**In-vitro Evaluation of the Antimicrobial Activities of Chitosan and Chitosan-PVP Linked Nanocomposite Films against Some Multidrug Resistant Bacteria**

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**Abstract**

Many polymeric materials are able to kill multidrug-resistant bacteria without inducing drug resistance or toxic side effects on the patient. A variety of antibacterial polymeric nano systems have exhibited great inhibition of multidrug resistant bacteria, prevent biofilm formation, enhance bacterial recognition and bind capabilities. The present study describes the effect of incorporating between two polymers; chitosan and polyvinyl pyrrolidone with CuO, TiO2, and Ag nanoparticles on some bacterial pathogens. The antimicrobial activity of the prepared polymers was determined by four methods; diffusion method, colony forming unit (CFU), Optical density and anti adhesive method. The plants beetroot was extracted by boiling water to obtain the extract. The results showed that the nanocomposite film was active against different pathogenic bacteria. In Gram negative Pseudomonas aeruginosa was the most sensitive to incorporating film. In Gram positive MRSA was effective with nanocomposite film. The blends of chitosan, PVP and Nanoparticles (CuO, TiO2, and Ag) were good antibacterial ability on gram positive and negative bacteria.

**Keywords:** Chitosan; Polyvinyl pyrrolidone; CuO; TiO2; Ag; Antimicrobial; Pseudomonas aeruginosa

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**Introduction**

Despite the helpful developments in medical and pharmaceutical technology, harmful bacteria, infecting millions of people annually, remain a great concern. Research into new antibiotics is not of interest to many large pharmaceutical companies, due to the facts that it is time consuming, expensive (around one billion USD annually) and risky, not to mention the short commercial life of such drugs (due to resistance acquisition by bacteria). Nonetheless, the rise of resistant pathogens coupled with the significant decrease in the rate of antibacterial-agent approval in recent decades has made the battle with bacterial infections one of the greatest health challenges facing the world. More attention and resources must be devoted to finding smart solutions to this problem that are both inexpensive and effective. Recent "bottom-up" approaches based in nanocomposite could help.

Chitosan, a copolymer of glucosamine and N-acetylglucosamine units linked by1,4-glycosidic linkages and good biodegradability, nontoxicity, biocompatibility and antifungal activity, and its derivatives have been widely used in the fields of medicine, cosmetics, agriculture, biochemical separation systems, tissue engineering and so on [1,2]. The chitosan promotes surface induced thrombosis and blood coagulation and accelerates coagulation in vivo by influencing the activation of platelets. The N-acetyl glucosamine (NAG) present in chitin and chitosan are a major component of dermal tissue which is essential for repair of scar tissues [3]. Chitosan’s inhibitory efficiency against different microorganisms is the subject of considerable debate. In some reports, its antimicrobial activity is stronger against Gram-negative bacteria than Gram-positive [4], while in another study, it is better against Gram-positive bacteria, due to the structure of the outer membrane barrier of Gram-negative bacteria [5]. Still other studies have found there are many articles demonstrated that the chitosan used as antimicrobial agents.

PVP, a synthetic polymer, has good biocompatibility and for many years has been applied as a biomaterial or additive to drug compositions, e.g. as a blood plasma expander [6]. The miscibility of chitosan and PVP in the films has been reported and is considered that carbonyl groups in the pyrrolidone rings of PVP interact with amino and hydroxyl groups present in chitosan by forming hydrogen bonding and produces material of a novel characteristics [7]. Since synthetic polymers are available at a lower price than biopolymer chitosan, substitution of chitosan by these synthetic polymers could reduce the price of chitosan-based films with safe effect on their functionality.

PVP and chitosan are promising for medical application because of ability of film forming structure, bioactivity, biocompatibility and biodegradability [8].

Now, the search for new treatment related to bacterial diseases
had become important because of the emergence of bacterial strains that resistant to antibiotics. Many researchers have suggested the nanocomposite film has been growing interest in medical research especially in antibacterial field. Therefore, in the present study, nanocomposite film investigated to study their anti-microbial properties of nanocomposite film to ensure their activity in vitro in order to discover resources of new pharmaceutical compounds.

Materials and Methods

Chemical Used

Chitosan (medium M.W) is purchased from ACROS ORGANICS Company (Geel, Belgium), Gluteraldehyde (GL), Polyvinyl pyrrolidone (PVP), Titanium oxide nanoparticles (TiO2), Copper Oxide nanoparticles (CuO) and silver nitrate (AgNO3) are purchased from Boss chemical industry C., LTD (Jinan, China).

Extraction of Plant Pigment

Different samples of plants will be extracted using different methods according to [9]. The extraction of the active compounds was carried out using boiling water and separating funnels. Fresh Beetroot (250 g) and red cabbage (40 g) were extracted with 2 L of boiling Distilled water. Similarly, the dried powder of Curcuma (30 g) was extracted with 150 ml ethanol using separating funnel and shaking for 72 hrs. at room temperature. The organic layer was filtered through Whatman filter paper No.1 and kept in small closed vials at low temperature 4°C.

Preparation of the Ag NPs

This solution was fresh prepared by dissolving 0.5 g Silver nitrate in 10 ml dist. water under constant magnetic stirring. Then, 10 ml of beetroot extract solution, A550=1.0, was added to the solution under continuous stirring until Ag NPs were formed. The Ag NPs formation was noticed by changing the solution color from red to brown [1].

Preparation of the TiO2 NPs

TiO2(0.5 g) was dissolved in 2% wt./v glacial acetic acid under stirring in 250 ml beaker for 24 hrs. at room temperature to ensure homogeneous solution. The homogeneous solution is filtrated through Whatman filter paper no. 0.1 and preserved at room temperature until final evaluations. Then 10g of solution transferred immediately into a Petri Dish plate (Dimensions: 90 mm length x 15 mm) (Sabean traders, Chennai, India). Afterward, 1 ml of TiO2 NPs, 1 ml Ag NPs or 0.0025 g of CuO NPs were gradually added (at the final concentration of 10 g and homogenized for 5 min at 12000 rpm (Ultra Turrax T25, IKA, Germany). Another film was prepared after adding 1 ml of both TiO2 NPs, 1 ml Ag NPs and 0.0025 g of CuO NPs. The resultant films were casted on the center of Petri dishes plates and dried at room temperature for 48 hrs. Dried films were gently peeled off and stored at 25°C and relative humidity until final evaluations. Then 10g of solution transferred immediately into a Petri Dish plate (Dimensions: 90 mm length x 15 mm) (Sabean traders, Chennai, India). Afterward, 1 ml of TiO2NPs, 1 ml Ag NPs or 0.0025 g of CuO NPs were gradually added (at the final concentration of 10 g and homogenized for 5 min at 12000 rpm (Ultra Turrax T25, IKA, Germany) and dried at room temperature (25°C) for 24 hrs. The formed cross-linked chitosan films are washed with double distilled water to neutralization and dried at room temperature.

Preparation of Chitosan-Polyvinyl pyrrolidone Nanocomposite Films

Film formation was carried out according to the method described by Archana D, et al. (2013) with some modifications. Chitosan and PVP (1% w/v) were hydrated in an aqueous solution of glacial acetic acid for 10 minutes using a magnetic stirrer at 400 rpm. Chitosan-PVP film was fabricated first with sulfonamide by cross-linked with GL (1% w/v); cross-linked composite film incorporated by beetroot extract. Afterwards, 1 ml of TiO2 NPs, 1 ml Ag NPs or 0.0025 g CuO NPs were gradually added at the final concentration of 10 g and homogenized for 5 min at 12000 rpm (Ultra Turrax T25, IKA, Germany). Another film was prepared after adding 1 ml of both TiO2 NPs, 1 ml Ag NPs and 0.0025 g of CuO NPs. The resultant films were casted on the center of Petri dishes plates and dried at room temperature for 48 hrs. Dried films were gently peeled off and stored at 25°C and relative humidity until final evaluations. Then 10g of solution transferred immediately into a Petri Dish plate (Dimensions: 90 mm length x 15 mm) (Sabean traders, Chennai, India). Afterward, 1 ml of TiO2NPs, 1 ml Ag NPs or 0.0025 g of CuO NPs were gradually added (at the final concentration of 10 g and homogenized for 5 min at 12000 rpm (Ultra Turrax T25, IKA, Germany) and dried at room temperature (25°C) for 24 hrs. The formed cross-linked chitosan films are washed with double distilled water to neutralization and dried at room temperature.

Antimicrobial activity

Tested bacteria: Six bacteria were obtained from King Abdul Aziz Hospital, Jeddah, Saudi Arabia. They were, Pseudomonas aeruginosa, Escherichia coli, Enterococcus faecalis, Streptococcus hemolyticus Staphylococcus aureus and Methicillin-resistant Staphylococcus aureus (MRSA).

Antimicrobial activity of nanocomposite films using diffusion method: Antimicrobial activities of the nanocomposite films were tested against different test microorganisms using agar diffusion method described by [11]. Petri plates were prepared by pouring 20 ml of Muller Hinton agar under sterile conditions. Optimally, within 15 min after adjusting the turbidity of the inoculum suspension, a sterile cotton swab was dipped into the adjusted suspension. The swab should be rotated several times and pressed firmly on the inside wall of the tube above the fluid level. This will remove excess inoculum from the swab. The dried surfaces of agar plates were inoculated by streaking the swab over the entire sterile agar surface. This procedure is repeated by streaking two more times, rotating the plate approximately 60° each time to ensure an even distribution of inoculum.

Using sterile forceps, films of 5 mm diameter put in agar plate. Plates were left for one hour at 4°C and then incubated for 24 hrs. at 37°C. Inhibition zones (including the diameter of disc) were measured.

Measurement of antimicrobial activity on film surfaces using colony forming unit (Contact Method): Surface contact method might be the main antimicrobial mechanism of some advanced materials, the contact method can provide very useful information about the antimicrobial capacity of these materials. In this method, the microorganisms are placed directly onto the material surface and their growth inhibition can be determined after a certain amount of time [12].

Place disk of films in separates wells of a sterile 24-well plate. Pipette 20 µL of the microbial suspension onto each disk surface. After that, aerobically incubate the 24-well plate at 37°C for 1 h. After 1 h of incubation, wash each disk with sterile water and transfer it to 15 mL
pre-sterilized tube containing 5 ml of sterile Phosphate buffer saline (with 800 ml of distilled water: Add 8 g of NaCl, 0.2 g of KCl, 1.44 g Na2HPO4, 0.24 g KH2PO4. Adjust the pH to 7.2 with HCl. Add distilled water to a total volume of 1 liter). Vortex the PBS with disk of film for 1 min at 50 Hz for 5 min to ensure that no viable microorganisms remain adhered to the material surface. Perform decimal serial dilutions of each sonicated culture in sterile microcentrifuge tubes containing MHB and spread 100 µl of the culture on MHA plates and incubate it aerobically at 37°C for 24 h. Count the number of colonies, which are the number of viable microorganisms on each sample and control disk surface. Express this number of viable cells in (CFU/mL). Each disk films were placed on MHA to test the antibacterial surface film.

The number of viable microorganism colonies was counted. The percentage of inhibition of each film was calculated with the following equation:

\[ \text{Mortality} \% = \frac{B - A}{B} \times 100 \]

Where B and A are the mean number of bacteria in the control samples (CFU/sample) and the treated samples after 24 h incubation (CFU/sample), respectively.

Measurement of antimicrobial activity on film surfaces using Optical density: The antibacterial activity on film surfaces were determined using the method reported by [13]. Gram-negative and Gram-positive bacteria had been grown on agar culture medium at 37°C for 12 hrs. The single colony was cultured in Muller Hinton Broth (MHB) at 37°C for 12 hrs. The bacteria were then seeded on 100mL NB to make the bacterium concentration at 10^5CFU/mL and 5mm disk films disinfected by alcohol and UV light were then added. The media were subsequently incubated at 37°C for 24 hrs., and their OD_{600} was determined. The inhibitory rate (%) was calculated by the following equation:

\[ \text{Inhibitory rate} = \frac{OD_{600, 1} - OD_{600, 2}}{OD_{600, 1}} \times 100\% \]

Contact-active and antibacterial properties of chitosan and chitosan-PVP nanocomposite films against S. aureus and MRSA: The tested nanocomposite films for previous experiment were tested using method described by [13], by take these films from suspension and immediately put them in the Muller Hinton Agar medium then incubation at 37°C for 24 hrs.

Statistical Analysis

Statistical analyses were carried out using SPSS version 16 and result was expressed as mean ± standard deviation (Mean ± SD).

Results and Discussion

Beetroot was collected and extracted using boiling water and the obtained extract of Beetroot was used for silver nitrate reduction (Figure 1). The UV spectra of the Beetroot extract and silver nanoemulsion were determined and the maximum absorption were obtained at 550 and 450 nm, respectively (Figures 2 and 3).

Antimicrobial Activity of Chitosan Films by using Colony Forming Unit

Seven different films were prepared from chitosan and PVP Nanoparticles (CuO, TiO₂, and Ag) (Figure 4). Antimicrobial activity of different chitosan-PVP nanoemulsions films was first tested to detect the optimal effect on S. aureus, MRSA and E. coli as model bacteria. Chitosan with beetroot film was the most effective against bacteria with just three colonies.

Figure 1: The prepared silver nanoparticles using beetroot extract (A: before and B: after Ag NPs preparation).

Figure 2: UV-Spectra of diluted Beetroot extract used for preparation on ag NPs.

Figure 3: UV-Spectra of silver nanoemulsions prepared by Beetroot extract.

Figure 4: Antimicrobial activity of chitosan cross link film with: beetroot film, A: control, a: treated film against S.aureus; B: control, b: treated film against MRSA and C: control, c: treated film against E. coli.
Antimicrobial Activity of Chitosan and Chitosan-PVP Nanocomposite Films by Diffusion Method

Antibacterial activity for a chitosan nanocomposite and chitosan-PVP nanocomposite films were determined using well diffusion agar methods. As showed in table 1 and table 2, chitosan with nanoparticles films was great antimicrobial activity against all tested bacteria with inhibition zone from (6.0±0.09 to 16.0±0.1 mm). Whereas, the chitosan-PVP film was showed great antimicrobial effect against some tested bacteria between (6.0±0.02 to 17.0±0.09 mm). On the other hand, no inhibition zone without nanoparticles on tested bacteria for two polymers. In case of Chitosan-PVP-CuO, TiO$_2$, and Ag nanoparticles film, the film showed high antimicrobial effect on all tested bacteria. The diameter of inhibition zone was ranged from 9-17 mm. Similarly, chitosan incorporation with three nanoparticles chitosan incorporation with three nanoparticles was get the same result with inhibition zone from (12.0±0.1 to 16.0±0.1 mm). In the present study, chitosan-PVP with CuO NP was active against *E. coli* and *E. faecalis*. Whereas, the antibacterial activity of chitosan-PVP with TiO$_2$ was against *S. aureus*, *E. faecalis* and *E. coli*. Chitosan-PVP-Ag NP show high activity against *P. aeruginosa* followed by *E. coli* (14 and 13 mm) respectively.

The all tested bacteria were susceptible to the chitosan and chitosan-PVP films just incorporated with one nanoparticle, CuO-TiO$_2$-Ag NPs gives composite films increase the antimicrobial activity as compared to the previous film was showed on *Streptococcus* sp. with inhibition zone 9 mm and high activity against *P. aeruginosa* with inhibition zone (17 mm). Results show antibacterial activity of the film in *S. aureus*, MRSA, *Enterococcus faecalis*, *P. aeruginosa* and *E. coli* (11, 11, 10, 17 and 15 mm) respectively (Figure 5-7).

Data are expressed as mean ± SD (n = 3), ND: Not detected; Film 4: chitosan-sulfa-beetroot- CuO NP; Film 5: chitosan-sulfa-beetroot - TiO$_2$ NP; Film 6: chitosan-sulfa-beetroot-Ag NP; Film 7: chitosan-sulfa-beetroot-(CuO, TiO$_2$, and Ag) NPs.

Data are expressed as mean ±SD (n = 3), ND: Not detected; Film 4: chitosan-sulfa-beetroot- CuO NP; Film 5: chitosan-sulfa-beetroot - TiO$_2$ NP; Film 6: chitosan-sulfa-beetroot-Ag NP; Film 7: chitosan-sulfa-beetroot-(CuO, TiO$_2$, and Ag) NPs.

Antibacterial Durability and Laundering Durability of Chitosan and Chitosan-PVP Nanocomposite Films by Colony Forming Unit

The antibacterial activity of chitosan and chitosan-PVP nanocomposite film was investigated against Gram-negative (*E. coli*, *P. aeruginosa*) and Gram-positive bacteria (*S. aureus*, MRSA, *E. faecalis*, *streptococcus* sp.) with the colony count method (CFU) by using Laundering Durability procedure as shown in figures 8 and 10, and the results were presented with the CFU (Figures 9 and 11). Figures 8 and 9 showed that chitosan and chitosan-PVP loading with nanoparticles films were very effective as antibacterial agents and achieved by the treatment using. Particularly, the incorporation of Nanoparticles into composite films increase the antimicrobial activity as compared to the films just incorporated with one nanoparticle, CuO-TiO$_2$-Ag NPs gives PVP with CuO, TiO$_2$, and Ag nanoparticles film. The lower activity of previous films was showed on *Streptococcus* sp. with inhibition zone 9 mm and high activity against *P. aeruginosa* with inhibition zone (17 mm).

Table 1: Antimicrobial activity of chitosan nanocomposite films by agar-well diffusion method.

<table>
<thead>
<tr>
<th>Tested Bacteria</th>
<th>Diameter of the inhibition zone (mm) ± SD</th>
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<tbody>
<tr>
<td></td>
<td>4</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>12.6±0.3</td>
</tr>
<tr>
<td>MRSA</td>
<td>8.0±0.2</td>
</tr>
<tr>
<td><em>E. faecalis</em></td>
<td>ND</td>
</tr>
<tr>
<td><em>Streptococcus</em></td>
<td>10.0±0.3</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>9.0±0.1</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>ND</td>
</tr>
<tr>
<td>Bacterial index</td>
<td>49.6</td>
</tr>
</tbody>
</table>

Table 2: Antimicrobial activity of chitosan-PVP nanocomposite films by agar-well diffusion method.

<table>
<thead>
<tr>
<th>Tested bacteria</th>
<th>Diameter of the inhibition zone (mm) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Film 4</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>ND</td>
</tr>
<tr>
<td>MRSA</td>
<td>ND</td>
</tr>
<tr>
<td><em>E. faecalis</em></td>
<td>8.0±0.09</td>
</tr>
<tr>
<td><em>Streptococcus</em></td>
<td>ND</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>ND</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>6.0±0.08</td>
</tr>
<tr>
<td>Bacterial index</td>
<td>14</td>
</tr>
</tbody>
</table>

Figure 5: Antibacterial effect of chitosan nanocomposite films against (A) Gram positive and (B) Gram negative bacteria.

Figure 6: Antibacterial effect of chitosan-PVP nanocomposite films against (A) Gram positive and (B) gram-negative bacteria.

Figure 7: (A) Chitosan nanocomposite film 7 (B) the antibacterial activity of the film against *P. aeruginosa*, (C) MRSA, (D) *E.coli* and (E) *S. aureus*.
a 100% kill rate against *Streptococcus* sp. in chitosan film, but film with CuO Np showed lower kill rate against *E. faecalis* as shown in figure 8. Chitosan-PVP nanocomposite films inhibit *P. aeruginosa* and *E. coli* with 100% (Figure 10).

**Antimicrobial Activity of Chitosan and Chitosan-PVP Nanocomposite Films by using Optical Density (OD) 600nm**

Inhibitory effect of chitosan and chitosan–PVP nanocomposite films against microbial strains are shown in table 3. The inhibitory effect was measured based on absorbent of treated bacterial suspension with tested films. Measurement of absorbent Compared by blank bacterial suspension without treated. the values were show good percentage on MRSA, *P. aeruginosa, Streptococcus* sp. and *E. coli*.

The effect of chitosan and chitosan–PVP nanocomposite film was tested by using optical density (OD600 nm) to demonstrate the inhibitory effect of tested nanocomposite films against tested bacteria. As shown in (Table 4) the inhibitory effect was measured based on absorbent of treated bacterial suspension with tested films and Compared by blank bacterial suspension without treated by films. Our results show *P. aeruginosa, E. coli, Streptococcus* sp. and *MRSA* that treated by different

![Figure 8](image1.png)

Figure 8: (A) Chitosan-PVP nanocomposite film 7, (B) the antibacterial activity of the film against *Streptococcus* sp., (C) *P. aeruginosa* and (D) MRSA.

![Figure 9](image2.png)

Figure 9: Antibacterial effect of washing durability of Chitosan nanocomposite films against (A) Gram positive and (B) gram negative bacteria.

![Figure 10](image3.png)

Figure 10: (A) Counts of MRSA and (B) *E. coli* from control, (a) no film is found, (b) chitosan-sulfa-beetroot-CuO NP Film, (c) chitosan-sulfa-beetroot-TiO2 NP Film, (d) chitosan- sulfa-beetroot-Ag NP Film, and (e) chitosan-sulfa-beetroot-(CuO,TiO2 and Ag) NPs Film.

![Figure 11](image4.png)

Figure 11: Antibacterial effect of washing durability of Chitosan-PVP nanocomposite films against (A) Gram positive and (B) Gram negative bacteria.

![Figure 12](image5.png)

Figure 12: Contact active antibacterial activity of chitosan and chitosan-PVP nanocomposite films against MRSA.

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**Table 3:** Antimicrobial activity of chitosan nanocomposite films (A) and chitosan-PVP nanocomposite films (B) by using optical density method.

<table>
<thead>
<tr>
<th></th>
<th>S. aureus</th>
<th>MRSA</th>
<th>E. faecalis</th>
<th>Streptococcus sp.</th>
<th><em>P. aeruginosa</em></th>
<th><em>E. coli</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>56.30%</td>
<td>90.85%</td>
<td>2.84%</td>
<td>97.33%</td>
<td>79.79%</td>
<td>91.38%</td>
</tr>
<tr>
<td>5</td>
<td>55.75%</td>
<td>93.15%</td>
<td>0.00%</td>
<td>94.73%</td>
<td>86.39%</td>
<td>83.23%</td>
</tr>
<tr>
<td>6</td>
<td>31.65%</td>
<td>83.81%</td>
<td>0.00%</td>
<td>98.87%</td>
<td>99.00%</td>
<td>100%</td>
</tr>
<tr>
<td>7</td>
<td>31.96%</td>
<td>93.19%</td>
<td>90.80%</td>
<td>100%</td>
<td>100%</td>
<td>98.94%</td>
</tr>
</tbody>
</table>


**Table 4:** Antimicrobial activity of chitosan-PVP nanocomposite films by using optical density method.

<table>
<thead>
<tr>
<th></th>
<th>S. aureus</th>
<th>MRSA</th>
<th>E. faecalis</th>
<th>Streptococcus sp.</th>
<th><em>P. aeruginosa</em></th>
<th><em>E. coli</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>60.96%</td>
<td>91.00%</td>
<td>71.91%</td>
<td>94.92%</td>
<td>80%</td>
<td>83%</td>
</tr>
<tr>
<td>5</td>
<td>85.00%</td>
<td>65.08%</td>
<td>75.64%</td>
<td>83.67%</td>
<td>70%</td>
<td>82%</td>
</tr>
<tr>
<td>6</td>
<td>78.05%</td>
<td>92.81%</td>
<td>94.35%</td>
<td>100%</td>
<td>100%</td>
<td>99%</td>
</tr>
<tr>
<td>7</td>
<td>95.51%</td>
<td>99.68%</td>
<td>74.31%</td>
<td>99.62%</td>
<td>100%</td>
<td>99%</td>
</tr>
</tbody>
</table>

nanocomposite films were the best bacteria-glessive absorbent when compared by bacteria that no treated with films.

Contact-Active Antibacterial Properties of Chitosan and Chitosan-PVP Nanocomposite Films against Methicillin Resistant *Staphylococcus aureus*

To evaluate antibacterial activities of the surface films against MRSA bacteria. Each film was cut to 6 mm and measurement conducted by contact active method. The NPs can reduce surface attached MRSA on chitosan –PVP films compared with chitosan films. As showed in figure 13, films with Ag NP was the good surface can kill MRSA and show no growth on and around the films. On the other hand, films with CuO and TiO2 NPs can kill MRSA as showed and no growth on the surface of films.

Discussion

In this study, chitosan and chitosan-PVP nanocomposite films were developed with three nanoparticles (CuO, TiO2, and Ag) and tested the abilities as antibacterial agents by four different methods.

Chitosan is a natural polymer with excellent physicochemical properties with higher antibacterial activity due to own positive charge on amino groups (NH+) that can be interaction strongly with bacterial negatively charge cell membrane that allow to disrupt the cell wall of bacteria cause to cell death. Moreover, the addition of Ag NPs to chitosan/ PVP solution, demonstrably increased the antimicrobial activity of the film. Since it is known the nano chitosan showed maximum antibacterial activity against *S. aureus* and *L. monocytogenes* with inhibition zone of 30mm (23μg/ml concentration) and the lowest 23mm with *E. coli* at the same concentration [15].

In diffusion method the inhibition zone was only showed when chitosan and chitosan-PVP incorporation with nanoparticles, which could be due to the stability of polymers and not solubility in media where nanoparticles can diffusion on media. A previous study produced ZnO/Chitosan bio nanocomposites films for packaging poultry meat and showed antibacterial against food borne pathogen *S. aureus* and *E. coli* [16].

By using CFU method, our result shows the mortality range of chitosan nanocomposite films were on the range between 0.0 to 100%. However, blend of chitosan with PVP and nanoparticles were showed excellent active films with mortality range between 60.96 to 100%. Moreover, Valizadeh S, et al. (2019) has showed that the chitosan carboxymethyl cellulose film was active against *P. aeruginosa* and *Listeria monocytogenes* and the colony forming unit were increased from 6.40 to 11.23 and 5.59 to 9.64 log CFU/ml in the blank samples, respectively [10]. Applerot G, et al. (2012) were seen the antibacterial activities of CuO NPs associated to their size so that the small CuO NPs having the higher inhibiting or killing activity than big NPs [17].

By using optical density ODabs our results show *P. aeruginosa* and *Streptococcus* sp. were the bacterium that give highly percentage of inhibitory rate (100%), since it is known bacterial growth creased by ZnO-chitosan nanocomposite film and the antimicrobial activity [13]. Contact active antibacterial activity of nanocomposite films were increased when film incorporation by Ag NPs. He W, et al. (2016) was achieved the same results with Gemini Quaternary Ammonium Salt Polyurethanes films on *S. aureus*.

Conclusion

In this study, novel nanocomposite films based on chitosan and PVP polymers, CuO, TiO2, and Ag NPs were successfully prepared using a simple casting method. Based on our research, it can be concluded that incorporation of three different nanoparticles with two polymers were increased the antibacterial activity of chitosan and chitosan-PVP films against both Gram-positive and Gram-negative bacteria, significantly. Thus, chitosan and chitosan-PVP nanocomposite films could be a promising application in wound healing and food packaging.

References

