CHAPTER IV
RESULTS AND DISCUSSIONS

4.1 Polyvinyl Acetate Solubility

Polyvinyl acetate usually dissolve in Esters such as methyl acetate, ethyl acetate etc. and partially dissolve in alcohol for instant ethanol, propanol and methanol (*et al.*, 2000).

The proper amount of ethyl acetate that can dissolve Polyvinyl acetate completely was investigated by varying the ratio of ethyl acetate in mixing solvent at 10%, 20%, 30%, 40% and 50%, respectively. The characteristic of PVAc solution were show in Figure 4.1.

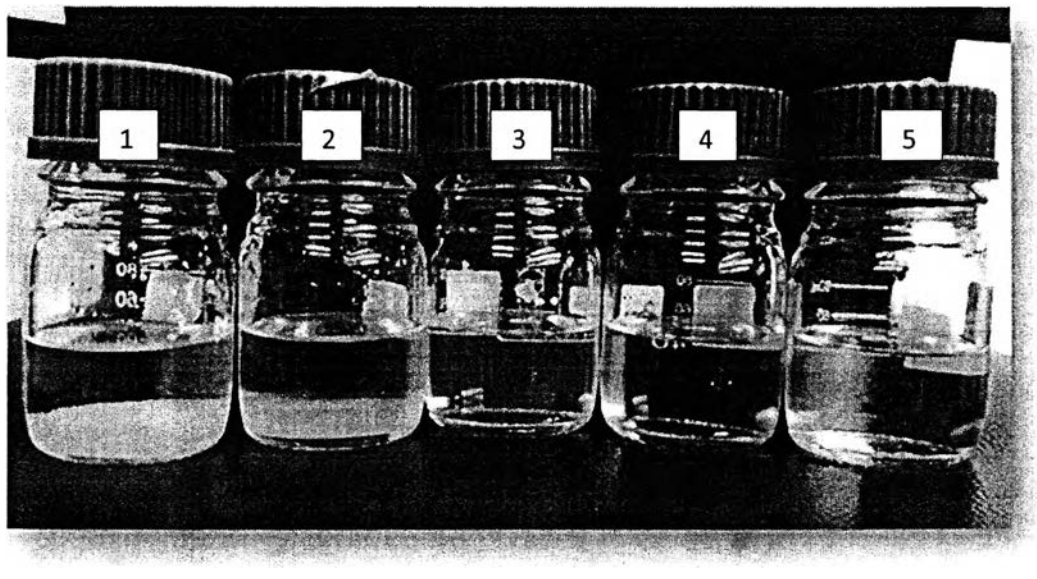


Figure 4.1 Characteristic of PVAc solutions by varyning the percentage of ethyl acetate solvent after left for 2 weeks, 1) 10% 2) 20% 3) 30% 4) 40% 5) 50%.

After left the solutions for 2 weeks, both 10% and 20% of ethyl acetate cannot dissolve PVAc, just swell and precipitated at the bottom. On the other hand,

at least 30% of ethyl acetate can dissolve PVAc completely due to apparent solution in the bottom number 3, 4 and 5.

Moreover, Turbidity measurement (JIS K0101) was used to perform turbidity of solution based on UV-absorbance at 660 nm (Gillett *et al.*, 1949). From Figure 4.2, the highest UV-absorbance has been detected at 10% and 20% of ethyl acetate due to incompletely dissolving PVAc. Whereas 30% of ethyl acetate showed low UV-light absorbance that mean no suspension solid of PVAc exist in solution.

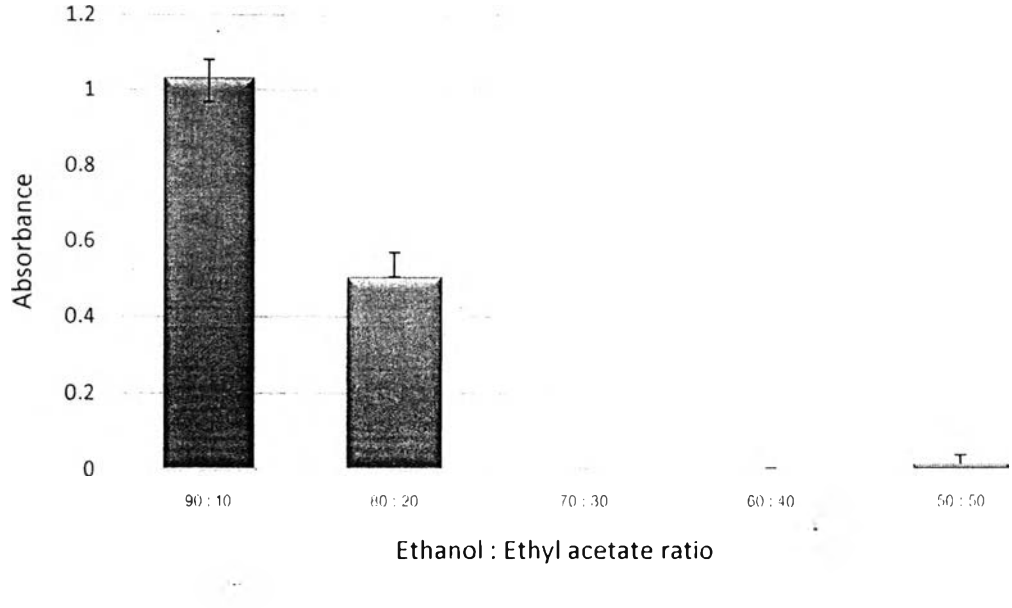


Figure 4.2 Absorbed light turbidity by varying amount of ethyl acetate.

4.2 Effect of Mixing Solvent on Spray Solution

The effect of mixing solvent on PVAc solubility was investigated by using turbidity measurement (JIS K0101). Figure 4.3 show absorbed light turbidity of mixing solvent by fix amount of ethyl acetate at 30% and varying the ratio of ethanol and propanol. In the progress, Propanol was added into solution at 0%, 10%, 20%, 30% and 40%, respectively. The solution performed low UV light absorbance until 20% of propanol then at 30% of propanol has higher UV-light absorbance and the highest at 40%. So the maximum amount of propanol that can be used is 20%.

Moreover the characteristic of mixing solvent of each solution has the same result which are showed in Figure 4.4.

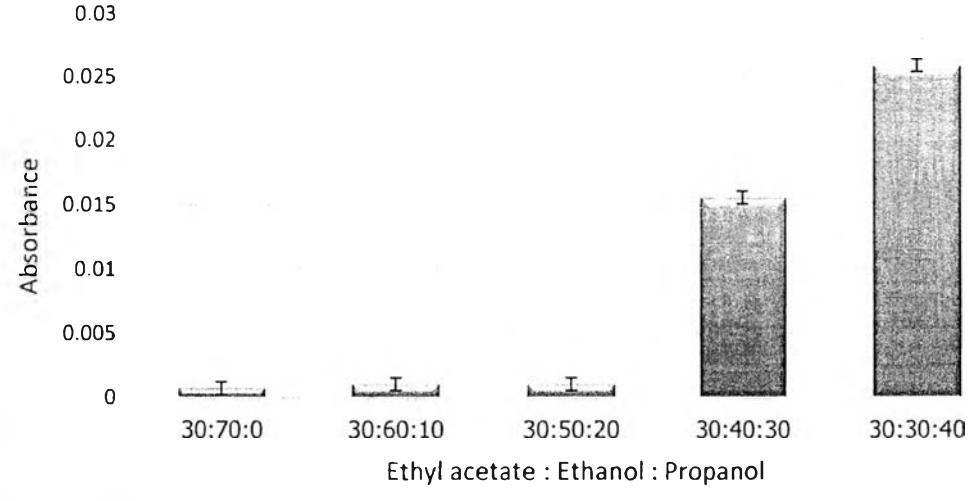


Figure 4.3 Absorbed light turbidity of mixing solvent.

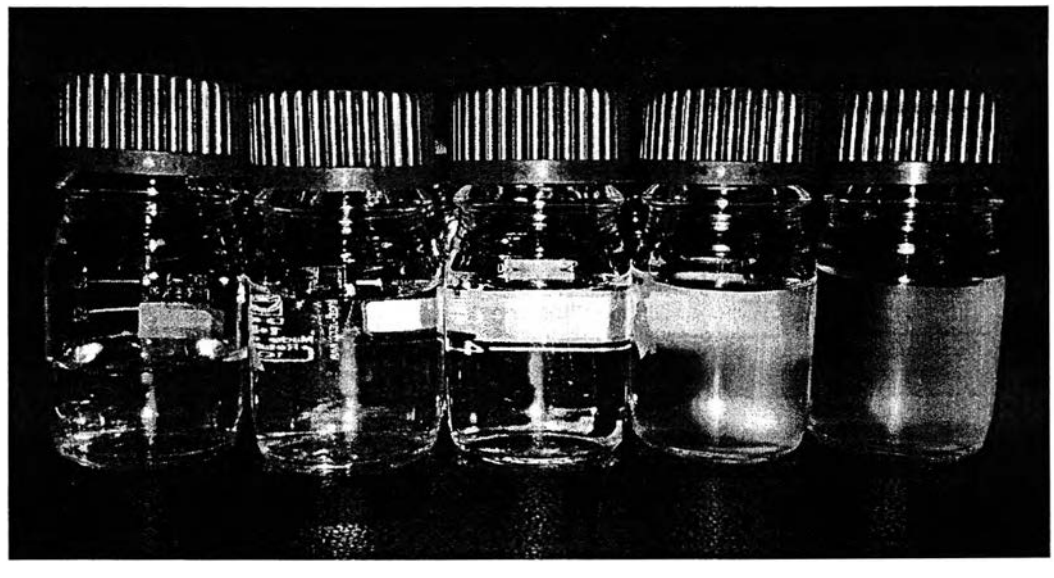


Figure 4.4 Characteristic of PVAc solutions after left for 2 weeks based on mixing solvent.

In order to fabricate spray dressing for wound, Evaporation rate will be concerned (Keshani *et al.*, 2015). In this work, the evaporation rate of mixing solvent was investigated by study on relationship between weight and time.

As the Figure 4.5 show the graph of the weight loss related to the function of time with slope scaling for the evaporation rate of the solution. From the curve, the percent weight rapidly decrease at the initial followed by a plateau with gradually loss.

Propanol concentration has an effect on evaporation rate. At mixing solvent without propanol (dot line) the weight gradually decrease until 6 minutes left. Whereas the highest component of propanol show a sharply decrease and steady at 5 minutes left. Thus, Propanol concentration is proportional to evaporation rate.

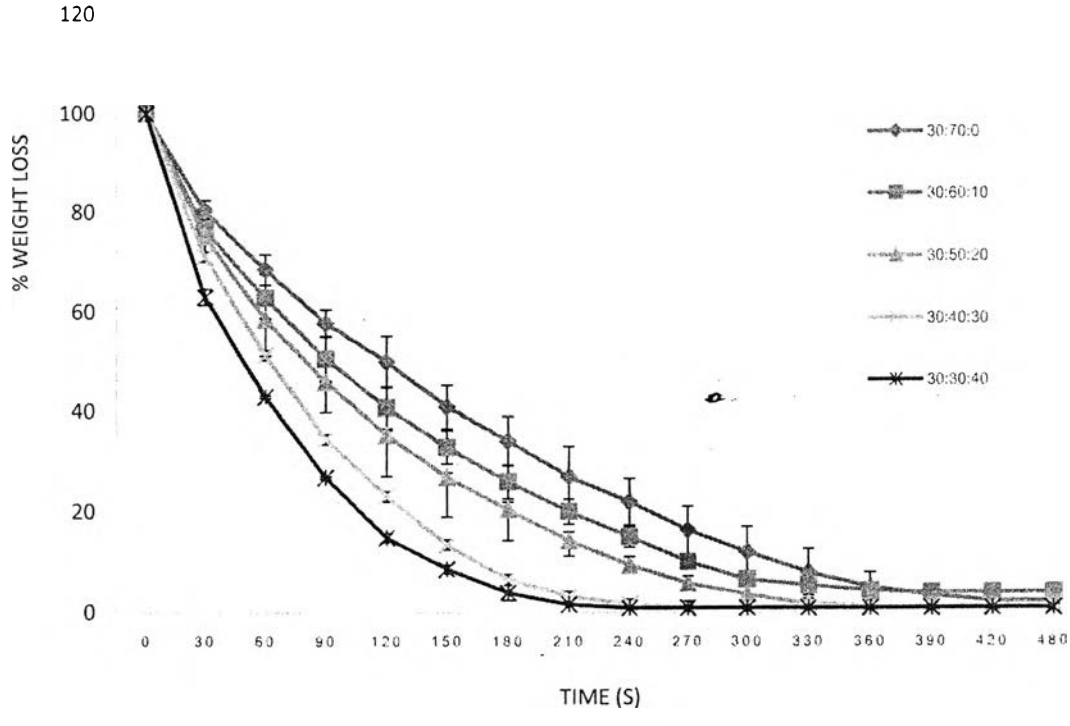


Figure 4.5 Evaporation rate of mixing solvent.

4.3. Polyvinyl Acetate Concentration

To Produce spray dressing product, solution viscosity has to concern (Keshani *et al.*, 2015). The specific concentration is the reciprocal of intrinsic viscosity of the solution (Bauer *et al.*, 1998). The intrinsic viscosity is the increase in restive viscosity caused by the dissolved polymer and thus is as indication of the hydrodynamic interaction between the polymer and the solvent (Nadkarni *et al.*, 1975).

As the Figure 4.6 shows the kinetic viscosity of several PVAc concentration. As the concentration of PVAc increased, solution viscosity increase. Viscosity influences droplet spreading, with more viscous solution not able to readily spread across the substrate surface before solvent evaporation occur (Felton *et al.*, 2013).

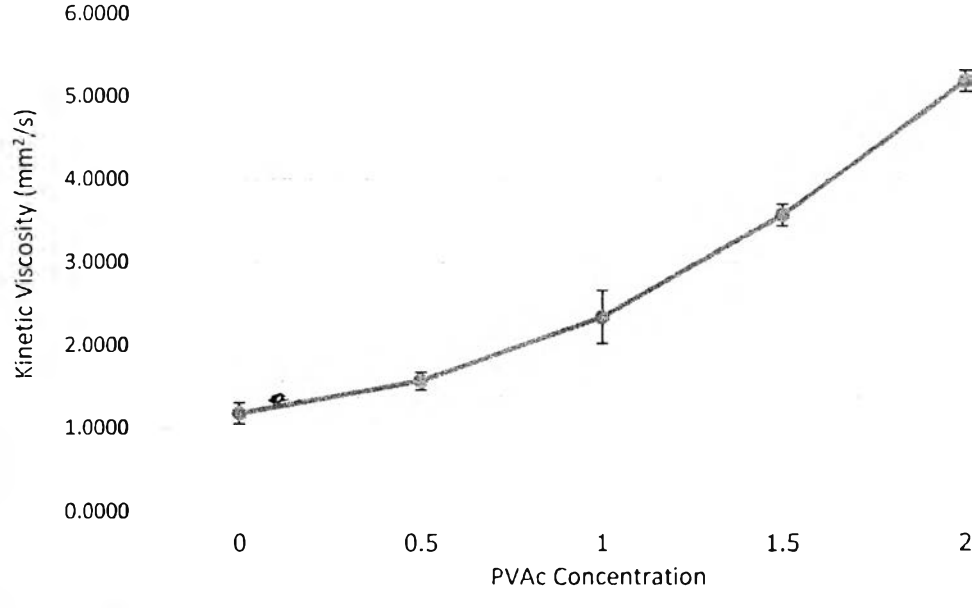


Figure 4.6 Solution viscosity of solution by varying polymer concentration.

Moreover, Polymer concentration also influences evaporation rate. In Figure 4.7 show the weight loss of several polymer concentration solution and commercial products. From the curves, as the slope of curve is evaporation rate, the percent weight rapidly decrease at the initial followed by a plateau with gradually loss.

Whereas PVAc concentration increase and evaporation rate decrease. At higher polymer concentration, the skin layer formed during evaporation step is resistance barrier between bulk of dressing and skin because of that the before drying will be longer.

The rate of solvent evaporation is critical in the film formation process for both polymeric solutions and dispersions. If a solvent evaporates too slowly, the substrates become overwetted and, in extreme cases, begin to dissolve. In contrast, if a solvent evaporates too quickly, the polymer-containing droplets may dry before either impinging on the substrate surface (spray drying) or spreading on the surface (orange peel effect). Solvent evaporation is dependent on temperature, atmospheric pressure, air movement, and in the case of water, relative humidity. Most of these variables can be adjusted by manipulating processing conditions (Felton *et al.*, 2013).

Furthermore, solid content of spray solution is proportional to polymer concentration. Finally, 20% of PVAc has a save solid content with both commercial products produced by 5 times spaying. However, solid content also depended on times to spray. Figure 4.8 shows weight of solid content after left 24hr by varying time to spay. Solid content increase due to an increase of time to spray

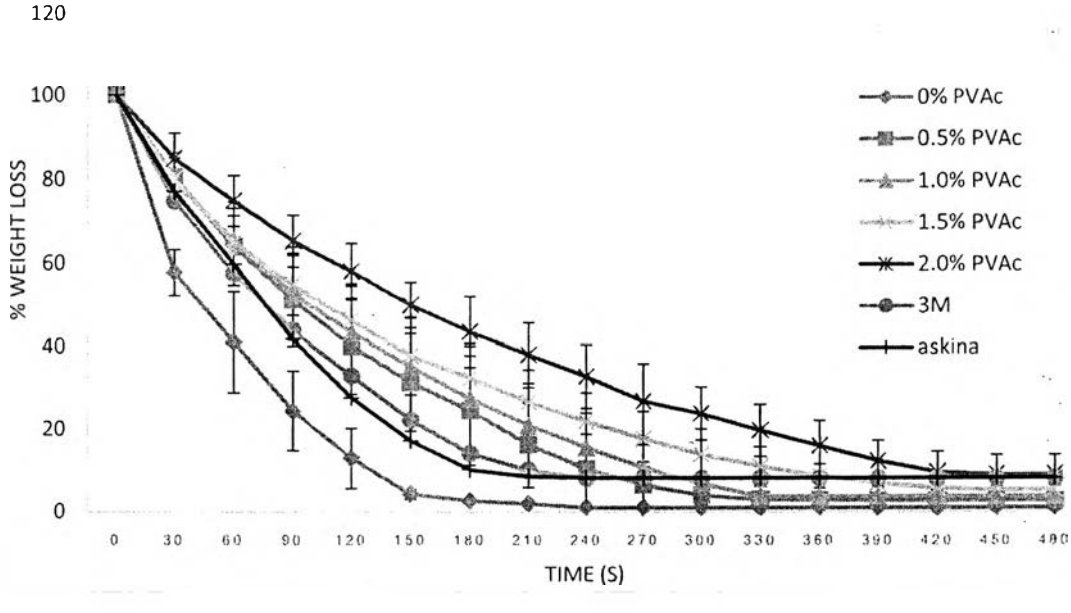


Figure 4.7 Rate of evaporation based on concentration of PVAc.

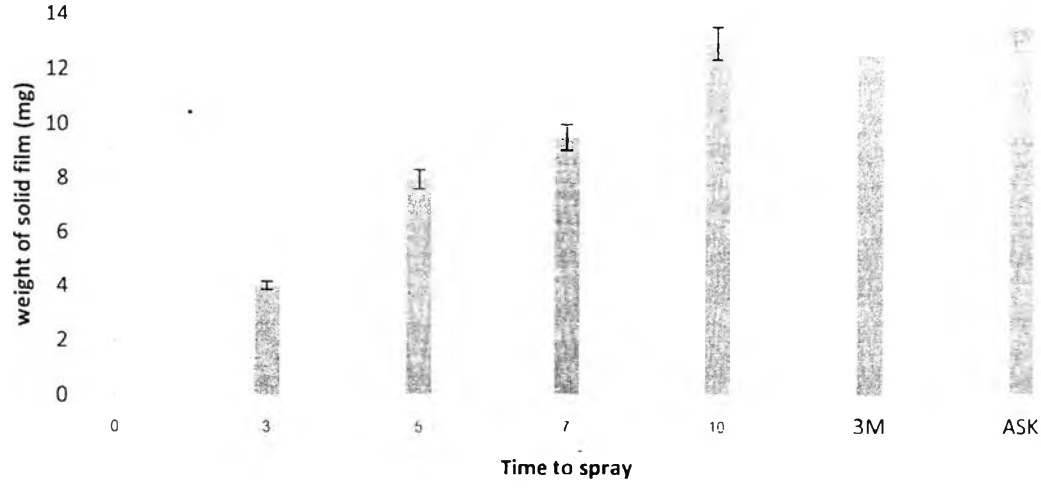


Figure 4.8 Solid content of each specimens by varying sprayed times.

4.4. Antibacterial Activity of Xantone

Table 4.1 MIC and MBC value of Mangosteen extract

Bacteria	MIC (µg/ml)	MBC (µg/ml)
Escherichia coli (E. coli)	1.56	100.00
Staphylococcus aureus (S. aureus)	0.39	12.50
Methicillin-resistant *Staphylococcus aureus* (MRSA)	1.56	6.25
Staphylococcus epidermidis (S. epidermisdis)	1.56	1.56
Acinetobacter baumannii (MDR)	12.50	50.00
Enterococcus faecalis (En. Faecalis)	12.50	-
Vancomycin-resistant *Enterococcus* (VRE)	-	-
Pseudomonas aeruginosa	50.00	-

The antibacterial activity of mangosteen extract have been reported against both gram-positive and gram-negative bacteria (Charernsriwilaiwat *et al.*, 2013). Table 4.1 shows the anti-bacterial activity of Xantone. Xantone is an active

compound founded in all part of mangosteen. More than 20 xantones were found in mangosteen. Both of α and β – mangostin (Pedraza-Chaverri *et al.*, 2008), especially in a peel of mangosteen. Xantone can inhibit several microorganisms.

MIC and MBC value of tested xantone extract ranged from 0.39 – 100 μg/ml against the 9 microorganisms (Table 4.1). This result ensure the strong antibacterial activity of extracted mangosteen on both gram positive such as (+) and gram negative for instant (-). From the table 4.1, the lowest amount of mangosteen extract which can inhibit all microorganisms equal to 100 μg/ml.

In this study, mangosteen extract was loaded by varying amount of extracts at 1%, 2%, and 3% w/w, respectively. The kinetic viscosity slightly increase deepened on the value of extracts as show in Figure 4.9.

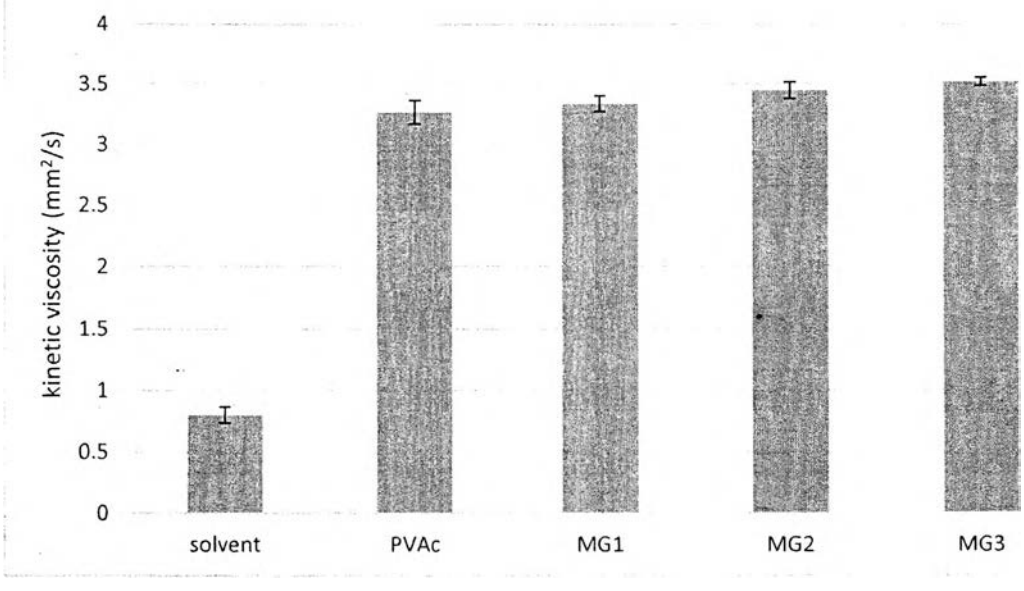


Figure 4.9 Solution viscosity of spray's solution due to varying amount of mangosteen extract.

Note: MG1 = PVAc solution was loaded with 1% w/w of mangosteen extract
MG2 = PVAc solution was loaded with 2% w/w of mangosteen extract
MG3 = PVAc solution was loaded with 3% w/w of mangosteen extract
CM1 = Commercial product 1
CM2 = Commercial product 2

Each spray solution was spray on spun-boned filter as a constructer. Then, Disk diffusion method ATCC147 was chosen to investigate the effectiveness of mangosteen extract releasing to kill microbial on both agar plate. As a result shows in Figure 4.10.

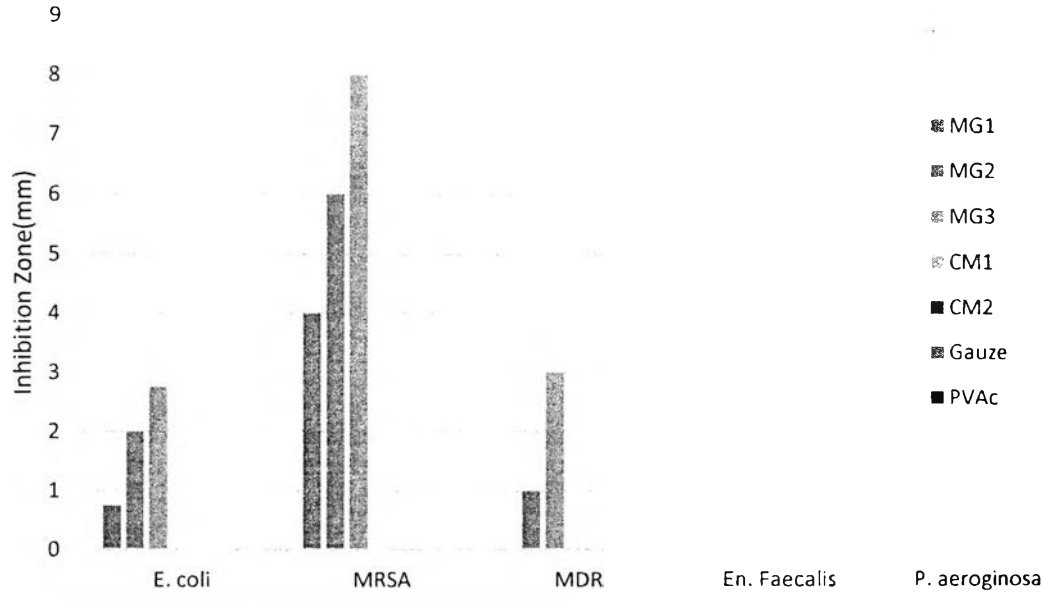


Figure 4.10 Inhibition zone of each sample.

The inhibition zone around the samples use to ensure that mangosteen extract can inhibit microorganism's growth. The highest effectiveness of mangosteen extract show on 3% w/w with broadest inhibition zone follow by 2%, and 1%, respectively. Some bacteria did not appear inhibition zone around the samples which are *Enterococcus faecalis* and *Pseudomonas aeruginosa.*

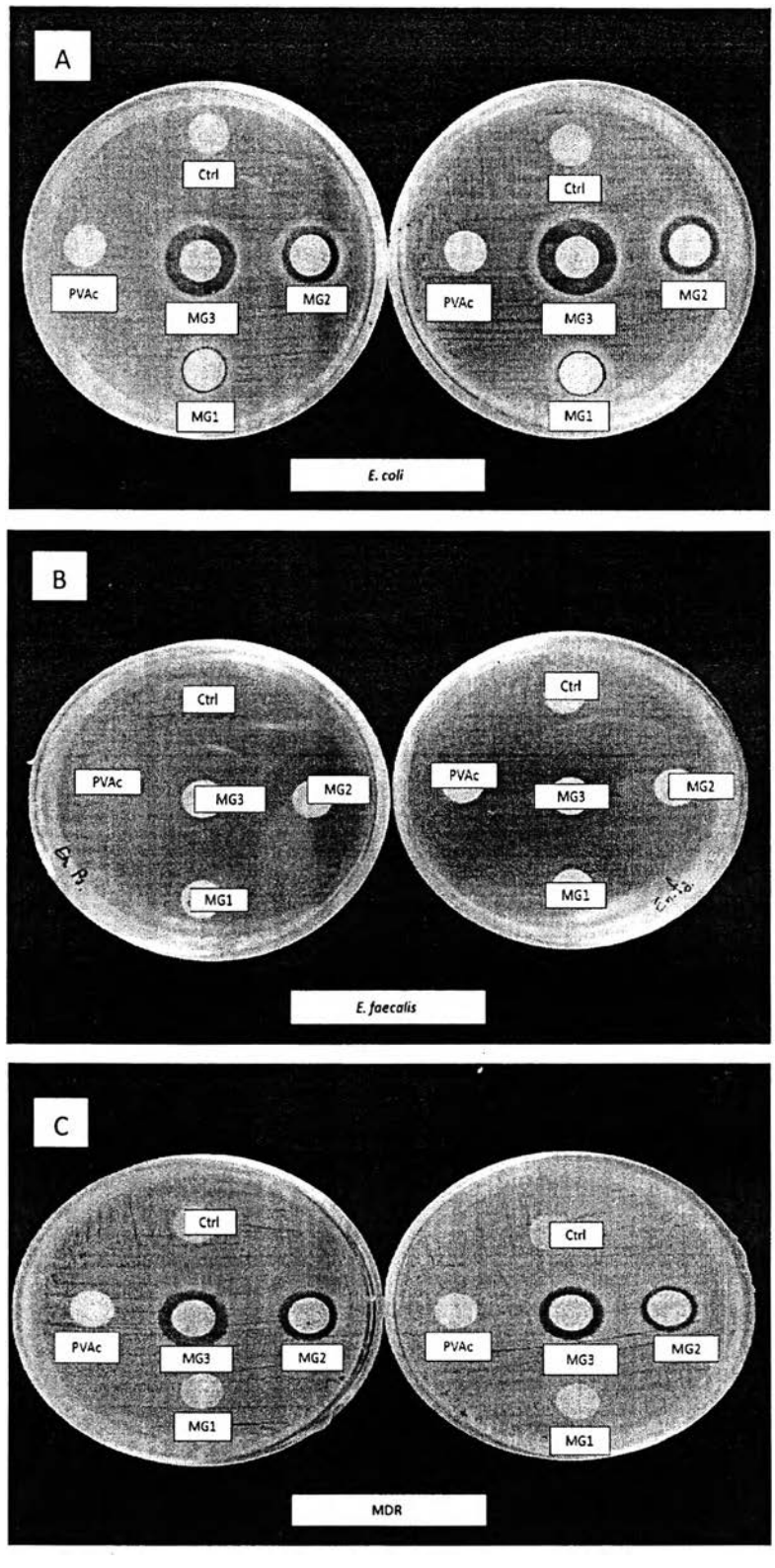


Figure 4.11 Illustration of inhibition zone around samples A) *E. coli* B) *E. farcalis* C) MDR D) MRSA E) *P. aeruginosa* F) *S.aureus* G) *S. epidermidis* H) VRE.

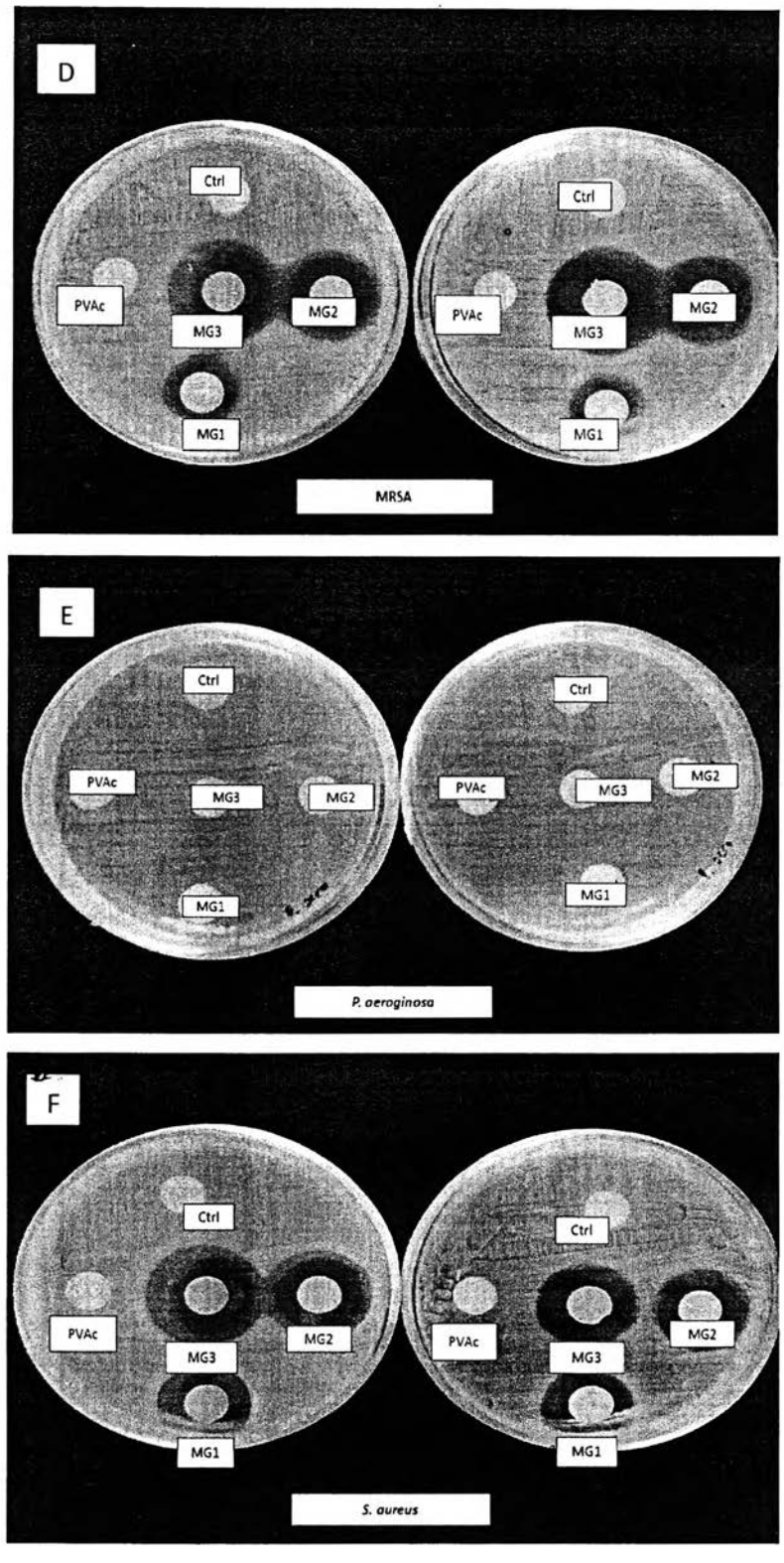
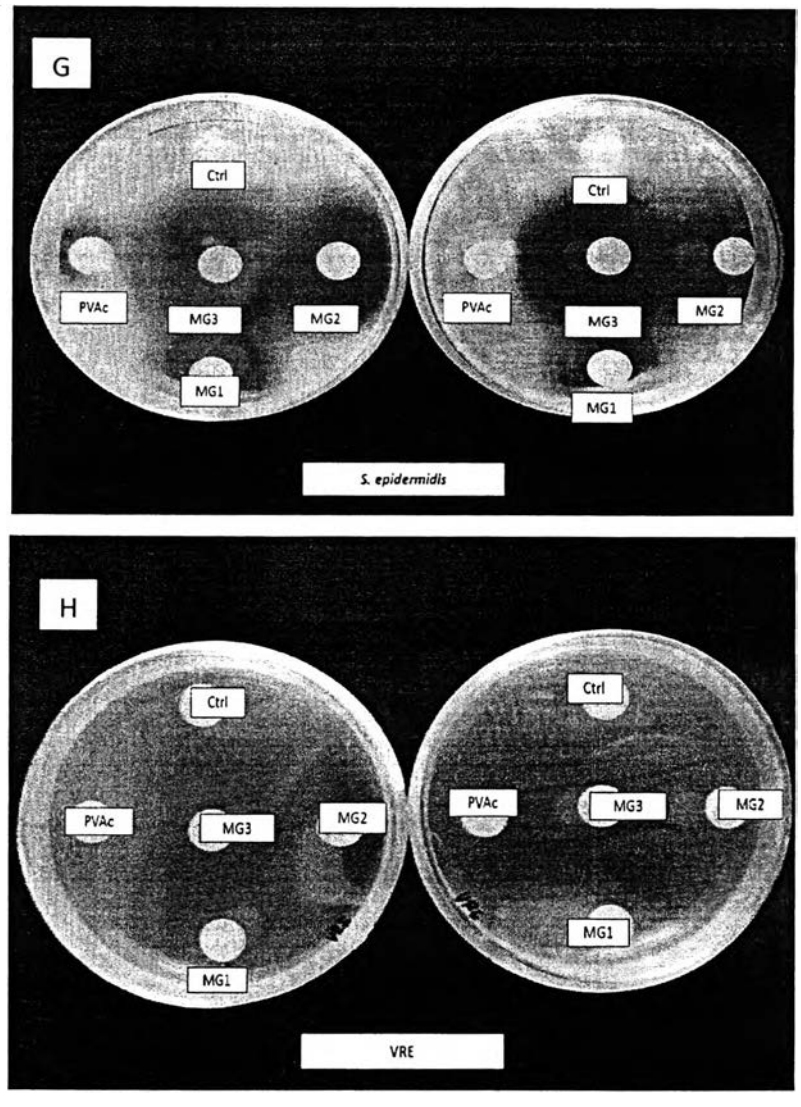


Figure 4.11 Illustration of inhibition zone around samples A) *E. coli* B) *E. farcalis* C) MDR D) MRSA E) *P. aeruginosa* F) *S.aureus* G) *S. epidermidis* H) VRE (con't.).



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In order to observe the nature of the action against gram-positive and gram-negative bacteria, Time-kill assay was chosen. The figure 4.12 show the bacterial survivors (CFU/ml) that exist on broth agar plates. Mangosteen extract can kill all microorganisms completely after dropping bacteria solution on the samples. At least 2% of mangosteen extract was required to kill *P. aeruginosa* in 24 hours (Figure 4.12A). For E. faecalis, it shows the same result (Figure 4.12E). On the other hand, the commercial products still have bacterial growth. As the result of MDR, bacterial viability gradually reduced based on time of each sample. Only 3% of mangosteen

extract can prohibit microbial completely after left samples in oven for 24 hours. In the contrast, MDR can grew on film of commercial products (Figure 4.12B). Then MRSA shows the same results (Figurs 4.12D). The lowest amount of mangosteen extract that can prohibit *E. coli* and the colony of *E. coli* disappeared on the broth agar plates is 1% w/w. of mangosteen extract. Whereas, the commercial products had more the number of *E. coli*'s colony that almost equal to controls (Figure 4.12). Thus, the antibacterial activities is proportional to the mangosteen extract concentration.

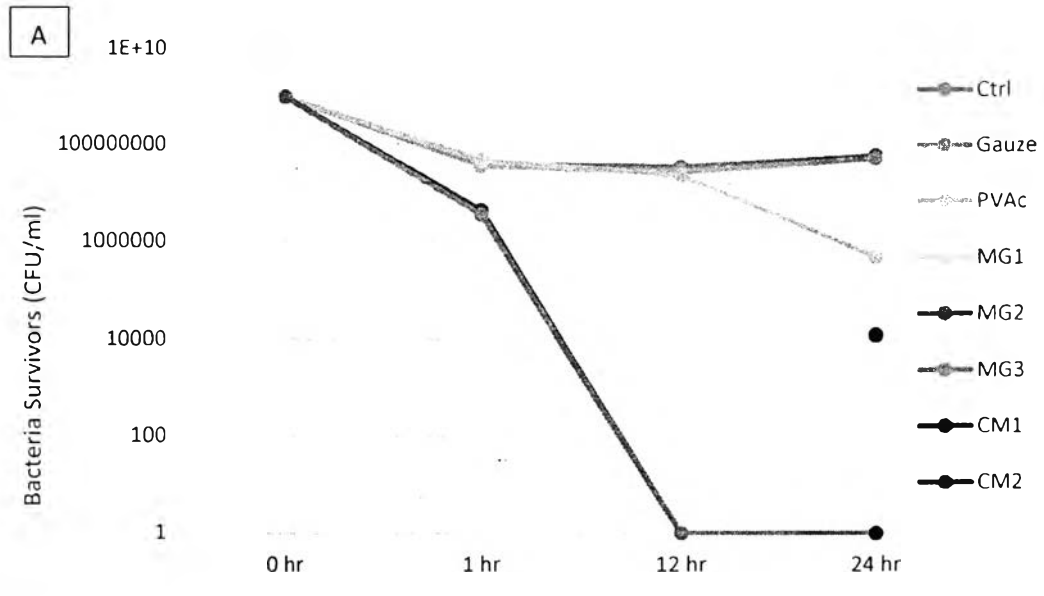
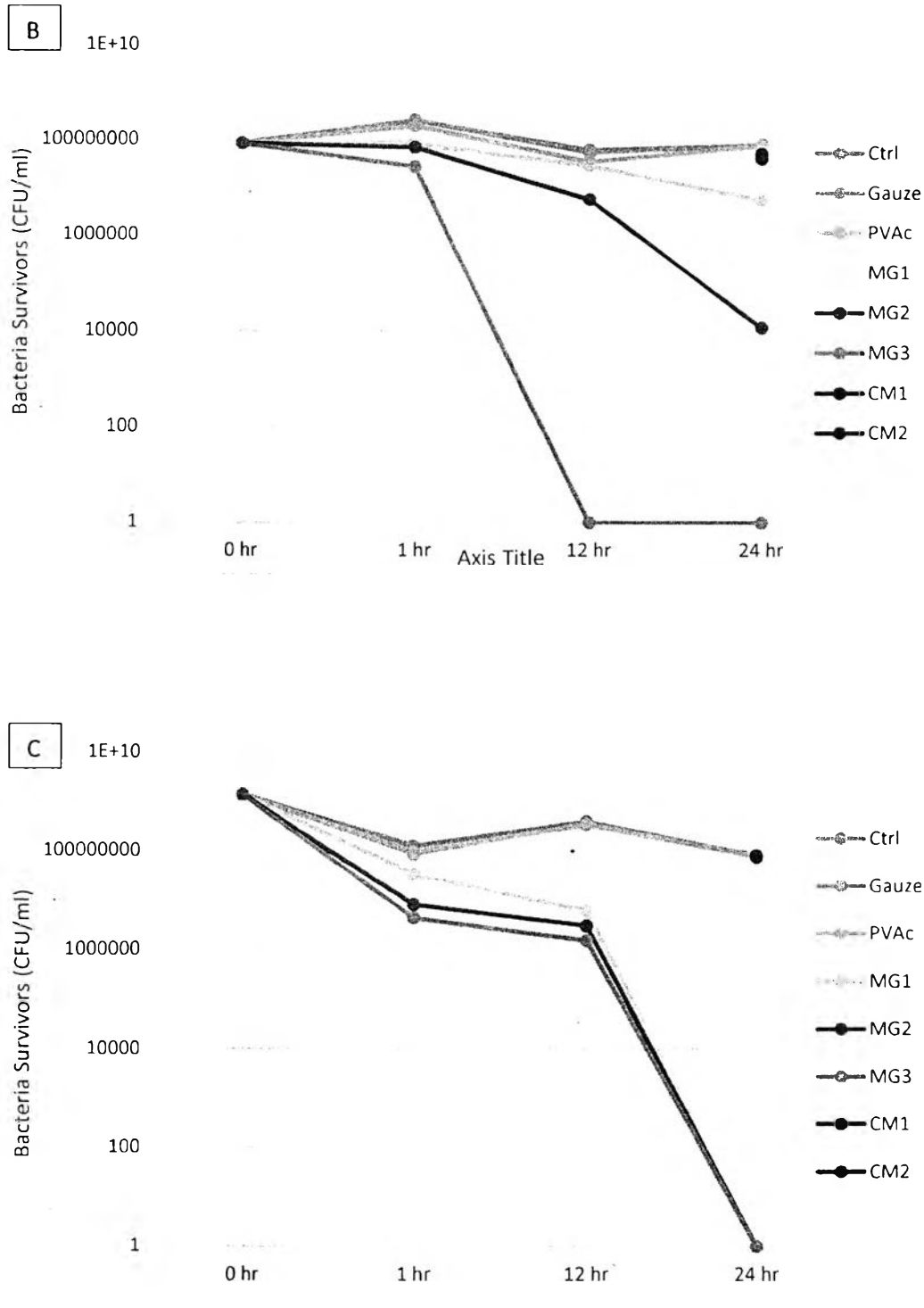


Figure 4.12 Effect of mangosteen extract concentration on bacteria reduction A) *P. aeroginosa* B) MDR C) E. coli D) MRSA E) *E. faecalis*.



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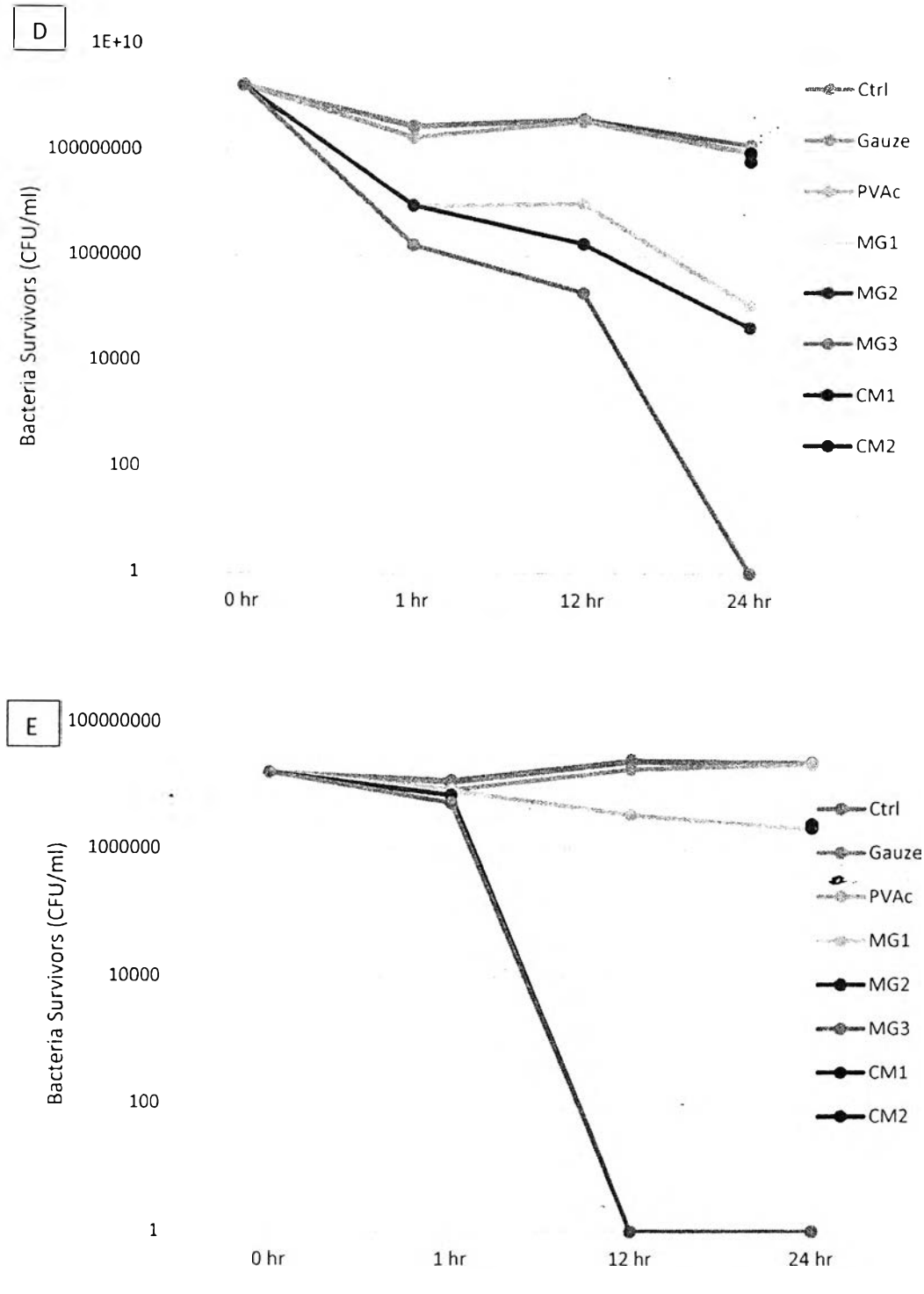

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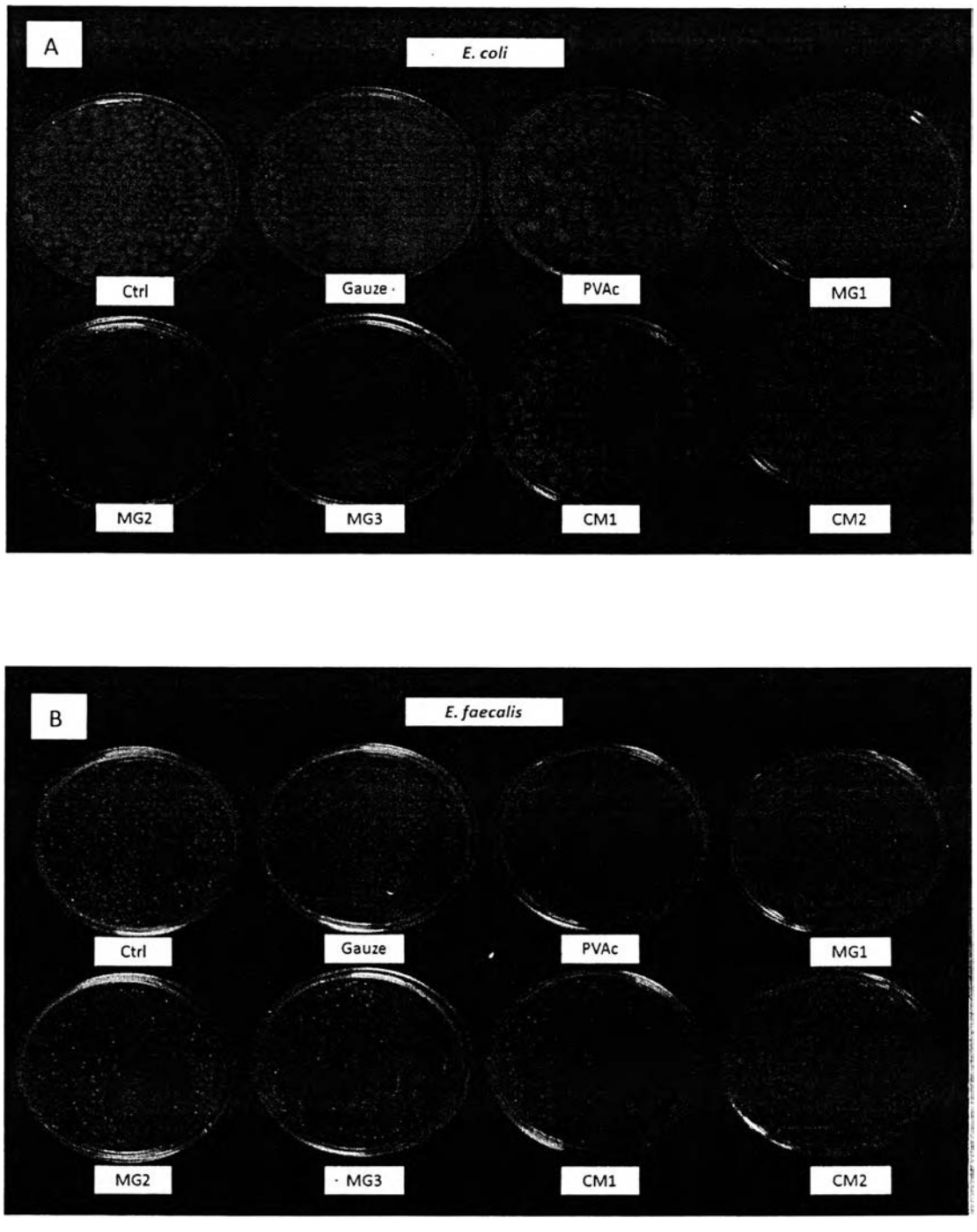

Figure 4.13 Bacteria on broth agar plate at the same dilution after dropping bacteria solution on each samples for 24 hours.

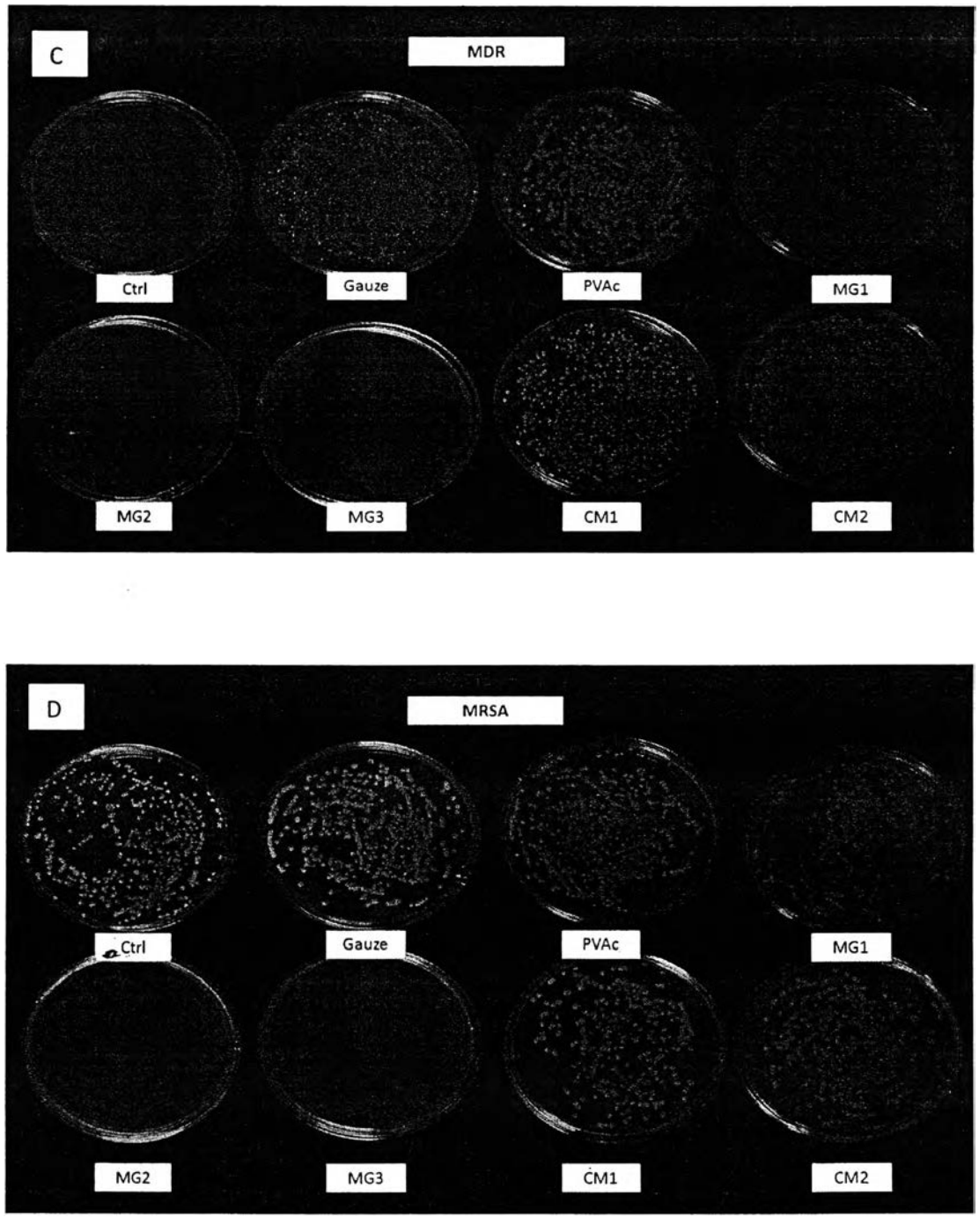

Figure 4.13 Bacteria on broth agar plate at the same dilution after dropping bacteria solution on each samples for 24 hours (con't.).

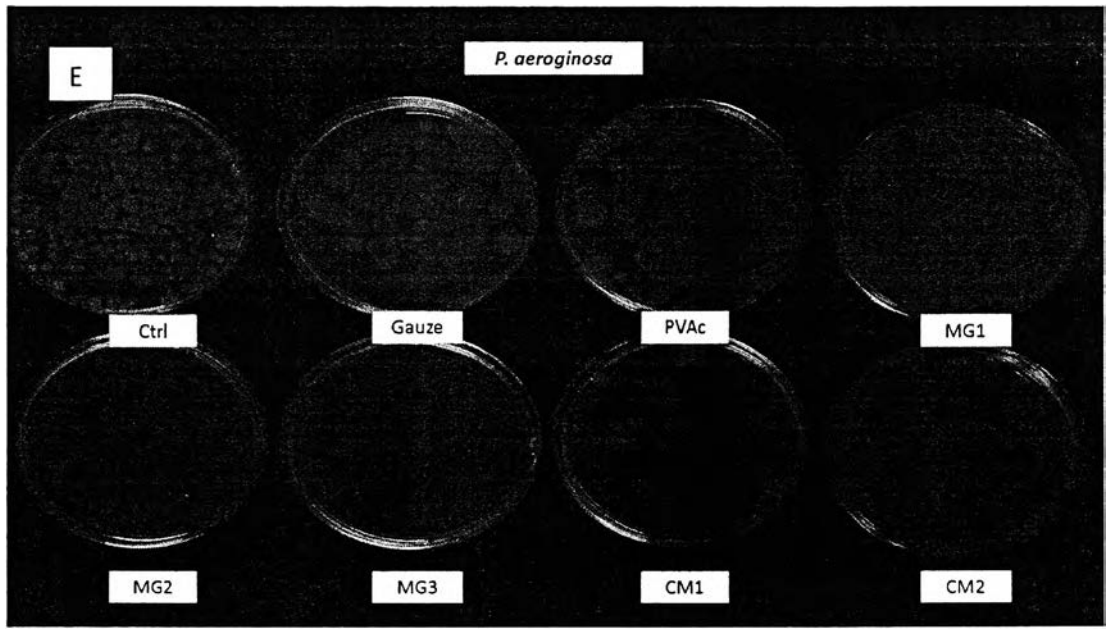

Figure 4.13 Bacteria on broth agar plate at the same dilution after dropping bacteria solution on each samples for 24 hours (con't.).

As the Figure 4.13 show bacterial colonies on the broth agar plates. From the picture it has been seen that the number of bacterial colonies declined when increase amount of mangosteen extract. The best concentration is 3% w/w of mangosteen extract that can prohibit bacterial growth both gram-positive and gram-negative bacteria when compare with control, only gauze, only PVAc, and few of commercial products.

4.5 Cytotoxicity

Although the mangosteen extract is good function on bacterial activities, the mangosteen extract dosages is effect on % cell viability (Wang *et al.*, 2011). The percentage of cell viability were investigated by indirect cytotoxicity method for 1 day and 3 days. As the Figure 4.14 show the percentage of L929 cell viability. The mangosteen extract is toxic to cell it can be seen form the graph. The percentage of cell viability of 3 days was lower than 1 day. In addition, the mangosteen extract dosages also effect on cell. The cell viability increase significantly until loading 2%

w/w of mangosteen extract and drop at higher concentration ($P < 0.05$). On 3 days, cell viability of commercial products is less than 80%. Thus, they are toxic and unsuitable to use as wound dressing.

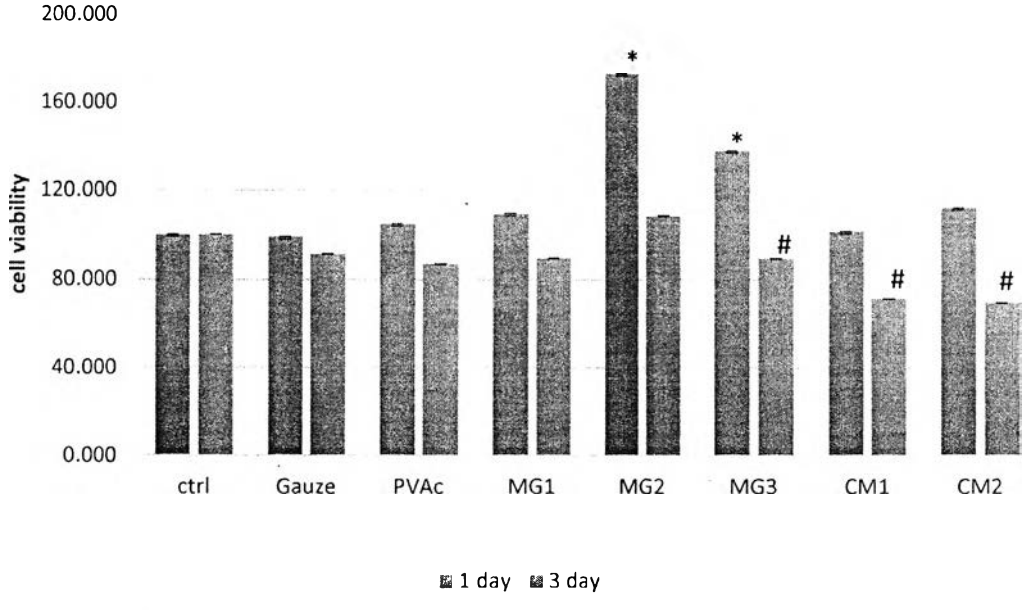

Figure 4.14 Cell viability of L929 of each sample.

For the HaCat cell (Figure 4.15), the cells can survive both 1 day and 3 days in each product. As 1 day results, the percentage of Hacat cell viability increase with mangosteen extract concentration. The results are same in commercial products. For 3 days results, it is the same except for 2% and 3% w/w of mangosteen extract. However, the Hacat cell viability are more than 80% for all dressing so it can be used for wound dressing.

The mangosteen extract is non-toxic to NHF cell that the result shows in Figure 4.16. The percentage of NHF cell viability both 1 day and 3 days is higher than control. Only 3% w/w of mangosteen extract is slightly effect on NHF cell.

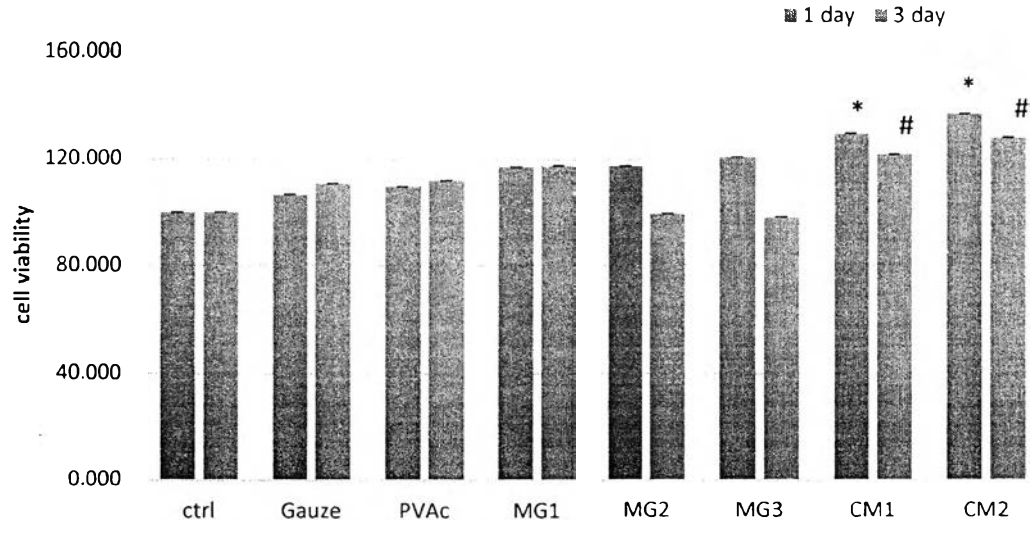

Figure 4.15 Cell viability of HaCat of each sample.

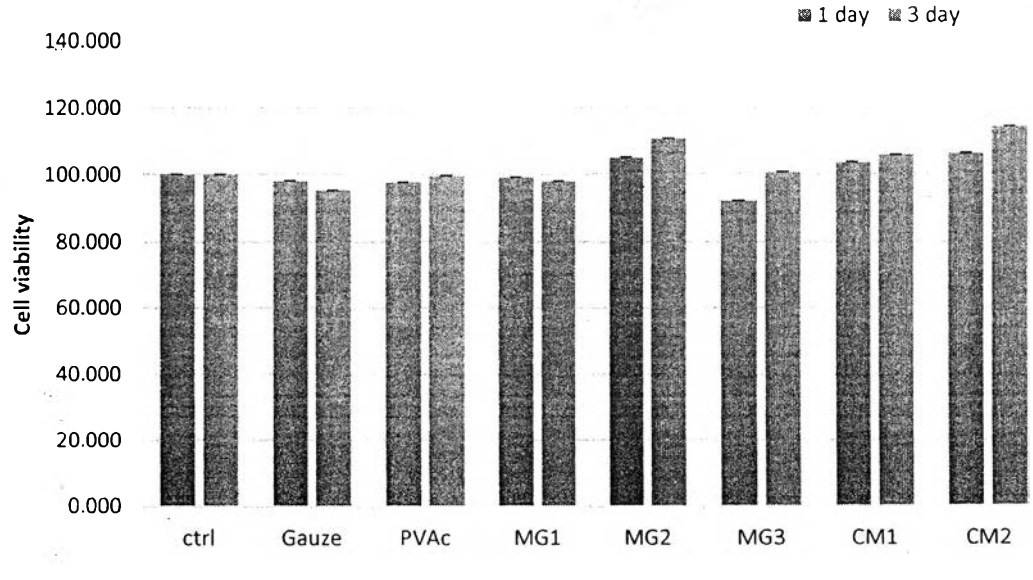

Figure 4.16 Cell viability of NHF of each sample.

4.6 Waterproof property

The contact angle results obtained by an image analyzing system. The contact angle on all sample including commercial product are given in Table 4.2. The waterproof property reduce when mangosteen extract concentration increase. Only PVAc has the highest waterproof property without effectiveness of gauze. In addition, after loading mangosteen extract, water contact angle results went down because the particles of mangosteen extract has more hydrophilicity than substrate when it combine with PVAc solution. The mangosteen extract will migrate to surface and irritate the system. So the waterproof property results gradually decreased. Moreover, in comparison with commercial products, the water contact angle results show the same case. The range of angles are around 100° – 110°.

Table 4.2 Water contact angle of samples and commercial products

Samples	Contact angle
Gauze	119.20
PVAc	116.13
MG1	110.70
MG2	109.46
MG3	105.86
CM1	105.10
CM2	106.80

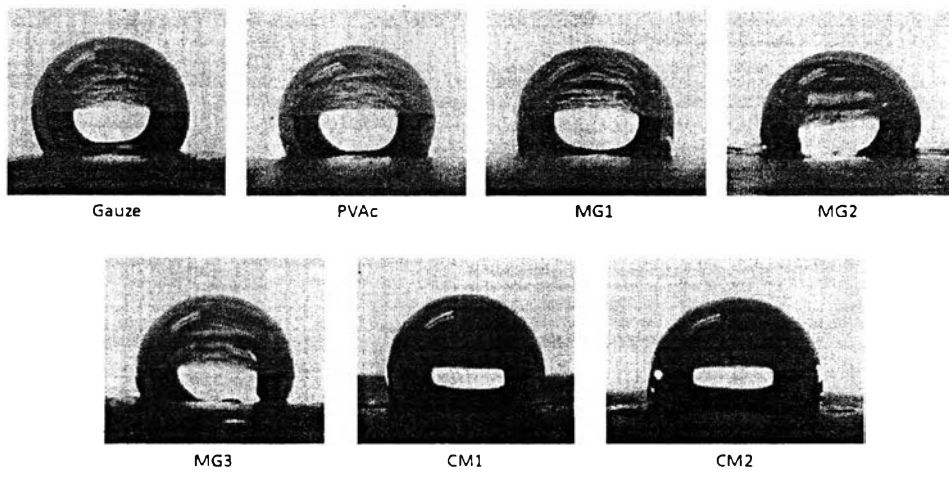

Figure 4.17 Contact angle of water on each samples.

4.7 *In vitro* Drug Release

In vitro drug release of mangosteen extract were observed by using UV-visible machine which are 1%, 2%, and 3% w/w of mangosteen extract in Phosphate buffer /Tween 80/Methanol (pH 7.4) and Acetate buffer/Tween 80/ Methanol (pH 5.5) at 380 nm. As the results show that the release of the amount of mangosteen extract is proportional to mangosteen extract concentration. At 3% w/w shows the highest cumulative release of mangosteen extract value based on actual weight of specimens following by 2% and 1%, respectively.

The mangosteen extract release in Acetate buffer/Tween 80/ Methanol (pH 5.5) faster than Phosphate buffer /Tween 80/Methanol (pH 7.4). It can be seen in Figure 4.18 and Figure 4.19 when comparing in the same time. The mangosteen extract spent the time for 10 hours to climb to the top in acetate buffer (pH 5.5). On the other hand, in phosphate buffer it spend more time for growing up to the plateau.

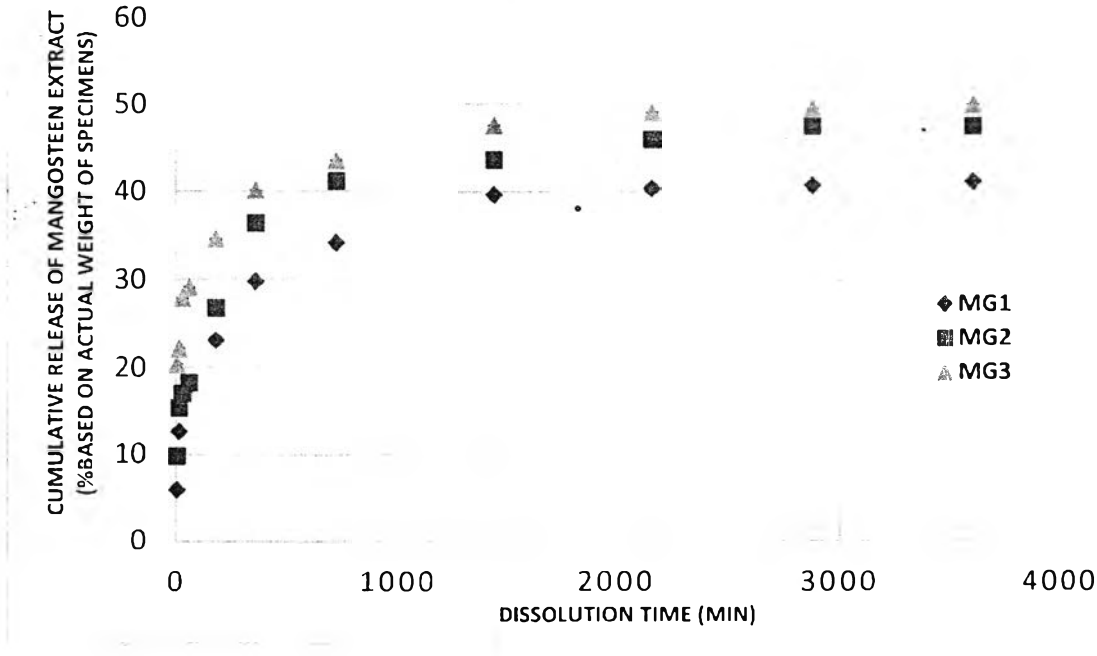

Figure 4.18 Illustration of mangosteen extract release in PBH buffer (pH 7.4).

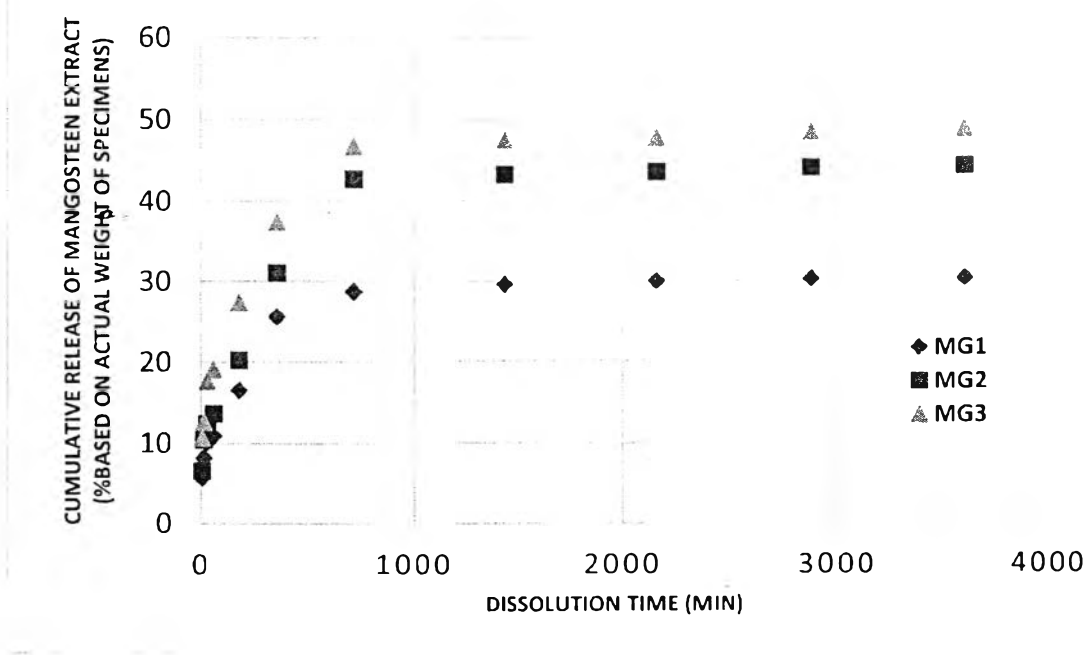

Figure 4.19 Illustration of mangosteen extract release in acetate buffer (pH 5.5).