ANTIMICROBIAL ACTIVITY OF CHITOSAN AND TANNIC ACID ON COTTON FABRIOUS MATERIALS

Kantima Juntarapun[1] & Chutimon Satirapipathkul[2*]

Chulalongkorn University, Faculty of Engineering,
Department of Chemical Engineering, Chemical Engineering Research Unit for Value Adding of Bioresources,
Bangkok 10330, Thailand
E-mail: kantima.j@student.chula.ac.th[1], Chutimon.s@chula.ac.th[2*]

Abstract: Chitosan and tannic acid were applied in alone and in combination on cotton fibrous materials for the assessment of their antimicrobial activity. The antimicrobial activity of these antimicrobial agents was better in peroxide treated cotton fibrous materials than their untreated materials. Tannic acid showed higher antimicrobial activity than chitosan when applied alone. The application of chitosan and tannic acid combination on peroxide treated cotton materials provides the highest antimicrobial resistance.
Keywords: Antimicrobial, Chitosan, Cotton, tannic acid

1. Introduction

Fabrics made from natural fibers are susceptible to attacks from micro-organisms, because some microbes can thrive under conditions provided by these fabrics. Infestation by microbes can cause cross infections by pathogens and development of odor where the fabric is worn next to the skin [1], especially for fabrics used in hospitals and hotels, which may damage the fabrics and harm the human body. With increasing awareness of the importance of a hygienic health lifestyle, antimicrobial finishing of natural textile products has received much attention in recent years. Several textile finishing techniques for antibacterial purposes have been developed [2-5].

Chitosan is a natural biopolymer that is derived from chitin, a major component of crustacean outer skeletons. It has a combination of many unique properties such as biodegradability, non-toxicity, cationic nature and antimicrobial activity. It is chemically called as beta-(1, 4)-2-(amino) -2-deoxy-Dglycopyranose. Chitosan provides inhibition of many microbes. In aqueous solution at pH < 6.5, its prorogated form (NH_3^+) behaves like a cationic polyelectrolyte. The interaction of this positively charged chitosan with the negatively charged residues at the cell surface of many fungi and bacteria causes extensive cell surface alterations and alters cell wall permeability [6, 7]. Tannins are water-soluble phenolic compounds having a molecular weight between 500 and 3000 Daltons. They can be classified into hydrolysable and condensed tannins. Hydrolysable tannins contain either gallotannins or ellagitannins. Condensed tannins are the polymerized products of flavan-3-ols and flavan-3,4-diols, or a mixture of the two. The polymers, referred to as "flavolans" are popularly called condensed tannins [8]. Tannins have been reported to have various health effects such as antimicrobial, anticarcinogenic and antimutagenic activities [9, 10].

The purpose of this research is to study the antimicrobial activity of cotton fabric treated with these antimicrobial agents in alone and in combination. The relation of the ingredient concentration and their antimicrobial activity on fabric sample was investigated.

2. Materials and Methods

2.1 Antimicrobial Agents

Chitosan with a degree of deacetylation of 95% and a molecular weight of 45,000 was obtained from Seafresh Lab (Bangkok, Thailand). Tannic acid was obtained from Sigma Chemical Company, St. Louis, Missouri.

2.2 Microorganism strains

The microorganism stains used in this study were all purchased from Thailand Institute of Scientific Technology (TIST), Bangkok: E*scherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923).

2.3 Fabric treatment

Plain cotton fabric, have the specification 75 g/m2 weight, 80's count, 78 EPI and 60 PPI, was used for this study.

The cotton fabrics were scoured with 1.0 % by weight of sodium hydroxide to eliminate impurities and washed with de-ionized water [11]. These fabrics were then bleached with hydrogen peroxide of 4.0 % volume and with other auxiliaries as per the standard procedure described in the textbook [12]. The distilled water was used for the above processes. Chitosan was dissolved with 2 % acetic acid solution and tannic acid was freshly prepared by dissolving in deionized water. These solutions were filtered before application. The antimicrobial agent was taken in a bath with material to liquor ratio of 1:100. The sample was entered into the bath and the pH is maintained to 5.0±0.2 with acetic acid solution. The bath temperature was raised to 70 ^{o}C for 30 minutes. After treatment, the bath was cooled to 30 ^{o} C. All samples were taken out and washed with warm water, rinsed with cold water and dried.

2.4 Determination of Add-on (%)

The cotton fabrics were dried in a vacuum oven at 70 o C until a constant weight was reached and oven-dried weight was determined before and after antimicrobial treatment and add-on value was calculated using the following formula.

$$\text{Add-on (\%)} = [(W_2 - W_1)/W1]x100$$

Where W_1: weight of fiber substrate before treatment (g)
W_2: weight of fiber substrate after treatment (g)

2.5 Antimicrobial assay

The antimicrobial activity of antimicrobial agent treated fabric samples was determined agar diffusion standard test method. The nutrient agar medium was prepared by dissolving 3.7 g in 100 ml distilled water and the conical flask was tightly closed by using non absorbent cotton [13]. The petriplates and the medium were sterilized by auto clave at 120 o C, 15 lbs for 20 min and then the plates were dried. The media when still hot, was poured in sterile petriplates (approximately 20 ml), the plates were allowed to cool, so that the agar is solidified. E*scherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923) were then grown overnight at 37 oC, 120 rpm in 10 ml nutrient broth. This broth was used for seeding the bacteria onto the agar plates. These bacteria suspensions (approximately105 cells/ml) were used for seeding the bacteria onto the agar plates. The fabric samples (5.0 mg) each were placed in agar medium. The

plates were incubated at 37 o C for 24 h and were examined for zone of inhibition around the samples. The zone was measured using a zone of inhibition measuring gauge. This test was carried out for about 4 days.

2.6 Morphological observations under scanning electron microscope (SEM)

The surface of the fabric and uniform distribution of polymers in the fabric samples were observed under scanning electron microscope.

3. Result and Discussion

3.1 Add-on of Antimicrobial Ingredient on Fiber Substrates

The amount of added antimicrobial agents on cotton fabrics is given in Table 1. The exhaustion of antimicrobial agents is higher in pretreated fabrics than the untreated fabric. The pretreatment increases wettability, critical surface tension and accessible regions. It also decreases the diffusion barrier of the fabric samples. These factors improve the adhesion and exhaustion of antimicrobial agents into the fibrous structures [14, 15].

Table 1 Add-on (%) of antimicrobial agent on cotton fibrous substrates

Antimicrobial agent (mg/ml)		Peroxide treatment	
Chitosan	Tannic acid	Untreated	Treated
3	-	0.82	2.31
-	6	0.76	1.94
3	6	-	4.26

3.2 Antimicrobial activity of cotton fabric

The higher add-on value in combined antimicrobial agent treatment enhanced their antimicrobial activity than their individual treatment.

The untreated and treated cotton fabrics were taken for antimicrobial effect against *S. aureus* and *E.coli* by agar diffusion test. It can be seen from Table 2. This study was carried out for about four days. The untreated fabric samples showed antimicrobial activity. The antibacterial activity of the untreated fabric shows, the zone of inhibition is 6.3 to 7.5 cm and 6.2 to 6.8 cm on the fourth day against *S. aureus* and *E. coli*. The residual impurities in untreated samples exhibit influence on the growth of microbial strains and also enhance the activity of antimicrobial agent.

All the fabric samples showed a significant inhibitory activity against both bacterial strains. Treated fabrics with tannic acid displayed higher activity against gram-positive *S. aureus* ATCC 25923 (17.4-19.2 mm) and gram-negative *E. coli* ATCC 25922 (14.4-16.3 mm). Treated fabrics with chitosan extracts showed lower activity against gram-positive *S. aureus* ATCC 25923 (12.4-17.4 mm) and gram-negative *E. coli* ATCC 25922 (10.8-13.2 mm). The result demonstrated that the gram positive bacteria like *S. aureus* ATCC 25923 was more susceptible to these antimicrobial agents than gram-negative *E. coli* ATCC 25922 was. On overall consideration, the antimicrobial activities of peroxide treated fabric samples were higher as compared to those of untreated samples in all cases.

The antimicrobial activity of tannic acid treated fabrics is higher than chitosan. Tannic acid has only phenolic groups, which form hydrogen bonding with hydroxyl groups of cellulose polymer in natural fibers and amino/hydroxyl groups of protein polymer. Gallic acid component present in tannic acid helps to promote antimicrobial activity by bonding with amino/hydroxyl groups of protein structure in microbial cellular wall [8]. The lower molecular weight of tannic acid makes it higher exhaustion than chitosan in fibrous structures. The antibacterial activity of tannic acid is slightly better than chitosan [14].

The antimicrobial activity of chitosan differs with respect to fabric samples. The sorption of chitosan on cellulose/protein fibrous structure is due to ionic interaction between negative charges and protonated amino groups of chitosan (NH_3^+), hydrogen bonding and van der Waals' forces. However, its' affinity can be generally regarded as weak. Therefore, its antimicrobial activity is weak when applied alone [15-17].

The strongest antibacterial activity is found in the peroxide treated fabrics samples with chitosan and tannic acid**.** When the antimicrobial ingredients are applied in combination form, the antibacterial activity is better. The interaction between chitosan/tannic acid and the functional groups such as hydroxyl, carboxyl and amino groups present in fibrous substrates make their antibacterial activity higher.

It is also observed that antimicrobial activity of chitosan and tannic acid treated fibrous substrates with pretreatment is better than their untreated samples. Peroxide treatment improves the accessible regions of cotton [18, 19]. These modifications improve swelling of fibrous structure in aqueous state and increase the adhesion and exhaustion of antimicrobial agents [20]. The exhausted antimicrobial agents further form chemical bonding with functional groups present in fibrous substrate. These chemical bonding further increases the fixation of antimicrobials and so improve their activity on fibrous substrates. The charged state of new functional groups present in antimicrobial treated fibrous substrate attacks the cell wall of the bacteria and inhibits the microbial growth in finished fibrous substrates [21].

Table 2 *Evaluation of antibacterial activity of cotton fabric samples*

Antimicrobial agent (mg/ml)		Diameter of inhibition zone (mm) ± S.D.[a]			
Chitosan	Tannic acid	*S. aureus*		*E. coli*	
		Untreated	Peroxide treated	Untreated	Peroxide treated
-	-	6.9±0.6	7.8±0.9	6.5±0.3	7.2±0.4
3	-	12.4±1.2	17.4±1.1	10.8±0.8	13.2±0.9
-	6	17.4±0.9	19.2±0.8	14.4±1.0	16.3±1.2
3	6	20.2±1.4	24.2±1.6	18.6±1.3	21.1±1.2

[a] Mean value of three determinations

3.3 Scanning electron microscope (SEM) analysis

It was clearly observed from the Figure 1 photograph, the chitosan polymer entered into the interior of the structure, which facilitates the antibacterial property of cotton fabric and also improves the absorbency of tannic acid. The polymer was uniformly distributed throughout the samples.

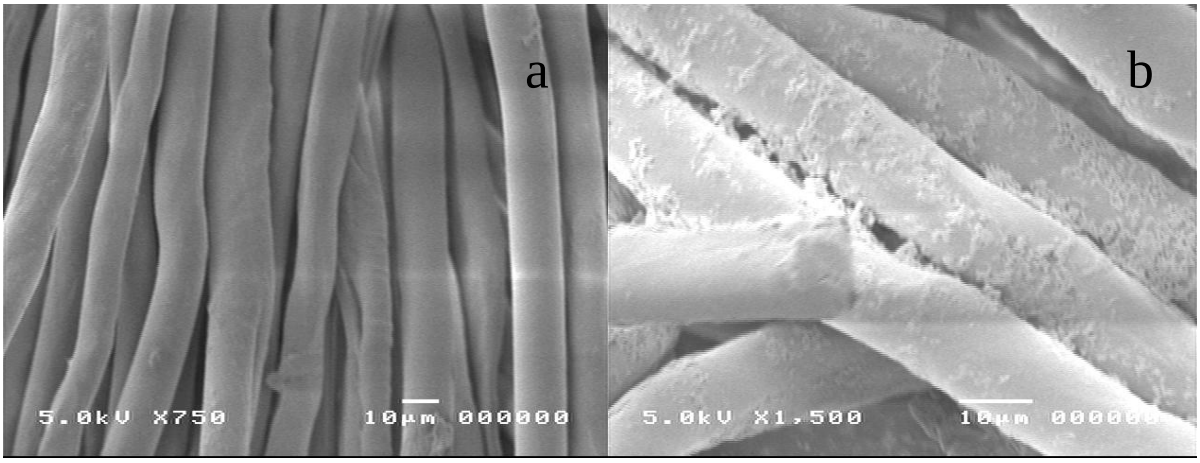

Figure 1. *SEM photograph of fabric samples (a) untreated sample, (b) peroxide treated sample with chitosan and tannic acid.*

4. Conclusion

The application of chitosan and tannic acid on peroxide treated cotton fabrics show better antimicrobial activity than their untreated fabrics. The pretreatment improves wettability, critical surface tension and accessible regions of fibrous substrate which enhances the fixation of antimicrobial agents. Tannic acid showed higher antimicrobial activity than chitosan when applied alone. The antimicrobial activity of treated samples was enhanced by addition of both chitosan and tannic acid. In combination treatment, the functional groups present in these antimicrobial agents further improve their fixation in fibrous structures .This synergistic action enhances the overall antimicrobial activity of cotton fabrics. These antimicrobial agents are eco friendly and so give good effect to human skin in addition to values on garments.

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