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Research Article
Antioxidant and Anticoagulant Activity of Microbial Nano Cellulose-ZnO-Ag Composite Components

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Abstract

Background and Objectives: Bacterial cellulose (BC) is a microbial extracellular biopolymer formed by microbial strains like *Gluconacetobacter xylinus*. The objective of this study was to determine the antioxidant and anticoagulant of a microbial nano cellulose-ZnO-Ag (CNCs) composite and its components separately. **Materials and Methods:** Three components were used for nano cellulose-ZnO-Ag composite synthesis, Ag-nanoparticles, ZnO-nanoparticles and BC. The DPPH method was used to calculate the scavenging of free radical behaviour of four different composite samples. **Results:** Results of silver nanoparticles were found to have the highest antioxidant activity with IC_{50} 65 μg mL^{-1}, followed by CNCs-ZnO-Ag composite (IC_{50} 88.98 μg mL^{-1}) but ZnONPs IC_{50} was 263 μg mL^{-1} and BC (IC_{50} 955 μg mL^{-1}). The CNCs-ZnO-Ag composite, BC and AgNPs at 25 μg mL^{-1} had clotting times that were nearly identical to the control. The APTT increased to 56 Sec at 75 μg mL^{-1} of CNCs-ZnO-Ag composite related to control that recorded 33 Sec. **Conclusion:** Bacterial cellulose acquired new activity in nano form and also when conjugated with nanoparticles. The CNCs-ZnO-Ag composite is ready for pharmaceutical application as an antioxidant and anticoagulant after *in vivo* study.

Key words: Bacterial cellulose, CNCs-ZnO-Ag composite, silver nanoparticles, oxide zinc nanoparticles, antioxidant

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Biopolymers, which have become valuable for use in a lot of applications, are superior to derived petrochemical in being biodegradable biocompatible and environmental.

Exopolysaccharides are long-chain polysaccharides composed of repeating sugars branched units or derivatives of sugar, fundamentally galactose, rhamnose and glucose in different ratios. They are divided into two groups: heteropolysaccharides (xanthan and gellan) and homopolysaccharides (curdlan, mutan, cellulose, dextran and pullulan)[1]. Homopolysaccharides are composed of only one kind of monosaccharides (D-fructose or D-glucose) connected either by a combination of a set number of linkage types or by a single linkage type. While, Heteropolysaccharides are composed of numerous oligosaccharides copies, including 3-8 residues, produced by a different microorganism. There are a lot of industrial applications for exopolysaccharides in pharmaceutical, food and other industries such as cosmetics, gelling agents, medicines for wound dressing, paper and textile[2].

The BC in a biomedical application offers a variety of enforcement. Applications include a biosensor, drug delivery and tissue culture engineering in which plant cellulose[3]. This is due to its high crystalline, mechanical strength in wet conditions, high purity and water absorption capacity[4,5].

Also, Nanotechnology incorporates a combination of different particles in structure, shape and size. These nanoparticles, being tiny in size, have a major surface area to volume ratio. Nanoparticles reflect different electrical, magnetic and optical properties associated with their bulk material. In nanotechnology, the synthesis of organic nanoparticles is one of the most thriving areas of interest[6]. The ZnO-Ag has received great attention due to ZnO can be synthesised by a simple process[5]. Ag nanoparticles have a perfect physicochemical property. Also, the ionization energy of acceptors in ZnO is reduced by silver leading to emission enhanced[7]. So, silver promotes ZnO antimicrobial activity[8,9].

On the other hand, reactive oxygen types are important for health due to they are incorporated in cell coding and are used by phagocytes for their antibacterial action. However, a tiny quantity of reactive oxygen, like oxidative stress, is thought to be closely linked to the ageing operation and some degenerative conditions, such as cancer, mental illness and heart disease[10]. Also, due to their ability as reactive oxygen species scavengers, antioxidative materials have recently gotten a lot of attention[11]. Natural antioxidants are of great attention due to their effects on health and good image as active substances against certain cancers and degenerative diseases. As a result of customer concerns regarding the protection of synthetic antioxidants, natural antioxidants have increased more than ever before[12].

This study was conducted to study, the antioxidant and anticoagulant behaviour of the nano Cellulose-ZnO-Ag composite and compounds made from, Bacterial cellulose, zinc oxide and silver nanoparticles.

MATERIALS AND METHODS

Study area: This study was divided into two parts, the first part: synthesis of nanomaterials carried out at Microbiology Laboratory, Department of Botany and Microbiology, Faculty of Science (Boys), Al-Azhar University, Cairo, Egypt. The second part: The activity analysis occurred at the Labs of Department of Chemistry of Natural and Microbial Products, Pharmaceutical and Drug Industries Research Institute, National Research Centre, Cairo, Egypt (2021).

Synthesis of different compounds

BC production: *Gluconacetobacter xylinus* NRRL B-43 purchased from Northern Regional Research Laboratory (NRRL), Agricultural Research Service culture collection (house research arm of the U.S. Department of Agriculture) culture was used for BC production in Sorbitol broth medium for 7 days at 28°C and pH 6.2. The BC was evaluated for their surface and shape characteristics by scanning electron microscopy (JEOL-JXA 840A, Model Japan) and confirmed by ultraviolet spectrum (T80+UV/VIS Spectrometer, PG Instrument Ltd., UK) analysis[13].

Production of CNCs-ZnO-Ag composite: El-Ghwas *et al.*[14], noted that BC boiled for 30 min in a 0.2 M aqueous NaOH solution and then mechanically disintegrated to a cellulosic paste at room temperature using a laboratory mixer set to 5000-6000 rpm for approximately 10 min. The enzyme activity was 700 units per gram and the mixture was centrifuged at 4°C for 20 min. Several times the cellulose was collected and rinsed. All chemicals used from (Sigma Chemical Co., St. Louis, Mo and U.S.A). Magnetic churning was used to distribute $ZnSO_4$ solution into CNCs suspensions and then NaOH was added dropwise under continuous stirring until pH 10 was reached. Following the observation of a milky color suspension, we added aqueous $AgNO_3$ solutions (20 mL, 10.0 wt percent) and continued the reaction for 2 hrs with vigorous stirring. Transmission electron microscopy (TEM) (Electron probe micro-analyzer JEOL-JXA 840A, Model Japan) was used to determine the CNCs-ZnO-Ag composite's size and form parameters.

Preparation of ZnONPs: *Aspergillus* sp., was cultivated in distilled water at a pH of 7 on MGYP medium (Biolife, Italy) comprising (g L^{-1}) malt extract, yeast extract, peptone and glucose. For seven days, the culture was incubated on an orbital shaker at 160 rpm and 32°C according to a pervious study by the same authors El-Ghwas *et al.*[15]. Filtration of the soup with Whatman-1 filter paper separated the mycelium from the broth and the filtrate was utilized to produce ZnO nanoparticles. About 1 mM $ZnSO_4$ was added to the filtrate in a 1:1 (v/v) ratio and incubated for 2 days at 150 rpm and 32°C. The development of ZnONPs is evidenced by the presence of a white precipitate. TEM and UV microscopy (T80+UV/VIS Spectrometer, PG Instrument Ltd., UK) were used to characterize the size, shape and optical characteristics of the ZnO nanoparticles[15].

Biosynthesis of AgNPs: *Streptomyces coerulescens* mycelium was employed to synthesize AgNPs. UV and FTIR (Jasco 6100, Model Japan) characterizations were conducted in parallel for the same authors[16]. At 37°C and shaking, a starch casein medium was employed for AgNPs production. After 6-7 days, the mycelia were thrice cleaned in sterile conditions using distilled water. The mycelium was used to synthesize the AgNPs. To prepare a solution of $AgNO_3$ (0.1 mM), 0.017 g of the chemical was dissolved in 100 mL of distilled water. Then, 50 mL of $AgNO_3$ solution was added to the mycelium and it was cultured for another 24 hrs under dark circumstances to examine colour change.

DPPH scavenging assay: The (2,2-diphenyl-1-picryl-hydrazyl-hydrate) (Sigma Chemical Co., St. Louis, Mo and U.S.A) free radical method provides a violet solution in ethanol and is an antioxidant assay dependent on electron transfer[17]. The DPPH method was used to calculate the scavenging of free radical behaviour of four different composite samples. In ethanol, a 0.1 mM DPPH solution was prepared. Then, 1 mL was applied to 3 mL of different extracts in ethanol at various concentrations (3.9, 7.8, 15.62, 31.25, 62.5, 125, 250, 500 and 1000 μg mL^{-1}).

Only those extracts that are soluble in ethanol are used and their different concentrations were prepared using the dilution process. The mixture was forcibly shaken and allowed to rest for 30 min at room temperature before being deliberate using a spectrophotometer at 517 nm (UV-VIS Milton Roy). Ascorbic acid was used as a reference model drug and the procedure was repeated three times. The Log dose inhibition curve was used to measure the sample's IC_{50} value. Also, the higher activity of free radical was demonstrated by the reaction mixture's lower absorbance and by utilizing the following formula, the percent DPPH was calculated:

$$\text{Inhibition or DPPH scavenging effect } (\%) = \frac{A0 - A1}{A0} \times 100$$

Where:

A0 = Control reaction absorbance

A1 = Absorbance in the presence of the test or normal sample[18]

Determination of anticoagulant: The prothrombin time (PT) and activity partial thromboplastin time (APTT) efficacy of BC, ZnONPs, AgNPs and CNCs-ZnO-Ag composite, were performed. The citrated plasma was collected from an adult, young people using centrifugation (Union-32R, Korean) at 6000×g at 4°C for 20 min. The concentrations of 25, 50 and 75 μg mL^{-1} of samples were tested and controlled and then an automated coagulometric was used to calculate the PT and PTT.

Statistical analysis: GraphPad Prism Version 6 (Graph Software Inc., La Jolla, CA) was used to analyse data from our practice.

RESULTS AND DISCUSSION

Synthesis and characterization of CNCs-ZnO-Ag composite and its materials: The SEM images of biosynthesized BC on the dried membrane surface reveal a network structure composed of aggregates of extended crystalline cellulose chains arranged in an ultrafine network structure composed of long nanofibers. The UV study of pure BC revealed no absorption at wavelengths greater than 500 nm. Authors' characteristics were thoroughly examined[13].

The CNCs ZnO-Ag was prepared using a novel stabilizing agent, cellulose nanocrystals, to inhibit nanoparticles aggregation and increase their stability. Zinc sulphate and $AgNO_3$ are both detrimental to the formation of AgNPs on the surface of ZnO. The solution disperses well in water and does not precipitate due to the presence of sulfate groups on the surface of the CNCs introduced during sulfuric acid hydrolysis and the abundance of hydroxyl groups. To begin, the Zn cations are absorbed. The (OH^-) functional groups are formed electrostatically by the interaction of metallic cations with the oxygen atoms in polar hydroxyls. The aggregation was avoided by controlling the particle size. Second, $Zn(OH)_2$

is slowly generated in CNCs via dropwise addition of NaOH solutions and ZnO is created under heat circumstances. Additionally, by adding $AgNO_3$ to an alkaline suspension, Ag^+ ions are converted to nanoparticles. The typical size of the CNCs-ZnO-Ag composite is between 6 and 50 nm, as determined by transmission electron microscopy[14].

The morphology of the ZnONPs produced with cell-free *Aspergillus* sp., revealed that they were large rods with a polycrystalline structure, in contrast to the uniform shape of ZnONPs synthesized chemically. The nanoparticles have a diameter ranging from 11.6-43.97 nm. The ZnONPs were expected to have absorption peaks between 340 and 385 nm[15].

The ultraviolet (UV) spectrum of bio AgNPs revealed strong absorption between 420 and 440 nm. The FTIR examination revealed a peak at 3445.21 and 3438.46 cm^{-1}, which is allocated to primary amines (N-H stretch group) and its strength indicated the formation of significant amounts of AgNPs. This is because proteins include an amide group, which has a high affinity for metal, implying that the proteins act as a capping agent, preventing agglomeration and so stabilizing the nanoparticles. Additionally, there is a peak between 1638.23 and 1644.98 cm^{-1} that is attributed to primary amines (N-H bond)[16,19].

Antioxidant activity of CNCs-ZnO-Ag composite and component: In Fig. 1-4, the synthesized CNCs ZnO-Ag composite and components displayed at all concentrations, DPPH scavenging activity was fairly good with activities ranging from 62-81.7% at a working concentration of 1000 mg mL^{-1}. On the other hand, the highest antioxidant activity was owned by AgNPs (IC_{50} 65 µg mL^{-1}), followed by CNCs-ZnO-Ag composite (IC_{50} 88.98 µg mL^{-1}), ZnONPs (IC_{50} 263 µg mL^{-1}) and BC (IC_{50} 955 µg mL^{-1}). Furthermore, CNCs-ZnO-Ag at 1 mg showed activity of antioxidants corresponding to 0.06 mg of ascorbic acid.

In this study, the highest antioxidant activity was owned by AgNPs, followed by CNCs- ZnO-Ag composite, Zinc-NPs and BC. The functional groups of the bio-reductant molecules clinging to the surface of the nanoparticles are thought to be responsible for the nanoparticle's free radical scavenging operation. Many scientists study the antioxidant activity of AgNPs as illustrated by Aina *et al.*[6] who proved that, the scavenging activities of the AgNPs range from 3.6-29.7% at a working concentration of 10-60 ug mL^{-1} and this is not comparable if compared to our results. Also, Akhtar *et al.*[20], mentioned that the AgNPs have good antioxidant activities of 20.13±0.14 and increased to 58.98±0.15 at a concentration of 20 mg L^{-1}. Furthermore, Nagajyothi *et al.*[21] demonstrated

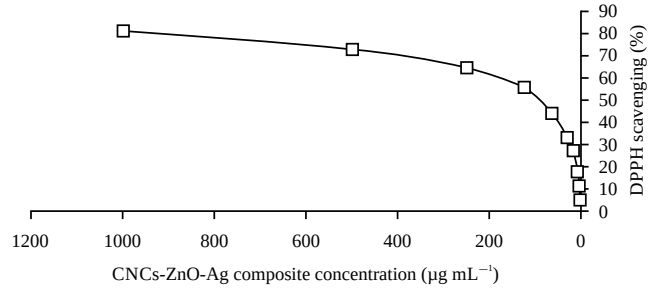

Fig. 1: Antioxidant activity of CNCs-ZnO-Ag composite

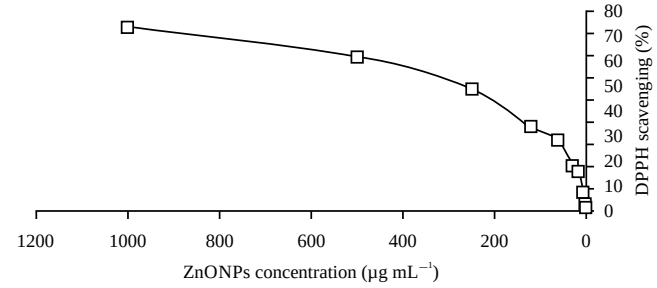

Fig. 2: Antioxidant activity of ZnONPs

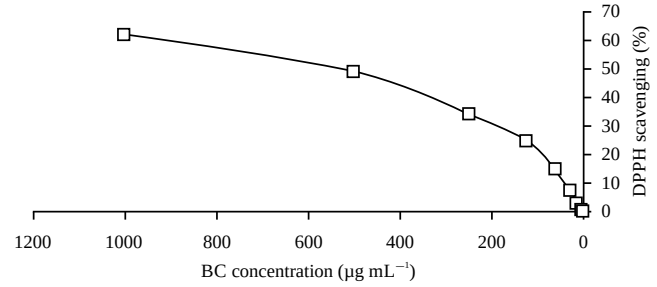

Fig. 3: Antioxidant activity of BC

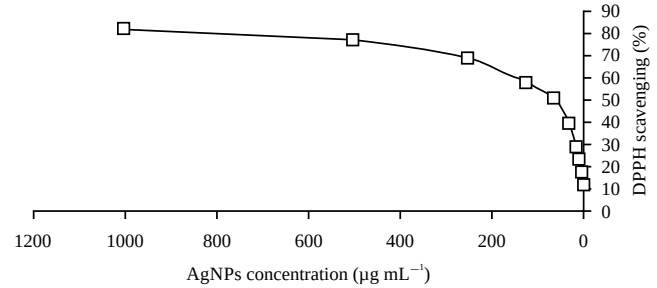

Fig. 4: Antioxidant activity of AgNPs

that ZnONPs were found to have mild antioxidant activity, of 45.47% at 1 mg mL^{-1}. On the other hand, Wheni *et al.*[3] examined the antioxidant activity of the BC with plant extracts

Table 1: Anticoagulant activity of CNCs- ZnO-Ag composite, BC, ZnONPs and AgNPs

	Prothrombin time (sec)				APTT (sec)			
Conc. (μg mL^{-1})	CNCs- ZnO-Ag	BC	ZnONPs	AgNPs	CNCs- ZnO-Ag	BC	ZnONPs	AgNPs
25	12.42	12.2	13.4	12.30	34	34	36	39
50	14.12	14.1	15.9	15.75	44	39	44	46
75	17.50	17.0	20.3	19.75	56	48	51	52

Control of normal PT 12 sec, Control of normal APTT 33 sec, Conc.: Concentration

and revealed that the green tea-BC extract had the highest antioxidant activity (IC_{50} 80.9 ppm), then red hibiscus-BC extract (IC_{50} 438.8 ppm), after that, roselle-BC extract (IC_{50} 505.1 ppm) and finally pink hibiscus-BC extract (IC_{50} 1015 ppm).

Anticoagulant behaviour of CNCs- ZnO-Ag composite, BC, ZnONPs and AgNPs: Table 1 proved that the PT increase as the concentrations of the samples increases up to 75 μg mL^{-1} for all. Also, The CNCs-ZnO-Ag composite, BC and AgNPs at concentration 25 μg mL^{-1} showed a clotting time nearly to the control. Also, when the intrinsic factor was increased, the clotting time was prolonged in a dose-dependent manner.

The APTT analyzed the coagulation pathway, so at 75 μg mL^{-1} of CNCs-ZnO-Ag composite plasma clotting time increased to 56 sec to the control recorded 33 sec. According to Mohan *et al.*[22] the total coagulation time and plasma deposition were increased and decreased, for multilayers of CNCs. Furthermore, Liu *et al.*[23] reported that an acid sulphated polysaccharide (Armatan), isolated from *Asparagopsis armata* (Harv.) a red alga, prolongs rat plasma coagulation period in an *in vivo* assay.

CONCLUSION

The CNCs-ZnO-Ag composite indicates antioxidant and anticoagulant activity. The AgNPs were observed the best in antioxidant activity, followed by CNCs-ZnO-Ag composite, ZnONPs and BC. Bacterial cellulose acquired new activity in nano form and also when conjugated with nanoparticles. The biosynthesized nanoparticles usually specialized in the structure that reflects on activity. The PT and PTT values of CNCs-ZnO-Ag composite, BC and AgNPs at 25 μg mL^{-1} were nearly to control.

SIGNIFICANCE STATEMENT

The present study discovers the possible effect of CNCs-ZnO-Ag composite treatments as antioxidants and anticoagulants. This study will help researchers to reveal the critical of CNCs-ZnO-Ag composite for pharmaceutical application as antioxidant and anticoagulant after *in vivo* study.

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