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# Antibacterial activity of cellulose/chitosan green composite for wound healing

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K E Y W O R D S	A B S T R A C T				
Cellulose	Cellulosic antibacterial fibers have great importance in the manufacturing				
Hemp	of healthcare products. In this research work, antibacterial hemp and bamboo fibers were selected for manufacturing two green composite for				
Bamboo	wound healing. The chitosan was selected to get gel formation and behave				
Green	as carrier in the manufacturing of cellulosic/chitosan green composite. Moreover, a water-soluble levofloxacin was included to give a release of				
Composite	antibacterial properties. The hemp and bamboo fibers were cleaned and				
Antibacterial	fiber-to-fiber separation was done with a manual comber machine. The				
Wound Healing	chitosan was solubilized using acetic acid and stirred for 12 hours to get a completely clear and viscous gel. Afterward, powder ground hemp and				
Infection	bamboo fibers were separately poured into two separate beakers. Two different types of composites (hemp/chitosan) and Bamboo/chitosan were transferred into petri dishes and dried in the oven at 40 <sup>o</sup> C for 24 hours. The samples were loaded with a levofloxacin antimicrobial agent. Both composites (hemp/chitosan, and bamboo/chitosan) were characterized for surface morphology, structural changes and antimicrobial testing against gram-positive and gram-negative bacteria. It was analyzed that without levofloxacin both cellulose/chitosan composites resisted bacteria growth and did not show inhibition zone, but samples loaded with levofloxacin displayed good antibacterial activity and showed excellent zone of inhibition against gram-positive and gram-negative bacteria. Futhermore, FTIR evidenced that there was no formation of cross linking composites, but composites were held together due to chitosan and fibers gel formation.				

### 1. Introduction

Cellulosic fibers are mainly divided into natural cellulosic [1] and regenerated cellulosic fibers [2] Nevertheless, natural cellulosic fibers [3] are more classified as seed fibers [4], bast fibers or plant fibers [5, © Mehran University of Engineering and Technology 2023

6] and woody fibers obtained from the wood pulp of trees like bamboo fibers [7]. Bast fiber is soft plant fiber collected from the stem of the plant. The most common types of bast fibers are flax, ramie, kenaf, jute, and hemp [8, 9]. These fibers are the most valuable raw materials<sup>[10]</sup> for fabrics preparations <sup>[7, 11]</sup>. Hemp fiber is one of the most extensively produced fibers with high cellulosic content. Among all the natural fibers, hemp fiber is the most widely used fiber as reinforced in composite because they are water permeable [12], antibacterial[13], UV resistant [14], non-irritant, and antistatic [15]. Among natural fibers, hemp fiber has higher tensile strength, flexibility, and fineness. Owing to these properties, hemp fibers are mainly used in high-quality textiles, composite materials, yarns, ropes[16], paper[17], and burlap[18]. Hemp has around 70-74% cellulose, 15-20% hemicelluloses, 0.8% pectin, 3.5-5.7% lignin, and low quantities of fat and wax [19]. The bamboo fiber is naturally found in bamboo trees [20]. The trees extracted bamboo fibers are the center of attraction and are commonly used in green composites. The bamboo fibers are antibacterial and can be used in medical applications to prepare antibacterial green composite for sensitive skin[21]. These fibers are soft, ultraviolet-protective, and moisture-absorben [22]. In recent years, innovative fabrics with antibacterial qualities have been created in response to the everincreasing need for cleanliness and hygiene textiles [23]. There are currently a slew of antimicrobial textile products on the market [24]. One of the fastest-growing segments of the textile business is antimicrobial textile [25]. Antibacterial textiles can also be utilized in a variety of medical applications[26], including wound healing, bandaging, and skin infection [27]. Excessive usage of antibiotics causes antimicrobial resistance effects to many bacteria strains [28, 29]. As a result, new natural antimicrobial medicines are urgently needed to replace current synthetic antibiotics in oral suspensions and powders [30].

Chitosan, N-halamine, quaternary ammonium compounds, and metal salts are some of the most popular antibacterial agents used in textiles. It can be employed in a variety of applications as solutions, gels, films, and fibers [31]. A good antibacterial product should be environmentally friendly [32]. Natural plants with antibacterial characteristics have received a lot of interest in recent years. Both the plant and its extract have the ability to inhibit the growth of certain bacteria, so they might be utilized as a new generation of antibiotics [33]. The plant-based antibiotics are environmentally friendly and non-toxic to human[34]. Hemp fiber-based products, such as powders, hemp textiles, and extracts, can be employed as the latest form of natural antibacterial material [35] Secondly, the physical application of antibiotics is a far better choice to reduce the infection of the wound without any side effects on the body. The work has been done on different © Mehran University of Engineering and Technology 2023

composites, such as silk/chitosan [36] or cyclodextrin [37] based composites. Many biomedical textile materials have been utilized for biomedical devices such as hernia mesh devices, stents [38, 39] and composites [40, 41].

However, in this research work, for the first time, we will prepare cellulose/chitosan green composites for wound healing. Two composites of different ratios with chitosan will be prepared. In the first composite, we will have antibacterial hemp and chitosan, while in the second composite, bamboo fibers will be mixed with chitosan. Levofloxacin will be incorporated in both composites for antibacterial release properties, as shown in Fig. 1. The detailed characterization and antibacterial activity will be analyzed to determine the inhibition zone of the two composites.

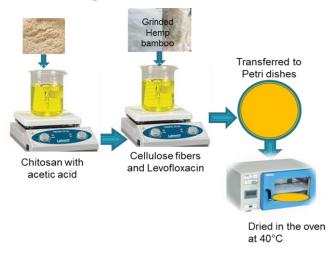


Fig. 1. Design of experiment for antibacterial green composites

### 2. Materials and Methods

The materials used in this study for the development of a composite scaffold are hemp fibers, chitosan (100-150 KDA), Bamboo fibers, acetic acid, levofloxacin (LVFX) HCL, and deionized water.

#### 2.1 Preparation of Composites

The composite scaffolds for wound dressing were prepared by using two different cellulosic fibers (hemp and bamboo) with chitosan, and levofloxacin HCL was loaded into the prepared samples. The hemp and bamboo fibers were first separated into individual fibers, then these fibers were oven dried at 45°C for 24 hours to remove the moisture content of the cellulosic fibers. Oven dried fibers were ground into powder form.

Chitosan was stirred in deionized water containing acetic acid for its solubility and gel formation. The chitosan was stirred in a water solution at room temperature for 12 hours. The cellulosic (hemp and bamboo) fibers are combed and ground to powder form. Afterward, the cellulosic fibers were mixed with chitosan and stirred for another 2 hours to get a homogeneous composite. A composite of chitosan with hemp and bamboo fibers was prepared separately. All parameters, like dwell time and temperature, were kept similar for both types of composites. The ratio of chitosan to cellulose (hemp and bamboo) fibers was set at 2:1, 1:1, 1:2 and 1:4 for hemp and bamboo fibers, respectively. The total weight for each sample was maintained at 3 grams. All solutions were transferred to petri dishes measuring 10 cm, and the samples were oven-dried at room temperature for 24 hours. However, for each ratio, three samples were prepared to get the final average values.

## Levofloxacin Loading:

The solution of 0.125% (500 ml) of levofloxacin HCL was prepared in a jar, and all samples were soaked for 12 hours. Levofloxacin drug was loaded for the release of antibiotics to get the inhibition zone of antibacterially loaded samples. For each category of cellulose fibers (hemp and bamboo), four samples of 2:1, 1:1, 1:2, and 1:4 were soaked in the levofloxacin HCL solution. A total of 4 samples (for hemp and bamboo) without levofloxacin and 4 samples with levofloxacin were prepared. The average weight increase was measured using the formula  $=V_I-V_2/V_2 \times 100$ . Where V<sub>1</sub> was the weight of the sample after loading levofloxacin and V<sub>2</sub> was the weight of the samples before levofloxacin loading.

## 2.2 Characterization

# SEM and EDX:

Cellulose composite samples were coated with gold, and then scanning electron microscopy (SEM) images of cellulose/cotton composite samples were taken at different pixel values (JEOL model JSM 6490). Furthermore, a unit of dispersive X-ray spectroscopy (EDX) was attached to test the element analysis of the cellulose composite.

# FTIR

Cellulose-based green composites were tested for structural analysis. The spectrum peaks for the cellulose composite were taken using Fourier transform infrared. FTIR model no. Nicolet IS10 FT-IR made in the USA was set to analyze the synthesized cellulose composite at a range of 1000 to 4000 cm<sup>2</sup> in ATR mode.

# **Antibacterial Activity**

The agar diffusion technique of testing is one of the officially utilized procedures in the majority of clinical laboratories for antimicrobial susceptibility testing [42, 43]. This approach is practically easy and wellstandardized [44]. A qualitative analysis method of agar antibacterial was utilized to analyze the inhibition zone of levofloxacin-loaded and non-loaded composite samples. A 400µL of bacteria suspension was spread on the Petri dish with the help of a pipette, and then a cotton swatch was used to spread the suspension of bacteria evenly in Petri dishes. Samples of hemp (1 cm circle) and bamboo (1.5 cm<sup>2</sup>) green composite were placed on the petri dishes. All samples were tested against two types of bacteria Staphylococcus aureus (gram-positive) and Escherichia coli (E. coli) (gram-negative). All petri dishes loaded with samples of bacteria suspension were put in the oven for 24 hours to confirm the antibacterial activity.

The inhibition zone was measured by the formula Z = X-Y/Y, where Z is the inhibition zone and X is the inhibition zone of antibacterial activity around the sample. Y is the original sample size placed before antibacterial activity. An average of three samples was made standard for recording the final average inhibition zone.

# 3. Results and Discussion

# 3.1 Preparation of Composites

The synthesis of cellulose with chitosan is shown in Fig. 2.

A total of 16 samples were characterized. Four samples of hemp (1-4) and bamboo (1-4) were without the loading of levofloxacin. However, in both types of cellulosic composites (hemp/chitosan and bamboo/chitosan), four samples (5-8) were loaded with levofloxacin, as shown in below Table 1. The initial total combined weights for chitosan and cellulose fibers were kep at 3 grams. However, the ratio of cellulosic fibers to chitosan was varied. It can be observed (Table 1) that the maximum ratio of chitosan samples showed reduced final weight with both types (bamboo and hemp) cellulosic fibers. Secondly, the weight increase for adding levofloxacin was very minor. The reduction in weight in the case of increasing chitosan may be due to the fiber moisture content. During the preparation of composites, it was also observed that increasing chitosan was increasing the stiffness feel of the bulk, and increasing the fiber content ratio was increasing the bulkiness of the composites. Adding more cellulosic fibers increased the softness and fluffy feel.

#### Table 1

Composition of composites

Sampl	C/H	C/B	LVF	Average weight (g)	
e Codes	ratio	ratio	X (g)	H/C	B/C
1	2:1	2:1	0	2.84±0.1	2.82 ±0.220
2	1:1	1:1	0	2.85±0.1	$2.84 \pm 0.121$
3	1:2	1:2	0	2.86±0.2	$2.84 \pm 0.162$
4	1:4	1:4	0	$2.87 \pm 0.15$	$2.86 \pm 0.191$
5	2:1	2:1	0.5	$2.87 \pm 0.15$	$2.85 \pm 0.230$
6	1:1	1:1	0.5	2.88±0.11	$2.86 \pm 0.190$
7	1:2	1:2	0.5	$2.88 \pm 0.23$	$2.87 \pm 0.111$
8	1:4	1:4	0.5	2.90±0.01	2.87 ±0.031

Note: C=Chitosan, H=Hemp, B=Bamboo.

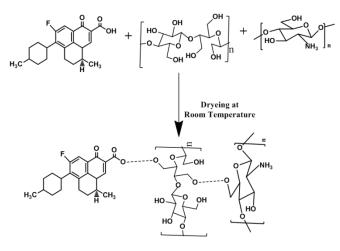


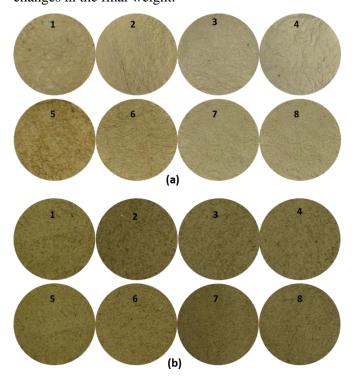
Fig. 2. Synthesis of cellulose and chitosan green composite

#### 3.2 Surface Morphology

Photos of bamboo/chitosan composites without levofloxacin (1-4) and with levofloxacin (5-8) are shown in Fig. 3 (a). It seems that by reducing the chitosan amount in samples 1-4, the original whiteness of bamboo fiber increased. Therefore, the surface of sample 4 was noticed as slightly bulky and white in comparison to sample 1. In other words, the color of the levofloxacin (5-8) loaded samples turned slightly too vellow, which indicates that levofloxacin was successfully added to the bamboo/chitosan composites. Moreover, It can be observed that the surface structure of samples (1-4) has cracks and pores. However, after levofloxacin loading, cracks in samples (5-8) are filled with levofloxacin HCl, and composites show swelling structure in comparison to unloaded levofloxacin.

As shown in Fig. 3 (b) Hemp/chitosan photos (1-4) have similar types of pores and cracks as shown in Fig. 3 (a) but samples 5-8 shown reduced cracks and pores are filled. This could be due to the loading of © Mehran University of Engineering and Technology 2023

levofloxacin HCL. Moreover, samples of Fig. 3(b) displayed dark brown color in comparison of light brown colors of Fig. 3 (a). This could be the reason that the original colors of hemp fibers were slightly brown and in the result sample (b) the shown darker surface colors than bamboo (a). Similarly, the swollen structure was also observed after levofloxacin addition. Overall, these fibers showed different colors as well as slight changes in the final weight.



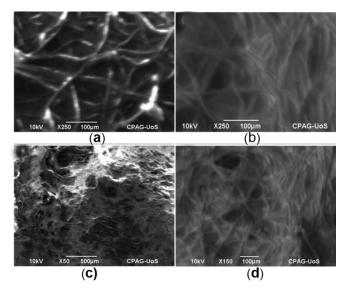
**Fig. 3.** (a) Bamboo/chitosan samples (1-4) and bamboo/chitosan/levofloxacin samples (5-8)

(b) Hemp/chitosan samples (1-4) and Hemp/chitosan/levofloxacin samples (5-8)

#### SEM

Scanning electron microscopy images are shown in Fig. 4. It can be noticed in the Fig. 4 (a) that fibers surface are clear but these fibers entangled with each other. However, in the Fig. 4 (c) all pores of composites are filled with gummy substances which could be levofloxacin adhered on the surface of hemp/chitosan green composites. Similarly Fig. 4 (b) shows a hemp/ chitosan image without levofloxacin loading while Fig. 4 (d) is a slightly swelled structure in comparison of Fig. 4(b).

Most important is that the bamboo/chitosan structure is Fig. 4 (b) (d) is swelled and white in color comparison to hemp/chitosan color. This could be result of witness and different surface lustre of two different composites.



**Fig. 4.** SEM images (a) (c) hemp/chitosan without and with levofloxacin HCL (b) and (d) bamboo fibers without levofloxacin and with levofloxacin.

# 3.3 Characterization

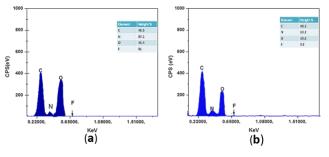
### **EDX Analysis:**

Fig. 5 shows the element analysis of the cellulose green composite. In Fig. 5 (a) Hemp/chitosan/ levofloxacin Carbon element, Nitrogen element, and Oxygen element are prominent at 0.26 KeV, 0.38 KeV, and 0.54 KeV respectively. Moreover, a small peak for levofloxacin is displayed at 0.68 KeV. This could be reason of showing loading of levofloxacin HCL.

Furthermore, Weight percentage for each atom is given in the Fig. 5(a). The carbon atom shares 46.5% of the total share, whereas, Oxygen is the second dominant atom with 45.4 % but the Nitrogen atom and fluorine displayed 7.1% and 1% respectively.

Nevertheless, Fig. 5 (b) Bamboo/chitosan /levofloxacin also shows similar elements at similar positions of KeV but different amount percentage of each element. The carbon atom shows 49.1%, similarly, the Nitrogen atom shows 10.2% but oxygen displayed the second highest amount of percentage 39.8 while the fluorine atom shows only 0.9%.

It can be observed that both composites displayed similar KeV but different percentages of atoms. This is due to the reason that only hemp and bamboo fibers are different in the two composites but both are cellulose in nature and both fibers shown almost similar trend of the EDX spectrum. The element amount of both composites is different due to reason that one bamboo/chitosan composite may contains different percentage of cellulose than hemp/chitosan composites.



**Fig. 5.** EDX spectra (a) Hemp/chitosan and levofloxacin (b) Bamboo/chitosan and levofloxacin.

#### **FTIR Analysis:**

6 shows FTIR spectra of composites Fig. hemp/chitosan/levofloxacin loaded and bamboo/chitosan/ with levofloxacin. Overall, 4 samples were tested to get FTIR spectra peaks. Sample 1 hemp/chitosan composite sample 2 was with levofloxacin. Whereas, sample 3 and sample 4 are of the bamboo/chitosan with and without levofloxacin HCL respectively. All samples were characterized at wavelength of 500cm<sup>-1</sup> to 4000 cm<sup>-1</sup>. FTIR peaks observed at 3446 cm<sup>-1</sup> were due to the reason of OH group. The peaks at 2901 cm<sup>-1</sup> were appeared due to the C-H stretch. Similarly, the peaks of chitosan were prominent at 1634 cm<sup>-1</sup> (C-C) and 1582cm<sup>-1</sup> (C-C). The peaks C-H stretch were also observed at 1435cm<sup>-1</sup>. The stretch of OH group was also displayed at 870 cm<sup>-1</sup> wavelength, in the finger print region.

It can be observed that FTIR peaks are similar for all samples. The reason could be that levofloxacin was not reacted but only trapped in the chitosan and cellulose viscous gel. Therefore, no bonding observed for composite formation. Secondly, hem/chitosan and bamboo/chitosan composites displayed similar peaks due to the reason that both composites contain similar functional groups because of the similar cellulosic nature of the composites.

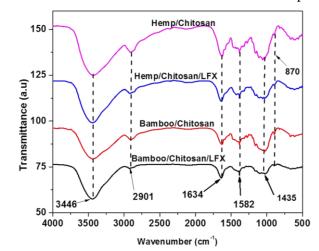
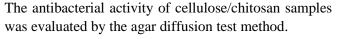
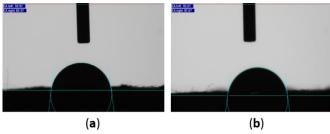


Fig. 6. FTIR spectra of cellulose green composites.

Contact Angle: The contact angle of cellulose and chitosan samples were was performed by the sessile drop method. the contact angles of the two composites were evaluated separately. As shown in Fig. 7 the average contact angle of hemp/chitosan ( $85^{\circ}\pm3$ ) 7(a) was greater than the average contact angle  $(82^\circ \pm 4)$  7(b) of bamboo/chitosan composites. The reason of this could be the difference in the absorbency of two cellulosic (hemp and bamboo) fibers. The regenerated bamboo fibers have greater absorbency than hemp fibers. This may affect the absorbency of the endproduct composites. Thus, hemp chitosan composites were slightly hydrophobic in nature in comparison to bamboo and chitosan composites. However, the overall difference was insignificant as both cellulose fibers were mixed with the same ratio of chitosan.

### **Antibacterial Activity:**





**Fig. 7.** Contact angle (a) hemp and chitosan composite (b) bamboo and chitosan composite.

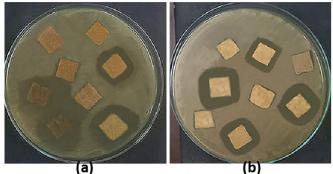
All samples were tested against gram-positive Staphylococcus aureus (S.A) and gram-negative Escherichia coli (E coli) bacteria. As shown in Fig. 8 (a) samples 1-4 showed slightly antibacterial activity and didn't show a visible inhibition zone. This could be the reason that these samples were not loaded with levofloxacin and their release is not good but chitosan resisted bacteria and slightly cleared samples. Nevertheless, Fig. 8 (a) samples 5-8 displayed a good zone of inhibition and loaded levofloxacin cleared composite samples very well. Sample 5 showed an average inhibition zone of 6 mm while sample 6 displayed a slightly greater zone of inhibition of 7.5 mm. Samples 7 and 8 showed excellent average zone of inhibition 7.8 mm and 7.78 mm respectively.

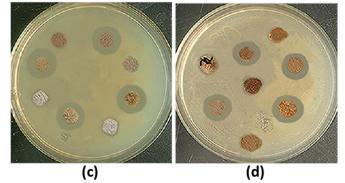
Fig. 8 (b) shows the antibacterial activity of bamboo/chitosan samples against E. coli. The same trend of the zone of inhibition was also observed as samples 1-4 displayed a limited zone of inhibition but samples loaded with levofloxacin showed excellent zone of inhibition. Samples 5 and 6 displayed an average

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inhibition zone of 6.1 mm and 7.2 mm while samples 7 and 8 shown increased zone of inhibition which was 7.45 mm and 7.67 mm respectively.

Fig. 8(c) shows the antibacterial activity of hemp/chitosan composites against S.A. The samples without loading of levofloxacin 1-4 again displayed a very small inhibition zone but samples loaded with levofloxacin HCL exhibited better antibacterial activity. Samples 5-8 demonstrated zone of inhibition 5.1mm, 5.40 mm, 5.41mm and 6mm respectively. The samples with more ratio of chitosan again displayed less inhibition zone and samples with more cellulose displayed better zone of inhibition.





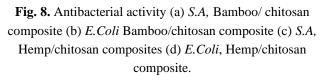


Fig. 8(d) displays the antibacterial activity of hemp/chitosan against S.A bacteria. Once again the samples without levofloxacin (1-4) demonstrated little antibacterial activity and just cleared the samples. Nevertheless, samples 5-8 displayed reasonable antibacterial activity and showing average zone of inhibition of 4.4 mm, 4.6 mm, 4.62 mm and 5.3 mm respectively. It was observed that the ratio of chitosan to cellulose was an important factor. As the cellulose portion, increased, the zone of inhibition was also increased. Secondly in the bamboo/chitosan composites E.Coli displayed a greater inhibition zone. Moreover, levofloxacin HCL loaded samples shown very good inhibition zone antobacterial activity.

In previous, different composites were prepared and their antibacterial activity have been disused. Suhail et al., reported antibacterial properties of silk/chitosan green composite for wound dressing. The author mentioned that as the amount of chitosan increased the inhibition zone decreased [45]. We observed results in same trend as the amount of chitosan decreased the inhibition zone increased. Moreover, Khan, B., et al., mentioned the antibacterial properties of cyclodextrin based scaffolds for infection prevention [37]. However, the drug carrier and antibacterial agent for such research work were different. The work was more focused in long term release of antibacterial drugs. Furthermore, Mehrabani et al: cross linked silver nano particles with chitin/silk composite. The release of cross-linked silver nano particles is not easier. which limits their drug release as well as antibacterial properties[46]. Thus, in our work we have incorporated soluble drug levofloxacin as antibacterial agent. The chitosan with natural antibacterial cellulose fibers were used as carrier. Furthermore, FTIR results proved that there is no additional peak for levofloxacin soluble drug but EDX results displayed peak of levofloxacin. Therefore, a composite with soluble drug without cross-linked may be more suitable to release antibacterial agent for long term antibacterial activity.

## 4. Conclusion

The green composites of cellulose/chitosan loaded with levofloxacin were successfully synthesized. The antibacterial cellulose/chitosan composites were incorporated with soluble antibacterial drugs showing excellent results of antibacterial activity. The selected cellulosic fibers for composites were commonly used natural antibacterial materials. The antibacterial inhibition zone increased with an increasing amount of cellulose fibers. In both types of cellulose/chitosan composites increasing fiber quantities also increased the final weight of composites. The composites without levofloxacin shown insignificant antibacterial properties which could be due to the reason that chitosan and cellulose didn't release any drug.

The infected wounds are difficult to cure. Thus, levofloxacin added natural antibacterial composites could be a useful product for the wound healing to prevent skin infection. The levofloxacin HCL could be released for 24 hours to heal up a wound and reduce the need for oral antibacterial suspension which may have lateral side effects.

# 5. Declarations

All authors declared that there is no conflict of interest.

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