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Cellulose Nanocomposite Fabrics with *In-situ* Generated Copper Oxide Nanoparticles Using *Aquilaria Malaccensis* (Agarwood) Leaf Extract as the Reducing Agent

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Abstract

Metallic nanoparticles often agglomerate when used as fillers in different matrices. *In-situ* generation of the nanoparticles in the matrices is suggested to overcome this problem. The present study aimed to use *Aquilaria malaccensis* leaf extract to synthesize copper oxide nanoparticles (CuONPs) on a cellulose cotton fabric. A surface coating of copper oxide was *in-situ* synthesized on the surface of cotton fabric using *A. malaccensis* leaf extract. Characterization of the copper oxide nanoparticle composite fabrics (CNCFs) was conducted using FESEM-EDX, and its antibacterial potential was assessed. The CuONPs formed on the surface of the cotton fabric were mainly spherical, ranging from 5 to 27 nm. The CNCFs exhibited good antibacterial activity against *Escherichia coli* and *Bacillus subtilis*, evaluated after 24 hours of incubation. Comparative $CuSO_4$-embedded fabrics showed lower antimicrobial inhibition. The CNCFs prepared using this environmentally friendly method showed prominent antibacterial properties and can be considered for medical and packaging applications.

Keywords

Cotton nanocomposites, Copper oxide nanoparticles, *In situ* generation, Antibacterial activity

Introduction

Copper is well known for its ability to serve as a broad-spectrum biocide, inhibiting the growth of many bacteria and fungi (Dollwet, 1985) and strong antibacterial activity even at the nanoscale (Raffi *et al*., 2010; Cady *et al*., 2011). Medical products with copper-containing surfaces have the potential to meet stringent hospital requirements of sterility (Mikolay *et al*., 2010). Copper oxide has also been widely used for food packaging applications, as biosensors and in the preparation of electrically conducting biodegradable paper (Tamayo *et al.*, 2016; Cady *et al.*, 2011; Yang *et al*., 2019). Copper is less expensive than silver, making it a cost-effective option (Jia *et al*., 2012).

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In a previous report, we established the synthesis of CuONPs using *Aquilaria malaccensis* leaf extract as a reducing agent. The X-ray diffraction (XRD) results confirmed the crystalline nature of the nanoparticle while scanning electron microscopy (SEM) and transmission electron microscopy (TEM) revealed its spherical shape (6 to 32 nm). The energy-dispersive X-ray spectroscopy (EDX) results confirmed copper and oxygen as the main elements in biosynthesized CuONPs. The Fourier Transform Infrared (FTIR) spectroscopy summarized the phytochemical compounds in the *A. malaccensis* leaf extract responsible for reducing CuONPs. Preliminary antimicrobial studies showed that CuONPs effectively inhibited Gram-positive bacteria such as *Bacillus subtilis* (Sathia *et al*., 2022).

When added to matrices (such as polymers and other matrix materials), nanoparticles with antimicrobial properties can act as active components, leading to a prolonged antimicrobial function of the material. In addition, nanoparticles improve the mechanical properties of the matrix material (Khan *et al.,* 2019). However, the addition of nanoparticles as fillers in different matrices often results in a severe agglomeration of particles on the matrix because of its large surface-area-to-volume ratio (Gao *et al*., 2016; Shah *et al*., 2016). Hence, *in-situ* nanoparticle generations of nanoparticles in a cellulose matrix were suggested (Sivaranjana *et al*., 2017). Vainio *et al*. (2007) and Li Qiuju *et al*. (2012) reported that the O-H group in the biopolymer cellulose could act as a mild reducing agent in the synthesis of nanoparticles *in-situ*. However, the process is time-consuming and, therefore, not viable. This present study aimed to hasten the process by utilizing leaf extract of *A. malaccensis* to synthesize CuONPs in a cellulose matrix. Also, the *in-situ* method attempted to achieve a homogeneous dispersion of nanoparticles in the cellulose matrix critical to attaining uniform and enhanced material properties.

Methodology

1000 g of fresh *A. malaccensis* leaves were collected from the garden of Nilai University, Malaysia and washed thoroughly with tap water. The rinsed leaves were then dried in the oven at 60°C for 48 h and powdered. Leaf extract was prepared by adding 2 g of dried leaf powder into 100 ml of water and boiling for 10 min. The extract was cooled to room temperature and centrifuged (UNIVERSAL 16R, Model LWB-122D) at 4000 rpm for 10 min. This centrifugation process was repeated at 4000 rpm for 20 min to remove tiny particles. The purified supernatant was then stored in the refrigerator at 4ºC.

Pure cotton fabric was bought from the local market in Nilai. The material was cut into 1 cm × 1 cm rectangular pieces, boiled at 100°C and rinsed with distilled water. The cotton fabric pieces were then dried for 1 h at 60°C in the oven before being immersed in *A. malaccensis* leaf extract for 2 h at room temperature (26°C to 30°C). Each piece of fabric was transferred into a flask containing different concentrations (100 mM, 200 mM, 500 mM, and 1000 mM) of $CuSO_4$ (Bendosen, Malaysia) and incubated at 70°C for 24 h. Control samples were prepared by treating cotton fabric separately on *A. malaccensis* leaf extract and 1000 mM $CuSO_4$. Afterwards, the fabric pieces were gently washed with distilled water and oven-dried at 60°C for 2 h before being stored in an airtight container.

FESEM/EDX was conducted to verify the generation of CuONPs on the fabric and assess its morphology, size, and elemental composition.

The potential antimicrobial activity of the CNCFs was evaluated using the agar well diffusion method against Gram-positive *B. subtilis* (ATCC 6051) and Gram-negative *E. coli* (ATCC 25922). Nutrient agar plates were lawn using a cotton swab dipped in 10^6 CFU 0.5 McFarland standard bacterial suspensions. The antimicrobial test was performed by placing the treatment discs of 100 mM, 200 mM, 500 mM, and 1000 mM CNCFs on the agar plates. 30 µg tetracycline disc was used as the positive control. The plates were sealed with parafilm and incubated at 37°C for 24 h. Antibacterial activities were evaluated by measuring the diameter of the inhibition zone around the discs. All data were expressed as means ± standard deviations (S.D.). Statistical analyses were carried out using analysis of variance (ANOVA), and means were separated statistically according to Tukey's honest significant difference test at P=0.05.

Results and Discussion

No colour change was observed on the fabrics treated separately with *A. malaccensis* leaf extract and aqueous $CuSO_4$. In contrast, the cotton fabric treated with aqueous $CuSO_4$ turned blue. However, dark brown colouration was observed in materials treated with leaf extract and $CuSO_4$ solution.

Figure 1d shows the image of the observed changes in the fabric after *in-situ* CuONP synthesis compared to plain fabric (Fig. 1a), fabric treated with *A. malaccensis* leaf extract alone (Fig. 1b) and aqueous $CuSO_4$ (Fig. 1c). After *in-situ* synthesis, the fabric colour changed from white to brown, indicating CuONPs on the material. The colour intensity of the fabric increased with the increase in $CuSO_4$ concentration (Fig. 1d, e, f, and g). Similar findings were observed by Sadanand *et al*. (2016), employing *Ocimum sanctum* leaf extract as a reducing agent with aqueous $CuSO_4$ as the precursor.

FESEM analysis was conducted to confirm the formation of CuONPs, morphology, and size distribution. Figure 2a shows the generated CuONPs at 50,000x magnification. The CuONPs were depicted with intense agglomeration, spherical morphology, and an average particle size of 5 to 27 nm. Further magnification could not be achieved as FESEM scans the material's surface, and in this case, the nanoparticles were embedded in the fabric, causing it to be hidden and unable to transmit electrons effectively. Electrons emitted from embedded sources are not accurately reflected in the detector. They would eventually burn the cellulose fabric sample, restricting the ability to observe the morphology of CuONPs in greater detail. Sadanand *et al*. (2016) reported that copper nanoparticles on composite film were spherical and had an average size ranging from 60 to 79 nm. Size, morphology, and dispersity of metallic nanoparticles are essential parameters in terms of bio-distribution (Owens and Peppas, 2006), internalization kinetics (Chithrani *et al.*, 2006), and cellular membrane deformability (Pegoraro *et al.*, 2014). Besides, spherical nanoparticles interact with cells differently than cylindrical nanoparticles, resulting in significant changes in bioavailability (Yoo and Mitragotri, 2010). Hence, coating of good nanoparticle shape, size, and distribution enhances the antibacterial effect of cotton fabric.

Elemental analysis was conducted to confirm the breakdown of elements in the synthesized CuONPs using EDX attached to the FESEM. The EDX spectrum of the CuONPs is shown in Figure 2b. The generated peaks correspond to the binding energies of copper (0.8%), oxygen (37.1%), and carbon (60%). The high peaks of carbon and oxygen could be due to the organic component of the cellulose fibre. This confirms the formation of CuONPs in the cellulose textile. The results also revealed the presence of 1.8 wt.% and 0.4 wt.% elemental N and Fe, respectively, which could be impurities originating from the cotton fibres used in the study.

The disc diffusion method was used to probe whether the CNCFs showed antibacterial activity against *B. subtilis* and *E. coli*. *A. malaccensis* leaf extract-coated fabric exhibited no growth inhibition, while 30 µg tetracycline inhibited *B. subtilis* (19.4 ± 0.10 mm) and *E. coli* (18.93 ± 0.13 mm). Also compared in the test were cotton fabric samples soaked at different concentrations of $CuSO_4$. *B. subtilis* was inhibited at all concentrations, with the largest diameter of the zone of inhibition at 1000 mM (38.22 ± 0.20 mm). For *E. coli,* however, only 500 mM and 1000 mM $CuSO_4$ depicted a zone of inhibition with a diameter of 10.22 ± 0.12 mm and 27.73 ± 1.00 mm, respectively. As shown in Figure 3, CNCFs samples have significantly higher inhibitory zones than $CUSO_4$-coated samples, indicating the importance of nano-size that corresponded to the increased surface area afforded by the CuONPs. CNCFs samples were more effective in inhibiting *B. subtilis* than *E. coli*.

Copper is known to produce reactive oxygen species, inactivate enzymes, modify cell walls, and alter nucleic acid synthesis, significantly inhibiting nosocomial infections (Zhai *et al*., 2016). However, CNCFs were more effective due to the extremely high surface area of nano-sized copper particles and unique crystal morphology (Klabunde *et al.,* 1996; Stoimenov *et al.,* 2002; Parikh *et al.,* 2014), which enhances the abovementioned antibacterial activity against both *B. subtilis* and *E. coli*.

According to Bondarenko *et al.* (2013), most common bacteria such as *E. coli* are well known for their mechanism involved in copper homeostasis due to the presence of two chromosomally encoded systems, which exhibit a critical role in copper resistance. *E. coli* can also survive in a copper-rich environment due to a plasmid-borne resistance system responsible for survival. However, the antibacterial effect was still recorded, which might be due to the presence of Cu^+ ions in CuNPs or CuONPs, which are well known to interrupt cell respiration and destroy DNA and RNA by puncturing the cell membrane. At the same time, the formation of reactive oxygen species causes considerable damage to different areas of the microorganism (Kumar *et al.,* 2021). Furthermore, because copper is released slowly and in small amounts, it is a type of long-lasting biocide (Drelich *et al*., 2011). According to the studies utilizing transmission electron microscope, laser confocal microscopy, and atomic force microscopy, the integrity of bacterial cell membranes changed substantially after exposure to nanoparticles, resulting in bacterial cell death (Tiwari *et al*., 2008).

Conclusion

The present work has demonstrated that copper oxide nanoparticle composite fabrics (CNCFs) could be synthesized through the *in-situ* surface coating of copper oxide using *A. malaccensis* leaf

extract on cellulose material. Spherical shapes of nanocrystals with dimensions of 5 to 27 nm in length were observed under FESEM. The copper nanoparticle composite fabrics were prepared using an environmentally friendly method that demonstrated potent antibacterial properties. The antibacterial effects of 100 mM, 200 mM, and 500 mM CNCFs were significantly higher than 100 mM, 200 mM, and 500 mM $CuSO_4$. Hence, CNCFs can be considered a possible biomedical or packaging material due to their remarkable antibacterial characteristics.

Figure 1. Representative images for plain cotton fabric (a), cotton fabric soaked in *A. malaccensis* leaf extract (b) and 1000 mM $CuSO_4$ (c), and CNCFs using 100 mM (d), 200 mM (e), 500 mM (f), 1000 mM (g) of $CuSO_4$ as a precursor during *in-situ* syntheses of nanoparticles.

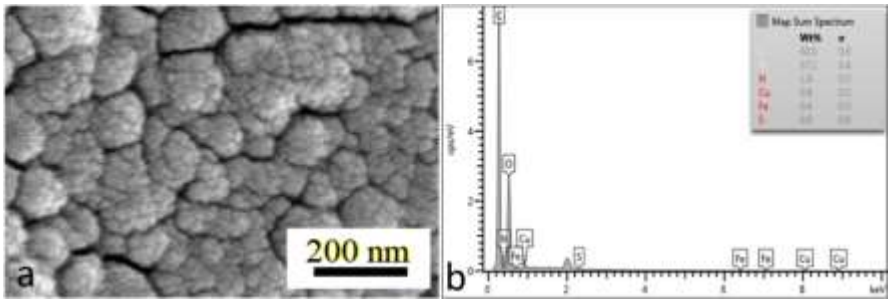

Figure 2. (a) Representative FESEM micrograph of CNCFs at 50,000x magnification and 3.00kv, and (b) EDX spectrum.

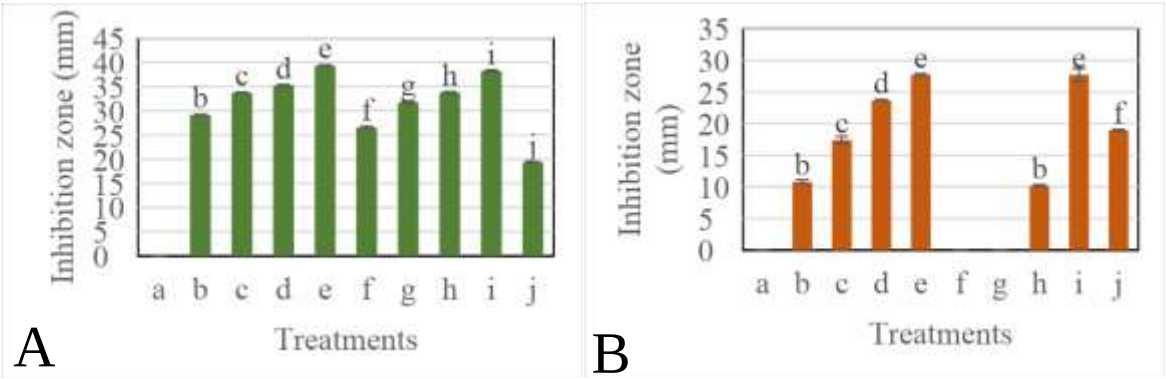

Figure 3. Antimicrobial activity against bacteria (A) *B. subtilis* and (B) *E. coli* of cotton fabric soaked in *A. malaccensis* leaf extract (a), CNCFs using 100 mM (b), 200 mM (c), 500 mM (d), 1000 mM (e) of $CuSO_4$ as a precursor during *in-situ* syntheses of nanoparticles, and fabric samples treated with $CuSO_4$ solution at 100 mM (f) 200 mM $CuSO_4$, (g) 500 mM $CuSO_4$, (h) 1000 mM (i).

30 µg tetracycline was used for comparison (j). Means with the same letter on bars are not significantly different at $P<0.05$.

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