract such as 1,6-octadien-3-ol, D-campher, eucalptol and 3-methyl-2-butenolic acid [48]. All these results suggested that CuO NPs coated latex gloves are highly effective in considerably decreasing bacterial contamination in short term applicable to both food supplying and clinical environment where the contamination typically arises within 20 s to 10 min of time.

Conclusions

In conclusion, CuO NPs were prepared using leaf extract of C. camphora by a green synthetic approach. Poly-disperse and branched CuO NPs with an average particle size of 30 nm were confirmed from TEM. FTIR confirmed that polyphenols were capped onto the surface of CuO NPs. On the other hand, the Gloves coated with CuO NPs were extremely successful in suppressing contamination of the outside glove surface with nosocomial-resistant microorganisms and hence beneficial of their use in the food sector or clinical context. However, based on in-vitro laboratory investigation, the applicability to an in-vivo clinical context is restricted. The effectiveness of CuO NPs-coated gloves in preventing microbial transmission or contamination in an in-vivo clinical environment is the subject of ongoing clinical research. In addition, research must be undertaken to assess the effectiveness of CuO NPs coated gloves in health care setting to know their effectiveness in protection from contaminated fluids that may infiltrate gloves.

Data availability statement

No new data were created or analysed in this study.

Table 1. Antimicrobial ability of CuO NPs coated gloves towards gram-negative, gram-positive bacteria and yeast (Data represented CFU/glove segment).

|          | Time | Uncoated gloves | CuO NPs coated gloves | P value |
|----------|------|-----------------|-----------------------|---------|
| MSA      |      |                 |                       |         |
|          | 30 s | \(10^8\)         | 0                     | .047    |
|          | 10 min | \(10^8\) | 0                     | .047    |
|          | 30 min | \(10^8\) | 0                     | .047    |
|          | 1 h   | \(10^8\)        | 0                     | .047    |
| VE       |      |                 |                       |         |
|          | 30 s | \(10^8\)         | \(2.58 \times 10^7\) | .018    |
|          | 10 min | \(10^8\) | \(2.50 \times 10^7\) | .018    |
|          | 30 min | \(10^8\) | 0                     | .013    |
|          | 1 h   | \(10^8\)        | 0                     | .013    |
| MDR-E. coli |      |                 |                       |         |
|          | 30 s | \(10^8\)         | 0                     | .047    |
|          | 10 min | \(10^8\) | 0                     | .047    |
|          | 30 min | \(10^8\) | 0                     | .047    |
|          | 1 h   | \(10^8\)        | 0                     | .047    |
| MDR-A. baumannii |      |                 |                       |         |
|          | 30 s | \(10^8\)         | \(6.67 \times 10^7\) | .059    |
|          | 10 min | \(10^8\) | 0                     | .047    |
|          | 30 min | \(10^8\) | 0                     | .047    |
|          | 1 h   | \(10^8\)        | 0                     | .047    |
| C. albicans |      |                 |                       |         |
|          | 30 s | \(10^8\)         | \(2.53 \times 10^7\) | .069    |
|          | 10 min | \(10^8\) | \(2.50 \times 10^7\) | .018    |
|          | 30 min | \(10^8\) | 0                     | .013    |
|          | 1 h   | \(10^8\)        | 0                     | .013    |
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