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# Preparation of biocompatible chitosan nanoparticles loaded with Aloe vera extract for use as a novel drug delivery mechanism to improve the antibacterial characteristics of cellulose-based fabrics

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**Abstract**

The primary goal of this study was to develop nanotechnology-based controlled drug delivery devices. As a nano carrier for Aloe vera extract, chitosan nanoparticles (CSNPs) were used. The ionic gelation method was used to make CSNPs from chitosan solution using tripolyphosphate (TPP). The generated high-performance CSNPs were then loaded with Aloe vera extract to create Aloe vera-laden chitosan nanoparticle nanocomposites. The nanocomposite is then employed as a superb antibacterial material with the least amount of toxicity. To impart antibacterial activity without cytotoxicity, cotton (100%) and viscose (100%) samples were treated with varied doses of this compound. The treated fabrics with chitosan nanoparticles and their nanocomposite with various concentrations prevented the growth of both Gram-positive and Gram-negative bacteria, according to the findings. The embedding of chitosan nanoparticles into fabrics and their bioactive material loaded were revealed using Fourier Transform Infrared spectroscopy (FTIR) and Scanning Electron Microscope (SEM) research. Finally, the cytotoxicity of CSNPs and Aloe vera extract loaded CSNPs nanocomposite was assessed using a cell viability assay, confirming that the produced nanocomposite is non-toxic and tissue compatible, just like CS.

**Keywords**: Chitosan nanoparticles, drug delivery, cotton, viscose fabrics, Aloe vera, antibacterial activity

## 1. Introduction

Chitosan is a cationic polymer made up of units of -1, 4 glucose amine and -1, 4-N-acetyl glucose amine. It is biocompatible, biodegradable, and non-toxic, making it ideal for biomedical uses such as protein delivery, medication delivery, and wound healing. [1-10]. Chitosan nanoparticles (CSNPs) have a huge surface area, a high zeta potential, and are very active. CSNPs are utilised as poly load agents in the administration of medications and have a wide range of applications in biomedical sectors. [11-14], vaccines [15-17], and genes [18, 19]. Chitosan nanoparticles (CSNPs) have a large surface area, a high zeta potential, and a high activity level. CSNPs are utilised as poly load agents in the administration of pharmaceuticals and have a variety of biomedical uses. [20]. Chitosan nanoparticles are a drug carrier having the benefit of gradual and controlled drug release, which enhances drug solubility and stability, as well as efficacy and toxicity. [14, 21-23]. Emulsion cross-linking, emulsion droplet coalescence, precipitation, ionotropic gelation, reverse micelle, template polymerization, and molecular self-assembly are some of the ways used to make chitosan nanoparticles. [24]. Particle size, thermal and chemical stability, and end product stability are all elements to consider when choosing a preparation process. [25].

Aloe vera is a medicinal plant whose peel (leaf) and gel contain numerous vitamins, polysaccharides, proteins, phenolic compounds, lignin, saponins, sterols, flavonoids, enzymes, and organic acids, which have been shown in several studies to play an important role in the reduction of nanoparticle cytotoxicity. [26].

For generations, the Aloe vera plant has been known and used for its health, beauty, medical, and skincare benefits. The term Aloe vera comes from the Arabic word "Alloeh," which means "shining bitter substance," and "vera," which means "truth" in Latin. Aloe vera was regarded as the universal panacea by Greek scientists 2000 years ago. Aloe was known as "the herb of immortality" by the Egyptians. The Aloe vera plant is now employed in dermatology for a variety of uses. For millennia, aloe vera has been utilised for therapeutic purposes in a variety of cultures: Greece, Egypt, India,

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Mexico, Japan, and China are just a few of the countries that have been mentioned. [27].

It was utilised by Egyptian princesses Nefertiti and Cleopatra as part of their daily beauty routines. It was used to cure soldiers' wounds by Alexander the Great and Christopher Columbus. John Goodyew's translation of Dioscorides' medical book De Materia Medica in A.D. 1655 was the earliest English mention to Aloe vera. [28, 29].

Aloe vera was utilised as a laxative in the United States by the early 1800s, but it wasn't until the mid-1930s that it was successfully employed to treat chronic and severe radiation dermatitis. Vitamins, enzymes, minerals, carbohydrates, lignin, saponins, salicylic acids, and amino acids are among the 75 potentially active elements in Aloe vera. [30-33].

Many applications have been employed to improve the functionalization of textiles in order to impart antibacterial activity, such as hygiene and health products, as well as barrier materials and infection control [34-40].

To increase the antibacterial activity of both cotton and viscose fabrics, chitosan nanoparticles were produced and loaded with Aloe vera extract. FT-IR, transmission electron microscope (TEM), and scanning electron microscope (SEM) were used to characterise CSNPs made by ionic gelation (SEM). Finally, these nanocomposites were employed to provide antibacterial activity to fabrics. These fabrics were characterised using FTIR, TGA, and SEM. A zone of inhibition approach was used to assess the antibacterial activity of various textiles. Finally, an assay for nanocomposite cytotoxicity testing was investigated to ensure that it is non-toxic and suitable for biomedical applications.

## 2. Materials and Methods

### 2.1 Materials

Chitosan (Alfa Aesar Company, Medium molecular weight, viscosity 1860 cps, degree of deacetylation 79.0 percent), and Penta sodium tripolyphosphate were the chemicals employed (TPP). All other chemicals used are analytical grade and were used without further purification. Sodium hydroxide (Modern Lab chemicals, Egypt), methyl alcohol, ethyl alcohol, and acetic acid (Sisco Research Laboratories, India), and all other chemicals used are analytical grade and were used without further purification. Aloe Vera plant leaves were gathered from a botanical garden in Cairo, Egypt. The fabrics were mill de-sized, scoured, and bleached before being used. EL-Nasr Company for Spinning, Weaving, and Dying, El-Mehalla Elkubra, Egypt, provided 100 percent cotton and 100 percent viscose fabrics. In the laboratory, the cotton fabrics were scoured by washing them at 100°C for 60 minutes in a solution containing 2 g/L Na2CO3 and 1 g/L Egyptol (non-ionic wetting agent based on ethylene oxide condensate). After that, the fabric was washed many times in boiling water, then in cold water, and finally dried at room temperature.

### 2.2 Methods:

#### 2.2.1. Extraction of Aloe Vera

The leaves of the aloe vera plant were chopped and cleaned with distilled water. The gel was removed from them and dried for three hours in an air-dry machine at 50°C. For one week, the dried gel was immersed in methanol. It was then filtered with filter paper. A rotary evaporator equipment was then used to distil the methanol. The extracted Aloe Vera gel solution was then obtained. Before applying the textiles, the aloe vera was dissolved in hot distilled water.

#### 2.2.2 Preparation of chitosan nanoparticles (CSNPs)

The improved ionotropic gelation process was used to make chitosan nanoparticles. [41]. Chitosan was dissolved in 1% (v/v) acetic acid and stirred for 24 hours. The pH was then adjusted to 5.5 using 0.01N NaOH, and tripolyphosphate (TPP) was dissolved separately in deionized water to a final concentration of 0.1 mg/ml. The TPP solution was then added to the chitosan solution dropwise at varying TPP: chitosan ratios at room temperature while being vigorously magnetically stirred. After that, the suspension was ultrasonically treated for 45 minutes.

#### 2.2.3. Preparation of Aloe vera bioactive material loaded chitosan nanoparticles

Different concentrations of antibiotics dissolved in distilled water were added to chitosan nanoparticles solution at the same molar ratio, stirred for 20 minutes, then ultrasonicated for 45 minutes, then stirred for another 20 minutes to obtain a final antibiotics-CSNPs poly load with concentrations ranging from 0.05 g/ml to 0.5 g/ml) [41].

#### 2.2.4. Finishing of cellulose-based fabrics with Aloe vera loaded CSNPs nanocomposite

Using the pad-dry-cure process, this solution was applied to washed and dried pure cotton and viscose fabrics. For all of the treatments, 30 × 30 cm of fabrics were soaked in an extract and chitosan nanoparticles solution including acrylate binder (1%) for 30 minutes before being processed through a padded mangle with 100 percent wet pick-up. The materials were then dried at 80°C for 5 minutes before being thermo-fixed at 140°C for 3 minutes. Finally, samples were washed and dried before being characterised and tested for antibacterial activity.

### 2.3. Testing and analysis

**2.3.1. Nitrogen content**

The ASTM Technique E258-67 method was used to determine the nitrogen content.

$$\text{Nitrogen content} = \text{Nitrogen\% X } 100/14 \text{ (mmole/100 g sample)}$$

**2.3.2. Tensile strength**

The ASTM Test Method D5035 was used to determine the tensile strength (TS) and elongation at break (EL) of treated and untreated fabrics. The tensile tester utilized was a Q-Test 1/5. Three specimens were evaluated in the warp direction for each treated cloth, with the average value being the fabric breaking load (Lb).

**2.3.3. FT-IR spectra**

The samples' FT-IR spectra were taken with an FT-IR spectrophotometer (JASCO FT-IR-6100) using the KBr pellet disc method for transmittance measurements in the range of 4000 - 400 cm-1, with a spectra resolution of 4 cm-1.

**2.3.4. Whiteness and yellowness index**

On Ultra scan Pro, the whiteness index (WI) and yellowness index (YI) were calculated for treated and untreated samples. Hunter's research facility.

**2.3.5. Transmission Electron Microscopy (TEM)**

Using a JEOL-JEM-1200 TEM, the shape and size of chitosan nanoparticles and their loaded antibiotics were examined. Placing a drop of colloidal solution on 400 mesh copper grids coated with an amorphous carbon film and evaporating the solvent in the air at room temperature were used to create specimens for TEM studies. The diameter of 100 nanoparticles observed in multiple arbitrarily chosen places in enlarged microphotographs was used to calculate the average diameter of the manufactured chitosan nanoparticles.

**2.3.6. Scanning Electron Microscopy (SEM)**

A Philips XL30 scanning electron microscope (SEM) with a LaB6 electron gun and a Philips-EDAX/DX4 was used to conduct microscopic research on fabric samples. Surface morphologies were photographed at various magnifications depending on picture clarity, with a 30kV accelerating voltage. To record images, fabric samples were glued using carbon adhesive and metalized with gold vapour deposition.

**2.3.7. Thermogravimetric analysis (TGA)**

Thermogravimetric analysis (TGA) was performed using the SDT Q600 V20.9 Build 20 instrument at temperatures ranging from 25 °C to 600 °C in an inert nitrogen environment with a heating rate of 10 °C min-1.

**2.4. Evaluation of Antibacterial Activity in vitro:**

**2.4.1. Materials**

*E. coli;* ATCC 11229 (Gram negative) and *S. aureus;* ATCC 6538 were the two bacteria strains used (Gram positive). Because they are the most common bacteria in wound infections, these bacterial strains were chosen as test cells. For antibacterial testing, fresh inoculants were prepared on nutritional broth for 24 hours at 37°C.

**2.4.2. Test methods**

To ensure consistency, all antibacterial activity tests were performed in triplicate. Using the colony counting method, the antibacterial activity of the completed fabrics against *S. aureus* and *E. coli* was assessed. [42] 0.5 g peptone and 0.3 g beef extract were mixed in 100 ml water to make a liquid culture. 1 cm diameter mixed film samples were sliced and placed in 10 ml of liquid culture, which was then injected with 10 l of microbe culture. At 37°C, all samples were incubated for 24 hours. 100 l of solution was extracted from each incubated sample, diluted, and spread on an agar plate. The colonies grown on all plates were counted after they had been cultured for 24 hours.

The percentage bacterial reduction was determined as follows:

$$Reduction\ in\ CFU(colony\ forming\ units)\% = \frac{C-A}{C}\ x100$$

Where CFU/ml is CFU/ml at zero contact time and A is CFU/ml after contact (end test).

**2.5. Determination of chitosan nanoparticles and their treated fabrics cytotoxicity on cells (MTT protocol)**

To create a full monolayer sheet, the 96 well tissue culture plate was inoculated with 1 X 105 cells/ml (100 ul/well) and incubated at 37°C for 24 hours. After forming a confluent sheet of cells, growth material was decanted from 96 well microtiter plates, and the cell monolayer was washed twice with wash media. In RPMI medium with 2% serum, two-fold dilutions of the tested material were prepared (maintenance medium). Three wells were left as controls, receiving only maintenance medium, and 0.1 ml of each dilution was tested in different wells. The plate was incubated and analyzed at 37°C. Any physical symptoms of toxicity, such as partial or total loss of the monolayer, rounding, shrinkage, or cell granulation, were examined in the cells. MTT solution (5 mg/ml in PBS) was prepared (BIO BASIC CANADA INC). Each well received a 20ul MTT solution. To completely mix the MTT into the media, place on a shaking table at 150rpm for 5 minutes. To allow the MTT to be metabolized, incubate for 1-5 hours at 37°C and 5% CO2. Remove the media from the equation. (If required, dry plate on paper towels to remove residue.) In 200ul DMSO, resuspend formazan (MTT metabolic product). To completely mix the formazan into the solvent, place on a shaking table at 150 rpm for 5 minutes. At 560nm, read the optical density and subtract the background at 620nm. The optical

density should be proportional to the number of cells. The MTT procedure, as published by Mosmann 1983, was used to evaluate cell viability with minimal changes. [43].

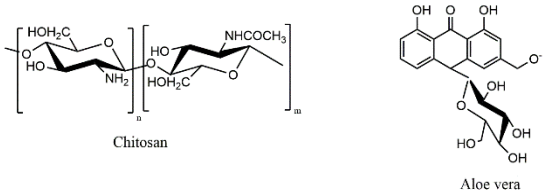
$$Precentage\ cytotoxicity = (1 - (Av(x) / (Av(NC))) \times 100$$

Where Av: average, X: absorbance of a sample well measured at 560 nm, and NC: absorbance of negative control measured at 620.

## 3. Result and discussion

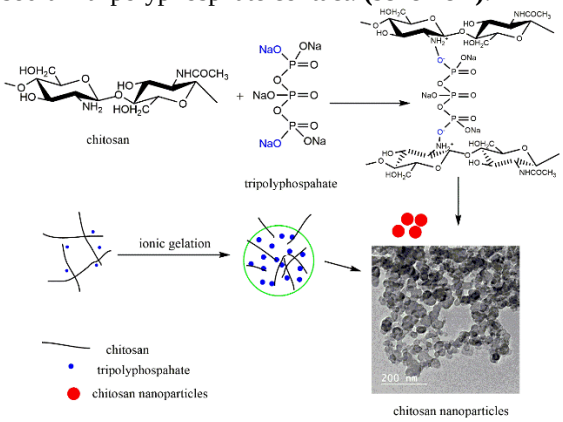
### 3.1. Preparation of chitosan nanoparticles and Aloe vera extract loaded CSNPs nanocomposite

Chitosan has many -$NH_2$ groups in its backbone that are changed to $NH_3^+$ in acidic media, allowing it to be physically and chemically cross-linked to form nanoparticles. [44, 45]. Physical cross-linking provides numerous advantages over chemical cross-linking, including the avoidance of harmful ingredients and decreased side effects, as well as improved biocompatibility. [25, 46, 47]. Complexes of chitosan positive charges and multivalent ions negative charges, such as sodium tripolyphosphate, form physical cross-linking (TPP) [48], citrate, and sulfate [49-51].




**Scheme 1.** Chemical structure of chitosan, and aloe vera

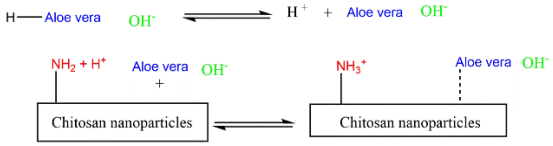
As indicated in our previous paper, chitosan nanoparticles were made by ionic gelation of chitosan and sodium tripolyphosphate at ambient temperature. [41]. Ionic gelation happens when chitosan and sodium tripolyphosphate are mixed together and the amino groups in chitosan and sodium tripolyphosphate contact. **(scheme 2)**.




**Scheme 2.** Ionic gelation and the production of chitosan nanoparticles: chemical structure of the chitosan-tripolyphosphate crosslinking complex

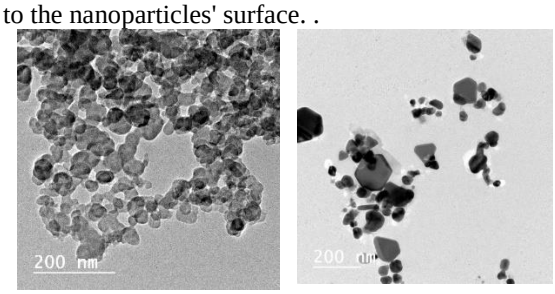
The produced chitosan nanoparticles (CSNPs) were then employed as a poly load for Aloe vera bioactive material to boost its biological activity without causing cytotoxicity, allowing it to be used in a variety of surgeries and targeted receptors across the human body. Finally, these nanocomposites are applied to cellulose-based fabrics (cotton and viscose) to create safe bandages for local infections and surgery. [52-54].

Chitosan nanoparticles have the same functional groups as chitosan but have the added benefit of having a higher surface area due to their nanophase structure, which allows them to have a greater number of amino groups on their surface. Furthermore, as illustrated in Scheme 3, Aloe vera bioactive substance can be adsorbed over chitosan nanoparticles. H-aloe vera-OH is the simplest way to represent aloe vera. As a result, they can be adsorbed onto chitosan nanoparticles via protonation and a chemical reaction (scheme 3).




**Scheme 3.** Proposed adsorption mechanism of aloe vera on chitosan nanoparticles

H-aloe vera-OH is the simplest way to represent aloe vera. As a result, they can be adsorbed onto chitosan nanoparticles via protonation and a chemical reaction (scheme 3). [55]. As a result, we assume that Aloe vera bioactive substance is linked to the nanoparticles' surface. .




a b

**Fig. 1.** TEM image of (a) chitosan nanoparticles (b) bioactive Alo vera-chitosan nanoparticles poly load

Figure 2 shows the FI-IR spectra of chitosan and chitosan nanoparticles. The FT-IR spectra of chitosan (CS) and chitosan nanoparticles are shown in Fig. 2. (CSNPs). Figure 2 shows an absorption peak for NH stretching in amine and amide at 3434 $cm^{-1}$, as well as two peaks for amide I (C=O) and amide II (N–H) at 1637 $cm^{-1}$ and 1564 $cm^{-1}$, respectively, and a peak for vibration of C–$CH_3$ at 1383 $cm^{-1}$ [56]. CSNPs have the same peak

absorption as CS with a shift in their FT-IR spectra. Due to increased hydrogen bonging, the $NH_2$ and OH group stretching vibration band occurs at 3434 $cm^{-1}$ in CS and shifts to 3399 $cm^{-1}$ in CSNPs [55]. In addition, the shoulder peak in CS at 1644 $cm^{-1}$ vanishes, with a new one developing at 1637 $cm^{-1}$ in CSNPs, and the $NH_2$ bending vibration peak shifts from 1602 $cm^{-1}$ in CS to 1534 $cm^{-1}$ in CSNPs [55]. TPP tri polyphosphoric groups bind with the CS ammonium group to generate CSNPs, according to FT-IR.

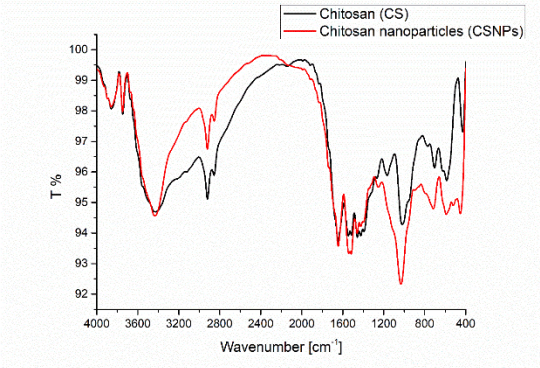
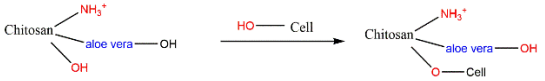



**Fig. 2.** FT-IR spectra of (a) chitosan (CS) and chitosan nanoparticles (CSNPs)

### 3.2. Finishing of cellulose-based fabrics (cotton and viscose) with chitosan (CS), chitosan nanoparticles, and Aloe vera – CSNPs ploy loaded

Cellulose-based fabrics were improved by the invention of a novel coating layer of aloe vera-loaded chitosan nanoparticles, resulting in an aloe vera bioactive delivery system with increased biological activity and minimal cytotoxicity as side effects. As demonstrated in Scheme 4, these nanocomposites can be coated on cellulose-based fabrics (cell-OH), "either cotton or viscose."




Scheme 4. The reaction of aloe vera-loaded chitosan nanoparticles with cellulose-based fabrics

Table 1 illustrates the influence of Aloe-vera-CSNPs Poly load concentration on the treated cotton fabrics' physicochemical and mechanical qualities. Regardless of the Aloe vera finishing agent utilised. Tensile strength of Aloe-vera-CSNP-treated cotton and viscose fabrics Poly load increases as the concentration of Aloe-vera utilised for antibacterial purposes increases, as do complexes between Aloe-vera and CSNPs. As concentration increased, the Elongation at Break dropped. The increased tensile strength of cotton and viscose fabrics coated with Aloe-vera and Aloe-vera -CSNPs Poly load composite was attributed to greater nanoparticle penetration and crosslinking of adjacent fibre molecules by various forces between the amino (-$NH_2$) and hydroxyl (-OH) groups of chitosan and the hydroxyl (-OH) groups of cellulose molecules. [57]. The percentage of CSNPs in the composites increases as the N percent increases, as does the antibacterial activity. The same results were obtained for viscose textiles, but due to the fibre structure and reactive functional groups present in viscose fabrics rather than cotton fibres, TS and all physicochemical parameters of COT treated fabrics were higher than those of viscose treated blended fabrics.

**Table 1.**

Nitrogen content, mechanical properties, and antibacterial activity via reduction percent of cotton fabrics treated with chitosan/chitosan nanoparticles and Aloe vera extract

| CS/CSNPs | Aloe vera | Cotton fabrics finished with chitosan and Aloe-vera extract | | | | | Cotton fabrics finished with chitosan nanoparticles and Aloe-vera extract | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Nitrogen content | Tensile strength | Elongation at break | Reduction percent; % | | Nitrogen content | Tensile strength | Elongation at break | Reduction percent; % | |
| wt.% | wt.% | % | KgF | mm | S. *aureus* | E. *coli* | % | KgF | mm | S. *aureus* | E. *coli* |
| Untreated fabrics | | 0.000 | 68 | 22 | 0.00 | 0.00 | 0.000 | 68 | 22 | 0.00 | 0.00 |
| 0.1 | 0.0 | 0.088 | 68 | 22 | 35.61 | 6.72 | 0.135 | 68 | 22 | 56.77 | 55.54 |
| | 0.1 | 0.109 | 64 | 21 | 47.21 | 22.60 | 0.145 | 68 | 20 | 67.55 | 62.77 |
| | 0.3 | 0.134 | 61 | 19 | 76.99 | 35.05 | 0.147 | 67 | 20 | 89.99 | 83.11 |
| | 0.5 | 0.146 | 59 | 19 | 89.44 | 55.76 | 0.152 | 67 | 19 | 91.00 | 90.01 |
| | 0.7 | 0.171 | 57 | 16 | 90.34 | 56.87 | 0.205 | 66 | 18 | 96.44 | 93.89 |
| 0.3 | 0.0 | 0.118 | 66 | 14 | 56.99 | 19.03 | 0.141 | 69 | 12 | 90.11 | 89.04 |
| | 0.1 | 0.129 | 64 | 13 | 88.79 | 40.98 | 0.145 | 68 | 12 | 94.23 | 92.22 |
| | 0.3 | 0.136 | 64 | 13 | 98.02 | 62.08 | 0.147 | 68 | 10 | 99.03 | 98.99 |
| | 0.5 | 0.159 | 58 | 10 | 99.02 | 76.89 | 0.168 | 67 | 9 | 99.97 | 99.94 |
| | 0.7 | 0.207 | 58 | 8.5 | 99.08 | 78.99 | 0.208 | 66 | 9 | 99.99 | 99.98 |

**Table 2.**

Nitrogen content, mechanical properties, and antibacterial activity via reduction percent of viscose fabrics treated with chitosan/chitosan nanoparticles and Aloe vera extract

| CS/CSNPs | Aloe vera | viscose fabrics finished with chitosan and Aloe-vera extract | | | | | Viscose fabrics finished with chitosan nanoparticles and Aloe-vera extract | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Nitrogen content | Tensile strength | Elongation at break | Reduction percent; % | | Nitrogen content | Tensile strength | Elongation at break | Reduction percent; % | |
| wt.% | wt.% | % | KgF | mm | S. *aureus* | E. *coli* | % | KgF | mm | S. *aureus* | E. *coli* |
| Untreated fabrics | | 0.000 | 59 | 23 | 0.00 | 0.00 | 0.000 | 59 | 23 | 0.00 | 0.00 |
| 0.1 | 0.0 | 0.106 | 56 | 23 | 23.04 | 6.98 | 0.114 | 58 | 19 | 33.91 | 28.87 |
| | 0.1 | 0.121 | 54 | 19 | 35.01 | 15.87 | 0.128 | 58 | 17 | 43.22 | 39.88 |
| | 0.3 | 0.142 | 54 | 18 | 54.19 | 21.01 | 0.145 | 56 | 17 | 59.02 | 50.01 |
| | 0.5 | 0.148 | 53 | 18 | 76.01 | 34.01 | 0.151 | 54 | 16.5 | 67.56 | 59.67 |
| | 0.7 | 0.170 | 49 | 17.5 | 89.91 | 46.09 | 0.171 | 54 | 13 | 90.21 | 56.67 |
| 0.3 | 0.0 | 0.119 | 58 | 19 | 81.01 | 56.09 | 0.124 | 59 | 20 | 88.91 | 87.22 |
| | 0.1 | 0.133 | 57 | 17 | 89.97 | 61.34 | 0.167 | 59 | 20 | 93.77 | 92.78 |
| | 0.3 | 0.152 | 55 | 14.5 | 92.11 | 67.99 | 0.168 | 57 | 19 | 96.91 | 94.77 |
| | 0.5 | 0.243 | 49 | 12 | 94.33 | 75.43 | 0.268 | 56 | 18 | 98.31 | 96.62 |
| | 0.7 | 0.301 | 49 | 9.5 | 97.45 | 78.61 | 0.377 | 55 | 15 | 98.01 | 97.99 |

### 3.3. FTIR Spectroscopy

The use of Aloe-vera loaded with CSNPs to finish cotton and viscose fabrics was confirmed using FT-IR spectra, as illustrated in Figs. 3 and 4. FT-IR spectra of untreated cotton fabrics reveal peaks at 3340, 2900, 1648, 1428, and 1057 $cm^{-1}$, which correspond to OH stretching, -CH stretching, -OH of absorbed water from cellulose, $-CH_2$ symmetric bending, and C–O stretching, respectively. [58]. The combined bands of cotton and Aloe-vera peaks are seen in the FT-IR of treated cotton fabrics with Aloe-vera loaded with CSNPs, with some shifted peaks in location and intensity due to physicochemical processes. In addition, with the red shift, the antibiotics' primary peaks show. .

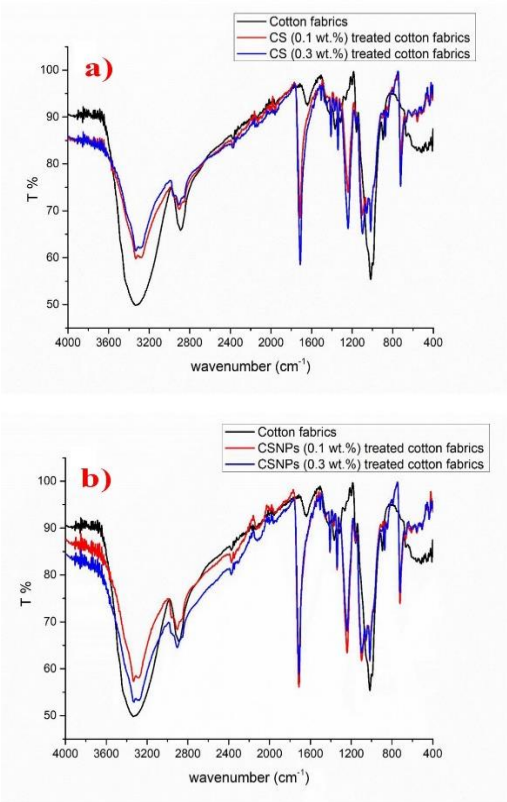



**Fig. 3.** FT-IR Spectra of untreated and treated cotton fabrics with chitosan (CS) and chitosan nanoparticles (CSNPs)

FT-IR spectra of treated and untreated viscose fabrics with Aloe-vera loaded with CSNPs are shown in Fig. 4. The FTIR spectrum of untreated viscose revealed a wide peak at 3320 $cm^{-1}$, which corresponded to cellulose's –OH stretching vibration. C–H asymmetric stretching was reported at 2892 $cm^{-1}$ while C=O had a peak at 1737 $cm^{-1}$. [59]. The combined bands of viscose and Aloe-vera peaks in the FTIR spectrum of viscose loaded with CSNPs, on the other hand, reveal some altered peaks in position and intensity due to specific physicochemical processes. In addition, with the red shift, the antibiotics' primary peaks show. [60, 61].

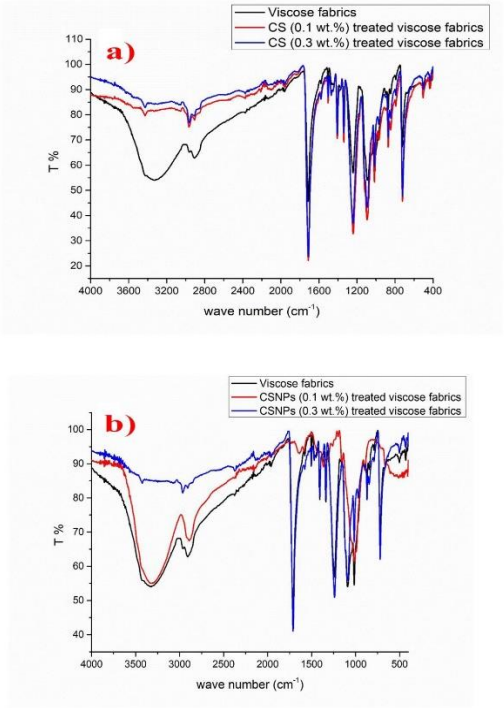



**Fig. 4.** FT-IR Spectra of untreated and treated viscose fabrics with chitosan (CS) and chitosan nanoparticles (CSNPs)

### 3.6. Antibacterial activity:

Antibacterial properties of cotton and viscose fabrics coated with chitosan nanoparticles and chitosan nanoparticles loaded with Aloe vera extract using antibiotic drugs as standard antibacterial material against Gram-positive and Gram-negative bacteria Staphylococcus aureus (*S. aureus*) and Escherichia coli (*E. coli*). Colony counting methods are used to evaluate the antibacterial activity of treated cotton fabric against tested microorganisms, as indicated in Table 2.

All treated fabrics demonstrated antibacterial activity against S. aureus and E. coli, as shown in Table 2. As the concentration of both CS/CSNPs and Aloe vera increases, so does the antibacterial action. Table 2 shows that antibacterial activity data from bacterial decrease percent follows the same pattern. Therefore, when the concentration of CS/CSNPs and Aloe vera rose, the antibacterial activity of cellulose-based fabrics (cotton and viscose) increased. Cotton

fabrics treated with CS/CSNPs and Aloe vera extract have bacterial reduction percent's of above 99.99 percent and 98.01 against *S. aureus*, and 99.98 and 97.99 against *E. coli*, respectively, indicating that treated fabric has better antibacterial action against diverse types of bacteria. Furthermore, its Gram-positive activity outnumbers Gram-negative activity by a little percentage.

Because of the presence of $-NH_2$ groups, chitosan has a strong ability to destabilise the bacterial outer membrane, infiltrate the plasma membrane, and kill bacteria in an acidic environment. [62-64]. The antibacterial activity of CS/CSNPs and Aloe vera against Gram-positive and Gram-negative *S. aureus* and *E. coli* increases, and the bacteriostatic to bactericidal action is converted due to the accumulation of larger amounts on the bacterial membrane. Furthermore, due to the bacterial membrane structure, these materials are more bactericidal against Gram-positive bacteria than Gram-negative bacteria.

Cotton-treated fabrics also have higher antibacterial activity than viscose-treated fabrics due to fibre structure, i.e. cotton has more functional groups capable of reacting with a larger quantity of |antibiotic, and its loaded CSNPs render more antibacterial activity. Because fibre affinity was reduced by the presence of CSNPs, which dimensioned the amount of absorbed antibiotic on the cotton and viscose fabrics, the antibacterial activity of Aloe vera extract-treated cotton and viscose fabrics was higher than loaded CSNPs treated cotton and viscose fabrics. Furthermore, CSNPs are employed as an antibacterial material carrier to restrict and control its release, improve solubility and stability, increase efficacy, and decrease toxicity. [22, 52].

For many Aloe vera-CSNPs, the sequence of antibacterial activity was determined. According to fabrics, cotton > viscose, and biomaterials drug carriers, cotton > viscose. Aloe vera-CS/CSNPs> CS/CSNPs

**Evaluation of cell viability (MTT assay)**

The effect of chitosan (CS), chitosan nanoparticles, and treated cellulose-based textiles loaded with Aloe vera extract on Hep G2 cells (control) was investigated using the MTT cytotoxicity assay. The studies were carried out in six different ways, and the % viability was calculated using the mean average. The goal was to develop antibacterial materials with a low cytotoxicity toward control cells (Hep G2).

Table 3 compares the IC50 values of chitosan nanoparticles (CSNPs), chitosan nanoparticles loaded Aloe vera (CH), and the antibiotic medication gentamycin sulphate (G) on Hep. G2 at various doses. The IC 50 values of both chitosan nanoparticles (CSNPs) and chitosan nanoparticles loaded with Aloe vera compared (CH) revealed that these materials had low toxicity towards the examined cell line, allowing them to be employed safely in biomedical applications.

**Table 3** IC50 values of chitosan nanoparticles (CSNPs), chitosan nanoparticles loaded Aloe vera compared (CH) with antibiotic drug gentamycin sulfate (G)

| ID | Conc. ug/ml | O.D | | | Mean O.D | ST.E | Viability % | Toxicity % | IC50 |
|---|---|---|---|---|---|---|---|---|---|
| HepG2 | ug/ml | 0.356 | 0.341 | 0.368 | 0.355 | 0.00781 | 100 | 0 | ug |
| Gentamycin | 10 | 0.168 | 0.197 | 0.182 | 0.182333 | 0.008373 | 51.36150235 | 48.63849765 | 10.1402 |
| | 5 | 0.359 | 0.35 | 0.354 | 0.354333 | 0.002603 | 99.81220657 | 0.187793427 | |
| | 2.5 | 0.349 | 0.352 | 0.358 | 0.353 | 0.002646 | 99.43661972 | 0.563380282 | |
| | 1.25 | 0.352 | 0.346 | 0.36 | 0.352667 | 0.004055 | 99.342723 | 0.657276995 | |
| | 0.625 | 0.361 | 0.348 | 0.353 | 0.354 | 0.003786 | 99.71830986 | 0.281690141 | |
| | 0.312 | 0.358 | 0.352 | 0.355 | 0.355 | 0.001732 | 100 | 0 | |
| CSNPs NP | 10000 | 0.023 | 0.034 | 0.028 | 0.028333 | 0.00318 | 7.981220657 | 92.01877934 | 3742.35 |
| | 5000 | 0.105 | 0.096 | 0.115 | 0.105333 | 0.005487 | 29.6713615 | 70.3286385 | |
| | 2500 | 0.241 | 0.236 | 0.225 | 0.234 | 0.004726 | 65.91549296 | 34.08450704 | |
| | 1250 | 0.342 | 0.359 | 0.353 | 0.351333 | 0.004978 | 98.96713615 | 1.03286385 | |
| | 625 | 0.359 | 0.351 | 0.357 | 0.355667 | 0.002404 | 100.1877934 | 0 | |
| | 312.5 | 0.363 | 0.354 | 0.352 | 0.356333 | 0.003383 | 100.3755869 | 0 | |
| CSNPs-loaded Aloe vera CH | 10000 | 0.023 | 0.018 | 0.019 | 0.02 | 0.001528 | 5.633802817 | 94.36619718 | 2125.79 |
| | 5000 | 0.042 | 0.063 | 0.05 | 0.051667 | 0.006119 | 14.55399061 | 85.44600939 | |
| | 2500 | 0.121 | 0.108 | 0.135 | 0.121333 | 0.007796 | 34.17840376 | 65.82159624 | |
| | 1250 | 0.295 | 0.304 | 0.316 | 0.305 | 0.006083 | 85.91549296 | 14.08450704 | |
| | 625 | 0.352 | 0.368 | 0.354 | 0.358 | 0.005033 | 100.8450704 | 0 | |
| | 312.5 | 0.352 | 0.36 | 0.357 | 0.356333 | 0.002333 | 100.3755869 | 0 | |

Fig. 5. At varied concentrations, the effect of chitosan nanoparticles (CSNPs), chitosan nanoparticles loaded Aloe vera (CH), and antibacterial medication gentamycin sulphate (G) on Hep. G2 was compared. These findings suggest that chitosan and loaded Aloe vera extract are non-toxic ingredients that can be safely employed in medical fields.

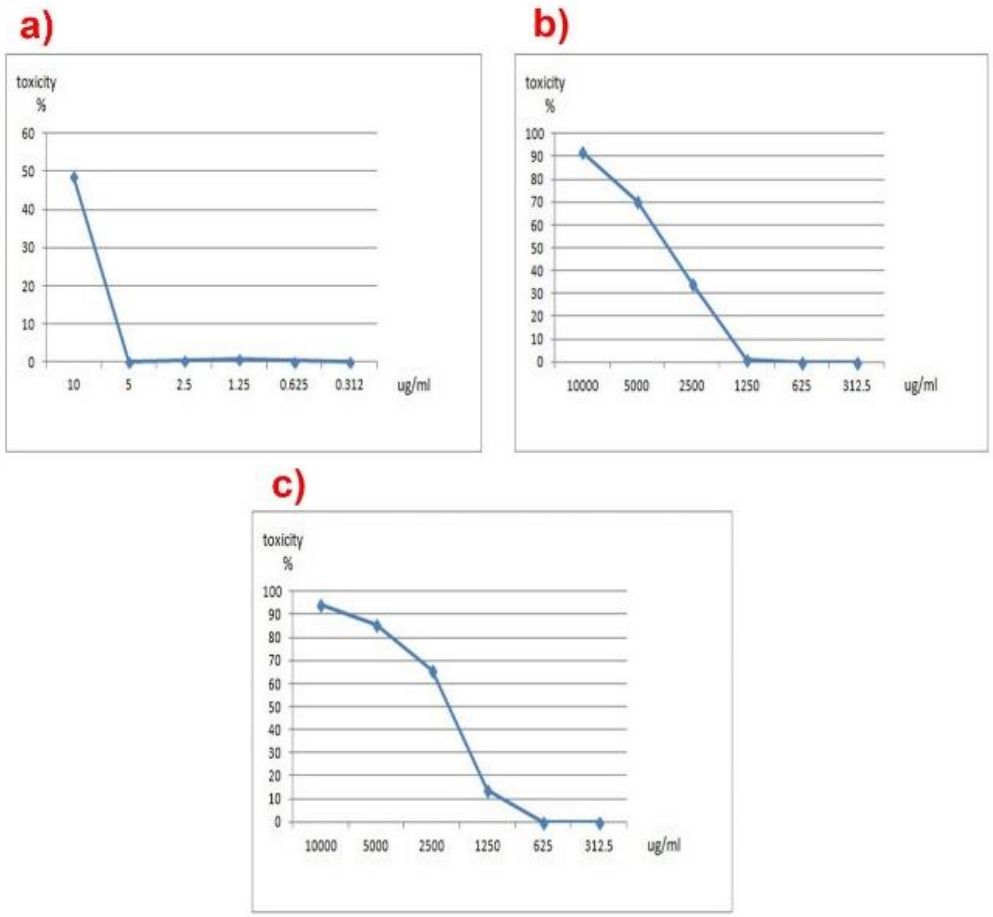



**Fig. 5.** Effect of chitosan nanoparticles (CSNPs), chitosan nanoparticles loaded Aloe vera compared (CH) with antibacterial medication gentamycin sulphate (G) at different concentrations on Hep. G2.

**Cellular uptake**

The treated HepG2 cells were observed using confocal microscopy to assess the uptake of chitosan nanoparticles (CSNPs), chitosan nanoparticles loaded Aloe vera compared (CH) in vitro. Hep. G2 cells were treated with chitosan nanoparticles (CSNPs) or chitosan nanoparticles loaded with Aloe vera (CH) for 72 hours. The uptake of chitosan nanoparticles (CSNPs), chitosan nanoparticles loaded Aloe vera (CH), and a native drug (gentamycin sulphate) in Hep. G2 cells is shown in Fig 6. The effect of different concentrations of chitosan nanoparticles (CSNPs) and chitosan nanoparticles laden Aloe vera compared (CH) on foul aroma intensity is shown in Fig. 6. As shown in the photographs, the encapsulation of both chitosan nanoparticles (CSNPs) and chitosan nanoparticles loaded with Aloe vera (CH) resulted in improved absorption in HepG2 cells. This is due to the medicines' increased ability to reduce HepG2 viability as compared to untreated HepG2 or native drug-treated HepG2.

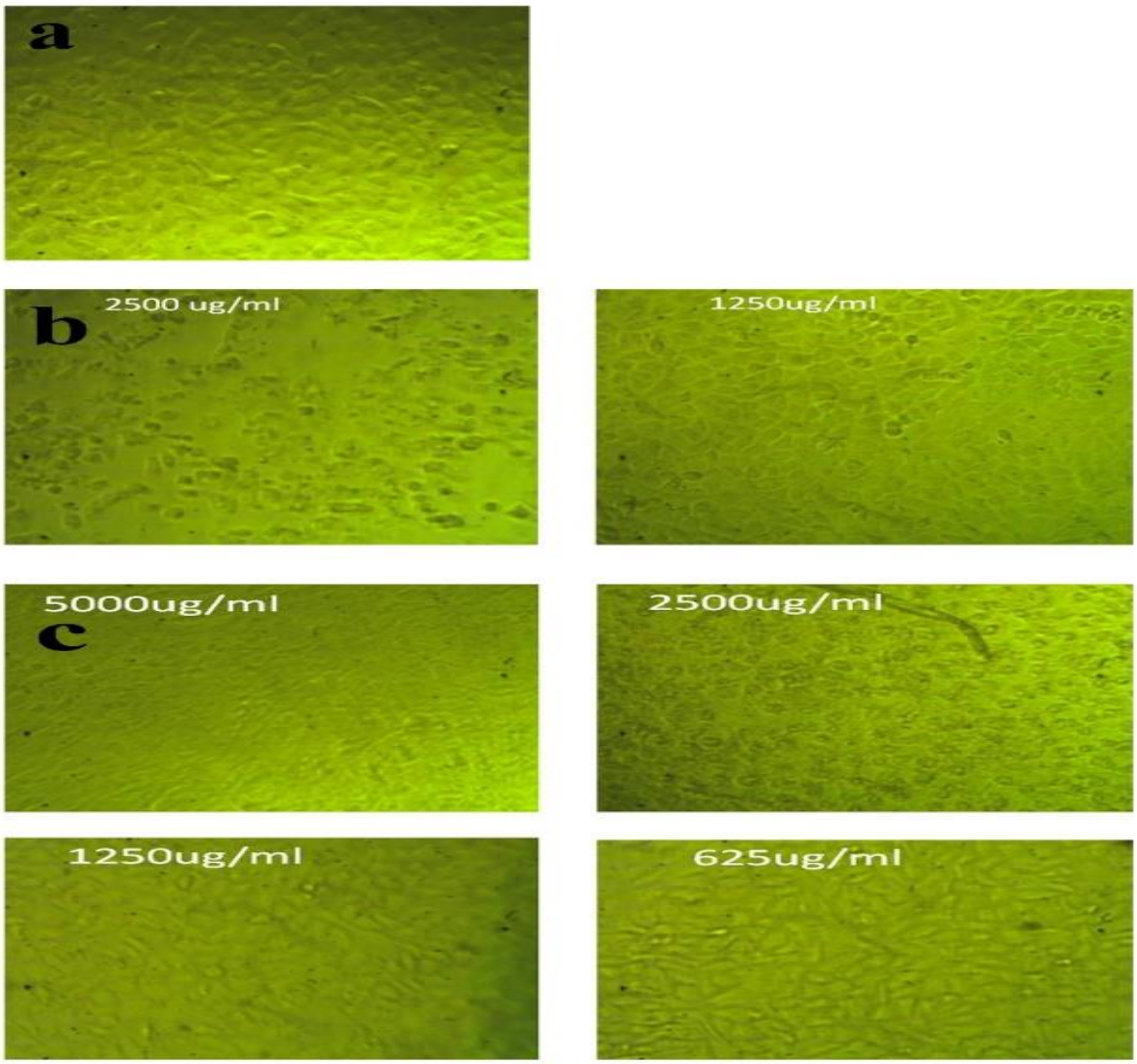



**Fig. 6.** cellular uptake (a) control HepG2 cells, Organism: homo sapiens, human; Tissue: liver; Cell Type: epithelial; Cultural properties: adherent; Disease: hepatocellular carcinoma; (**b)** Effect of G on HepG2 cells at different concentration; (**c)** Effect of NP on HepG2 cells at different concentration; (**d)** Effect of CH on HepG2 cells at different concentration

Table 4 indicates the cytotoxicity of fabrics treated with chitosan nanoparticles and Aloe vera extract (cotton and viscose) and CSNPs and Aloe vera extract. The greater viability of HepG2 cells suggests that cellulose-based materials (cotton or viscose) are not hazardous to them. Furthermore, viscose fibres have a higher cytotoxicity than cotton materials.

**Table 4.** cytotoxicity of chitosan nanoparticles/Aloe vera extract treated fabrics (cotton and viscose) with CSNPs and Aloe vera extract

| ID | O.D | | | Mean O.D | ST.E | Viability % | Toxicity % |
|---|---|---|---|---|---|---|---|
| HepG2 | 0.324 | 0.348 | 0.339 | 0.337 | 0.007 | 100 | 0 |
| Treated cotton | 0.268 | 0.237 | 0.252 | 0.252333 | 0.00895 | 74.87636004 | 25.12363996 |
| Treated viscose | 0.274 | 0.263 | 0.265 | 0.267333 | 0.003383 | 79.32739862 | 20.67260138 |

After 48 hours, HepG2 cells were treated with chitosan nanoparticles loaded with Aloe vera extract on treated cotton fabrics (a) and treated viscose fabrics (b). These findings are also consistent with live cells,

indicating that cotton and viscose prevent cell deformation after 28 hours. As a result, these fabrics can be securely used as bandages.

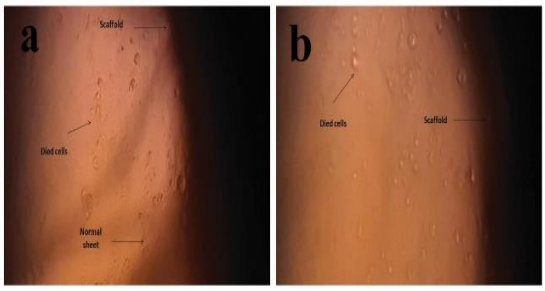



**Fig 7.** Effect of chitosan nanoparticles loaded with Aloe vera extract on HepG2 cells after 48 hours on treated cotton fabrics (a) and treated viscose fabrics (b).

## 4. Conclusion

Using tripolyphosphate, chitosan nanoparticles (CSNPs) were produced and characterised using the ionic gelation process. Several natural bioactive substances were found in aloe vera leaf extract. The loading of Aloe vera extract on CSNPs poly load, resulting in a CSNPs/Aloe vera composite, was employed to increase bioactivity without toxicity, hence improving their biomedical uses. This nanocomposite was applied to cotton and viscose fabrics to give them extraordinary antibacterial properties. The fabrics treated with chitosan nanoparticles, as well as their composites containing varying concentrations of CSNPs and Aloe vera extract, were found to prevent the growth of a variety of bacteria. The increased penetration of nanoparticles and crosslinking of adjacent fibre molecules by various forces between amino (-NH2) and hydroxyl (-OH) groups of chitosan and hydroxyl (-OH) groups of cellulose molecules resulted in improved physicochemical and mechanical properties of cotton and viscose fabrics coated with CSNPs and Aloe vera Poly load nanocomposite. The MTT technique has no cytotoxicity for Aloe vera-CSNPs Poly load. The bactericidal activity results for Aloe vera-CSNPs Poly load were as follows:

cotton > viscose for textiles, and viscose > cotton for cytotoxicity.

To summarise, the discovered formulation might be considered an essential instrument in the fight against melanoma drug resistance, and more research is needed.

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