Synthesis, Characterization of Chitosan-PVA Films Embedded with Curcumin-Capped CuO Nanomaterials for Antibacterial Activity Againts *Escherichia Coli*

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Abstract. The biogenesis of curcumin-capped CuO nanoparticles (cur-CuO NPs) has attracted a lot of interest lately, especially in relation to its interactions with chitosan and PVA. Cur-CuO NPs are suggested for biofabrication in this work, and physicochemical characteristics are documented. Using ethanol extract of *Curcuma longa*, cur-CuO NPs were biosynthesized. A synthesis film of chitosan-PVA-cur-CuO NPs was made using the casting technique. To characterize biosynthesis and the synthesis result with FT-IR and XRD spectrophotometry and evaluate their antibacterial activity with the agar diffusion method. The presence of Cu–O was revealed by bands at 593-602 cm^{-1} for the Cu–O group in the film of chitosan-PVA-cur-CuO NPs. FT-IR studies revealed that the cur-CuO NPs had Cu-O functional groups at 597 cm^{-1}. The cur-CuO NPs had crystallite sizes of 4.43 nm. Antibacterial investigations showed that the film of chitosan-PVA-cur-CuO NPs type C had a better inhibitory effect than types A and B.

Keywords: Antibacterial, chitosan, PVA, curcumin, CuO.

1 Introduction

A nanomaterial is a substance with at least one dimension in the range of 1 to 100 nanometers, which gives it unique properties [1] that differ from its bulk form, such as altered optical, magnetic, electrical, and chemical behaviors [2]. These materials can occur naturally or be intentionally produced through "top-down" or "bottom-up" manufacturing processes [3].

Biosynthesis of metal oxide nanoparticles is an environmentally friendly and cost-effective green synthesis method that utilizes biological entities like plants, bacteria, or fungi to reduce

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and stabilize metal ions, forming metal oxide nanoparticles [4]. These biological sources contain phytochemicals or enzymes that act as reducing and capping agents, producing NPs with superior biocompatibility and controlled size and shape for applications [5].

The primary advantages of biosynthesized CuO nanoparticles include their eco-friendly and cost-effective production by avoiding harsh chemicals, their enhanced stability and control over properties like size and morphology inherited from biological precursors, and their numerous uses in a variety of industries, including energy storage, environmental remediation (catalysis, adsorption), and medicine (antimicrobial, anticancer), motivated by their distinct physicochemical properties [6]. CuO nanoparticles possess a high surface area and a narrow band gap of around 1.2-1.4 eV, making them effective semiconductors for electronic and solar applications. Their chemical stability, catalytic activity, and antimicrobial properties are also significant [7,8].

Combining chitosan and copper oxide (CuO) nanoparticles creates nanocomposites with enhanced antibacterial activity. The chitosan matrix protects the CuO, while the synergistic interaction provides extended, long-acting antimicrobial effects and better overall performance than either component [9]. Combining chitosan, polyvinyl alcohol (PVA), and CuO nanoparticles creates a versatile material with enhanced antibacterial activity [10,11] due to synergistic interactions. The chitosan and PVA blend offers biocompatibility and improved mechanical properties compared to pure chitosan, while CuO nanoparticles provide strong bactericidal and ROS-generating effects [11], making the composite material effective for wound dressings, packaging, and other biomedical applications.

Biosynthesis of CuO NPs using *curcuma longa* extract offers advantages like eco-friendliness, reduced toxicity, lower production costs, and the inherent biological properties of the turmeric extract [12], such as antimicrobial effects [13], which can enhance the final CuO nanoparticles' overall efficacy for biomedical applications. Chitosan-cur-CuO NPs offer enhanced antimicrobial and cytotoxic activities, increased antioxidant and antidiabetic effects, and improved biocompatibility compared to uncapped CuO nanoparticles. Curcumin (from *Curcuma longa*) adds strong antioxidant, anti-inflammatory, and antibacterial qualities to the chitosan's positive charge and biocompatibility, creating a multipurpose substance for a range of uses [14].

The chitosan-PVA-cur-CuO NPs offers several advantages, including superior antibacterial and antioxidant activities, enhanced wound healing, and potential as a bioactive agent due to the synergistic effects of chitosan, PVA, and curcuma. The curcuma (turmeric) increases the stability and bioavailability of the nanoparticles, while the chitosan and PVA provide a biocompatible matrix. This combination also exhibits potential for drug delivery, as well as for creating advanced materials like antibacterial food packaging and wound dressings, improving properties like mechanical strength, moisture resistance, and cell proliferation [14,15,16].

In this paper, the film of chitosan-PVA-cur-CuO NPs used as antibacterial of *Escherichia coli* with agar diffusion method. Characterization of this film and cur-CuO NPs using FTIR and XRD spectrophotometry.

2 Method

2.1 Material

All of Merck's reagents, including pottasium hydroxide, acetic acid, copper sulfate pentahydrate, absolute ethanol, and nutritional agar, are extremely pure. Distilled water (aquadest) and *Escherichia coli* from our lab. *Curcuma longa* from the Palembang area. FTIR (Shimadzu Prestige-21) spectrophotometer and X-ray diffraction (Shimadzu XRD-6000).

2.2 Procedures

Preparation of ethanolic extract of *turmeric.* A 96% v/v ethanol solution is used to macerate freshly grated turmeric (*Curcuma longa*). In the black bottle, 25 g of freshly grated turmeric were submerged in a 250 mL ethanol solution (96%, v/v). This procedure is run for one night. The combination was separated after a night, and the macerate was saved for further use. For the residue, the maceration process is repeated using 96% (v/v, 250 mL) ethanol overnight. The filtrate (macerate) is then mixed with the first filtrate for the subsequent experiment.

Fabrication of cur-CuO NPs. This procedure is based on previous research with a few small modifications [17]. 0.1 M, 100 mL solutions of copper sulphate pentahydrate were mixed with 100 mL of *curcuma longa* ethanolic extract in a 500 mL beaker. Then, for one and a half hours at 80°C, the mixture was continuously stirred. 0.2 M KOH solution was added to the mixture (30 minutes) to bring its pH up to 9. The leftover mixture was left to precipitate for one night at room temperature. The precipitate (cur-CuO NPs) was thoroughly cleaned using aquadest and a 96% v/v ethanol solution. The precipitate was then allowed to dry completely (in a vacuum oven set to 80°C). Cur-CuO NPs were ultimately calcined dry at 300°C for an hour in a muffle furnace.

Fabrication of film chitosan-PVA-cur-CuO NPs. The film was made using the method described in our previous study [11]. To make a 0.5 g chitosan solution, 15 mL of a 2.5% v/v aqueous acetic acid solution was added to a 250 mL glass beaker. Following that, the liquid was constantly stirred (100 rpm) to produce a homogenous solution using a hotplate set at room temperature (25°C) (on the hot plate, 100 rpm). The mixture was then continuously stirred for an hour at room temperature (25°C, 100 rpm) while 0.05 g of cur- CuO NPs was added to 15 mL of the chitosan solution above. Lastly, the mixture was constantly spun at room temperature for thirty minutes (25°C, 100 rpm) while being combined with 10 mL of the 5% (w/v) PVA solution. After being equally spread on a petri dish, the resultant solution was left to dry at room temperature (25°C). The film was completely dry and usable after seven days. As shown in Table 1, the other movies were made for different comparisons. The prepared film and cur-CuO NPs images are shown in **Figure 1** and **Figure 2**.

Table 1. Materials used in film synthesis.

Chitosan (g)	Cur-CuO NPs (g)	PVA 5% w/v (mL)	Film type
0.5	0.05	10	A
0.5	0.10	10	B
0.5	0.15	10	C

2.3 Characterization

The product (cur-CuO NPs, Film types A, B, and C) is identified by its physical structure (Shimadzu XRD 6000 spectrophotometer), functional groups (FTIR Spectrophotometer, Shimadzu Prestige-21), and wavenumber (cm^{-1}), which was selected at a range of 4500–500. The crystallite size is determined from the product's XRD pattern.

The antibacterial evaluation of chitosan-PVA-cur-CuO NPs film against *Escherichia coli.* The inoculum suspension was made by introducing a sterile 0.9% NaCl solution into a fresh culture, shaking it, and keeping an eye on the optical density at 580 nm, adjusting it until the inoculum's transmittance reached 25% [18]. After the microbial inoculum had solidified in 10 millilitres of nutritional agar (the first layer), it was inoculated into the second layer of the Petri plate using the Agar well diffusion method. The second layer was adhered using film types A, B, and C, film of chitosan, film of PVA, and chloramphenicol solution 0.1% w/v. The diameter of each film is ± 6 mm. The plate was incubated at 37°C for 24 hours. Lastly, a caliper was used to quantify the zone of inhibition in millimetres, which was then compared to the standard chloramphenicol. The film's ability to effectively stop bacterial growth is demonstrated by the greatest inhibition zone.

3 Results and discussion

3.1 Biosynthesis of cur-CuO NPs and synthesis film of chitosan-PVA-cur-CuO NPs

The pH has an impact on the reduction of metal ions during the biosynthesis of cur-CuO NPs. Since the color of the solution changed colloidally more quickly at a higher pH (pH 8) than it would in a lower pH solution, it was demonstrated that the rate of reduction was accelerated [19]. According to the aforementioned claim, cur-CuO NPs was biosynthesized in an alkaline medium. The biomolecules are inactive in acidic environments, according to the earlier study [20]. The biomolecule's electrical charge is altered by the addition of pottasium hydroxide solution, and this biomolecule can function as a stabilizing and capping agent [21].

Because of the presence of electrostatic repulsion upon OH^- ion adsorption, nanoparticles exhibit stable characteristics [22]. The ethanol extract of Curcuma longa contains secondary metabolites such as terpenoids and flavonoids, which may function as stabilizing and reducing agents during the production of nanoparticles [23]. **Figure 1** depicts the solid result of biosynthesis.

Fig. 1. The solid of cur-CuO NPs.

The basic principle of a chitosan (CS)-PVA-cur-CuO NPs film relies on the synergistic combination of the properties of its individual components, integrated into a stable polymer matrix to create a functional nanocomposite material [24] as shown in **Figure 2**.

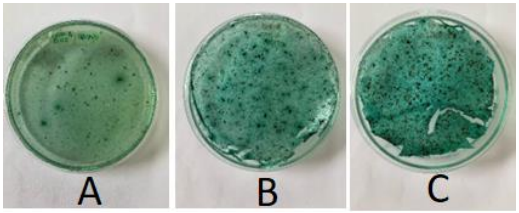

Fig. 2. The chitosan-PVA-cur-CuO NPs A, B, and C film.

3.2 FTIR analysis

FTIR spectra of cur-CuO NPs and all films as seen in **Figure 3**.

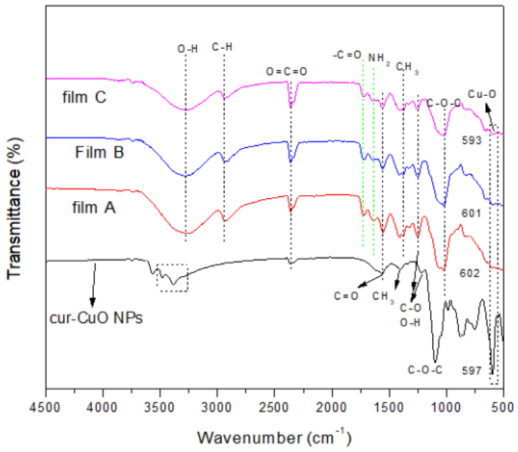

Fig. 3. FTIR spectra of product biosynthesis and synthesis.

Because of the phenolic components in the turmeric extract or the presence of intermolecularly bound alcohol, the spectra of curcumin-capped CuO nanoparticles exhibit a rounded broad peak at 3481-3563 cm^{-1}, which is linked to the phenolic O–H stretching vibration [14, 25, 26]. The O=C=O group observed at 2364 cm^{-1} as the asymmetric stretching vibration [14]. The heavily mixed vibrations of v(C=O) were identified as the cause of the band in the cur-CuO NPs spectra at 1563 cm^{-1} [26]. The curcuminoid components' bending phenolic OH group and the aromatic C–O enol's weak stretching band are responsible for the peak at 1219 cm^{-1} [14]. The chemical structure of curcumin and other components (such as curcuminoid) extracted from turmeric was confirmed by the strong stretching band (1098 cm^{-1}), which is associated with the *v*(C–O–C) of phenyl alkyl ether [14]. The stretching band (sharp) at 597 cm^{-1} belongs to Cu–O [26, 27].

Figure 2 shows the chitosan-PVA-cur-CuO NPs film (A, B and C), and these spectra were analyzed using the previous results [14,26,27]. The stretching vibrations of –OH and –C–O–C–

matched to the characteristic peaks for chitosan, which were seen at 3279, 3297, 3277, and 1023,1058; 1022; 1021 cm^{-1}. The $–NH_2$ bending vibration peak in chitosan was discovered at 1638, 1645, and 1646 cm^{-1}. The peaks at 2934, 2937, and 2936 cm^{-1} represent $\nu(–CH_3)$ and $\nu(–NH)$. The band at 1410; 1410; 1409 cm^{-1} is caused by ν(C–H) bond. The peak (medium) was observed at 2361; 2361; 2361 cm^{-1} due to $\nu(–O=C=O)$ groups present in curcumin and primary alcohol (1°). The NH_2 (chitosan), –C-H, C=O (R(C=O)R'), and –O-H group (R-COOH) were responsible for the difference between film chitosan-PVA-cur-CuO NPs and cur-CuO NPs at 3279, 3287, 3277, 2934, 2937, 2936, and 1724, 1722, and 1723 cm^{-1}. The curcuminoid components' bending phenolic OH group and the aromatic C–O enol's weak stretching band are responsible for the peak at 1251, 1250, and 1252 cm^{-1}.

The film chitosan-PVA-cur-CuO NPs's FTIR spectra showed notable distinctive peaks at 602, 601, and 593 cm^{-1}. These peaks were indicative of Cu–O single bonds, whereas the peak in the 500–700 cm^{-1} region should indicate a stretching of Cu–O. The higher wave number of the Cu-O group in the chitosan-PVA-cur-CuO NPs film (593-602 cm^{-1}), compared to the Cu-O group in cur-CuO NPs (597 cm^{-1}), is primarily due to the formation of strong interactions, such as hydrogen bonds and complexation, between the CuO NPs and the functional groups (hydroxyl and amine) of the chitosan and PVA polymer chains [28,29].

3.3 XRD analysis

Difractogram of cur-CuO NPs and all film as presented in **Figure 4** and **Figure 5**, respectively.

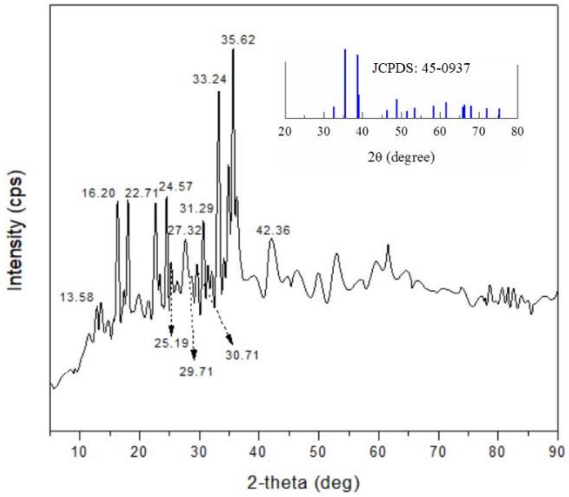

Fig. 4. Cur-CuO NPs' XRD pattern.

The X-ray diffraction patterns of the cur-CuO NPs are shown in **Figure 4**. CuO has its strongest peaks at 2θ° values of 33.24° and 35.62° [14,30]. This peak agreement with JCPDS card number 45-0937 is demonstrated by the sharp and strong peak observed at 35.62° position (1 1 1) [31,32]. Another peak appeared at 2θ = 30.71°; 31.29°, 42.36° [29,30,31][33,34,35]. Curcumin showed its characteristics peaks at 2θ=13.58°,16.20°,22.71°,24.57°, 25.19°, 27.32° and 29.71°

[27,36,37]. The cur-CuO NPs calculated crystallite size is 4.43 nm based on the Debye Scherrer equation [38].

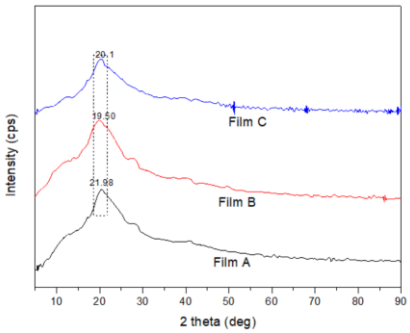

Fig. 5. XRD pattern of film chitosan-PVA-cur-CuO NPs.

The amorphous nature from the XRD patterns of the film chitosan-PVA-cur-CuO NPs (Figure 5) showed at $2\theta \approx 19.50°$-$21.98°$ (broad diffraction peak). The polymer blend of PVA and chitosan demonstrates how of the PVA (semi-crystalline) is diminished and impacted by blending it with chitosan polymer, and the properties of each polymer vanish as a result of hydrogen bonding between -O-H group (PVA) and NH_2 or O-H group (chitosan). This indicates that there is a substantial interaction between PVA and chitosan [39]. The peaks of curcumin were found to overlap with the film peak (broad), and the film of chitosan-PVA-cur-CuO NPs indicated as amorphous nature [40]. Amorphous formed in the chitosan-PVA-cur-CuO NP film due to the strong electrostatic interactions between curcumin and chitosan molecules. This prevented the former from assembling into ordered crystalline structures [41].

3.4 Antibacterial analysis

The antibacterial investigation was conducted using films A, B, C, chitosan (D), PVA (E), and chloramphenicol solution (0.1% w/v). **Figure 6** shows the antibacterial study's outcome, and **Table 2** summarizes the zone inhibition diameter.

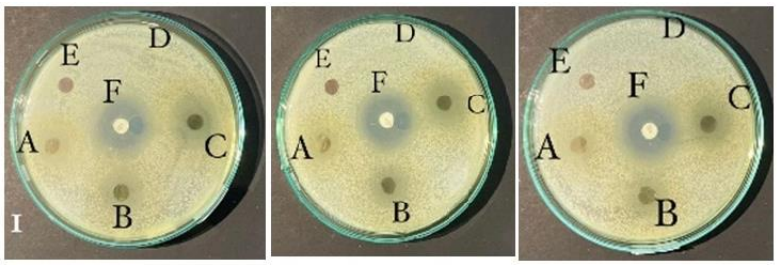

Fig.6. The result of the antibacterial study.

Table 2. Inhibition zone calculation result.

No.	The Petri dish	Diameter of zone inhibition (mm), film:					
		A	B	C	D	E	F
1	I	11.01	18.06	20.80	0.00	0.00	33.16
2	II	10.56	18.71	23.01	0.00	0.00	33.93
3	III	10.54	17.06	20.43	0.00	0.00	33.17
Average ± SD		10.70 ± 0.26	17.94 ± 0.83	21.41 ± 1.39	0.00	0.00	33.42 ± 0.44

A distinct zone of inhibition (clear zone) surrounding film C is larger than surrounding films A and B, as seen in **Figure 6** and **Table 2**. Increasing the concentration of metal nanoparticles leads to a larger (wider) clear zone of inhibition, indicating enhanced antibacterial activity [42]. The chitosan-PVA-cur-CuO NPs film acts as an antibacterial agent due to a synergistic effect of its individual components, which employ multiple mechanisms to disrupt and kill bacteria. Chitosan is a natural, polycationic polymer. Its primary antibacterial mechanism involves the electrostatic interaction between its positively charged amino groups and the negatively charged surface of bacterial cell walls. This interaction disrupts the cell membrane, increases its permeability, and leads to the leakage of intracellular components and eventual cell death [43].

According to Zheng et al., [44], curcumin, a natural polyphenolic compound, is a potent antibacterial agent with multiple modes of action such as membrane disruption **(**Its lipophilic structure allows it to insert into bacterial cell membranes, altering their integrity and permeability), Production of Reactive Oxygen Species (ROS**,** curcumin can induce the production of ROS within the bacterial cell, leading to oxidative stress, damage to DNA, proteins, and lipids, and ultimately cell death), Inhibition of cell division and virulence **(**It inhibits bacterial cell division by targeting proteins like FtsZ and interferes with bacterial communication systems (quorum sensing), which limits biofilm formation and the production of virulence factors)

CuO nanoparticles have potent antibacterial action via a number of different mechanisms. The release and retention of Cu^{2+} ions in a chitosan-polyvinyl alcohol (PVA) matrix are regulated by the interactions between the metal ions and the functional groups of the polymer as well as the physical structure of the matrix. The nanoparticles gradually release copper ions (Cu^{2+}), which can penetrate the bacterial cell and disrupt vital biological processes like DNA replication, protein synthesis, and enzyme functioning, ultimately leading to cell death. CuO NPs produce ROS, which oxidatively damages the biological components of bacteria. The integrity of the bacterial cell membrane may be physically harmed by direct contact between the nanoparticles and the membrane [8,45].

4 Conclusion

Interaction between the ethanolic extract of *curcuma longa* and Cu^{2+} ion in alkaline solution can form a cur-CuO NPs. The film of chitosan-PVA-cur-CuO NPs can be synthesized from chitosan, PVA, and cur-CuO Nps. The functional groups of Cu-O in cur-CuO NPs and film of chitosan-PVA-cur-CuO NPs were detected at 597 and 593-602 cm^{-1} on FTIR spectra,

respectively. The cur-CuO NPs has a crystallite size of 4.43 nm. The physical structure of all films (chitosan-PVA-cur-CuO NPs) has an amorphous nature. The inhibition zone of film chitosan-PVA- cur- CuO (type C) is higher than film chitosan-PVA-cur-CuO NPs (type A and B).

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