CHAPTER VI

PREPARATION AND CHARACTERIAZATION OF ELECTROSPUN POLYACRYLONITRILE FIBER MATS CONTAINING *GARCINIA MANGOSTANA*

6.1 Abstract

Garcinia mangostana-loaded Electrospun polyacrylonitrile (PAN) fiber mats has antibacterial and antituberculosis properties that were fabricated from PAN solution containing *G. Mangostana* extract in dimethylformamide (DMF). 10 % PAN solution was mixed with the *G. mangostana* 10, 15, 20 and 30 wt.% used in the experiment. The PAN solutions were fabricated by electrospining process. Both the neat and the *G. mangostana*-loaded PAN fibers were successfully fabricated and the average diameters of the fibers were ~215 nm and ~245 nm, respectively. Morphologies, release characteristics, antimicrobial efficiencies (against *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* (MRSA) DMST 20654, *Staphylococcus epidermidis* ATCC 12228, *Strephylococcus agalactiae* DMST 17129, *Strephylococcus pyogenes* DMST 17020], and anti-drug-resistant tuberculosis (TB-MDR) properties of the neat electrospun and 10 % to30 % *G. mangostana*-loaded e-spun fiber mats were investigated. The release characteristics of *G. mangostana* from the e-spun PAN fiber mats were determined in acetate buffer and phosphate buffer saline solutions. The cumulative released amount of *G. mangostana* from these samples proportionally increased with the increase of *G. mangostana* incorporated in the spinning solutions. This study demonstrated a convenient procedure and the potential to develop antimicrobial and antituberculosis properties of electrospun fibrous membranes containing *G. mangostana,* which are beneficial in filtration applications for respirator, face mask, and air conditioning filter.

(**Key words**: Electrospinning; Polyacrylonitrile; *Garciniamangostana*)

6.2 Introduction

Tuberculosis is still one of the world's infectious diseases that can cause death. According to the World Health Organization, an estimated 8–10 million are infected and about 2 million die from it each year[1]. Tuberculosis patients can spread the disease to other people by coughing and sneezing small infectious *tuberculosis* droplets in the air[2]. Mangosteen is a tropical fruit with medicinal properties that is fairly found in Thai, Malaysia, India and Sri-Lanka. People in these countries often use *G. mangostana* for skin infection treatment, diarrhea, leucorrhoea, and gonorrhea.[3-7] The chemical composition of *G. mangostana* pericarp has been identified as phenolic compounds including xanthones, tannins, and flavonoids.[8-12]. In addition, *G. mangostana* exhibits antitumor and antioxidant abilities[13-18] as well as antibacterial properties that combat*Staphylococcus epidermidis and Propionibacterium acnes*[19]. Recently, the prenylatedxanthones from the hull of *G. mangostana,* in an *in-vitro* test, were found to be active against *M. tuberculosis*[20].

At present, nanofibers have received popular into membranes due to its high permeability and small pore size. In addition, nano fiber membranes offer amazing characteristics like large surface area, excellent mechanical properties and the potential to incorporate active chemistry on nanoscales compared with traditional membranes[21]. The electrospun fibers have potential for use in applications such as filter membranes, nano-sensor, chemically protective clothing, wound dressings, and tissue engineering[22,23]. The electrospining method was used to prepare ultrafine fibers. Polyacrylonitrile (PAN) is widely use in the filtration applications due to its superior mechanical properties, good thermal stability, and chemical resistivity[24,25]. Several studies have reported their functional nanofibers, through electrospinning by loading active compounds, can provide antibacterial properties. Lala and coworker reported that three types of electrospun fibers, cellulose acetate (CA), polyacrylonitrile (PAN), and polyvinylchloride (PVC) with silver nanoparticles, showed antibacterial activity with *E. coli* and *P. aeruginosa*[26]. Electrospun polyacrylonitrile fibers containing silver nanoparticles also showed high antibacterial activity with *S. aureus* and *E. coli*[27]. Therefore, incorporation of *G. mangostana*

within PAN nanofibers may serve as a promising scaffold in providing effective antibacterial and antituberculosis properties. The release characteristics of *G. mangostana*-loaded PAN electrospun fiber mats were studied. Furthermore, the efficacy of *G. mangostana*-encapsulated PAN nanofibers in inhibiting bacterial and tuberculosis activities was investigated.

6.3 Experimental Details

6.3.1 Materials

Polyacrylonitrile (PAN) (Mw $\approx$ 55.5 kDa) containing a 91.4 wt % acrylonitrile monomer (CH_2=CHCN) and 8.6 wt.% methylacrylatecomonomer (CH_2=CH(CH_3)COOH) was received from Thai Acrylic Fiber Co., Ltd. (Thailand). Mangosteen extract was prepared was prepared by Department of Chemistry and Center of Excellence for Innovation in Chemistry, Srinakharinwirot University. The solvents *N,N*-dimethylformamide (DMF) and methanol were purchased from Labscan (Asia) (Thailand). Glacial acetic acid was purchased from Carlo Erba (Italy). Sodium acetate and disodiumhydrogenphosphateheptahydrate ($Na_2HPO_4 \cdot 7H_2O$) were purchased from Ajax Chemicals (Australia). Tween 80 was purchased from Sigma-Aldrich (USA). All of these chemicals were of analytical reagent grade and used without further purification.

6.3.2 Preparation of Mangosteen Extract

Mangosteen extract was prepared by soaking the dried and pulverized fruit husk skin hull in ethyl alcohol for 48 hrs. The solvent was then removed to give a brownish residue. Water was then added, stirred occasionally and the resulting yellow solid was filtered and then dried in vacuum.

6.3.3 Fabrication of Neat and *G. Mangostana*-Loaded PAN Fiber Mats

A PAN solution was dissolved in DMF at 10 % w/v. Mangosteen extract was added into the PAN solution in amounts of (10, 15, 20, 30) wt.% to form a homogeneous solution. The shear viscosity of the PAN solutions was characterized by using a Brookfield rheometer. The measurement was carried out in triplicate. The solution was electrospun at 15 kV, with a collector wrapped in an aluminium sheet at a distance of 20 cm. The collection time was 48 h and the thickness of the fiber mats was 148 ±12 μm. The fibers were keep in a vacuum oven before further characterization.

6.3.4 Characterization of Neat and *G. Mangostana*-Loaded PAN Fiber Mats

The electrospun fiber morphology was investigated using scanning electron microscope (SEM). Diameters of the 50 individual fibers were estimated using a SemAphore 4.0 software. Stress at maximum load (MPa), stress at break (MPa), and Young's Modulus (MPa) of both the neat and the fix of *G. mangostana*-loaded PAN fiber mats were investigated by a Lloyd LRX universal testing machine with a 50 mm gauge length and crosshead speed of 100 mm min^{-1}. The samples were prepared into 10 mm ×100 mm pieces.

6.3.5 Release of *G. Mangostana* from *G. Mangostana*-Loaded PAN Fiber Mats

6.3.5.1 Actual G. mangostana content

In order to measure the actual content of *G. mangostana* in the *G. mangostana*-loaded PAN fiber mats, each specimen was cut into circular discs, and mixed with DMF 10 ml. The solution was diluted with the releasing media. The amount of the *G. mangostana* was measured with a UV-vis spectrophotometer (Shimadzu UV-2550, Japan) at 320 nm. The *G. mangostana* concentration was determined using a calibration curve in each of the releasing media.

6.3.5.2. G. Mangostana-Releasing Assay

The release characteristics of *G. mangostana* from the *G. mangostana*-loaded PAN fiber mats was determined in each of the two releasing

medium, including acetate buffer and phosphate buffer solutions. The *G. mangostana* extract was limited in solubility therefore acetate buffer with Tween 80 and 3% v/v methanol at 32 °C and 96.5% v/v phosphate buffer with 0.5% v/v Tween 80 and 3% v/v methanol at 37 °C were used. Each specimen was incubated in 30 ml of releasing medium. The sample solution (1 ml) was taken and was replaced with the fresh medium. The amount of *G. mangostana* was measured by the UV-vis spectrophotometer at 320 nm. The cumulative amount of *G. mangostana* was calculated at each submersion time interval between 0 and 48 hrs.

6.3.6 Antibacterial Activity

6.3.6.1 Agar Disk Diffusion Method

The antibacterial activity of the *G. mangostana*-loaded electrospun fiber mats was investigated using the agar disk diffusion method[28].The 18 h bacterial culture of *Staphylococcus aureus* ATCC 25923 (*S. aureus)*, *Staphylococcus aureus* (MRSA) DMST 20654 (*S.aureus MRSA)*, *Staphylococcus epidermidis* ATCC 12228 (*S.epidermidis*), *Strephylococcusagalactiae* DMST 17129 (*S. agalactiae)*, and *Strephylococcus pyogenes* DMST 17020 (*S.pyogenes)* were diluted in Mueller-Hinton broth and adjusted to 0.5 McFarland standard turbidity. The inoculum (about 10^6 CFU/ml) was uniformly and aseptically spread on the agar plate with a cotton swab. The plate was allowed to dry for 5 min in a sterile workstation. The cylindrical e-spun fibers (15 mm in diameter) were gently placed over the surface of the agar plates. The neat PAN e-spun fibers were used as a control and the e-spun fiber with varying the amounts of *G. mangostana*were used as test samples. Before using, the samples were sterilized with UV for 2 h. Ampicillin and chloramphenicol were used as positive control drugs. The reaction of the microorganisms to the *G. mangostana*-loaded PAN fibers was determined by the size of the inhibition zone. The clear zone (including the diameter of the material) was measured in four different directions and the mean value was calculated. The experiments were completed in triplicate.

6.3.7 Multidrug-Resistant Tuberculosis (MDR-TB)

6.3.7.1 Preparation of Multidrug-Resistant Tuberculosis

Rifamipicin, Isoniazid, Ethambutol and Streptomycin (TB-MDR: Mc. No.1) were added to the broth medium and incubated at 37 °C until the concentration of culture 10^7-10^8CFU. Next, 100 µl of culture was mixed with 9.9 ml of sterilized distilled water to get aconcentration of 10^5-10^6 CFU. The whole samples were sterilized with UV for 2 h before use.

6.3.7.2 Disc Diffusion Method

Using an inoculating loop loaded with one full loop of the diluted inoculums (10^5-10^6 CFU), the solution was streaked to the surface of agar plate. The neat and *G.mangostana*-loaded e-spun PAN fiber mats were placed on the plate and then incubator at 37 °C for 3 weeks. The inhibition zone were measured in terms of inhibition zone.

6.3.7.3 Percentage of Inhibition

The neat and *G.mangostana*-loaded PAN fibers (15 mm in diameter) absorbed the 20 µl and 50 µl of culture medium (10^5-10^6 CFU/ml), and left no liquid in the small plastic bag after incubation for 24 h. Bacteria were eluted from the samples. These samples were transferred into a vial containing 2 ml of sterilized water and the flasks were shaken for 5 minutes, and then spiralled on plates of nutrient agar. For the control, culture medium (10^5-10^6 CFU/ml) was spiralled on plates on nutrient agar.

6.4 Results and Discussion

6.4.1 The electrospinning of PAN solution containing *G.Mangostana*

Before electrospinning, the viscosity of PAN solutions were measured and the results are summarized in Table 6.1. The shear viscosity slightly increase when the addition of the initial amount of *G.mangostana*. The PAN solution with and without *G.mangostana* was fabricated at 15 kV/20 cm by electrospinning.

Representative SEM images illustrating the electrospun fiber from both the neat and the *G.mangostana* containers are shown in Fig 6.1. The average diameter of the neat PAN fibers was ~242 ± 0.12 μm, while that of the 10%, 15%, 20%, 30% *G.mangostana* -loaded PAN fiber mats were ~241 ± 0.17 μm, 239 ± 0.33 μm, 223 ± 0.17 μm and 215 ± 0.24 μm, respectively. The fiber diameters were decreased with the increase of *G.mangostana* which may be because the increasing *G.mangostana* enhanced the viscosity of the spinning solution leading to straighter and finer fibers observed for PAN e-spun fibers with varying amount of *G.mangostana*. Rujitanaroj *et al.* [27] illustrated the incorporation of silver nanoparticles (AgNPs) in e-spun polyacrylonitriles (PAN) fibers showed smooth surface and the diameter of PAN electrospun fibers with silver nanoparticles ranged between 194 nm to236 nm. With increasing concentrations of $AgNO_3$, decreased diameters of AgNPs-loaded PAN e-spun fibers were present.

6.4.2 Mechanical Properties of Neat and *G.Mangostana*-Loaded PAN Fibers

Stress at maximum load, the stress at break, and the Young's modulus for the PAN fiber mats were 1.14 ± 0.24, 0.81 ± 0.17, and 161.40 ± 9.20 MPa, respectively (Table 6.2). The mechanical properties of 10 % wt. to 30% wt. *G.mangostana* –loaded PAN electrosspun fibers increased when compared with the neat materials. Stress at maximum load, the stress at break, and the elongation at break of the fiber mats containing 10 wt.% to30 wt.% *G.mangostana* ranged between 2.15 ± 0.03 and 5.67 ± 1.34 MPa, 1.99 ± 0.39 and 4.39 ± 0.48 MPa and 175.91 ± 6.62 and 239.33 ± 10.18 MPa, respectively.

6.4.3 Release of *G.Mangostana* from *G.Mangostana*-Loaded PAN Fiber Mats

The amount of *G.mangostana* in the *G.mangostana*-loaded e-spun PAN fiber mats were determined and followed by investigated the release characteristic of *G.mangostana* from electrospun fibers as summarized in Table 6.3. After the drug assay experiments, the actual amount of *G.mangostana* in the PAN -loaded fiber mat samples containing *G.mangostana* ranged between ~71 % and ~84

% in the acetate buffer solution and ~77 and ~89% in the phosphate buffer solution, respectively.

The release characteristics of *G.mangostana* from the samples was determined by using the total immersion method in acetate buffer and phosphate buffer solutions with Tween 80 0.5 % v/v and methanol 3% v/v. In the study, Suwantong and coworker[29] studied the release characteristic of curcumin from the cellulose acetate electrospun containing curcumin bin acetate buffer solution containing Tween and methanol. Moreover, Taepaiboon*et al.* [30] fabricated vitamin A and vitamin E loaded cellulose acetate fiber mats and studied the release characteristic of vitamin A and E by using 0.5 % v/v Tween and 10 % methanol in acetate buffer solution. In this work, the percentage of cumulative release profiles of *G.mangostana* were reported and at any time point, the amount of *G.mangostana* rapidly increased and gradual increase in the cumulative amount of *G.mangostana* release followed for the next hours (Fig. 2). The release profile was investigated a plateau value at any submersion time.

At each time interval, the cumulative of *G.mangostana* released from these materials was increased with increasing amount of *G.mangostana.* Specifically, in the acetate buffer solution with 0.5% v/v Tween 80 and 3% v/v methanol, the higher the *G.mangostana* in the e-spun fibers, the greater the percentage of *G.mangostana* released (Fig. 6.2a). The maximum cumulative amounts of the *G.mangostana* from (10, 15, 20, 30) wt% of *G.mangostana* were ~(21.25, 26.61, 60.81, and 70.87)% respectively. Figure 6.2 b reveals that neat and *G.mangostana*-loaded electrospun fibers in the phosphate buffer saline solution (PBS) (P/T/M medium) presented a similar release profile. The maximum amount of mangosteen extract released from 10, 15, 20, 30 wt% was (18.64, 29.72, 65.80, and 89.87) % respectively.

6.4.4 Antibacterial Activity

The antibacterial activity of neat and *G. mangostana*-loaded PAN e-spun fiber mats were evaluated against *S. aureus*, *S. aureus* (MRSA), *S. epidermidis*, *S. agalactiae*, *S. pyogenes* (Table 6.4). The neat e-spun fiber mats was used as a control. No inhibition zones were observed around the bacteria the neat PAN fiber

mats where as all *G.mangostana* in the *G.mangostana*-loaded PAN fibers were found to be active against all bacteria. The strongest antibacterial activity was seen against *S. epidermidis* followed by *S. pyrogenes*, *S. agalactiae, S. aureus* (MRSA) and *S. aureus*. The clear zones of the specimens generally increased with increasing concentrations of *G. mangostana* in the PAN e-spun fiber mats. The major composition of *G.mangostana* was a variety of prenylatedxanthone derivatives[20], such as α-, β- and γ-mangostins, garcinones B-E, gartanin and 9-hydroxycalabaxanthone[10]. This is agreement with Chomnawang *et al*, who reported that *G.mangostana* exhibited potent inhibitory effects against methicillin-resistant*S.aureus (S.aureus* ATCC 25923 and clinical isolated MRSA)due to the large amout of prenylated xanthones[32].

6.4.5 Multidrug-Resistant Tuberculosis (MDR- TB)

The multidrug-resistant tuberculosis test (MDR-TB) was carried out to investigate the potential inhibition with *G.mangostana*-loaded PAN electrospun fiber mats. The clear zone increased with increasing loadings of *G.mangostana* in the electrospun fiber mats. The strongest multidrug-resistant tuberculosis was observed at 30 % *G.mangostana*-loaded PAN fibers as indicated in Fig 6.3 a. Moreover, the inhibition was determined and summarized in Figure 6.3 b. It demonstrated that the loading of *G.mangostana* more than 15 % showed 99.99 % inhibition of Tuberculosis. Therefore, *G.mangostana*-loaded PAN electrospun fiber mats served as a platform for the inhibition of tuberculosis.

6.5 Conclusion

G.mangostana was successfully incorporated into electrospun PAN fiber mats at 15 kV electric field and distance 20 cm. *G.mangostana* exhibited gradual release in the cumulative amount of *G.mangostana* released throughoutthe testing period. The release characteristic of *G.mangostana* increased with increasing *G.mangostana*-loading content in the fibers. Lastly, with the antibacterial and multidrug-resistant tuberculosis test properties of *G.mangostana*-loaded

onelectrospun PAN fiber mats, the zone of inhibition increased with increasing loading of *G.mangostana* in the electrospun fiber mats. The combination of these excellent antibacterial and antituberculosis properties of *G.mangostana* and the unique structural features of the electrospun fiber mats could be a suitable fabrication method for respirator, face mask, and air conditioning filters.

6.6 Acknowledgements

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6.7 References

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Table 6.1 Shear viscosity of neat, 10 wt % to 30 w t% of *G.mangostana*-loaded PAN solutions (n = 3)

Type of PAN solution	**Shear viscosity (mPa s)**
Neat	149.0 ± 1.00
With 10% *G.mangostana*	156.6 ± 0.58
With 15% *G.mangostana*	163.7 ± 1.21
With 20% *G.mangostana*	176.7 ± 0.57
With 30% *G.mangostana*	185.0 ± 1.73

Table 6.2 Mechanical integrity of neat and 10 % to 30 % *G.mangostana*-loaded electrospun PAN fiber mats (n =10)

Type of materials	**Stress at Maximum Load (MPa)**	**Stress at Break (MPa)**	**Young 's Modulus (MPa)**
Neat PAN e-spun fiber mats	1.14 ± 0.24	0.81 ± 0.17	161.40 ± 9.20
10% *G.mangostana* - loaded PAN e-spun fiber mats	2.15 ± 0 .03	1.99 ± 0.39	175.91 ± 6.62
15% *G.mangostana* - loaded PAN e-spun fiber mats	2.74 ± 0.30	2.32 ± 0.22	188.76 ± 9.17
20% *G.mangostana* - loaded PAN e-spun fiber mats	3.56 ± 0.15	4.02 ± 0.54	201.11 ± 6.38
30% *G.mangostana* - loaded PAN e-spun fiber mats	5.67 ± 1.34	4.39 ± 0.48	239.33 ± 10.18

Table 6.3 Actual amount of *G.mangostana* incorporated in the *G.mangostana* - loaded electrospun PAN fiber mats in acetate buffer solutions containing 0.5% v/v Tween 80 and 3% v/v methanol (A/T/M medium) (pH= 5.5) and in phosphate buffer saline solutionscontaining 0.5% v/v Tween 80 and 3% v/v methanol (P/T/M medium) (pH= 7.4) (n=3)

Sample	**Drug assay in A/T/M medium (pH= 5.5) (%)**	**Drug assay in P/T/M medium (pH= 7.4) (%)**
10% *G.mangostana* -loaded PAN e-spun fiber mats	84.51 ± 11.05	77.07 ± 9.35
15% *G.mangostana* -loaded PAN e-spun fiber mats	82.78 ± 5.22	85.06 ± 6.42
20% *G.mangostana* -loaded PAN e-spun fiber mats	75.16 ± 9.79	88.35 ± 1.04
30% *G.mangostana* -loaded PAN e-spun fiber mats	71.85 ± 3.27	81.34 ± 6.79

Table 6.4 Antibacterial properties of the neat and 10 % to 30 % *G.mangostana*-loaded PAN fiber mats determined by disk diffusion method (measured from the edge of the samples to the edge of the clear zones) (mm) (n=3)

Samples	Inhibition zone (mm)				
	S. aureus	***S. aureus* (MRSA)**	***S. epidermidis***	***S. agalactiae***	***S. pyrogenes***
10% *G.mangostana* /PAN fiber mats	15.77 ± 0.25	16.23 ± 0.4	17.67 ± 0.57	16.5 ± 0.5	16 ± 0
15% *G.mangostana* /PAN fiber mats	16.5 ± 0.5	16.83 ± 0.28	18.17 ± 0.76	16.83 ± 0.28	17.17 ± 0.28
20% *G.mangostana* /PAN fiber mats	17.16 ± 0.76	17.27 ± 0.64	19.5 ± 0.5	17.7 ± 0.51	17.73 ± 0.46
30% *G.mangostana* /PAN fiber mats	17.66 ± 0.57	18 ± 0.5	22.33 ± 1.15	18.83 ± 1.25	19 ± 1

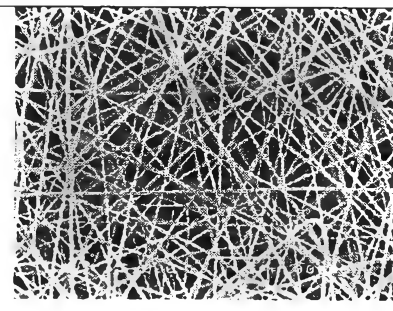
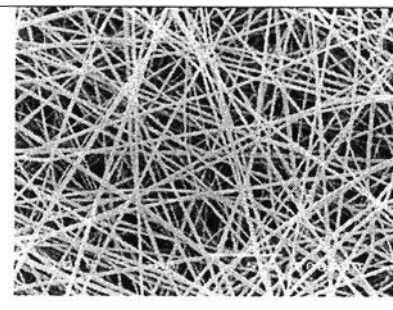
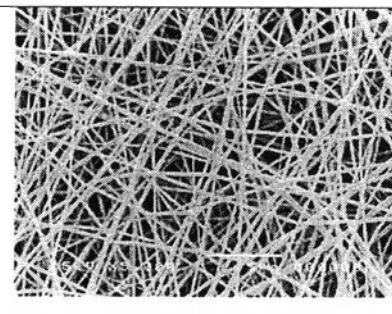
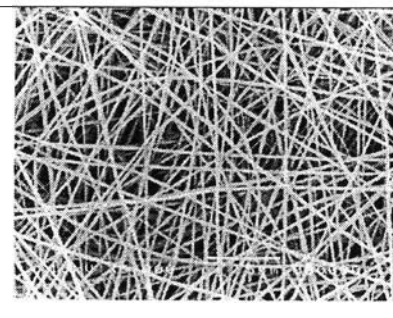
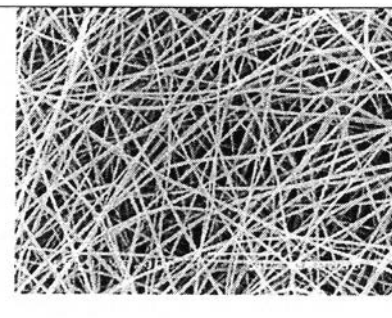
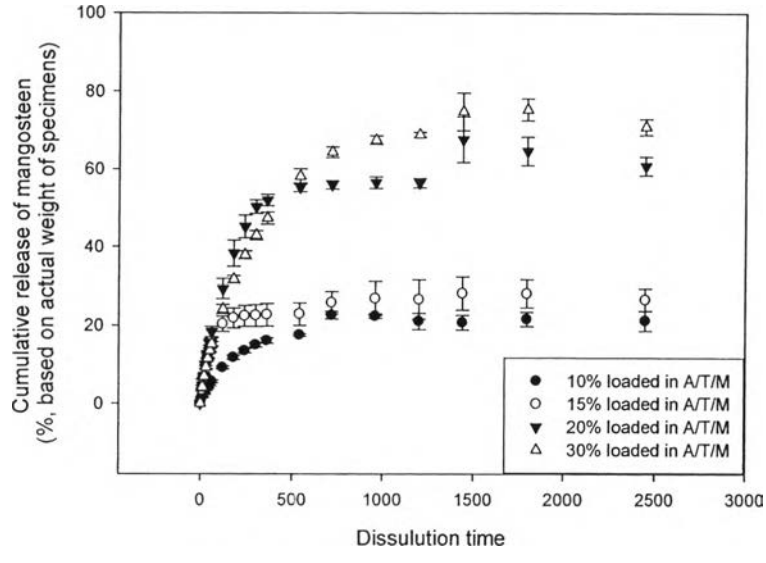
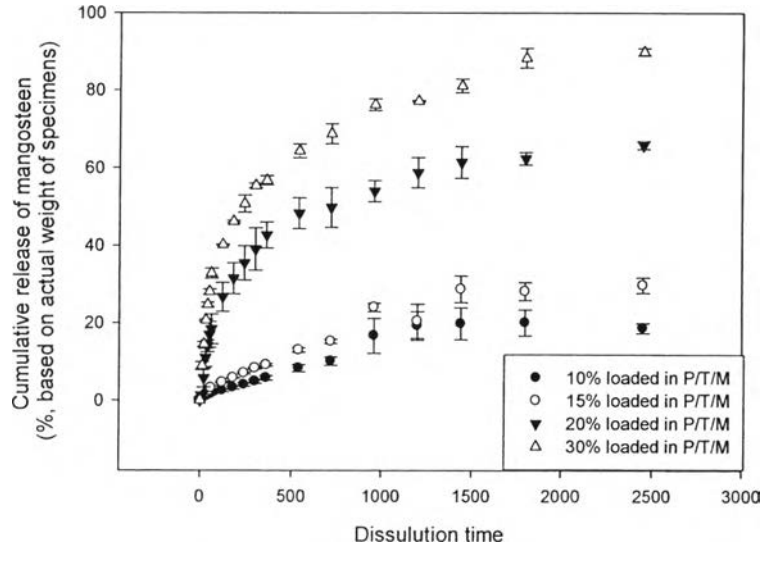
Type of materials	Electrospun fiber mats
Neat PAN e-spun fiber mats	
10 % *G.mangostana* -loaded PAN e-spun fiber mats	
15 % *G.mangostana* -loaded PAN e-spun fiber mats	
20 % *G.mangostana* -loaded PAN e-spun fiber mats	
30 % *G.mangostana* -loaded PAN e-spun fiber mats	

Figure 6.1 Representative SEM images illustrating morphology of electrospun fibers from 10% w/v PAN Solution in DMF and the amounts of 10 % to30 % *G.mangostana* by weight of PAN solution.



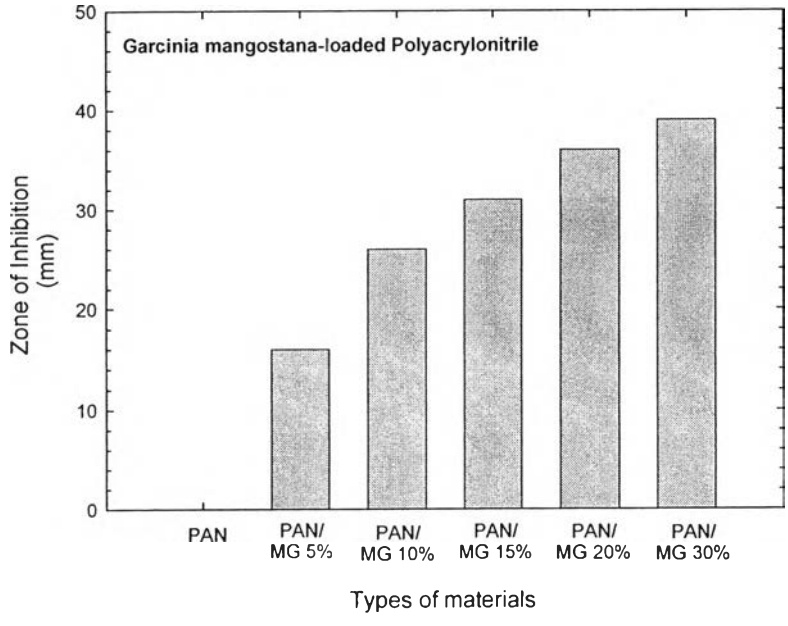
(a)



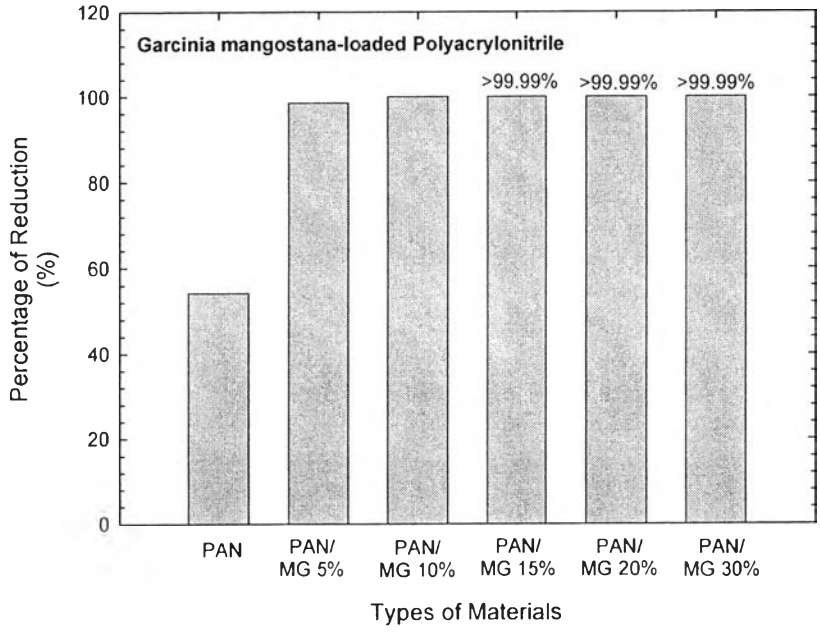
(b)

Figure 6.2 Cumulative release profiles of *G.mangostana* from *G.mangostana*-loaded electrospun PAN fiber mats reported as a percentage of the weight of *G.mangostana* released by total immersion method in

a) acetate buffer/tween 80/methanol (A/T/M medium) (pH 5.5) at 32 °C,and b) Phosphate buffer/tween 80/methanol (P/T/M medium) (pH 7.4) at 37 °C for various time intervals (n=3).



(a)



(b)

Figure 6.3 Average lengths of the inhibition zones of multidrug-resistant tuberculosis of the neat and *G.mangostana* -loaded e-spun PAN fiber mats.