CHAPTER VI

DEVELOPMENT OF A NOVEL ANTI-TUBERCULOSIS MELT-BLOWN POLYPROPYLENE FILTER COATED WITH MANGOSTEEN EXTRACTS FOR MEDICAL FACE MASK APPLICATIONS

6.1 Abstract

This present research reports the development of three-layer mask, which is made of low cost polypropylene filter containing multifunctional properties such as antibacterial, antimicrobial, high bacterial filtration efficiency (BFE) and so on. The PP filters were prepared by spray coating with various mangosteen extract concentrations from 2 to 5 %(w/v) in absolute ethanol. The physical properties of filters were evaluated. The BFE experiments were performed by spraying the biological aerosol through the filters. Breathability of face masks were also measured as a pressure drop parameters. The coated filters were then challenged with anti-multidrug-resistant tuberculosis (MDR-TB), *Escherichia coli* (*E.coli*) and *Staphylococcus aureus* (*S.aureus*) as the representative bacteria. From the results show that with increase the MG concentration for coating, caused fiber diameter, hydrophilicity, %BFE (>95%) and pressure drop of filters were also increased. The MG coated filter exhibited good antibacterial performances for *S.aureus* and MDR-TB whereas showed low activities against *E.coli.* This study demonstrates that the filters coated with MG significantly play an important role in achieving antibacterial face mask.

Keywords: Mangosteen extracts, face mask, tuberculosis, respiratory system

6.2 Introduction

The protection of the human respiratory system against the aerosol of bacterial cells from the high-risk environment is one of most public health-care attentions these days. Because of their small sizes, bacterial cells could easily spread in the air and penetrate though the human respiratory system and eventually cause disease (Anna Balazy, 2006), especially infections of Mycobacterium tuberculosis (TB) cause infectious diseases which are mainly the global problem.(A. Carducci, 2011) When tuberculosis patients cough or sneeze, some bacterial droplets with particle diameters ranging from 1-5 μm are expelled into the environment (Diaz *et al.*, 2010). The recommended advice to protect ourselves from bacterial particles is using of face mask which has been used in broad range of hospital and health care setting. Face masks today are usually composed of spun bond or melt-blown polyprolylene filters, which has a good chemical stability and good thermal stability. However, they technically lack of an antibacterial property which is considered important for health. Therefore, the use of face mask with an antibacterial properties gains an increasing interest from both academic and industrial point of view.

The technics to enhance antibacterial properties to filters in many methods such as the dipping (Dubas *et al.*, 2006, Shateri Khalil-Abad *et al.*, 2010, Xue *et al.*, 2012), coating (Tiliket *et al.*, 2011), physical and chemical bonding (Alonso *et al.*, 2009, Li *et al.*, 2011, Lim *et al.*, 2004, Wang *et al.*, 2008) of antimicrobial molecules onto surface. Spray-coating technique is one of a common techniques to spray antibacterial suspension solution onto the surface and subsequently evaporate the solvent out (Appendini *et al.*, 2002). These methods can be applied to a wide range of industrial filter applications. Coated filters can be a self-cleaning surface because they can continuously comfront the biological attacks.

The commercial synthesized antibacterial agents for coating such as silver ions (Guzman *et al.*, 2012, Kaali *et al.*, 2010), ciprofloxacin (Kwok *et al.*, 1999, Kwok *et al.*, 1999), benzalkonium chloride (Muñoz-Bonilla *et al.*, 2012) and triclosan (Kandelbauer *et al.*, 2009, Yang *et al.*, 2011) have high biological activities but they are also a potent threat to human health and environment. There have been more interests in antibacterial filter especially those from natural sources with various bioactive compounds due to their lower toxicity and gentle to the environment. Several studies have reported that xanthone derivatives from mangosteen extracts (MG), espectially α-mangostin, has called much attention since it has desirable properties (Vishnu Priya V, 2010) including antifungal (Geetha Gopalakrishnan, 1997), antioxidant (Yu *et al.*, 2007), antiplasmodial (Elfita *et al.*, 2009) cytotoxic activities (Ajayi *et al.*, 2007) antibacterial (Arunrattiyakorn *et al.*. 2011, Pedraza-Chaverri *et al.*, 2008, Vishnu Priya V, 2010) and anti-multidrugs resistant tuberculosis (MDR-TB) (Gale *et al.*, 2007, García *et al.*)

The objectives of the present study were to modify the surface of low cost commercial polypropylene melt-blown filter with various concentrations of mangosteen extract by spray coating. Physical properties of filters were studied. Bacterial filtration efficacy (BFE) and pressure drop were performed by NELSON Laboratory. Further, the antibacterial activities of coated filter against Gram-positive bacteria *Staphylococcus aureus*, Gram-negative bacteria *Escherichia coli* and MDR-TB were evaluated quantitatively as a function of exposure times.

6.3 Experimental

6.3.1 Materials

The commercially available filters ; front(SP-14) and back(SP-30) layers made of polypropylene spunbond non-woven fabric (fiber diameter: 20.88±2.79 μm ,14 g/m^2) and (fiber diameter: 20.81±3.12 μm, 30 g/m^2), respectively were perchased from Narula Nonwoven Company Limited Thailand. The middle layer (MB-0) was made of melt-blown polypropylene filter (fiber diameter: 5.30±2.16 μm, 20 g/m^2). Mangosteen extract (α-mangostin and γ-mangostin contents approximately 46.36% and 5.45% of dry weight of extracted crude, respectively) was supported by Department of Chemistry, Faculty of Science, Srinakrarinwirot University, Thailand Absolute ethanol(99%) was purchased from Sigma Aldrich. Media for bacterial culture, soybean casein digest broth (SCDB), Triptic Soy Broth (TSB), (TSA) were purchased from Diffco. All other reagents and solvents were of analytical grade and used without further purification.

6.3.2 Spray Coating and Filter Embedding

The coating solution 2% and 5% (w/v) were prepared by dissolving MG 20 and 50 g in 1000 ml of absolute ethanol, respectively and stirring for 4 hours. The MB-0 filter was carried out in ultrafine spray coating (collector speed:10 rpm, nozzle diameter: 2 mm, spraying pressure: 110 psi). After coating, the coated MB filter were passed through the hot air (65 °C) blower unit to evaporate the residue ethanol. The filters coated with 2% and 5% (w/v) of MG concentrations, were labeled as MB-2 and MB-5, respectively. Finally, to embed the 3 layers of SP-14, middle layer (MB-0, MB-2, MB-5) and SP-30 were sealed with heater to produce a 3-layer face mask.

6.3.3 Determination MG Content on Filter

This method following to Chaivisuthangkura (Apinya Chaivisuthangkura, 2008), coated filters were cut in circle shape with diameter 1.5 cm in random position. The filter samples were dissolve in ethyl acetate and then, All samples were performed on HPLC Thermo (Thermo Finnigan, USA) which consists of Chrom Quest software, Degasser Thermo separation product, Quaternary gradient spectra system P400 and UV detector spectra system 2000. The MG extract was separated on a 150 mm 9 4.6 mm i.d., 4-lm particle, Synergi Hydro column (Phenomenex, Torrance, CA, USA) with a 4.0 mm 9 2.0 mm i.d. C_{18} (ODS) guard column. The mobile phase was a gradient prepared from acetonitrile (component A), 2% (v/v) acetic acid in water (component B), and n-butanol (component C). The gradient program was: A:B:C from 30:70:0 to 45:45:10 in 2 min, from 45:45:10 to 80:15:5 in 23 min, from 80:15:5 to 80:20:0 in 2 min, from 80:20:0 to 95:5:0 in 8 min, and isocratic at 95:5:0 for 25 min. The flow rate was 0.5 mL min^{-1} and the total separation time was 60 min. Chromatography was performed at ambient temperature.

6.3.4 Filtration Performance

Bacterial filtration efficiency (BFE) was evaluated following by ASTM F2101-01 Standard Test Method for Evaluating the Bacterial Filtration Efficiency (BFE) of surgical masks using a Biological Aerosol of *Staphylococcus aureus* ATCC 6538 diluted in 1.5% peptone water to an accurate concentration to yield challenge level counts of 2200±500 colony forming units (cfu) per test sample. The samples were prepared from complete face masks 100 mm x 100 mm including all layers of mask which were installed on the top of Andersen sampler(six-stage viable particle cascade impact sampling system containing one agar(SCDA) plate for each stage). The microorganism are generated into aerosol with nebulizer at the vaccum flow rate through the Andersen sampler maintained at 1 CFM (28.3±5% l/min) and fixed air pressure for one minute. The aerosol droplets with mean particle size (MPS) of approximately 3.0±0.3 µm. The tests were repeated 5 times for each sample. All plates were incubated at 37 °C for 48 hours. The colonies formed were counted and calculated as in equation 1

$$\text{Bacterial filtration efficiency (\% BFE)} = [(C - T)/T] \times 100$$

Where C is Average of control values and T is count total for testing. Differential pressure (Delta P or ΔP) test which also relate to the breathability of face-mask materials. The equipments need to be calibrated prior to using them. Install the sample holder and adjust manometer level to a reference mark. Allow the air flow rate to be 8 l/min pass through the reference material into sample holder and take manometer measuring at five different sites. ΔP values were calculated from

$$\Delta P = M / A$$

Where M is the average mm of the water of the test replicates and A is the area of test cell (4.9 cm^2).

6.3.5 Physical Properties of Coated Filter

6.3.5.1 Surface morphology

The surface morphology of filters and fiber diameters were characterized by Hitachi S-4800 Field Emission Scanning electron microsope (FE-SEM) at 10kV and SemAphore 4.0 software.

6.3.5.2 Water contact angle

Static water contact angles of the filters were carried out with a KrÜss DSA 100 drop shape analysis system. Ten droplets of distilled water (10 µl) were dropped on random test areas on each sample. The projected pictures of the droplets, after they had been captured to stay on the filter surface until no change in their shapes was observed and analyzed the contact angles.

6.3.6 Antibacterial Evaluation

The antibacterial activities against gram-positive bacteria *Staphylococcus aureus* (*S. aureus*, ATCC 25923) and *Escherichia coli* (*E.coli*, ATCC) were selected as a model for the Gram-negative and Gram-positive bacteria carried out according to a modified procedure of the testing method followed by AATCC test method 100-2004 assessment of antibacterial finished on textiles. The filters were cut in disc shape (15 mm in diameter, 0.24 ± 0.10 cm in thickness) and sterilized under UV lamps of FUNA-UV-LINKER FS800 for 60 min. A colony of *S.aureus* and *E.coli* were cultured in TSB, 100 ml at 37 °C for 24 hours under a shaking (Orbital incubator shaker, GYROMAX^{TM} 737) of 120 rpm washed with normal saline solution (0.85 % NaCl solution) subsequently evaluate the antibacterial reduction tests as a function of times, a portion of saline solution containing the bacteria was diluted to 10^5 cfu/ml. 50 µl of the prepared bacterial suspensions were placed onto the sample surfaces contained in vials. After a certain period of contact time (5, 15, 30, 60 and 1440 min),then pipetted 2 ml of sterile normal saline to the vials and vortexed for 5 min to remove adherent bacteria to saline solution. The 100 µl of each diluents were placed onto agar plates. The colony of bacterial growths were counted after incubation at 37 °C for 24 hours. Each test was repeated for 3 times. The percentage of reduction was calculated according to the following equation:

$$\text{Percent reduction of bacterial (\%)} = [(C - T)/C\,] \times 100$$

Where C is Cells number of Blank at time intervals and T is cells number of added at time intervals. For anti-multidrug-resistant tuberculosis (MDR-TB) tests, *M. tuberculosis* H37Rv ATCC 27294 and clinical isolate of multidrug-resistant *M. tuberculosis* (MDR-TB) were used for this study. The strains were cultured on Lowenstein-Jensen (LJ) medium and incubated aerobically at 37°C for 3 weeks. The mycobacterial suspension was prepared in 0.04% Tween 80 and diluted with sterile distilled water to a turbidity of the McFarland no. 1. The suspension of 1.0

McFarland was then diluted 1:100 with sterile distilled water and 50 μl was placed onto each of at least 3 replicate of the coated filters and 6 replicate of the uncoated filters. Two milliliters of sterile distilled water was added immediately to each of the 3 replicate of the uncoated filters in individual containers and vortexed. One hundred microliters of each samples was drawn and spread onto a 7H11 agar plate and incubated at 37°C for 3 weeks for viable counts to provide base-line data.

6.4 Results and Discussion

The content of α-mangostin and γ-mangostin coated on the various filters, characterized by HPLC technic is shown in **Table I.** . The SEM images of these filters before and after coating are shown in **Fig 6.1.** The influence of MG solution concentration from 0 to 5% (w/v) on structural and hydrophobicity of various filters were observed. The morphology of filters can be seen from **Fig. 6.1(a)** the pristine polypropylene melt-blown fibers show typical fibril structure with smooth surface. After coating, the melt-blown fiber surface shows more rougher and covering with MG particles, as shown in **Fig. 6.1(b).** With increasing the MG solution concentration, the average diameter of the melt-blown filters were slightly increased. Wettability of coated(MB-1, MB-2) and uncoated(MB-0) filter were performed by water contact angle measurements. **Table 6.1** shows the water contact angle of MB-0 of about 138±0.11. Polypropylene exhibited a hydrophobic surface. When the filters were coated with MG solution, they showed high hydrophilic properties and much higher by than those using high concentration of MG solution.

The diameter of filter fibers BFE and △P, which are the important parameters that can reveal the performance of face mask shown in **Table 6.2**. For BFE test, the penetration of bacterial aerosol droplets (3 μm) through the filters may differ from nonbiologic simulants. These biological particles will be captured by the 3 layers of filters. With increase the fiber diameter, can promote the BFE of filters due to the lower free volume between coated fibers. Theses phenomena was also found in the pressure drop evaluations that was concerned with breathability of user. According to a high level of coated filter diameters, the air can difficultly pass through the filter layer and lead the pressure drop to increase. Therefore, the pressure drop will be increased (Patanaik *et al.*, 2010). Despite, the commercial face masks contain pressure drop in range of 7-30 mmH_2O / cm^2 (Viscusi *et al.*, 2009).

In the antimicrobial study, the protocol of testing was simulated for the real use of face mask (bacilli are carried through airborne droplet nuclei produced from coughing and talking) was useful as an indicator of surface antimicrobial activity(Andrea, 2006). The α-mangostin and γ-mangostin contents coated on filters were shown in **Table 6.1** which are the most important roles to inhibit bacterial growth. Some researchers reported that the minimum inhibitory concentration of bacteria (MIC) of α-mangostin was between 1.57 to 50 μg/ml(Pedraza-Chaverri, 2008) for bacteria and 6.25 μg/ml for MDR-TB. However, the action mechanism of antibacterial activities of α-mangostin there is not entirely understand(Hemaiswarya *et al.*, 2008). Anti- bacterial and mycobacterial activities of coated melt-blown filters were evaluated in term of % bacterial reduction as a function of exposer times shown in **Fig 6.2-6.4.** The results showed that the MB-0, exhibited slightly antibacterial activities of *E.coli*, *S.aureus* and MDR-TB. The percent reduction of Gram-positives bacterial *S.au*reus and MDR-TB clearly increased on both MB-2 and MB-5 filters within the exposer time compared to Gram-negatives like *E.coli.* At the same exposure times 60 mins, The melt-blown coated with MG showed more exhibited inhibitory effect againt *S.aureus* than MDR-M TB. Since, TB is a mycobacteria containing a high degree of intrinsic resistance to most antibiotics and chemotherapeutic agents (Andersen *et al.*, 2010). The low permeability of the mycobacterial cell wall (consisting of 60%, N-glycolylmuramic acid and mycolic acid of dry weight), with its complex structure, is now known to be a major factor in this resistance (Jarlier *et al.*, 1994). Despite, the Gram-negative (*E.coli*) showed low activities and at 24 hours, *E.coli* growth was over than control. The results of present research revealed the more useful information for the filter applications.

6.5 Conclusions

The three-layer face mask was successfully developed by the assemble of three layers filters which were spun bond and melt-blown PP media. The coated melt-blown PP layer with MG extracts resulting to increase the %BFE and breathability. In addition, MG coated filter also exhibited highly antibacterial activities against MDR-TB and *S. aureus* which are the gram positive bacteria caused the inspiration diseases. . This study demonstrates that the filters coated with MG significantly play an important role in achieving antibacterial filter in many various applications such as the filters used in air condition systems.

Acknowledgements

This research was supported by the Petroleum and Petrochemical College, Chulalongkorn university Thanks are also extended to faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand, provided MDR-TB culture laboratory, Faculty of Medicine, Srinakharinwirot University, provided MG extract

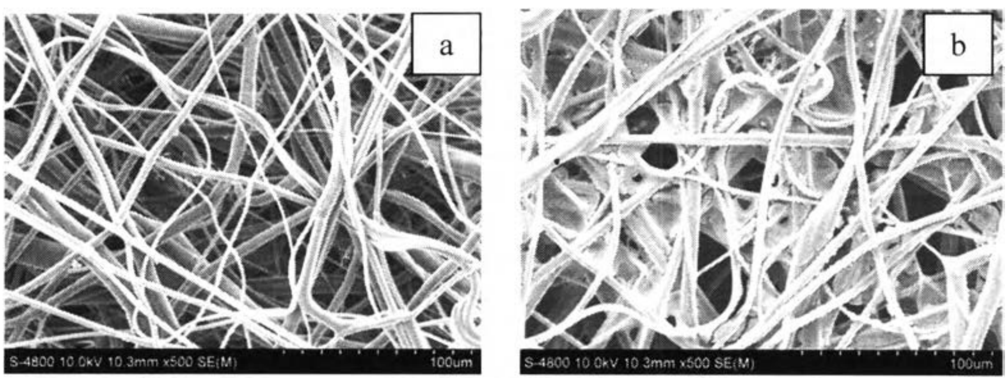

Figure 6.1 SEM images of polypropylene melt-blown filter (a) before coating and (b) after coating with 5% (w/v) of MG solution.

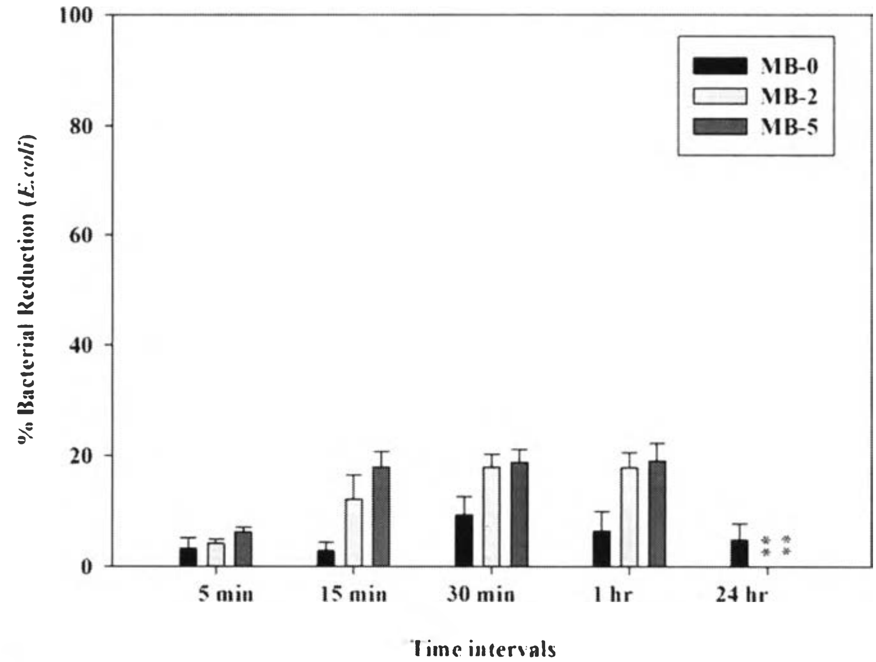

Figure 6.2 Percentage of *E.coli* reduction as a function of time intervals.

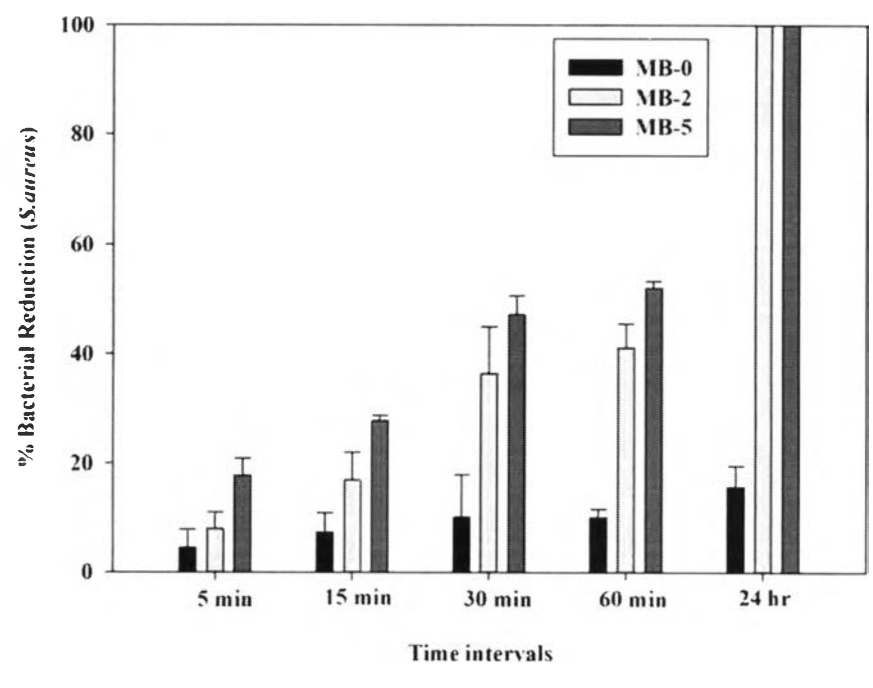

Figure 6.3 Percentage of *S.aureus* reduction as a function of time intervals.

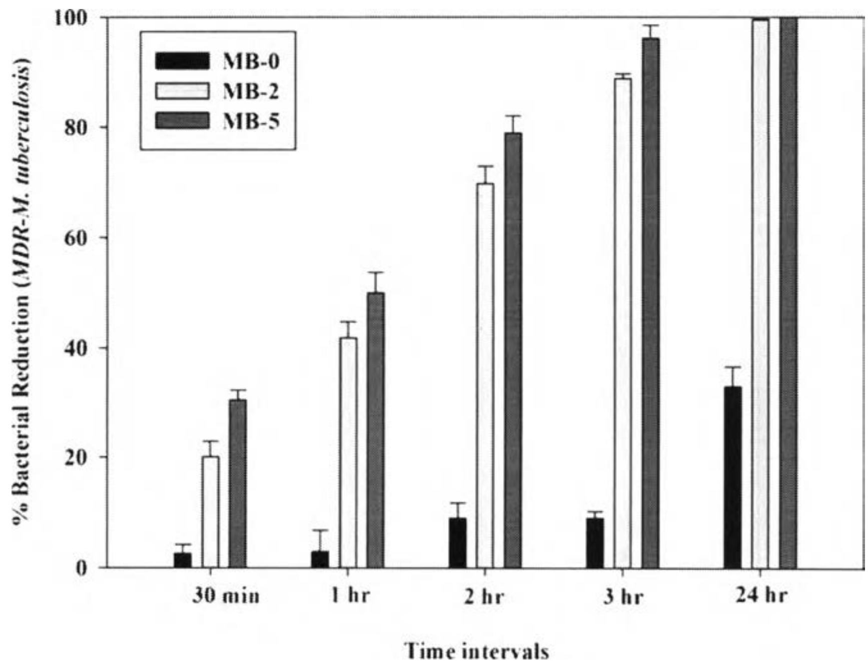

Figure 6.4 Percentage of MDR-M. tuberculosis reduction as a function of time intervals.

Table 6.1 The characteristic properties of polypropylene melt-blown filters

Sample	Fiber diameter (μm)	Water contact angle °	Drug content on the melt-blown filter mg/ sample area (1.76 cm^2)	
			α-mangostin	γ-mangostin
MB-0	5.30±2.16	138±0.11	0	0
MB-2	5.40±2.15	110±0.43	0.0634 ± 0.0011	0.0053 ± 0.0018
MB-5	5.62±1.48	57.4±2.76	0.1087 ± 0.0003	0.0100 ± 0.0005

Table 6.2 The filtration performances of complete face mask

Complete face mask embedded with three layers	Percentage of bacterial filtration efficiency (% BFE)	Pressure Drop (mmH_2O / cm^2)
SP14+MB-0+SP30	95.38±0.78	2.90±0.10
SP14+MB-2+SP30	96.54±0.61	3.36±0.16
SP14+MB-5+SP30	97.88±0.20	4.70±0.10