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ORIGINAL PAPER

Antimicrobial activity of nanocellulose conjugated with allicin and lysozyme

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Abstract In this study, cellulose nanoparticles were prepared by acid hydrolysis, separately conjugated with allicin and lysozyme by a carbodiimide cross-linker, and characterized by scanning electron microscopy, dynamic light scattering, and Fourier transform infrared spectroscopy. Then, their antimicrobial properties were evaluated by the microdilution method and compared with allicin, lysozyme, and nanocellulose alone. The results showed that nanocellulose had few antimicrobial activities, but allicin-conjugated nanocellulose (ACNC) and lysozyme-conjugated nanocellulose (LCNC) had good antifungal and antibacterial effects against standard strains of *Candida albicans, Aspergillus niger, Staphylococcus aureus,* and *Escherichia coli*. Noticeably, although allicin and lysozyme had different minimum inhibitory concentrations (MICs) against all strains, the same quantity of MIC_{50} and MIC_{90} was observed for both ACNC and LCNC. The authors suggest that both ACNC and LCNC can be used in industries as an antimicrobial agent in food packaging, inside foodstuffs, and in textile materials.

Keywords Antimicrobial agent · Cellulose · Nanoparticles · Lysozyme · Allicin

Introduction

Cellulose, as a glucose polymer, is the main component of the plant cell wall. Its monomers bind together with β (1–4) bands. Although cellulose is not water soluble, it has many hydroxyl groups, which lead to strong hydrogen bands. Generally, the native form of

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cellulose is called type I, and the alkaline-treated cellulose is known as type II; they have different crystallite and thermodynamic properties (Park et al. 2010). Nanocellulose has a high surface:volume ratio, crystallization ability, and activity. Its good stability against proteolytic enzymes, acids, and temperatures, and high biodegradability are amazing (Pandey et al. 2012). According to these properties, different applications have been suggested for nanocellulose, e.g., as a reinforcing filler in nanocomposites, a strengthening element in paper, an adsorbent, as a carrier of genes and drugs in medicine, and a degradable film in packaging (Habibi et al. 2010). Technically, nanocellulose can be prepared by different methods, but acid hydrolysis has been used in most studies. After hydrolysis, disintegration is achieved by a high-pressure homogenizer, ultrasound device, and/or ball miller (Loelovich 2012; Bondeson et al. 2006). Regarding its chemical structure, nanocellulose has many active hydroxyl groups, which can be modified by different molecules for various applications (MacLeod et al. 2001). Antimicrobial activity is an important property that can be achieved by modification or conjugation of nanocellulose with different antimicrobial agents such as metal nanoparticles, metal oxide nanoparticles, organic compounds, etc. (Janes et al. 2002).

An important natural antimicrobial agent is the enzyme lysozyme, which is found in human secretions, e.g., saliva, mucus, tears, and milk. Other animals, plants, and some microorganism also produce high amounts of it. Other names of this natural antimicrobial protein are muramidase, *N*-acetylmuramide glycanhydrolase, and glycoside hydrolase (Blake et al. 1965), because it hydrolyzes peptidoglycan of the bacterial cell wall. Lysozyme can be used as a preservative in foodstuffs and in food packaging for prevention of bacterial growth (Hughey and Johnson 1987) because of its wide range of antimicrobial activities (Samaranayake et al. 2001; Lee-Huang et al. 2005). Unlike most of the other antibacterial compounds and traditional preservatives, this enzyme is not toxic to human cells.

Another natural antimicrobial agent is allicin (diallyl thiosulfinate), which inhibits a wide range of gram-negative and -positive bacteria, fungi, parasites, and viruses (Ankri and Mirelman 1999). In garlic clove cells, alliin is converted to pyruvate, ammonia, and allicin by the pyridoxal 5′-phosphate (PLP)-dependent alliinase. Allicin can react with thiol groups of various enzymes, such as thioredoxin reductase, alcohol dehydrogenase, RNA and DNA polymerase, and cysteine proteinase (Stoll and Seebeck 1951). Moreover, allicin has antioxidant, anticarcinogenic, antiinflammatory, antithrombotic, antiatherosclerotic, antihyperlipidemic, and procirculatory effects (Dethier et al. 2012; Bagiu et al. 2012).

The conjugation of lysozyme and cellulose has been described for the production of antimicrobial textiles, which can be achieved by different procedures (Edwards et al. 2011). Generally, cellulose must be treated, modified, and then conjugated with the enzyme. In the case of allicin, since it has no active functional groups, its attachment to other chemical molecules is not easy, necessitating modification before conjugation. Amine-allicin is a modified form that can conjugate with other molecules by its amine group. Although alliin has carboxyl and amine groups that can be used for conjugation, no antimicrobial activity has yet been reported for it (Bagiu et al. 2012; Harris et al. 2001). In the literature, there is no study on the conjugation of nanocellulose with allicin or lysozyme. Thus, the aim of this study was to prepare allicin-conjugated nanocellulose (ACNC) and lysozyme-conjugated nanocellulose (LCNC), after which its antifungal and antibacterial properties were investigated by microdilution method.

Experimental section

Materials

For preparation of nanocellulose, cotton (raw cellulose) manufactured by My Baby Company, Iran, was used. RPMI1640 was purchased from Invitrogen, UK. Allicin, lysozyme, bovine serum albumin (BSA), hydroxybenzotriazole (HOBT), and *N*-ethyl-*N*-(dimethylaminopropyl) carbodiimide (EDC) were provided by Sigma-Aldrich Co. (St Louis, MO, USA). Other chemicals including nitric acid, sulfuric acid, formaldehyde, sodium hydroxide, amine-allicin, and dimethylsulfoxide (DMSO) were sourced from Zyst Fannavar Shargh Co., Yazd, Iran.

Nanocellulose preparation

To prepare nanocellulose, the acid hydrolysis method was used according to the study of Loelovich et al.

with some modifications (Loelovich 2012). Initially, 5 g cellulose was treated with 25 mL of 5 M NaOH at 37 °C for 1 h and then rinsed with distilled water (DW). Then 25 mL of 1 M DMSO was added to the washed cellulose and incubated at 37 °C for 1 h, too. In the next step, DMSO-treated cellulose was washed three times with DW and used for preparation of nanocellulose. Finally, serial concentrations (90, 80, 70, 60, and 50 %) of the acid mixture were prepared. The initial acid mixture had sulfuric acid (85 %), nitric acid (5 %), and water (10 %). Then, 1 g of washed cellulose was separately added to 1 mL of serial concentrations of the acid mixture and incubated at room temperature for 30 min. A completely hydrolyzed cellulose with milky color was chosen, and 2 mL of 5 M NaOH was gently added to it. In the final step, tubes were centrifuged at 3,000 rpm for 5 min, and then nanocellulose pellets were washed by DW three times. Nanocellulose was suspended in DW, shaken for 5 min, and stored at 5 °C.

Preparation of ACNC

Briefly, 500 mg of nanocellulose was added to 5 mL of 7 % citric acid and 5 % sodium hypophosphite monohydrate, and shaken for 30 min at room temperature. Then, modified nanocellulose was centrifuged for 5 min at 5,000 rpm and washed with DW. Then, 1 mL of EDC (233 mg/mL) and 1 mL of amine-allicin (100 mg/mL) were added to 500 mg of modified nanocellulose, incubated at 37 °C for 1 h, centrifuged at 5,000 rpm, and washed with DW. The schematic of this reaction is shown in Fig. 1a. In the final step, serial concentrations (1,000, 500, 250, 125, 62.5 μg/mL) of nanocellulose, ACNC, and allicin alone were prepared in RPMI1640 medium.

Preparation of LCNC

At first, 500 mg of nanocellulose was added to 5 mL of BSA at a concentration of 500 mg/mL and shaken for 5 min. Then, 1 mL of 10 % formaldehyde and 1 mL of HOBT (250 mg/mL) were added to the BSA and cellulose nanoparticle mixture, and incubated at 37 °C for 1 h, in order to allow the esterification reaction. After incubation, the contents of the tube were centrifuged at 5,000 rpm for 5 min and washed with DW. Then, 1 mL of lysozyme at a concentration of 100 mg/mL and 1 mL of EDC at a concentration of 233 mg/mL were added to 500 mg of BSA-nanocellulose, incubated at 37 °C for 1 h, centrifuged at 5,000 rpm, and washed with DW. The schematic of this reaction is shown in Fig. 1b. Finally, serial concentrations (1,000, 500, 250, 125, 62.5 μg/mL) of LCNC, lysozyme, and nanocellulose were prepared in RPMI1640.

Characterization of nanocellulose

The structure, size distribution, and surface composition of nanocellulose, ACNC, and LCNC were studied by scanning electron microscopy (SEM) (Hitachi, S-2400), dynamic light scattering (DLS) (Malvern Instruments, Italy), and Fourier transform infrared spectroscopy (FTIR) (ELICO, India), respectively. For SEM investigation, all samples were coated with gold by a sputtering apparatus and then studied at 15 kV. The FTIR was used to confirm conjugation. In this experiment, the spectrums of allicin, lysozyme, nanocellulose, ACNC, and LCNC were investigated at 500–3,500 cm^{-1}.

Evaluation of antimicrobial properties

The broth microdilution method was used for evaluation of the antimicrobial susceptibility of allicin, lysozyme, nanocellulose, ACNC, and LCNC, according to Clinical and Laboratory Standards Institute (CLSI) guidelines. Four standard microbial strains including *Candida albican (C. albicans)* ATCC 10231, *Aspergillus niger (A. niger)*ATCC 16888, *Staphylococcus aureus (S. aureus)* ATCC 25923, and *Escherichia coli (E. coli)* ATCC 25922 obtained from the Iranian Research Organization for Science and Technology were used in this study. First, fungal and bacterial strains were inoculated onto Sabouraud dextrose agar and nutrient agar, respectively. Molds, yeast, and bacterial plates were incubated for 48 h at 25, 35, and 35 °C, respectively. Then, one colony of each strain was separately added to 5 mL of RPMI1640 plus 2 % glucose medium. The final density was 2×10^4 cells/mL, and its optical density (OD) was 0.1 at 260 nm. In the next step, serial concentrations of 100 μL of allicin, lysozyme, nanocellulose, ACNC, and LCNC were separately incubated with 100 μL of microbial suspension. Then, mold, yeast, and bacterial strains were incubated at 25, 35, and 35 °C for 48 h, respectively.

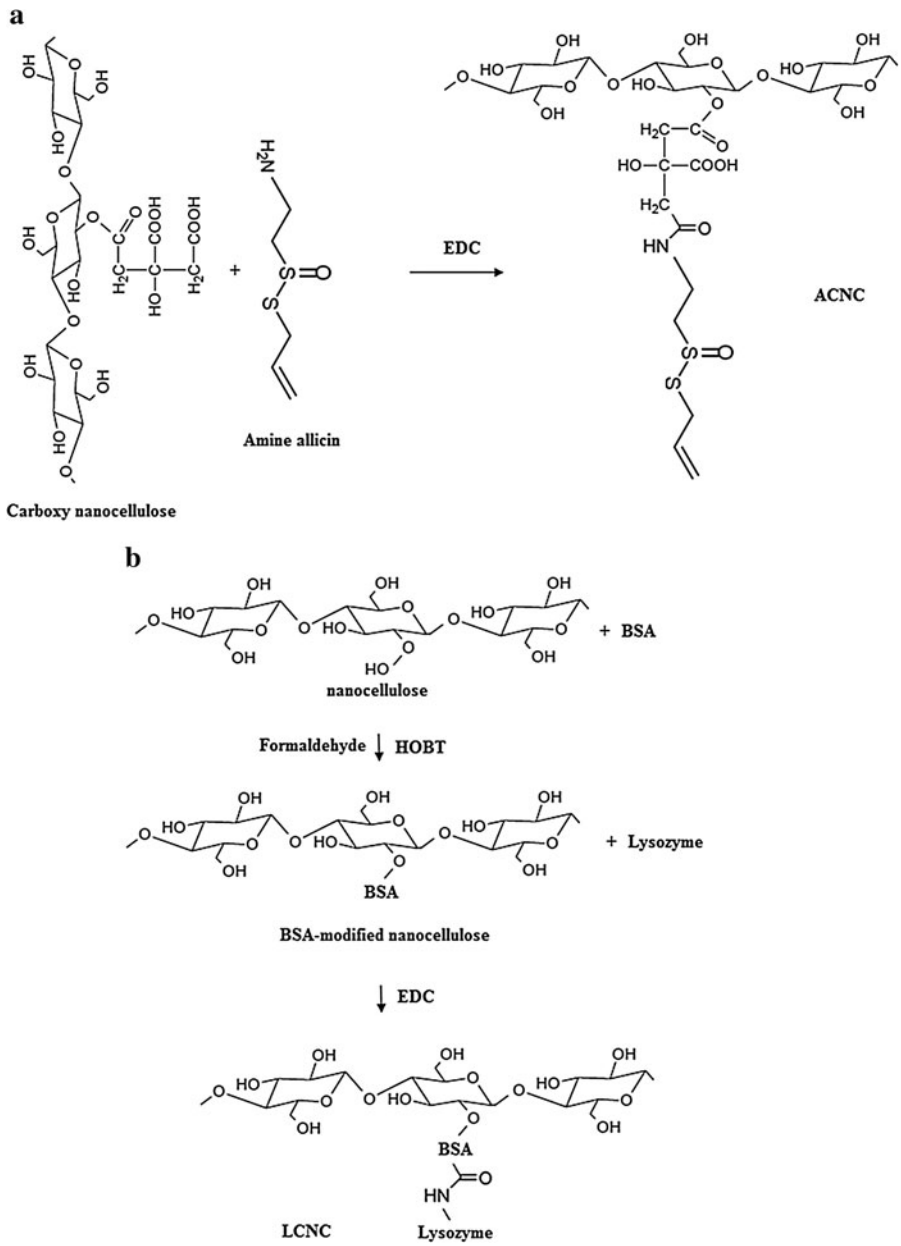

Fig. 1 Schematic representation of conjugation between carboxy-nanocellulose and amine allicin (**a**), and between BSA-nanocellulose and lysozyme (**b**) by EDC cross-linker

In this experiment, negative and positive controls were included. Microbial suspensions not treated with allicin, lysozyme, nanocellulose, ACNC, or LCNC were considered as negative control. For positive control, fungal strains were exposed to 2 μg/mL nystatin, and bacterial strains were treated with 1 μg/mL ceftriaxone. After incubation, the OD of each well was read at 405 nm by an ELISA reader (Novin Gostar, Iran), and minimum inhibitory concentrations (MIC) of allicin, lysozyme, nanocellulose, ACNC, and LCNC against different strains were calculated. In this study, MIC_{50} and MIC_{90} were measured, in comparison with controls.

Statistical analysis

The results are shown as the mean ± standard deviation (SD) with three replicates. A parametric test (Student's *t* test) was applied to evaluate significant

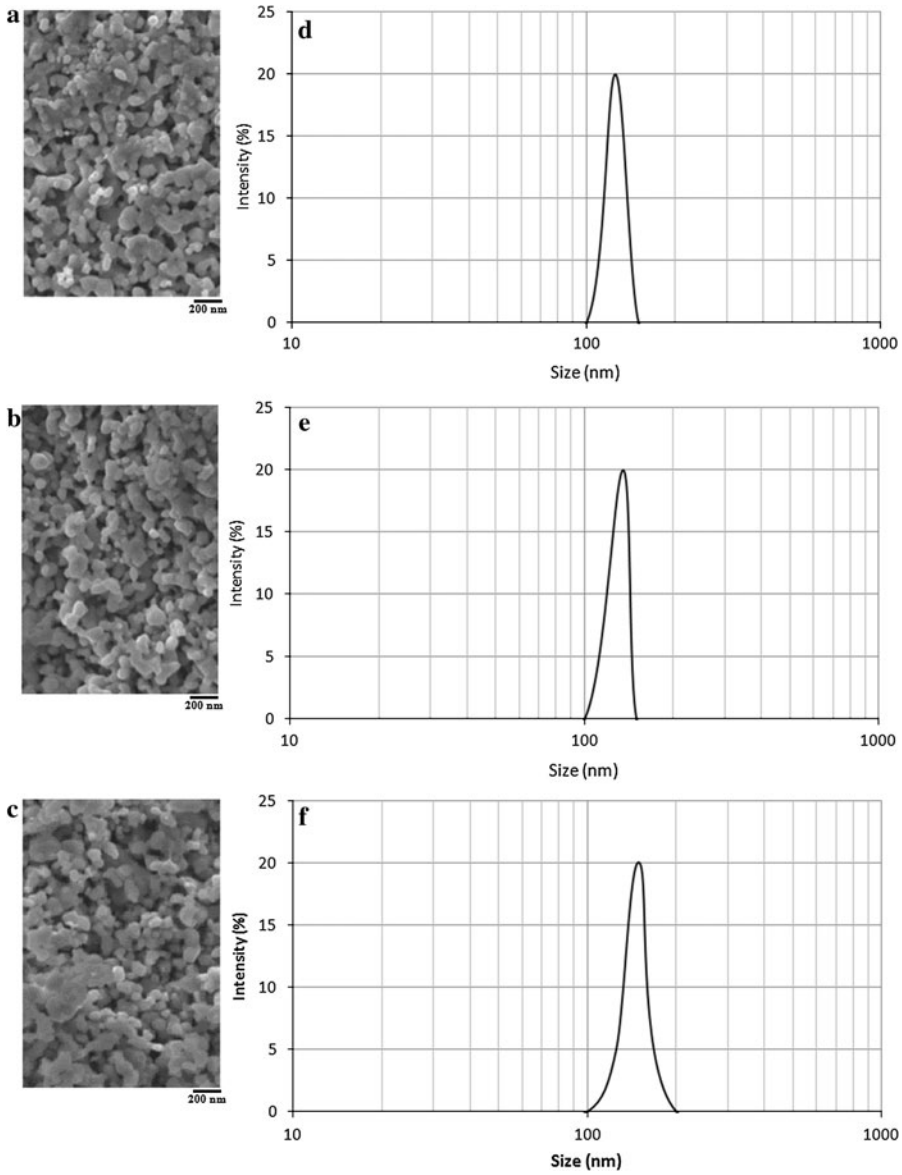

Fig. 2 SEM images of nanocellulose (**a**), ACNC (**b**), and LCNC (**c**), and the distribution size of nanocellulose, ACNC, and LCNC

differences using SPSS software, V.16.0, for Windows (SPSS Inc., USA). $P < 0.05$ was considered a significant difference.

Results

Characterization of nanoparticles

SEM images of nanocellulose, ACNC, and LCNC are shown in Fig. 2a–c, respectively. According to these figures, both the nanocellulose and conjugates are approximately spherical. Figure 2d–f shows the size distribution of nanocellulose, ACNC, and LCNC. As is shown, the sizes of nanocellulose, ACNC, and LCNC are about 100–150, 100–170, and 100–200 nm, respectively. Also, the FTIR spectrum of nanocellulose (a), citric acid-modified nanocellulose (carboxy nanocellulose) (b), amine allicin (c), and ACNC (d) is observed in Fig. 3a. Generally, the spectrum of ACNC confirmed attachment of amine allicin to modified nanocellulose, i.e., both specific spectrums of allicin and modified nanocellulose were seen in the spectrum of ACNC. Also, the FTIR results showed that

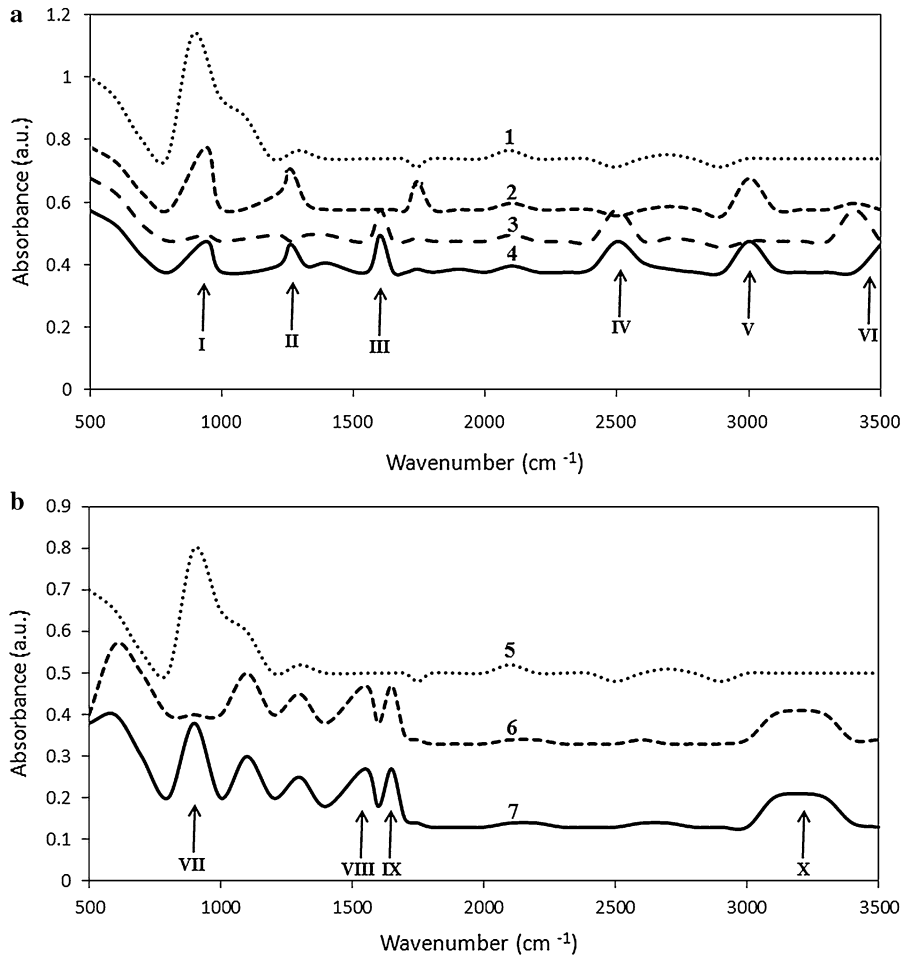

Fig. 3 **a** FTIR spectrum of nanocellulose (*1*), citric acid-modified nanocellulose (*2*), amine allicin (*3*), and ACNC (*4*). **b** FTIR spectrum of nanocellulose (*5*), lysozyme (*6*), and LCNC (*7*) (**b**). *I* (940 cm^{-1}), *II* (1,260 cm^{-1}), *III* (1,600 cm^{-1}), *IV* (2,500 cm^{-1}), *V* (3,000 cm^{-1}), *VI* (3,500 cm^{-1}), *VII* (940 cm^{-1}), *VIII* (1,550 cm^{-1}), *IX* (1,650 cm^{-1}), and *X* (3,170–3,300 cm^{-1}) are vibration peaks

lysozyme and LCNC (Fig. 3b) had amide band I (1,650 cm^{-1}), amide band II (1,550 cm^{-1}), and amide A and B (3,170–3,300 cm^{-1}). These specific bands were not observed for nanocellulose.

MIC results

The MIC_{50} and MIC_{90} of allicin, lysozyme, nanocellulose, ACNC, and LCNC against two standard fungal and two bacterial strains are shown in Table 1. Figure 4a–d demonstrates the dose–response graph of allicin, nanocellulose, and ACNC against *E. coli*, *S. aureus*, *A. niger,* and *C. albicans*, respectively. Figure 5a–d shows the dose–response graph of lysozyme, nanocellulose, and LCNC against *C. albicans*, *A. niger*, *S. aureus*, and *E. coli*, respectively.

As demonstrated, nanocellulose had few antifungal and antibacterial properties, and the highest effect was seen at a concentration of 1,000 μg/mL. In general, the

Table 1 The MIC_{50} and MIC_{90} of allicin, lysozyme, nanocellulose, ACNC, and LCNC against standard fungal and bacterial strains

	Isolates			
	C. albicans	*A. niger*	*S. areus*	*E. coli*
MIC_{50} (μg/mL)				
Nanocellulose	>1,000	>1,000	>1,000	>1,000
Lysozyme	250	500	125	>1,000
LCNC	500	500	500	500
Allicin	250	125	62.5	125
ACNC	500	500	500	500
MIC_{90} (μg/mL)				
Nanocellulose	>1,000	>1,000	>1,000	>1,000
Lysozyme	1,000	>1,000	250	>1,000
LCNC	1,000	>1,000	1,000	1,000
Allicin	1,000	500	250	500
ACNC	1,000	1,000	1,000	1,000

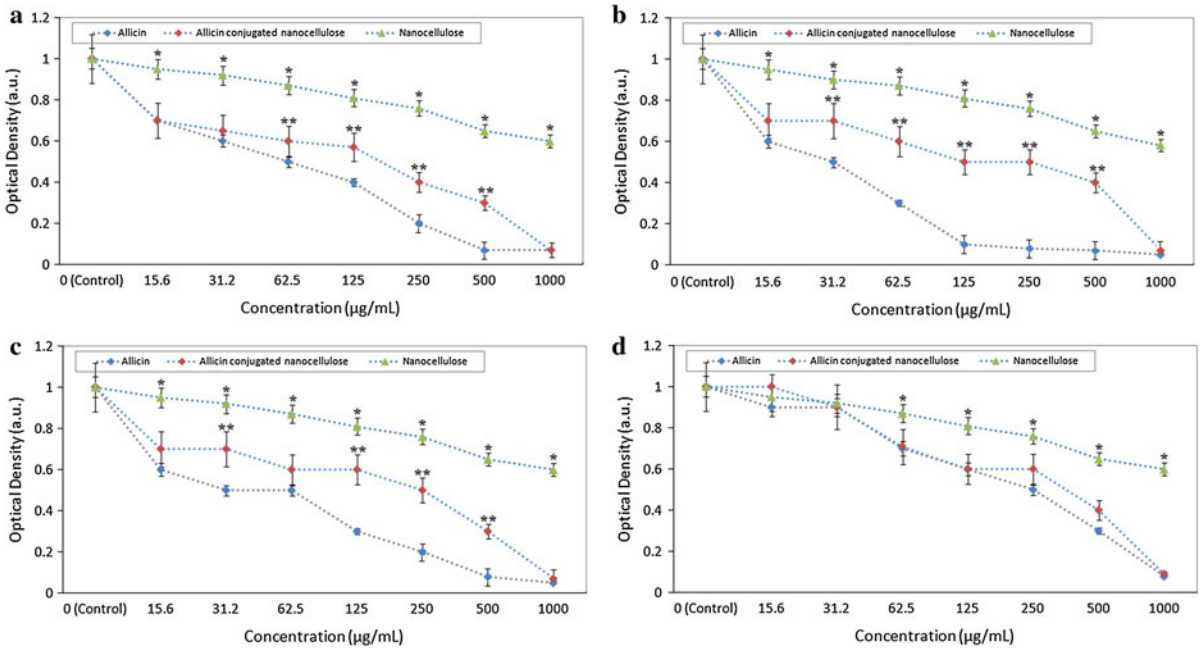

Fig. 4 Dose–response graph. Different concentrations of nanocellulose, ACNC, and allicin were separately added to *E. coli* (**a**), *S. aureus* (**b**), *A. niger* (**c**), and *C. albicans* (**d**) suspensions, and incubated 48 h at 25, 35, and 35 °C for fungal, yeast, and bacterial strains, respectively. The OD of each well was read at 405 nm by an ELISA reader. All data are shown as mean ± SD with $n = 3$. $^{*}P < 0.05$ compared with allicin and allicin-conjugated nanocellulose at the same concentration. $^{**}P < 0.05$ compared with allicin at the same concentration

MIC_{50} and MIC_{90} of both ACNC and LCNC against all strains were 500 and 1,000 μg/mL, respectively. The MIC_{50} of allicin was less than the MIC_{50} of ACNC ($P < 0.05$) in all strains, and the same pattern was seen for MIC_{90} ($P < 0.05$), i.e., the MIC_{90} of allicin was less than the MIC_{90} of ACNC (except of *C. albicans*). In case of lysozyme and LCNC, the MIC_{50} of lysozyme was less than the MIC_{50} of LCNC against *C. albicans*, *S. aureus*, and *E. coli* ($P < 0.05$). But in the case of MIC_{90}, this pattern was seen only against *S. aureus* ($P < 0.05$). Overall, an inverse relationship was observed between OD and concentration of allicin, lysozyme, nanocellulose, ACNC, and LCNC against all strains. As shown, the lowest MIC_{50} (62.5 μg/mL) was seen for allicin against *S. aureus*. Overall, although allicin had less MIC_{50} and MIC_{90} than lysozyme, but both ACNC and LCNC had the same MIC_{50} and MIC_{90} against all strains.

Discussion

In the present study, cellulose nanoparticles were prepared by the hydrolysis method, modified by citric acid, and then conjugated with amine allicin by EDC (Fig. 1a). Also, after the preparation of nanocellulose, it was coated with BSA and conjugated with lysozyme by the EDC method (Fig. 1a). Then, antibacterial and antifungal properties of allicin, lysozyme, nanocellulose, ACNC, and LCNC against standard microbial species were evaluated by the microdilution method.

As noted, acid hydrolysis was used to prepare nanocellulose, because this method is easy and inexpensive (Habibi et al. 2010; Bondeson et al. 2006). For this purpose, crude cellulose was first washed with NaOH and DMSO in order to eliminate cellulose impurity. We believe that different impurities may affect the preparation of nanocellulose, just like in other chemical reactions. In the second step, washed cellulose was incubated with serial concentrations of the acid mixture (90, 80, 70, 60, and 50 %), because the preparation of nanocellulose depends mainly on the acid concentration. We found that acid mixtures at concentrations of 80 and 90 % were not suitable for preparation of nanocellulose, since they led to a dramatic reduction of cellulose. Also, the concentrations of 60 and 50 % did not have enough power to prepare nanocellulose and led to partial

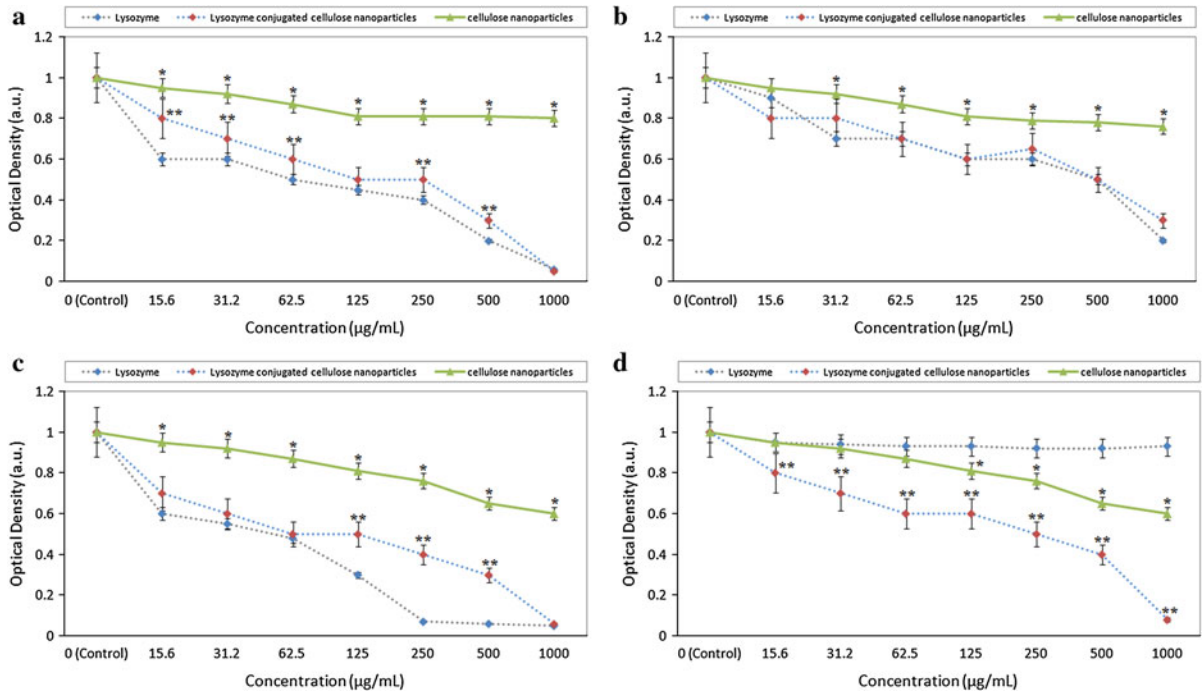

Fig. 5 Dose–response graph. Different concentrations of nanocellulose, LCNC, and lysozyme were separately added to *C. albicans* (**a**), *A. niger* (**b**), *S. aureus* (**c**), and *E. coli* (**d**), and incubated 48 h at 25, 35, and 35 °C for fungal, yeast, and bacterial strains, respectively. The OD of each well was read at 405 nm by an ELISA reader. All data are shown as mean ± SD with $n = 3$. *$P < 0.05$ compared with lysozyme and conjugated cellulose nanoparticles at the same concentration. **$P < 0.05$ compared with lysozyme at the same concentration

hydrolysis. We observed that a concentration of 70 % was exactly suitable, and it produced nanocellulose at room temperature with the desired size. In the previous studies, preparation of nanocellulose has been reported at different concentrations of sulfuric acid (44–70 %), temperatures (25–70 °C), and hydrolysis times (0.5–24 h) (Bondeson et al. 2006; Habibi et al. 2010).

In the second step, nanocellulose was first modified with citric acid. The reason for the modification was to add carboxyl groups to the nanocellulose. As mentioned, allicin has no active functional groups, and its attachment to other chemical molecules is difficult. Amine-allicin is a modified molecule that can conjugate with other molecules by its amine group. EDC, as a known cross-linker, conjugates carboxyl and amine, and it was used to conjugate modified nanocellulose and amine allicin (Fig. 1a). As noted in the Results section, the FTIR spectrum confirmed attachment of these molecules. On the other hand, nanocellulose was coated with BSA as a linker between cellulose nanoparticles and lysozyme, and then the conjugation of BSA and lysozyme was carried out by the EDC method (Fig. 1b). As demonstrated in Fig. 6, BSA is a large molecule, and several lysozyme moieties can bind to one BSA molecule. According to the DLS results (Fig. 2d–f), the size distribution of nanocellulose, ACNC, and LCNC was about 100–150, 100–170, and 100–200 nm, respectively. The bigger size of conjugates is due to attachment of linker and antimicrobial agents on the surface of nanoparticles, which leads to larger hydrodynamic size.

The microdilution results showed that nanocellulose had the same antifungal and antibacterial activities, but these properties were not as powerful, i.e., the MIC_{50} and MIC_{90} of nanocellulose were >1,000 μg/mL against all strains. We hypothesize that nanocellulose cannot target and disturb the cell wall, cell membrane, or active enzymes of bacterial and fungal strains. However, allicin and ACNC had powerful antibacterial and antifungal properties (Fig. 4). In case of *A. niger*, *S. aureus,* and *E. coli*, allicin had higher antibacterial and antifungal activity (with less MIC_{50} and MIC_{90}) than ACNC. The reason for this pattern

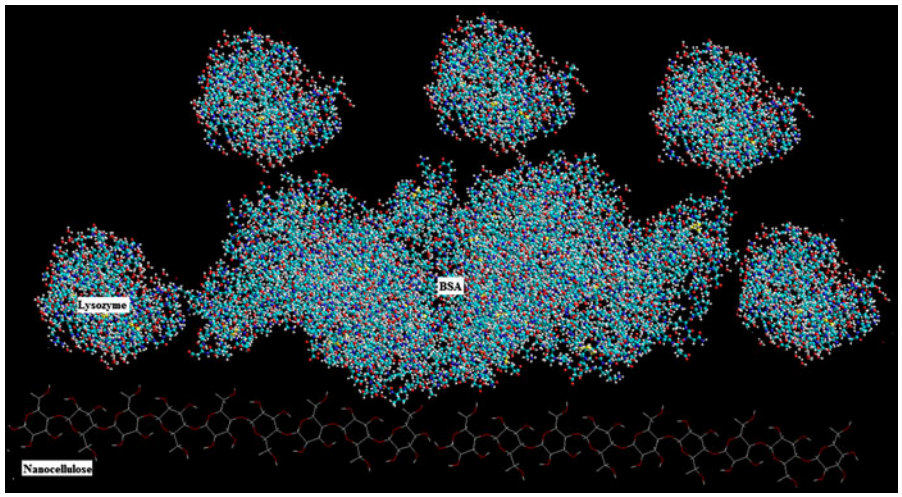

Fig. 6 Schematic images of lysozyme, BSA, and nanocellulose. One BSA conjugate is together with some lysozyme molecules. The reaction took place on the second OH group of cellulose that is randomly drawn. The position of substitution is depicted as C–2 only for convenience. The source of the files is the protein data bank

may be the different quantity of active molecules in the same concentrations of allicin and ACNC. On the other hand, ACNC might not damage cytoplasmic enzymes as effectively as allicin, because of its large size. Rather, ACNC might only damage surface enzymes and proteins. In the case of *C. albicans* (Fig. 4d), the same pattern of the antimicrobial property was seen for ACNC and allicin. The authors hypothesize that ACNC and allicin may have different mechanisms of inhibition on *C. albicans*. Although ACNC has allicin as an integral part, this structure may have an affect on bacterial and fungal strains with different routes, as described for allicin. The related mechanisms and their uptake must be investigated in future studies. There have been no studies on the conjugation of allicin and nanocellulose, and also no report was found on its antimicrobial activity. Regarding allicin alone, findings of this study were consistent with previous works. According to other studies, allicin exhibits a powerful antibacterial activity against different gram-negative and -positive bacteria such as *Escherichia, Staphylococcus, Streptococcus, Salmonella*, *Proteus, Klebsiella*, *Clostridium, Bacillus,* and *Mycobacterium* (Uchida et al. 1975). Also, allicin has an antifungal activity against *Aspergillus*, *Cryptococcus*, *Candida*, *Trichophyton*, *Epidermophyton*, and *Microsporum* (Davis et al. 1994; Hughes and Lawson 1991; Yamada and Azuma 1997). In terms of mechanism, the rapid reaction of thiosulfinate with thiol groups of enzymes leads to their inhibition and therefore biocidal activity. This reaction destroys some vital microbial enzymes, e.g., thioredoxin reductase, RNA and DNA polymerase, alcohol dehydrogenase, cysteine proteinase, and others.

In the case of lysozyme (Fig. 5), it could not inhibit *E. coli*, but LCNC inhibited its growth. Lysozyme disrupts peptidoglycan molecules of the bacterial cell wall and hydrolyzes the linkage between *N*-acetylmuramic acid and *N*-acetyl-D-glucosamine residues. Gram-positive bacteria are susceptible to lysozyme, because of the high proportion of peptidoglycan, but less susceptibility is observed in gram-negative bacteria because of an outer membrane and a lower proportion of peptidoglycan. Therefore, lysozyme cannot damage the cell wall of *E. coli*. However, in the case of LCNC, the authors hypothesize that the activity of lysozyme is changed by conjugation with BSA and nanocellulose. We suggest that LCNC may bind to other compartments of *E. coli*, leading to cell damage. This finding is a new result and must be studied further in future studies. The antimicrobial mechanism of conjugated nanocellulose may help us in developing new antimicrobial agents. As demonstrated in Fig. 5b, both LCNC and lysozyme have comparable antifungal properties against *A. niger*. We hypothesize that conjugation of lysozyme could not affect antifungal activity. It must be mentioned that LCNC and lysozyme may damage *A. niger* by different mechanisms, related to their different chemical formulations and conformations. This has been

indicated for *E. coli*, too. In the case of *C. albicans* and *S. aureus*, LCNC has fewer antifungal and antibacterial properties than lysozyme alone. It can be explained that at the same concentration of lysozyme and LCNC (e.g., 500 μg/mL), the number of lysozyme molecules in the lysozyme solution is more than in the LCNC solution. This fact may justify the lower antifungal and antibacterial properties of LCNC. The lower antimicrobial activity of LCNC may also be attributable to the conjugation method. On the other hand, LCNC cannot affect *C. albicans* and *S. aureus* as potently as *E. coli* and *A. niger*. This variation in the antimicrobial activity of LCNC may be due to the cell wall and membrane composition. Here, we showed that MIC_{50} and MIC_{90} of LCNC and ACNC were 500 and 1,000 μg/mL, respectively, for all strains. It must be noted that these quantities are quite high compared with traditional antibacterial and antifungal drugs. Although there is no study on antimicrobial properties of LCNC, some related studies may be mentioned here. Kandemir et al. (2005) showed the antibacterial activity of biodegradable films composed of exopolysacharide and lysozyme. In another study, good antimicrobial activity of conjugated film (containing chitosan and lysozyme) was presented (Duan et al. 2008). Mascheroni et al. (2009) demonstrated that cellulose fibers could be modified by lysozyme. They declared that modified cellulose has good antibacterial properties against *Micrococcus lysodeikticus*.

Regarding its application, the authors suggest that both ACNC and LCNC can be used as a preservative in food or as an antimicrobial agent in food packaging or textile materials. Of course, the safety of ACNC and LCNC should be evaluated in future studies. Taken together, nanocellulose can be conjugated with allicin and lysozyme, and has antifungal and antibacterial activity against *C. albicans*, *A. niger*, *S. aureus*, and *E. coli* strains.

Conclusion

This study showed that although cellulose nanoparticles have few antibacterial and antifungal activities, but both ACNC and LCNC have high antimicrobial effects against *C. albicans*, *A. niger*, *S. aureus*, and *E. coli*, and may be used in industry as an antimicrobial agent in packaging, inside foods, and on textile materials.

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Conflict of interest No conflict of interest exists.

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