Advances in Life Science and Technology
ISSN 2224-7181 (Paper) ISSN 2225-062X (Online)
Vol.67, 2018

Assessment of Antimicrobial by Nanocomposite of Bacterial Cellulose Loaded with Nanoparticles Sodium Alginate and Chitosan

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Abstract
Antimicrobial membrane disc of BC with sodium alginate and chitosan were fabricated through doping nanoparticles as antibacterial agents. . Nanoparticles was densely arrayed inside BC fibers composites as confirmed by field emission scanning electron microscopy (FE-SEM). Fourier transform-infrared (FT-IR) spectra of the BC membranes exhibited characteristic peaks for specific functional groups of ch and sd alg beside swelling test and antimicrobial property of these nanoparticles sodium alginate and Chitosan doped cellulose membrane against *E. coli* and *S. aureus* was evaluated by the diffusion disc method. Effects of antimicrobial agent quantity, volume-related properties (nanoparticles) on the antimicrobial activity of cellulose membrane were studied. Antimicrobial activity was observed Chitosan nanoparticles were compared when compared with sodium alginate. Maximum *E. coli* inhibition 85% was achieved with only 5% of amphetamine from the nanoparticles chitosan in cellulose membrane. The resulting bio composites membrane have high antibiotic activity against *Staphylococcus aureus.* These membranes can potentially be used in medicine as a wound healing.
Keywords: Bacterial cellulose, chitosan, nanoparticles, antibacterial activity, *Staphylococcus aureus E. coli*.

1-Introduction

The microbes often are harmful and can cause many infectious diseases such as whooping cough, diarrhea, respiratory diseases, and fever [1]. Noble metals (silver, copper, zinc) and natural products (essential oils, bio-polymers, and organic acids) are among the antimicrobial agents available to prevent microbial infections [2, 3]. Antimicrobial cellulose were needed to prevent the growth of microbes in food for the manufacture of food packaging , the wound dressing in medical devices, clothing in the textile industry and the footwear industry [4, 5].

The use of chitosan in the field of packaging showed excellent results because of its unique energetic characteristics such as lower level of toxicity and advanced bio-degradation. The inhibitory effectiveness of Chitosan is high toward a wide range of microorganisms (seen in both negative and positive Gram stain) and can be used in various applications such as pharmaceutical, food processing and textile industries, water treatment, and cosmetics. In addition, Chitosan can either be used in its pure state or mixed with natural polymers such as starch, gelatin and cellulose [6, 7]. However, chitosan was not used in the form of nanoparticles. The small size of the nanoparticles of chitosan make them unique physiochemical properties such as large surface area (providing more cationic sites) and highly interactive and thus can enhance the reaction of the charge on the surface of the microbes and lead to a stronger antimicrobial effect [6]. Some researchers have synthesized nanoparticles in starch films and hydroxypropyl methyl cellulose (HPMC) to prepare antimicrobial films. However, their work has focused on the impact of nanoparticles on the membrane barrier and its mechanical properties. They concluded that the improvement of the properties of antimicrobial films was due to the good interaction between chitosan nanoparticles and polymeric cellulose [8]. However, it is also useful to investigate the effectiveness of antimicrobial membrane against nanoparticles against microbial activity.

In the current study, we prepared a BC-ch/BC/ sd alg membrane disc composite in situ method to enhance the effectiveness of inhibitory anti-microorganisms towards a number of pathogenic bacteria. BC-ch/sd composite was prepared by doping of nanoparticles on BC fibers. The morphological changes occurring during the composite process were monitored by FE-SEM, FTIR analyses, evaluation of their antimicrobial activity via diffusion assay. The effect of the particle size properties of evaluate long-term killing ability of BC/ch, and BC/sd al NPs. We found that, beyond the intrinsic antibacterial activity of either BC/sd-NPs, the loaded carrier showed a potent, and prolonged bactericidal ability against various *E. Coli* & S. aureus Bacteria. . This combination of BC with nanoparticles chitosan and sodium alginate may serve as excellent antimicrobial membrane material for potential applications in wound healing.

Materials and Methods

2.1 Bacteria *Gluconacetobacter xylinum*

Gluconacetobacter xylinum (ATCC53582) was used for the synthesis of BC Sheet as reported previously [10]. Briefly, the bacterium was cultured in a Hestrin and Schramm (HS) medium containing glucose 20 gL^{-1}, yeast extract 5 gL^{-1}, peptone 5 gL^{-1}, disodium phosphate 2.7 gL^{-1}, and citric acid 1.15 gL^{-1}. The pH of the medium was

adjusted to 6.0 with 1.0 M NaOH. The medium was sterilized for 15 min at 15 psi and 121°C. 1ml colonies from *G. xylinum* culture tube were inoculated into 100 mL of broth medium in a 250 mL Erlenmeyer flask and incubated for 24 h at 30°C under statically conditions. Thereafter, a small volume (3-5% v/v) from this freshly prepared pre-culture was inoculated into a new HS medium and incubated statically at 30°C for 6-10 days. As a result BC membrane was produced at the surface of the culture medium. The BC produced was harvested and treated with 0.3 M NaOH for 15 min at 15 psi at 121°C to disrupt and dissolve the cell debris as reported previously. The treated BC was then washed with deionized distilled water until the pH became neutral, and was stored in distilled water at 4°C for synthesis of composite and analysis.

2.2 Pathogenic bacteria

S. aureus & *E. coli* Bacteria were obtained from the university laboratory.

2.3 Preparation of Ch Nanoparticles- and Doped Cellulose fiber

Chitosan (ch) -doped cellulose films solutions were prepared by adding various amounts (0.5, 1, 1. 5, 2, % (v/v) [11] of chitosan nanoparticles in to 1% acetic acid and stirred until it solubility is achieved. Then into cellulose solution. The mixtures were then magnetically stirred for 30 minutes, transferred into petri dish, and dried in oven at 60°C to obtain cellulose film. Subsequently, the dried film was rinsed with UPW before drying at room temperature**.**

2.4 Preparation of sd al Nanoparticles- and Doped Cellulose fiber

Sodium alginate was acquired from the manufacturer (Sinopharm chemical Reagent) from china, were prepared by adding various amounts (0.5, 1, 1. 5, 2, % (v/v) [10] of sodium alginate nanoparticles in to 1% acetic acid and stirred until it solubility is achieved. Then into cellulose solution. The mixtures were then magnetically stirred for 30 minutes, transferred into petri dish, and dried in oven at 60°C to obtain cellulose film. Subsequently, the dried film was rinsed with UPW before drying at room temperature

2.5 Characterization

2.5.1 Scanning Electron Microscopy.

The surface morphology of Bacterial cellulose membrane with and without nanoparticles were analyzed using FE-SEM. Briefly, samples were fixed on a brass holder and coated with gold on a Cu SEM disk and analyzed through a Nova NanoSEM450 FE-SEM (Nova NanoSEM450, FEI, Holand).

2.5.2 Fourier Transform Infrared Spectroscopy (FTIR).

Bacterial cellulose membrane with and without nanoparticles were analyzed using Fourier Transform Infrared Spectroscopy (FTIR) (Shimadzu, FTIR IRAfnity-1S, Kyoto, Japan) using transmittance modes. Membranes were kept in an oven at 30 °C, after drying they were ground. Approximately 2 mg of the sample was mixed with 300 mg of KBr to form the pellet. Spectra were obtained in the 4000 to 400 cm−1 wavelength range after 64 scans, with a resolution of 4 cm−1. The spectra were normalized and the vibration bands were associated with the main chemical groups [12].

2.5.3 Swelling analysis.

The swelling ability of materials was also studied. The membranes were cut into square shaped of 2 cm × 2 cm and dried to obtain the constant weight. After that the water was eliminated, they were submerged in unionized water at room temperature [13].

The swelling ratio (% SR) was calculated using the following formula;

$$\% SR = \frac{Wt - Wd}{} * 100$$

The *Wt* is weight of swollen hydrogel. The Wd is the weight of dried, the swelling tests were done in three replicate and average results were defined [14].

2.5.4 Agar disc diffusion method

In this method, the antibacterial activity of BC/ch, and BC/sd al Nanocomposites was examined by agar petri dishes prepared using *E. coli,* (ATCC 9721), *s. aureas* (ATCC 10390). The frozen dried samples were then cut from a rod and a hook-in-disc into disk shapes with a diameter of 1.5 cm and sterilized at 121 ° C for 15 min. The fresh *E. coli* and *S. aureus* was then spread onto the plates, the discs were placed on top, and the samples were incubated for 24 hours at 37 ° C, at which time the inhibition zones were measured**.**

3. Results and Discussion

Cellulose membranes were produced by using bacteria as shown in Figure 4-1. The amounting thickness of cellulose was 7.5 mm and after drying, it was 0.80 mm.

Production of BC-ch and BC- Sd al membranes

BC-Chiosan and BC-Sodium alginate membranes were produced by using bacteria as shown in Figure (4-1). The thickness of cellulose was 7 mm after drying was 0.75 mm for BC-ch thickness was 4 mm after drying was

0.70 mm. The thickness of cellulose was 5 mm after drying was 0.75 mm for BC- Alg.

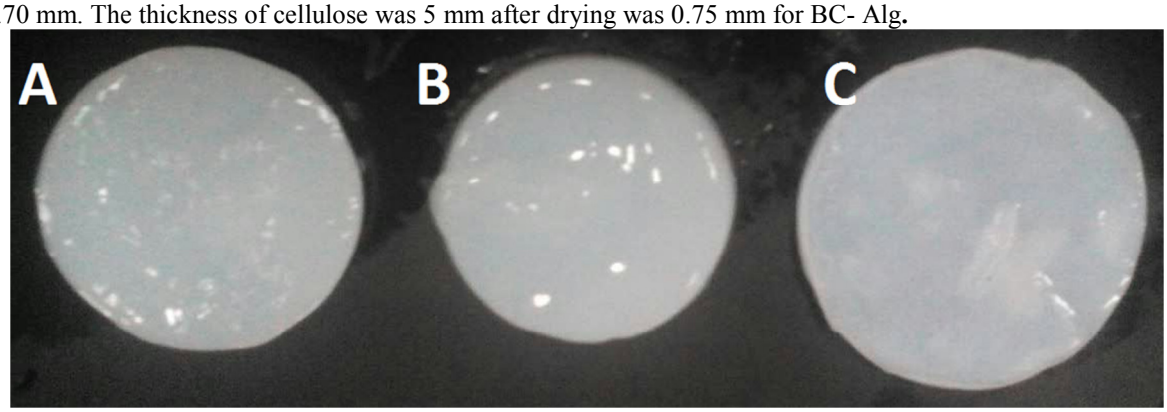

Fig. 4-1 A- Cellulose membrane, B- Cellulose membrane modification by sodium alginate, and C- Cellulose membrane modification by chitosan

3.1. Characterization of BC & BC-ch & BC- sd al membranes

Morphological analysis of BC, BC-ch, and BC-sd al membranes (SEM)

In the Fig. 2 shows the SEM micrographs of pure BC and BC/ch, BC/sd al composites nanoparticles. SEM micrograph of pure BC showed a three dimensional arrangement of long chain web-shaped cellulose [15]. As well as with other researchers, the characteristic tridimensional fibre network of BC was clearly observed on the film surfaces. The surface morphology of BC was changed after ch and sd al treatment. A thick layer of ch covered the film, so it was difficult to observe individual BC nanofibers. BC and BC/Ch microfibrils are further joining together into one millionth centimeter thick ribbon-like fibers. Besides, the presence of uniformly distributed fibers of the carcass is evident, which ensures high strength of the films. Interaction of s d al and BC molecules is also noticeable. A high amount of hydrogen bonds formed by the hydroxyl groups caused the pure BC fibres to aggregate together.

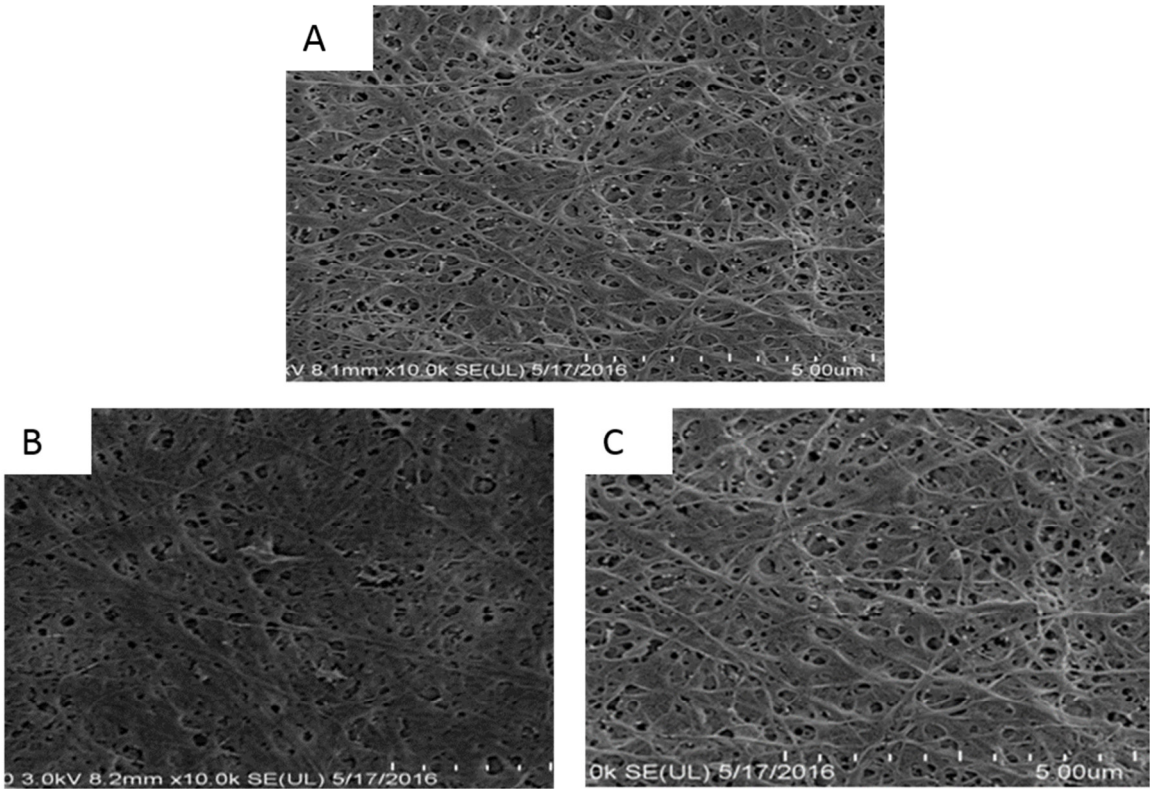

Fig. (4-2) : (A) BC fiber **(B)** Cellulose Fibers with chitosan **(C)** Cellulose Fibers with sodium alginate.

3.2. FTIR characterization of pure BC & BC-ch & BC- sd al membranes composites

FTIR is important spectroscopic technique to analysis the existence of target functional groups and the nature of chemical bonds linking them together in a molecule, polymer, or a composite [16 Ul-Islam et al., 2013]. In the current

study pure BC, BC/ch, BC/sd nanoparticles were investigated through FTIR to see the successful impregnation of nanoparticles into the BC matrix.

Fig. 4-3 shows the FTIR spectra of pure BC, BC/ch, BC/sd nanoparticles. The FT-IR Represents the spectrum of the BC membrane impregnated with chitosan solution. even in although bacterial cellulose membrane only impregnated with chitosan, and some variations visible in the spectrum of the composite material. In several papers mentioned that for the compound Interference of materials and / or absorption transformation. The peaks are possible [17 Ostadhossein]. Absorption band At 3200-3500 cm - 1 turned to 3034-3491.6 cm – 1 The boundary has become, indicating the possibility of overlap The span of hydrogen is limited –NH2. Featured bands at 2987.1 cm - 1 for BC typical for CH extension, turning to 2899 cm - 1. In cellulose containing chitosan, and vibrations of the amino groups in 1560.3 cm - 1 feature chitosan is visible. The peaks at 1.650 cm - 1 and 1560.3 cm - 1, which correspond to the structure of chitosan, is also present in the compound spectrum. These new bands are noted at 1650 cm – 1. The 1560.3 cm-1 is attributed to Amid-I, Amidi- The second row, which is found in the particles of separation. Two more characteristic peaks appeared at 1,427 cm−1 and 1,371 cm−1 which demonstrate the symmetric deformation and bending vibration of CH group, respectively. It is assumed that the chemical modification of bacterial cellulose consists in introducing the glucosamine and N-acetyl glucosamine units into the cellulose chain [18]. The BC/sd which Sodium alginate and its distinctive peaks. From this figure one can observe that the absorption ranges are about 1610 cm, 1416 cm -1 -1 and 1306 cm are attributed to the expansion vibrations of asymmetric and symmetric bands of carboxylic anions, respectively. The peak at 3430 cm corresponds to hydroxyl expansion vibrations. FTIR investigation reveals the films obtained that there is a possible chemical reaction between Bio-polymers.

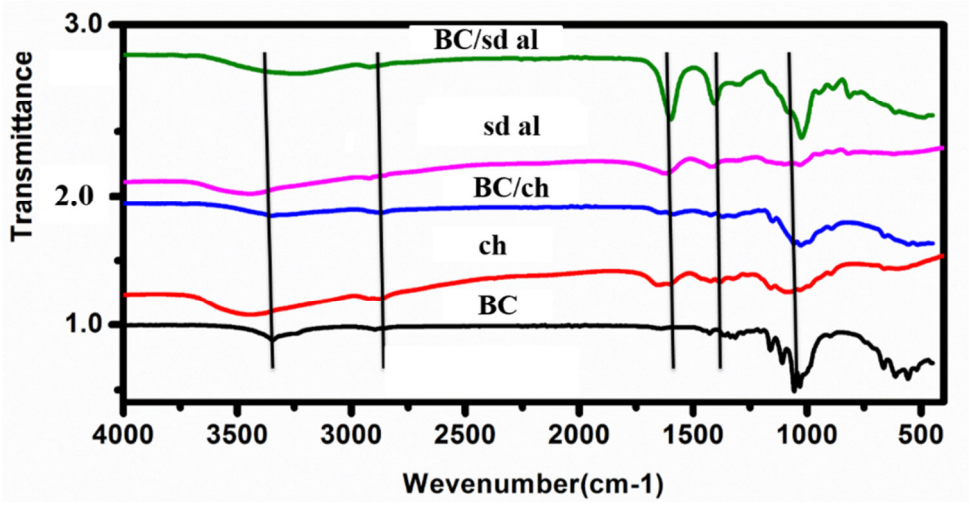

Fig. 4-3. FTIR spectrum of pure BC, chitosan, BC/ch, sodium alginate and BC/sd al nanoparticles.

3.3. Swelling analysis of BC, BC-ch and BC-sd al membranes

The swelling behavior of the compound materials in the distilled water was studied through the weight and the result of swelling for BC, BC-Ch and BC-S-Alg membranes were obtained for BC the degree of swelling was 64.8%, BC/ch 57.6%, BC/sd al 62.8 % . Thus, as demonstrated by the SEM images, all the scaffolds were highly porous, which allowed for high water uptake suggesting their ability to efficiently absorb wound exudate or nutrients for effective wound dressing or tissue engineering applications, respectively [19].

3.4. Antibacterial properties of BC, BC/ch and BC/sd al membranes

The chitosan and sodium Alginate BC / Ch membrane disc has been placed on the agar surface plate is fixed on microorganisms. After applying the composite membrane, The plates are incubated at 37 ° C for 24 ° h. Clear areas inhibit the growth of the target The microorganisms were then measured (Figure 1). [20]. as the results showed the contrasting effect of the inhibitory cellulose membranes when soaked in different concentrations of chitosan toward bacteria *E Coli* & *staphylococcus aureus*. As the results showed the effectiveness of good inhibitory membranes cellulose soaked in concentrations (1.5, 2) % chitosan towards all types of pathogenic bacteria. In 0.5% chitosan is used as modified membranes by sodium Alginate but did not have any effect toward these bacteria. Sodium alginate does not have inhibitory effects towards *E. Coli* & *Staph aureus*, because sodium alginate does not possess ions associated with the cell wall, unlike with chitosan.

The highest effective inhibition obtained in the concentration of 2.0% to the bacteria *E. coli* & *staph aureus* with diameter of inhibition zone of 21.5 mm and 18 mm respectively. While in 1.5% concentration, the diameter of inhibition zone was 14 mm and 12 mm respectively (Table 1.). While the 1.5% concentration the inhibition

zone was 3mm and 2mm respectively and diameter of inhibition for 0.5% did not show much inhibitory effect toward *E. coli & staph aureus*. These Results were compared with other disks of the antibiotic (Penicillin, Ampicillin, Tetracycline, and Chloramphenicol). Similar data were obtained by other researchers [21, 22]. In (figure 2) of BC/ sd al showed no antibacterial effect in agar diffusion test in any concentration because the sodium alginate does not possess ions associated with the cell wall, As a compare with Manufactured disc Penicillin, Ampicillin, Tetracycline, Chloramphenicol have good inhibition zoon on organism target. As a showed in (Table 2).

Table. 1 Inhibitory effects of cellulose membranes antibiotic disc manufacture toward bacteria *E. coli & staph aureus,* soaked in different concentrations of chitosan

Concentrations chitosan% and antibiotic disc manufacture		Diameter the inhibition zone(mm)	
		E. coli	*Staph aureus*
1	0.5	-	-
2	1	3	2
3	1.5	14	12
4	2	21.5	18
5	**Penicillin**	3	5
6	**Ampicillin**	17	15
7	**Tetracycline**	20	19
8	**Chloramphenicol**	27	29

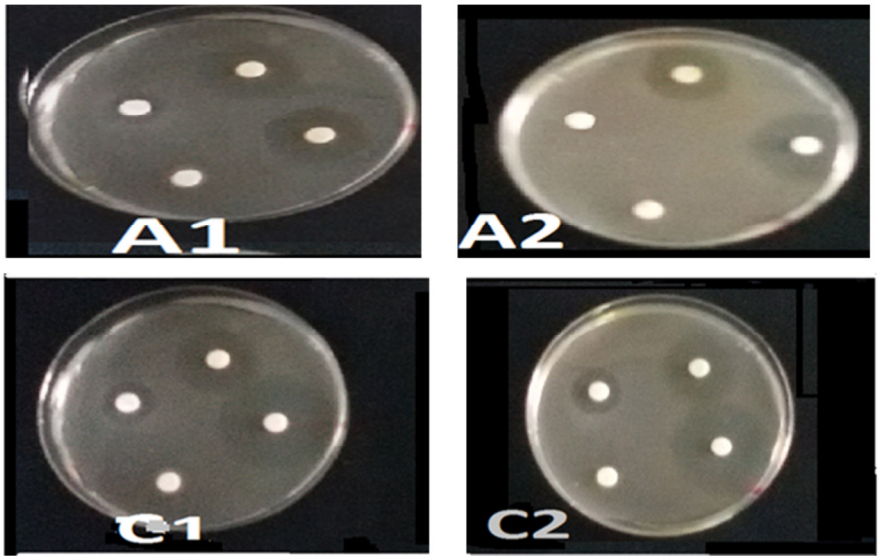

Fig. 1. (A1, A2) Inhibitory affectivity of cellulose membranes soaked in different concentrations of chitosan toward bacteria *E.coli & staph aureus* by antibiotic discs, and (C1 C2) Inhibitory affectivity of Manufactured disc Penicillin, Ampicillin, Tetracycline, Chloramphenicol.

Table 2. Inhibitory effects of cellulose membranes and antibiotic disc manufacture toward bacteria *E. coli& staph aureus,* soaked in different concentrations of sodium alginate

Concentrations sodium Alginate % and antibiotic disc manufacture		Diameter the inhibition zone(mm)	
		E.coli	*staph aureus*
1	0.5	-	-
2	1	-	-
3	1.5	-	-
4	2	-	-
5	**Penicillin**	3	5
6	**Ampicillin**	17	15
7	**Tetracycline**	20	19
8	**Chloramphenicol**	27	29

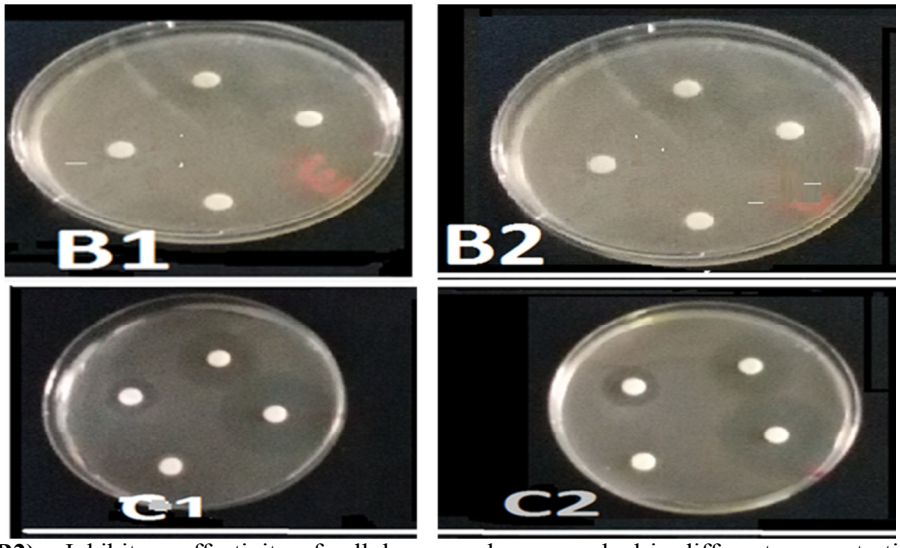

Fig. 2. (B1, B2) Inhibitory effectivity of cellulose membranes soaked in different concentrations of sodium alginate toward bacteria *E.coli & staph aureus* in antibiotic disc **and** , and (C1 C2)Inhibitory affectivity of Manufactured disc Penicillin , Ampicillin, Tetracycline , Chloroamphenicol.

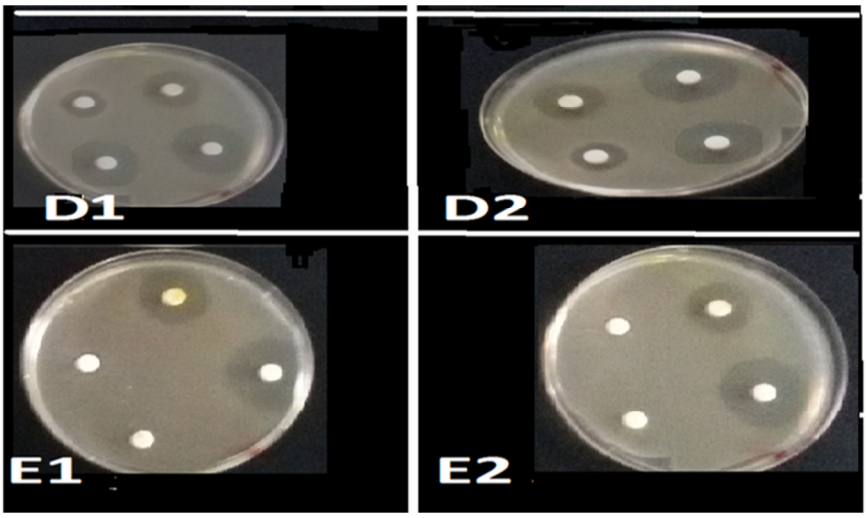

Fig. 3. (D1, D2). The antibiotic discs have chitosan concentrations (1.5 & 2.0) % with manufactured discs **Tetracycline, Chloramphenicol). (E1, E2)** The antibiotic discs of sodium alginate concentrations (1.5 & 2.0) % with manufactured discs (**Tetracycline, Chloramphenicol).**

CONCLUSION

In summary, nanoparticles chitosan and sodium alginate loaded BC bio composites with antibacterial activity were prepared and investigated, through a new strategy to improve the biomedical applications of BC. The composites were synthesized using an economically friendly technique and showed impressive enhancements in the antibacterial properties of nanoparticles. BC nanoparticles synthesized are showing antibacterial activities against both Gram Negative and Gram Positive bacteria but the activities more in Gram (-) than Gram (+) bacteria. BC/chitosan nanoparticles synthesized in situ method are showing inhibitory effect as the degradation of the wall cells in microorganisms and it was present that chitosan derivatives for E .coli bacteria and sodium alginate does not have inhibitory effects towards *E.coli* & *Staph aureus*, because sodium alginate does not possess ions associated with the cell wall, unlike with chitosan.

References

1. M.F. Adegboye, O.O., Babalola, and D.A., Akinpelu, “Issues of resistance of pathogens to antimicrobial agents,” Scientific Research Essays, vol. 7, no. 41, pp. 3468–3478, 2012.
2. N. Luo, K. Varaprasad, G. V., Reddy, A.V. Rajulu, and J. Zhang, “Preparation and characterization of cellulose/curcumin composite films,” Royal Society of Chemistry, vol. 2, no. 22, pp. 8483–8488, 2012.
3. S. Naz, S. Jabeen, F. Manzoor, F. Aslam, and A. Ali, “Antibacterial activity of Curcuma longa varieties

against different strains of bacteria," Pakistan Journal of Botany, vol. 42, no. 1, pp. 455–462, 2010.
4. A. Pielesz, Machnicka, A. and Sarna, E. "Antibacterial activity and scanning electron microscopy (SEM) examination of alginate-based films and wound dressings," Ecological Chemistry and Engineering S, vol. 18, no. 2, pp. 197–210, 2011.
5. M.C. Barros, I.P. Fernandez, V. Pinto, M.J. Ferreira, M. F. Barreiro, and J.S. Amaral, "Chitosan as antimicrobial agent for footwear leather components," in Biodegradable Polymers and Sustainable Polymers, A. Jimnez and G. E. Zairov, Eds., pp. 151–162, Nova Science, 2011.
6. L. Zhang, D. Pornpattananangkul, C.-M. J. Hu, and C.-M. Huang, "Development of nanoparticles for antimicrobial drug delivery," Current Medicinal Chemistry, vol. 17, no. 6, pp. 585–594, 2010.
7. P. R. Chang, R. Jian, J. Yu, and X. Ma, "Fabrication and characterisation of chitosan nanoparticles/plasticised-starch composites," Food Chemistry, vol. 120, no. 3, pp. 736–740, 2010.
8. M. R. de Moura, F. A. Aouada, R. J. Avena- Bustillos, T. H. McHugh, J. M. Krochta, and L. H. C. Mattoso, "Improved barrier and mechanical properties of novel hydroxypropyl methylcellulose edible films with chitosan/tripolyphosphate nanoparticles," Journal of Food Engineering, vol. 92, no. 4, pp. 448–453, 2009.
9. D.R. Solway, M. Consalter, D.J. Levinson, *Wounds: a Compendium of Clinical Research and Practice* 22 (1) 17–19, 2010.
10. Shi, Z., Gao, X., Ullah, M.W., et al. Electro conductive natural polymer-based hydrogels. Biomaterials, 111: 40-54, 2016.
11. R.M. Silverstein, G.C. Bassler, and T.C. Morril, Spectrometric Identification of Organic Compounds, John Willy and Sons, New York, NY, USA, 4th edition, 1981.
12. Luan LQ, Ha VT, Nagasawa N, Kume T, Yoshii F, Nakanishi TM Biological effect of irradiated chitosan on plants in vitro. Biotechnol. Appl. Biochem., 41: 49-57, 2005.
13. A.O.A.C. Official Methods of Analysis 16th ed. Association of Official Analytical Chemists International Arligton, Virginia, U.S.A, 2008.
14. Tomoda Y, Umemura K, Adachi T. Promotion of barley root elongation under hypoxic conditions by alginate lyase-lysate (A.L.L.). Bios. Biotechnol. Biochem., 58: 203-203. 1994.
15. Weihua ,T.; Shiru , J.; Yuanyuan, J. and Hongjiang, Y.. The influence of fermentation conditions and post-treatment methods on porosity of bacterial cellulose membrane., World J Microbiol Biotechnol ., 26:125-131, 2010.
16. Ul-Islam, M., Khattak, W. A., Kang, M., Kim, S. M., Khan, T., & Park, J. K. Effect of post-synthetic processing conditions on structural variations and applications of bacterial cellulose. *Cellulose*, 20, 253–263, 2013.
17. F. Ostadhossein, N. Mahmoudi, G. Morales- Cid, E. Tamjid, F.J. Navas-Martos, B. Soriano- Cuadrado, J.M. López Paniza, A. Simchi,. Development of Chitosan/Bacterial Cellulose Composite Films Containing Nanodiamonds as a Potential Flexible Platform for Wound Dressing. *Materials* 8 6401–6418, 2015. DOI: 10.3390/ ma8095309.
18. Akturk, O., Tezcaner, A., Bilgili, H., et al., Evaluation of sericin/collagen membranes as prospective wound dressing biomaterial. Journal of Bioscience and Bioengineering, 112, (3), 279-28, 2011.
19. J.M. Swenson, J.A. Hindler, L.R. Peterson. Special tests for detecting antibacterial resistance. In: Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH, eds. Manual of Clinical Microbiology. Washington DC: American Society for Microbiology, 1356–1367, 1995.
20. M.T. Postek, W.C. Lin, C.C. Lien, H.J. Yeh, C.M. Yu, S.H. Hsu. Bacterial cellulose and bacterial cellulose-chitosan membranes for wound dressing applications *Carbohydrate. Polymer.* 94, 603–611, 2013.
21. I.S. Savitskaya, A.S. Kistaubayeva, I.E. Digel, D.H. Shokatayev. Physicochemical and Antibacterial Properties of Composite Films Based on Bacterial Cellulose and Chitosan for Wound Dressing Materials. *Carbohydrate. Polymer.* 78, 169–174. 2009.