CHAPTER IV

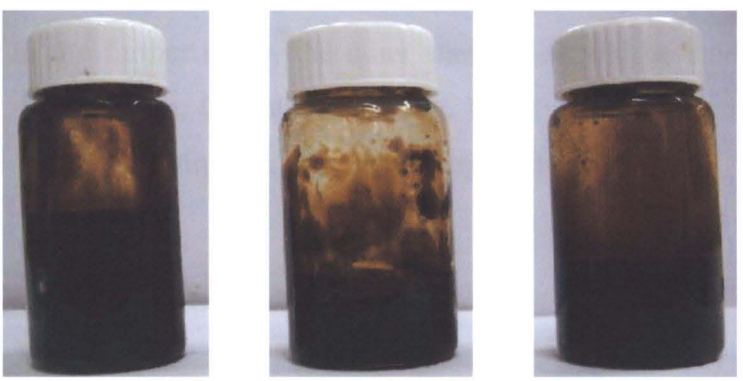
RESULTS AND DISCUSSION

4.1 Extraction of beehive

From 3 different solvents extraction: DI water, 50% ethanol and 95% ethanol. The results showed that the beehive extract from water (W) had the highest percentage yield 16.25% followed by the beehive extract from 50% ethanol (WE) 14.42% and the beehive extract from 95% ethanol (E) 8.67% respectively as shown in Table 4.1. The physical appearances of the extracts were dark brown color and honey like smell. (Figure 4.1)

Table 4.1 Beehive extracted by using different solvents

Extract	Weight of beehive (g)	Weight of extract (g)	% yield	Physical appearance
W	420.56	68.34	16.25	Dark brown, viscous and honey-like smell
WE	448.14	64.62	14.42	Dark brown, viscous and honey-like smell
E	416.39	36.10	8.67	Dark brown, viscous and honey-like smell



W WE E

Figure 4.1 Appearance of the beehive extracts

4.2 Quality control of chemical analysis

4.2.1 Determination of chromatographic finger print of the beehive extract by HPLC

HPLC is used almost exclusively for the qualitative and quantitative analysis. Retention times were utilized as primary criterion for peak identification. The mass spectrometer used as chromatographic detector offers additional data for the identification of separated compounds. The most frequent identification method is the comparison of recorded spectra with an MS library and/or reference standard compound.

The chromatographic finger print of W, WE and E extracts found that they had the similar pattern of chromatogram, five major peaks (A, C, D, E and H) were found as shown in Figure 4.2, but different in peak height. At the same concentration, the W extract exhibited the higher peak than WE and E extract. The results of this study might correspond to the antibacterial activity that the W extract revealed the higher activity than WE and E extracts.

In addition, the analyze of the W, WE and E extracts compared with standards such as Chlorogenic acid, Chysin, Ellagic acid, Gallic acid, Hesperidine, Morin, Pinocembrin, Quercetin, Rutin and Trans-cinamamide as shown in Figure 4.3 to Figure 4.4.

The results showed that all samples had the similar chromatogram pattern as of Morin (Figure 4.5 to Figure 4.7).

Althought E extract was move separated compounds than W and WE extract, the results could not identify when compared them with all of standard.

In the further study, the other standard should be studied comparing such as Pinobanksin, Galangin, Luteolin, Kaempferol, Benzamide, Galangin, Coumaric acid for indentifying the unknown compounds of E extract.

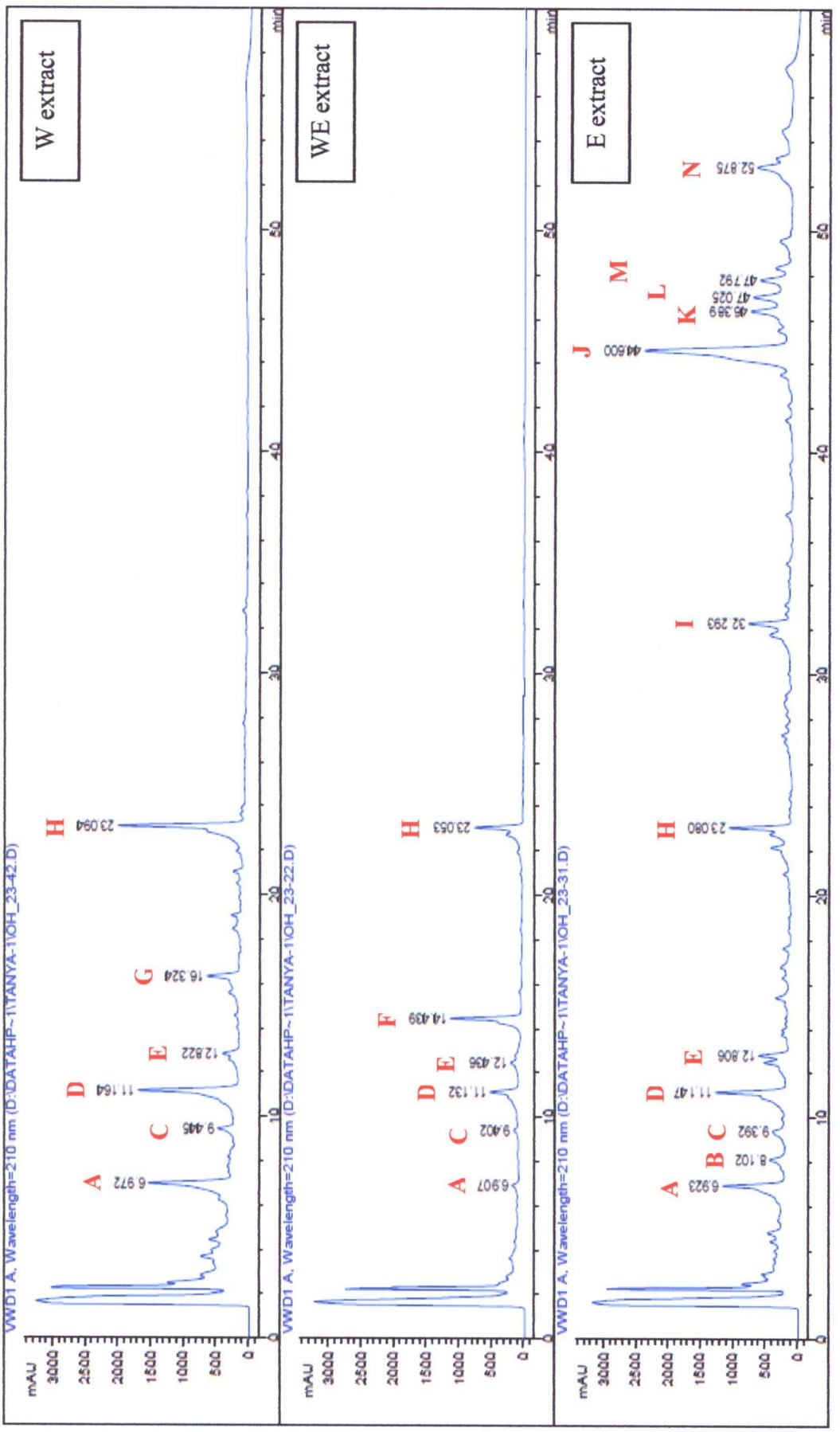


Figure 4.2 HPLC chromatogram of the W, WE and E extract at 210 nm

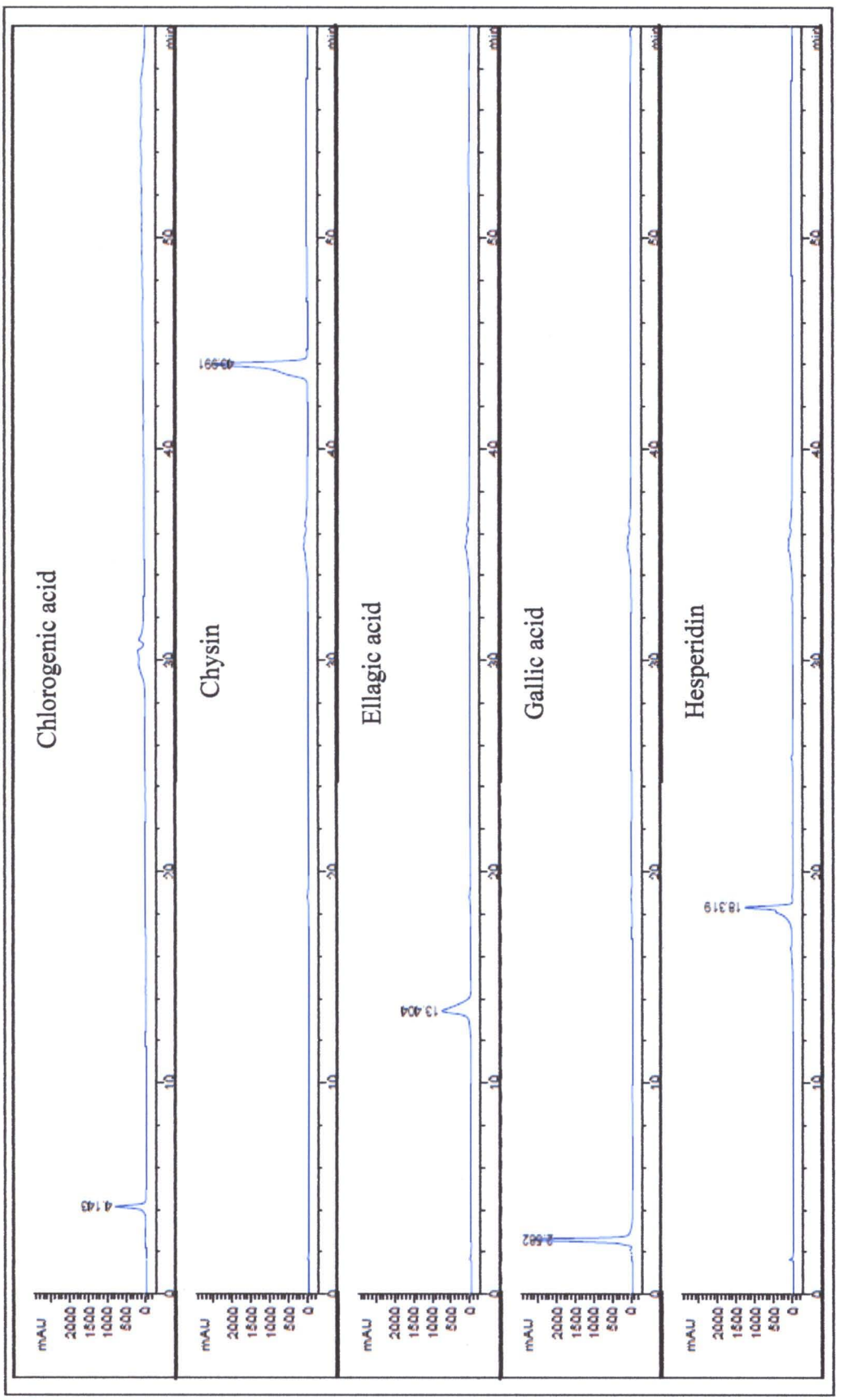


Figure 4.3 HPLC chromatogram of Chlorogenic acid, Chysin, Ellagic acid, Gallic acid and Hesperidin at 210 nm

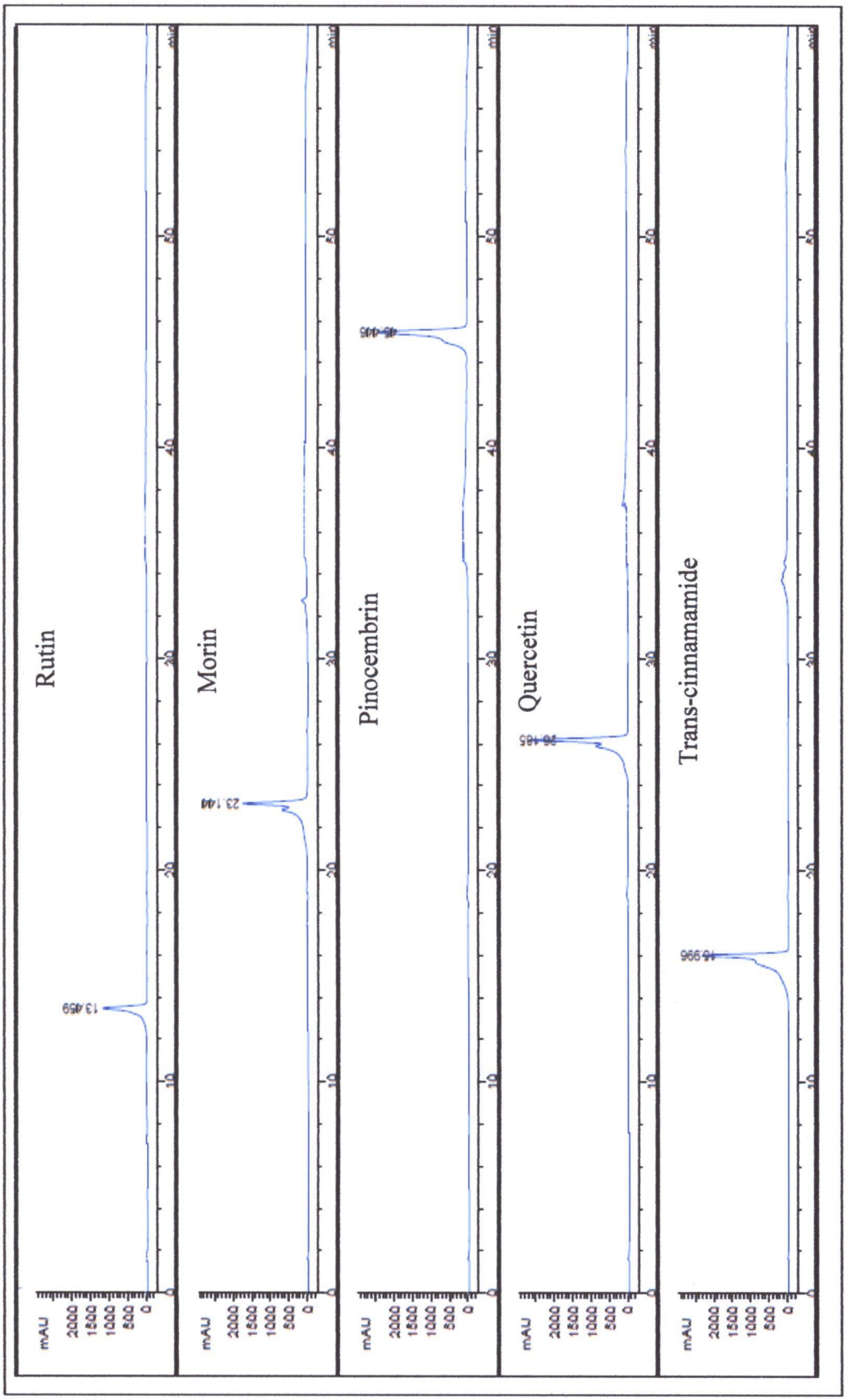


Figure 4.4 HPLC chromatogram of Rutin, Morin, Pinocembrin, Quercetin and Trans-cinnamamide at 210 nm

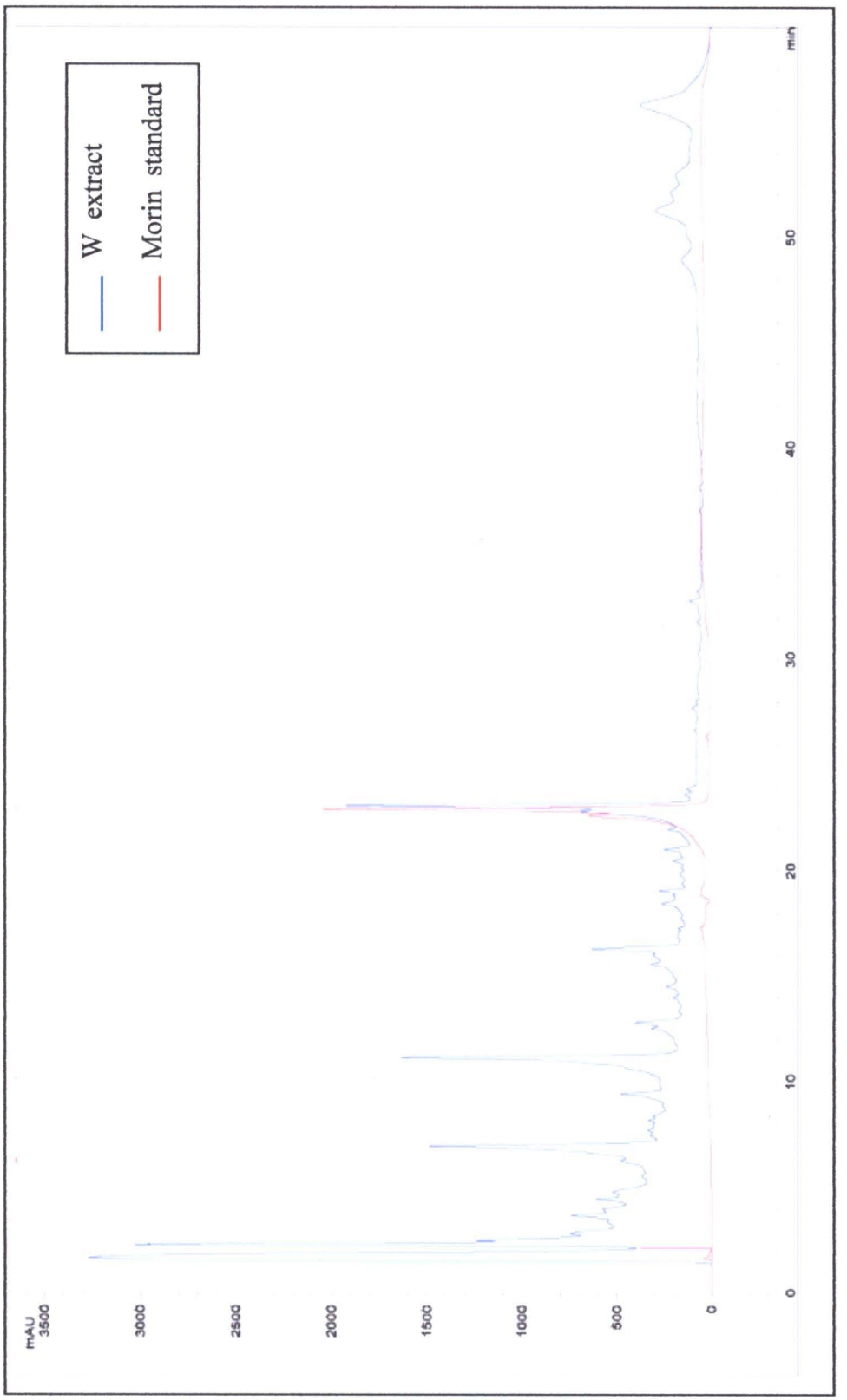


Figure 4.5 HPLC chromatogram of the W extract compared Morin standard

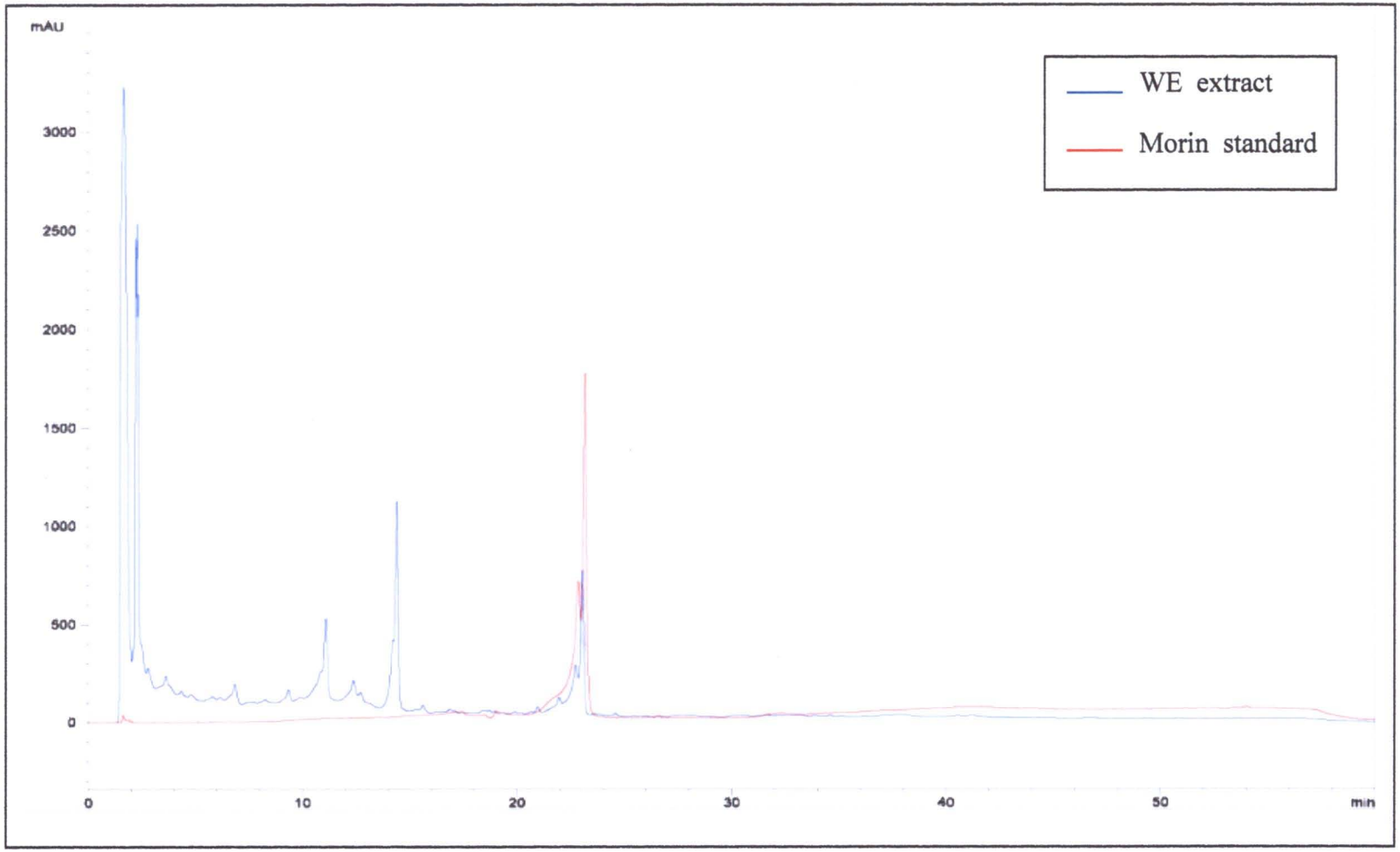


Figure 4.6 HPLC chromatogram of the WE extract compared Morin standard

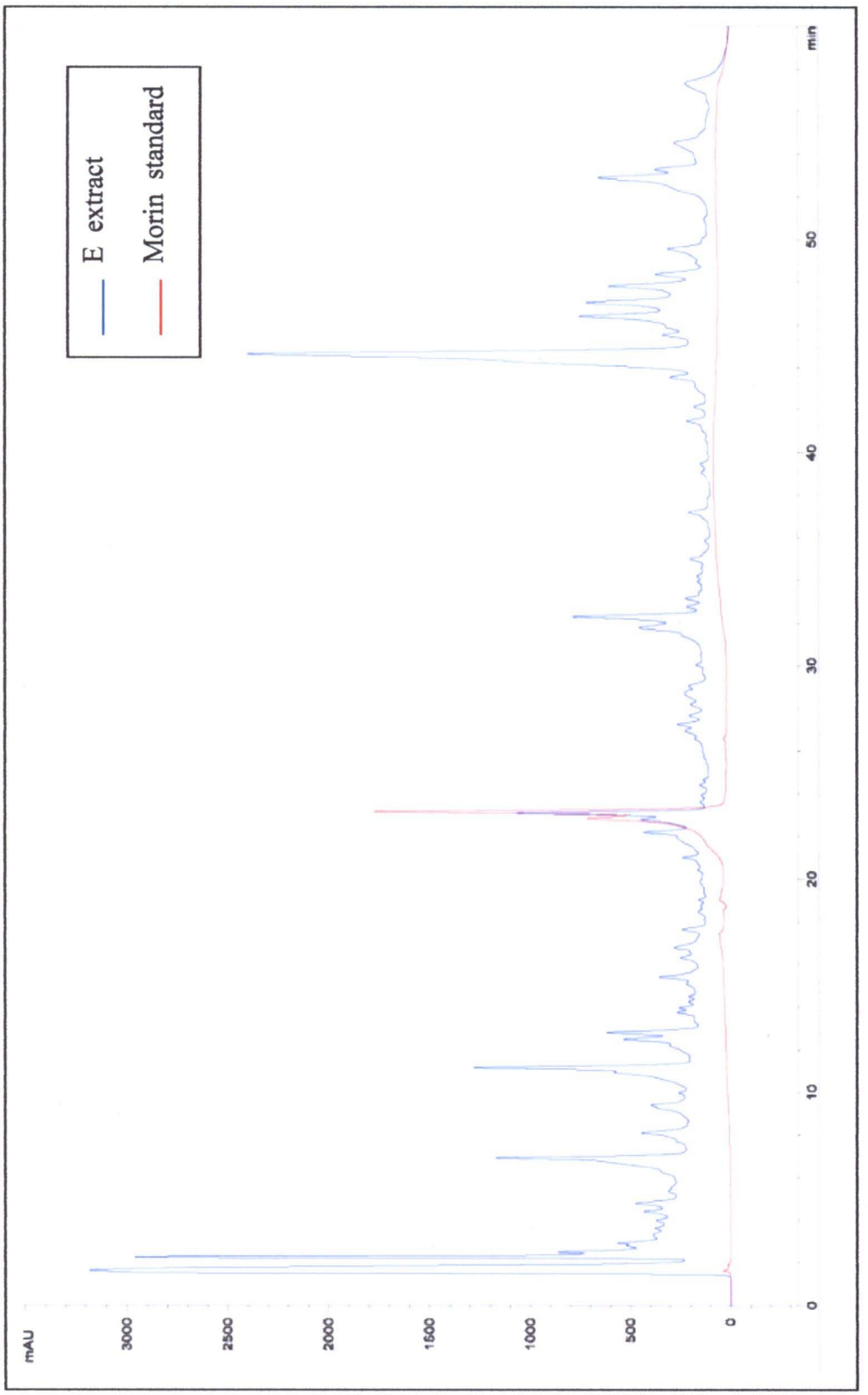


Figure 4.7 HPLC chromatogram of the E extract compared Morin standard

4.2.2 Preformulation study of beehive extracts

Solubility test

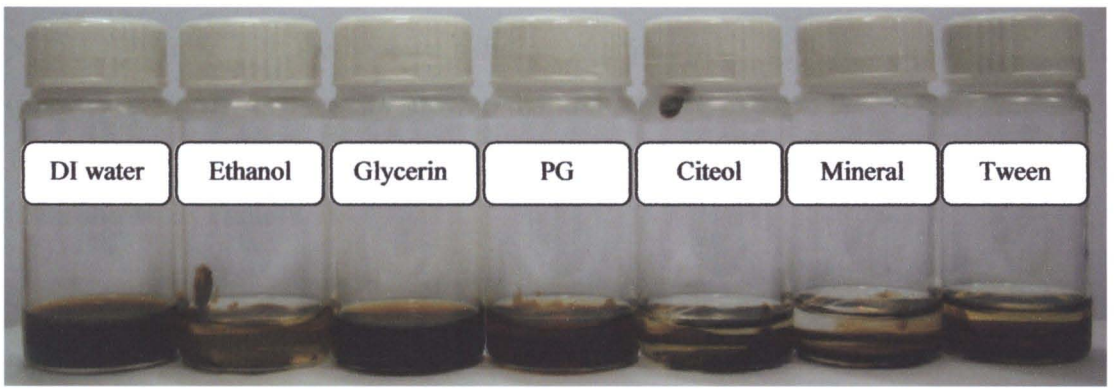
The W and WE extracts were better soluble in higher polar solvent such as water and slightly soluble in glycerine and propylene glycol but E extract insoluble in ethanol and all of samples insoluble in citeol HE, mineral oil and tween 80. (Table 4.2)

From this results DI water was chosen for further study.

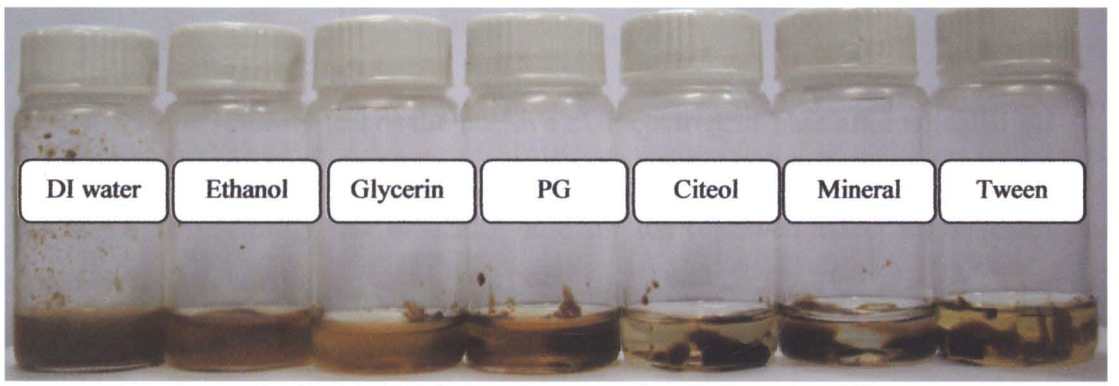
Table 4.2 The solubility of beehive extracts in the solvents

Extract	Physical characteristics	Solvents						
		DI water	Ethanol	Glycerine	Propylene glycol	Citeol HE	Mineral oil	Tween 80
W	Dissolution	+++	+	++	+	-	-	-
	Color	DB	LB	DB	B	C	C	LB
WE	Dissolution	+++	+	+	-	-	-	-
	Color	B	B	LB	B	C	C	LB
E	Dissolution	+++	-	+	++	-	-	-
	Color	B	C	B	B	C	C	LB

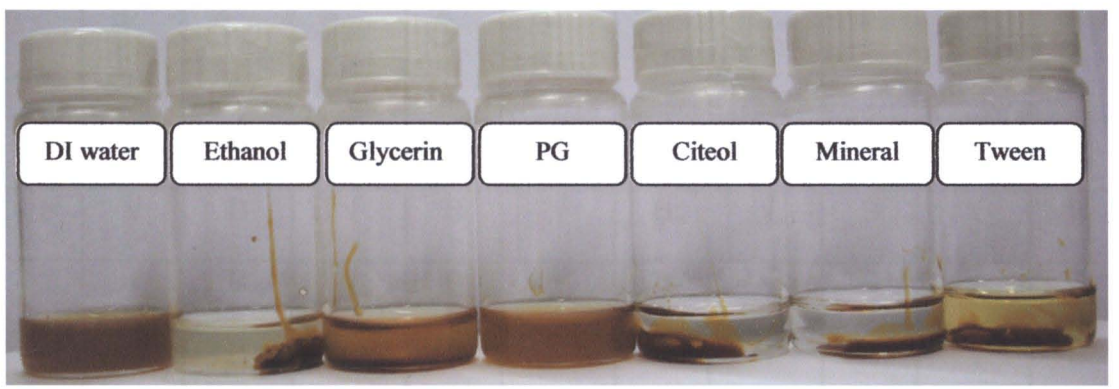
Color		Dissolution	
B	Brown	+++	Very good
LB	Light Brown	++	Good
BD	Dark Brown	+	Little
C	Clear	-	Insoluble



W



WE



E

Figure 4.8 Characteristic solubility of W, WE and E extract in various solvents

The compatibility and stability to acid-base

The pH of beehive solution was 3.37, when addition of HCl 1 N and NaOH 10% w/v into the beehive solution to adjust pH 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 (C is the control). The results showed that the beehive extracts were precipitate at pH 1-4, stable at pH 5-7 and the color of the extracts changed to a little darker at pH 8-10. (data shown in Table 4.3)

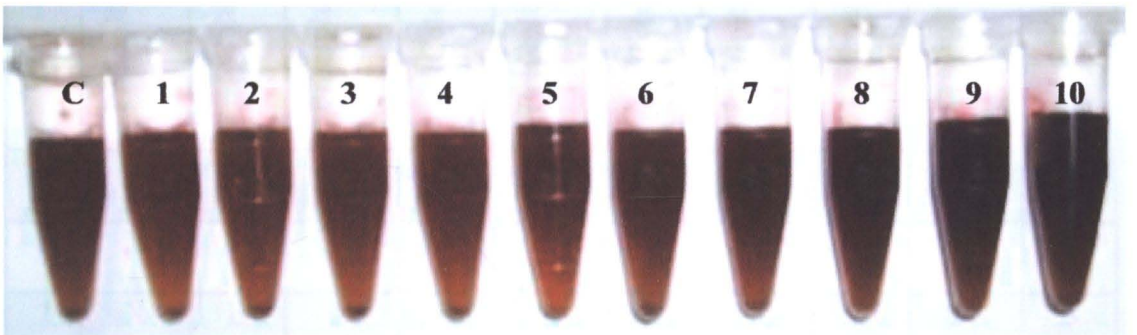


Figure 4.9 The stability test of the beehive extracts to acid-base after adjusted pH

Table 4.3 The stability test of acid - base for the observed effect immediately and stored at various condition

pH	Conditions									
	Observed immediately		RT		RT-D		45°C		H/C (6 cycle)	
	Precipitation	Color	Precipitation	Color	Precipitation	Color	Precipitation	Color	Precipitation	Color
C	X	B	X	B	X	B	X	B	X	B
1	√	B	√	B	√	B	√	B	√	B
2	√	B	√	B	√	B	√	B	√	B
3	√	B	√	B	√	B	√	B	√	B
4	√	B	√	B	√	B	√	B	√	B

Table 4.3 (Continued) The stability test of acid - base for the observed effect immediately and stored at various conditions

pH	Conditions									
	Observed immediately		RT		RT-D		45°C		H/C (6 cycle)	
	Precipitation	Color	Precipitation	Color	Precipitation	Color	Precipitation	Color	Precipitation	Color
5	X	B	X	B	X	B	X	B	X	B
6	X	B	X	B	X	B	X	B	X	B
7	X	B	X	B	X	B	X	B	X	B
8	X	DB	X	DB	X	DB	X	DB	X	DB
9	X	DB	X	DB	X	DB	X	DB	X	DB
10	X	DB	X	DB	X	DB	X	DB	X	DB

RT: room temperature, RT-D: room temperature in the dark, H/C: heating/cooling
B: brown, DB: dark brown, √: precipitation, –: little precipitation, X: no precipitation

4.3 Antibacterial activity of the extracts

4.3.1 Antibacterial activity of beehive extracts by Agar well diffusion method

Screening for antimicrobial activity of various concentrations of beehive extracts were tested against three gram-positive bacteria (*S. aureus*, MRSA, GAS) and two gram-negative bacteria (*E. coli, P. aeruginosa*)

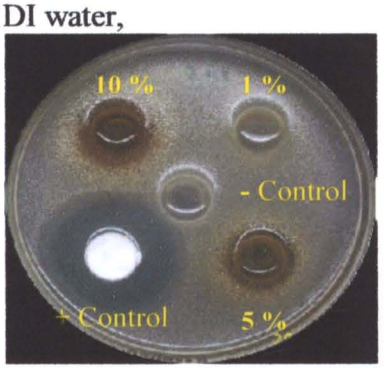
The W extract showed antimicrobial activity against *S. aureus*, MRSA, GAS and *P. aeruginosa* at concentration of 5% and 10% with inhibition zone diameter ranged from 17.00 to 31.00 mm. At the concentration of 1% the W extract showed less activity against GAS and *P. aeruginosa* but not to the other. All of the concentrations of W extract could not inhibit *E. coli* as shown in Table 4.4, Figure 4.10.

Table 4.4 Antibacterial activity of various concentrations of W extract by agar diffusion method

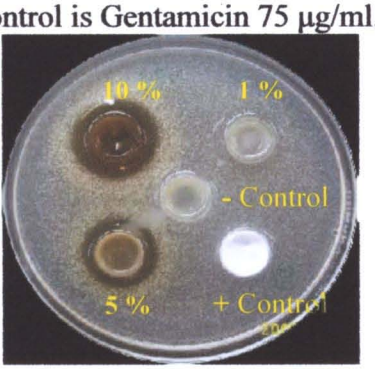
W extract (concentration)	Inhibition zone (mm) ± SD				
	S. aureus ATCC 25923	MRSA	GAS	*E. coli* ATCC 25922	*P. aeruginosa* ATCC 27853
1%	-	-	18.00±2.82	-	16.50±0.70
5%	18.50±0.70	19.83±1.17	25.33±0.94	-	17.17±0.23
10%	22.00±0.00	22.33±0.94	31.66±0.70	-	19.33±0.94
- control	-	-	-	-	-
+ control	40.00±0.00	-	24.00±0.00	38.83±1.17	37.00±1.41

Diameter of ring=12 mm., - is not inhibit,

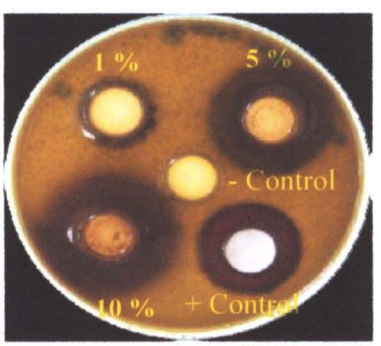
- control is DI water, + control is Gentamicin 75 µg/ml.



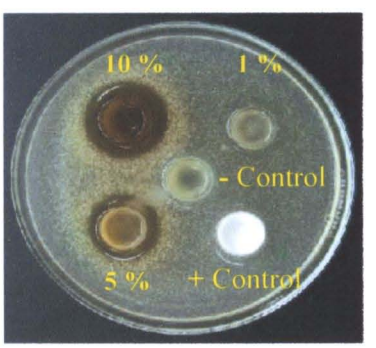
S. aureus



MRSA



GAS



P. aeruginosa

Figure 4.10 Inhibition zone of various concentrations of W extract against *S. aureus*, MRSA, GAS and *P. aeruginosa*

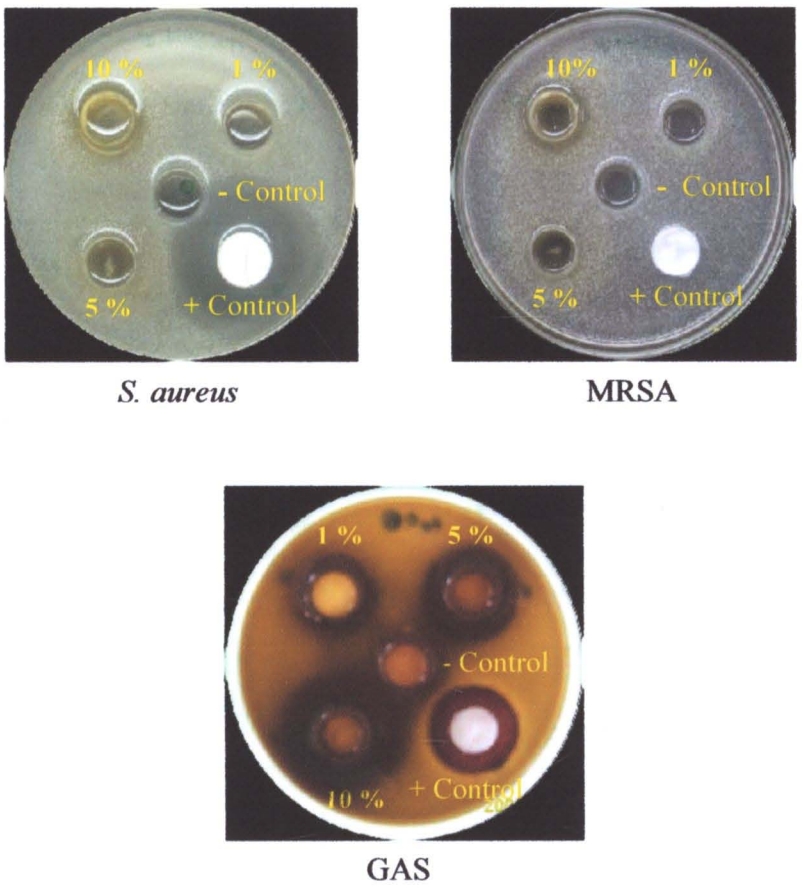
The WE extract showed antimicrobial activity against *S. aureus*, MRSA and GAS at the concentration 10% with inhibition zone diameter ranged from 18.00 to 34.00 mm. At the 5% and 1% concentration of the WE extract could inhibited MRSA, GAS and only GAS, respectively. All of the concentrations could not inhibit *P. aeruginosa* and *E. coli* as shown in Table 4.5 and Figure 4.11.

Table 4.5 Antibacterial activity of various concentrations of WE extract by agar diffusion method

WE extract (concentration)	**Inhibition zone (mm) ± SD**				
	***S. aureus* ATCC 25923**	**MRSA**	**GAS**	***E. coli* ATCC 25922**	***P. aeruginosa* ATCC 27853**
1%	-	-	17.50±0.70	-	-
5%	-	18.00±0.00	29.67±0.47	-	-
10%	20.50±0.70	18.00±0.00	34.00±1.41	-	-
- control	-	-	-	-	-
+ control	40.50±0.70	-	26.83±0.24	41.16±0.70	37.83±0.24

Diameter of ring=12 mm., - is not inhibit,

- control is DI water, + control is Gentamicin 75 μg/ml.



S. aureus MRSA

GAS

Figure 4.11 Inhibition zone of various concentrations of WE extract against *S. aureus*, MRSA and GAS

The E extract showed antimicrobial activity against *S. aureus*, MRSA, GAS and *P. aeruginosa* at the concentration of 5% and 10% with inhibition zone diameter ranged from 17.00 to 30.00 mm. The 1% concentration of the E extract showed less activity against GAS and *P. aeruginosa* but not to the other as shown in Table 4.6 and Figure 4.12.

Table 4.6 Antibacterial activity of various concentrations of E extract by agar diffusion method

E extract (concentration)	**Inhibition zone (mm) ± SD**				
	***S. aureus* ATCC 25923**	**MRSA**	**GAS**	***E. coli* ATCC 25922**	***P. aeruginosa* ATCC 27853**
1%	-	-	21.00±1.41	-	19.33±1.88
5%	18.00±1.41	17.66±2.35	26.00±1.41	-	20.33±0.46
10%	18.50±0.70	18.00±0.70	30.00±0.00	-	20.66±0.00
- control	-	-	ND	-	-
+ control	42.83±0.24	-	27.67±0.94	40.77±1.78	41.55±0.70

Diameter of ring=12 mm., - is not inhibit,

- control is DI water, + control is Gentamicin 75 μg/ml.

ND is not determined

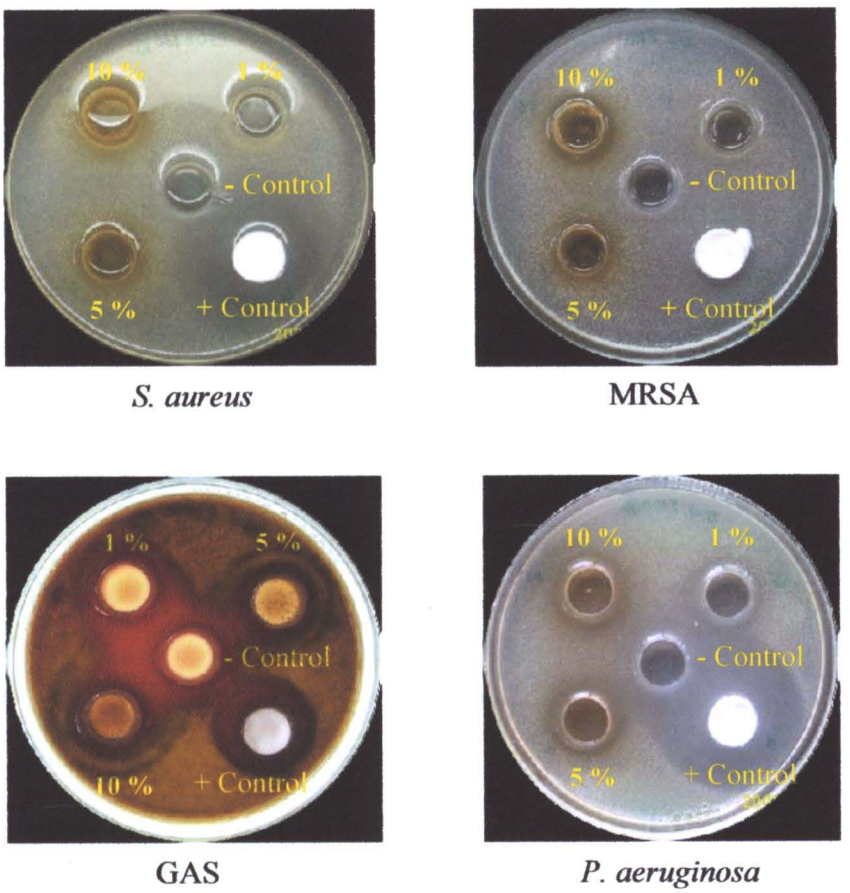


Figure 4.12 Inhibition zone of various concentrations of E extract against *S. aureus*, MRSA, GAS and *P. aeruginosa*

The results revealed that the W and E extracts inhibited *S. aureus*, MRSA, GAS and *P. aeruginosa* at the concentration 10% while the WE extract could not inhibit *P. aeruginosa*. However, it was interestingly found that all of the samples inhibited MRSA while Gentamicin could not.

Siriwong *et al.*(2009) that reported *S. aureus* strains are resistant to methicillin and other beta-lactam antibiotics. And also found that MRSA can be resistant to other types of semi-synthetic antibiotics, such as ampicillin, tetracycline, erythromycin, streptomycin, cephalosporin and vancomicin [89]. Streptomycin and gentamicin are in the same aminoglycosides group. Therefore, they had the similar of the mechanism effect to antibacterial.

The results of gentamicin could not inhibit MRSA, shown that this may be resistant to gentamicin.

The results revealed that the W and E extract were found to be effective against *S. aureus*, MRSA, GAS and *P. aeruginosa*, but WE extract was ineffective against *P. aeruginosa* and all of the extracts were ineffective against *E. coli*. Similarly to Sumonthip (2004) that reported the beehive extracts were effective against *S. aureus* and *E. coli* [5], but in this study all samples were ineffective against *E. coli*. It also attributed to the different chemical composition in the beehive which depended on the geographic origin.

In addition, all of the extracts could not inhibit the *E. coli*. Therefore, all of the extracts were selected to further study base on MIC and MBC against *S. aureus*, MRSA, GAS and *P. aeruginosa*.

The beehive extracts could not inhibit *E. coli* (gram-negative bacteria) because cell wall of gram-negative has outer membrane contain lipid 11-22%. The outer membrane surrounding the peptidoglycan could protect chemicals and enzymes from outside to destroyed cell wall of bacteria [90]. Then gram-positive bacteria are easy to destroyed than gram-negative bacteria.

4.3.2 Determination of MIC and MBC

The W extract exhibited the highest antimicrobial activity with MIC value of 62.50 mg/ml against *P. aeruginosa*, *S. aureus* and MRSA and 31.25 mg/ml against GAS, where its MBC value against all tested microorganism is 62.50 mg/ml as shown in Table 4.7 and Figure 4.13 to Figure 4.15). The results presented the corresponding to the chromatographic fingerprint of W extract which exhibited the higher peak (the higher amount of active ingredients) and higher activity than WE and E extract. Therefore, the W extract was selected to incorporate for the topical antimicrobial gel.

These results of the MIC and MBC are difference only 1 dilution it is that no significantly. Because the value could deviation in the range of dilution at ±2 dilution [85, 91-93].

This result claimed that the concentration of the extract in gel preparation should not be less than 6.25% (calculated from MIC of W extract) in order to get the most effective antibacterial gel preparation.

Table 4.7 Determination of MIC and MBC of the beehive extracts

Extracts	Concentration (mg/ml)							
	***S. aureus* ATCC 29213**		**MRSA**		**GAS**		***P. aeruginosa* ATCC 27853**	
	MIC	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
W	62.5	62.5	62.5	62.5	31.25	62.5	62.5	62.5
WE	62.5	62.5	31.25	31.25	15.63	15.63	-	-
E	62.5	62.5	125	125	125	125	62.5	62.5
Gentamicin	2.344×10^{-4}	2.344×10^{-4}	-	-	1.172×10^{-4}	2.344×10^{-4}	5.86×10^{-5}	1.172×10^{-4}

- is not inhibit

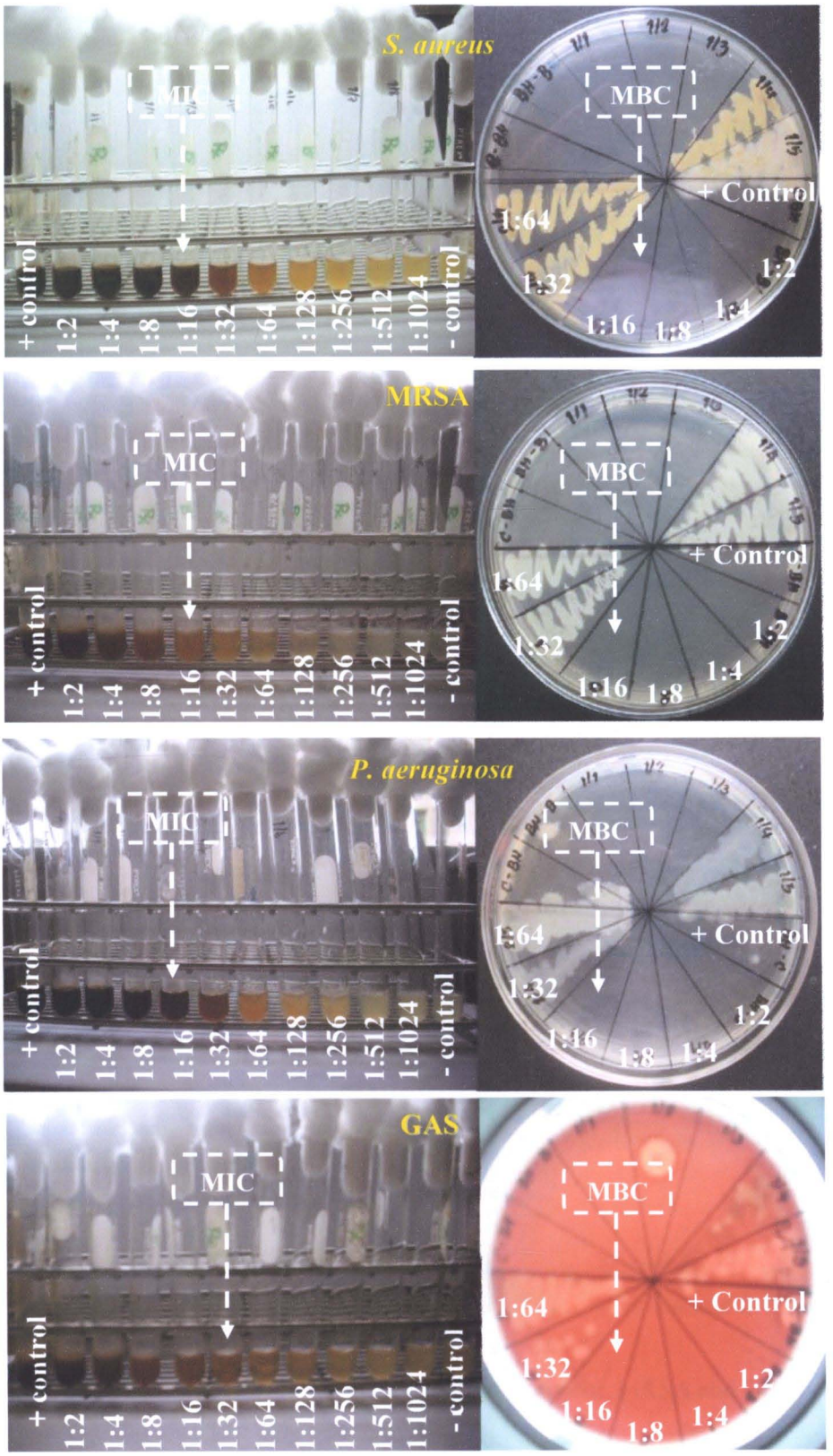


Figure 4.13 MIC and MBC of W extract against *S .aureus*, MRSA, *P. aeruginosa* and GAS

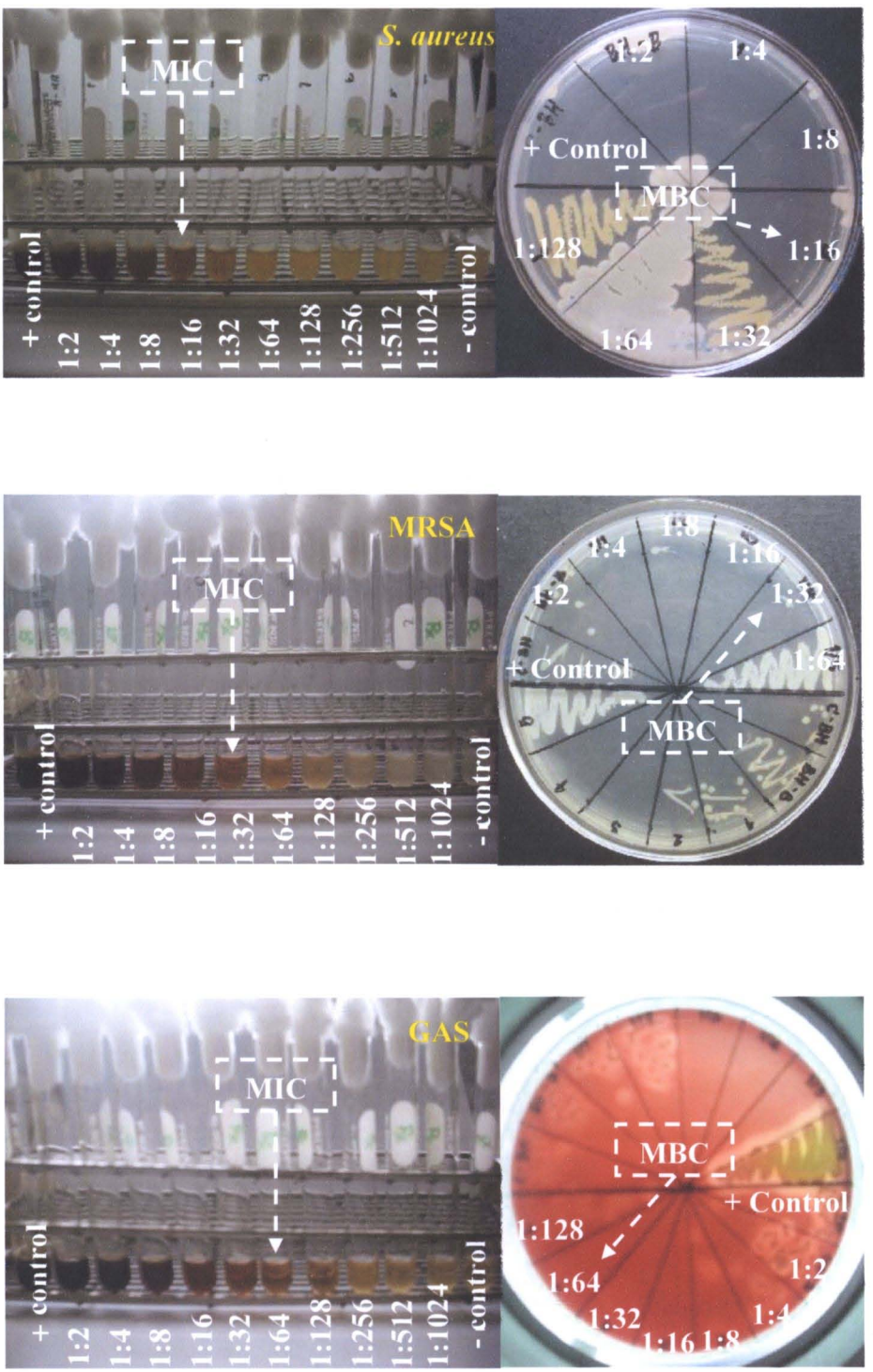


Figure 4.14 MIC and MBC of WE extract against *S .aureus*, MRSA and GAS

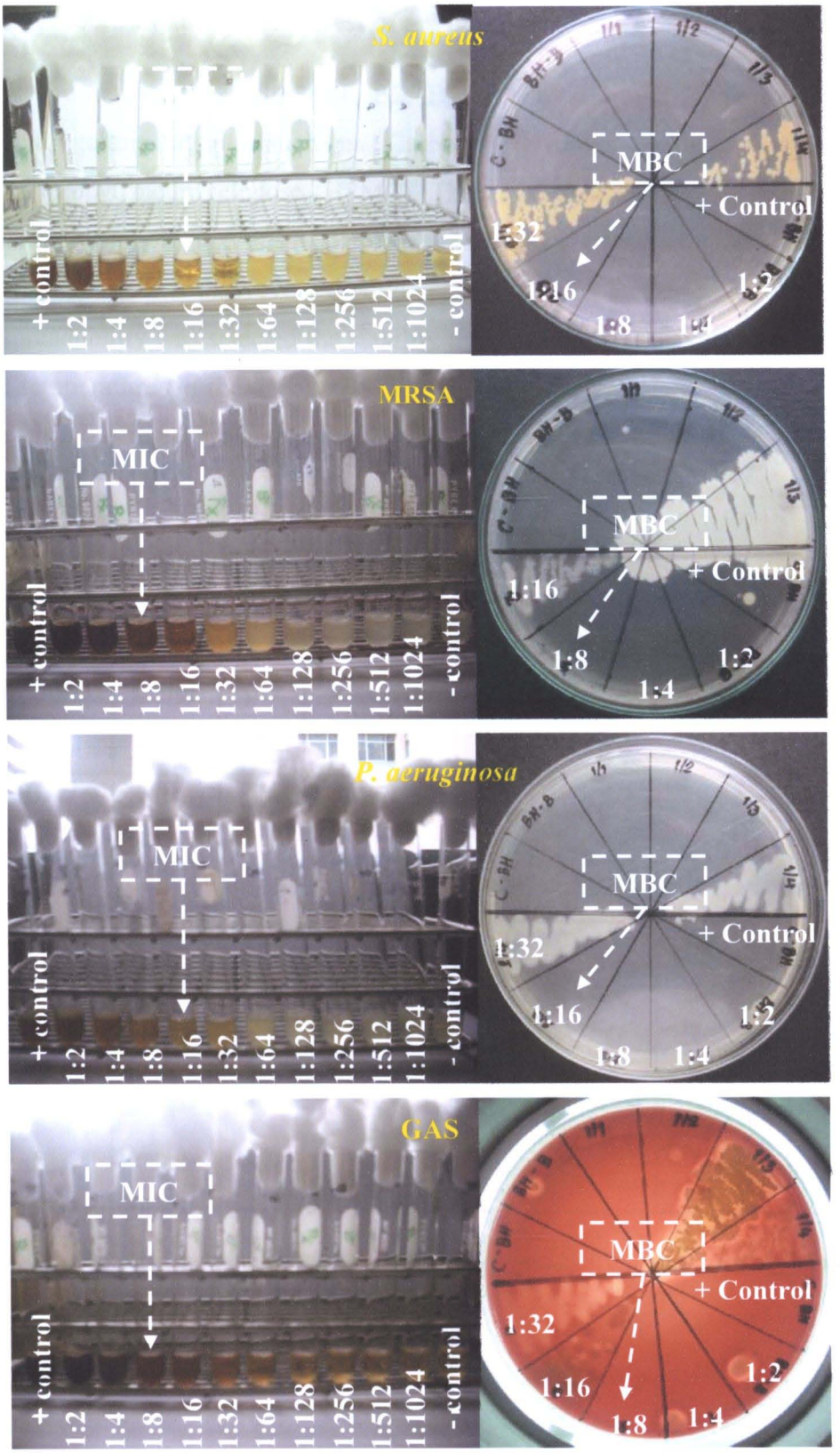


Figure 4.15 MIC and MBC of E extract against *S .aureus*, MRSA, *P. aeruginosa* and GAS

4.4 Formulation and stability test of gel base

From seven formulas (B1, B2, B3, C, D, D1 and E) of gel base using different gelling agents such as Carbopol, Hydroxyethylcellulose (HEC) and Hydroxypropyl methylcellulose (HPMC). Then evaluated for their physical properties appearance, smooth texture, pH, spreadability and stability as shown in Table 4.8. The fresh gel base E that revealed good appearance with a clear color, smooth texture and spreadability was selected the stability test under the storage at room temperature, room temperature (in the dark), 2-8 °C, 45 °C for 1 month and then accelerated test on heating-cooling 6 cycles, the results are as shown in Figure 4.16.

Table 4.8 The physical properties of freshly prepared gel base

Formula	Color	pH	Viscosity	Separation and Precipitation	Smooth texture	Spread ability
B1	Clear	7.5	++	X	+++	+++
B2	Clear	5.5	+++	X	+	+++
B3	Clear	5.5	+	X	+++	++
C	Clear	5.5	++	X	++	+++
D	Clear	5.5	+++	X	+	+
D1	Clear	5.5	+++	X	+	+
E	Clear	5.5	++	X	+++	+++

+ : Moderate, ++ : Good, +++ : Very good,

X : Not Separation and Precipitation

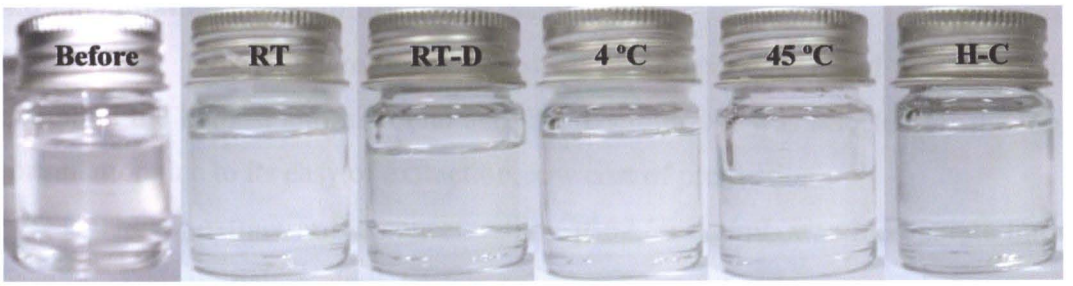


Figure 4.16 Characteristics of before and after stability test of E gel

4.5 Selection of good gel base

From the stability test of gel base, E gel showed no change after 6 cycles of heating-cooling cycling stability test was found that color, odor, smooth texture and pH showed nearly no difference from freshly prepared as shown in Table 4.9, then E gel was chosen for incorporating the beehive extract.

Table 4.9 The physical properties of E gel after stability test for 1 month

Storage conditions	Color	pH	Viscosity (Pascal)	Separation-Precipitation	Smooth texture	Spread-ability
RT	Clear	6.0	0.758	X	+++	+++
RT-D	Clear	6.0	0.731	X	+++	+++
2-8 °C	Clear	5.5	0.930	X	+++	+++
45 °C	Clear	5.5	3.062	X	+++	+++
H/C (6 cycle)	Clear	5.5	1.276	X	+++	+++

RT : Room temperature, RT-D : Room temperature in the dark,

H/C : Heating-Cooling cycling, X : Not Separation and Precipitation

+ : Moderate, ++ : Good, +++ : Very good

4.6 Formulation and stability test of beehive gel

From the antimicrobial study of beehive extracts found that W extract exhibited the highest antimicrobial activity. Therefore, it was selected for the topical formulation due to its easy of extraction, low cost of production.

The W extract at the concentration of 62.50 mg/mL was incorporate into gel base E. The W gel is dark brown color and very good of smooth texture and spreadability. After the stability test at various conditions (5 months), the results were as following:

- pH

The pH of W gel were determined and the results were showed in Table 4.10. The pH of all conditions after passed stability test was decrease but showed no significant difference ($p>0.05$, pair t-test) from freshly prepared gel.

Table 4.10 pH values of W gel freshly prepared and after the stability test

Conditions	pH			
	Freshly prepared	**1 month**	**3 months**	**5 months**
RT	5.50	4.68	4.79	4.96
RT-D		4.88	4.90	4.96
2-8 °C		4.90	4.92	5.06
45 °C		4.43	4.47	4.50
H/C (6 cycles)		4.88	ND	ND

RT : Room temperature RT-D : Room temperature in the dark

H/C : Heating/Cooling cycling ND : No determined

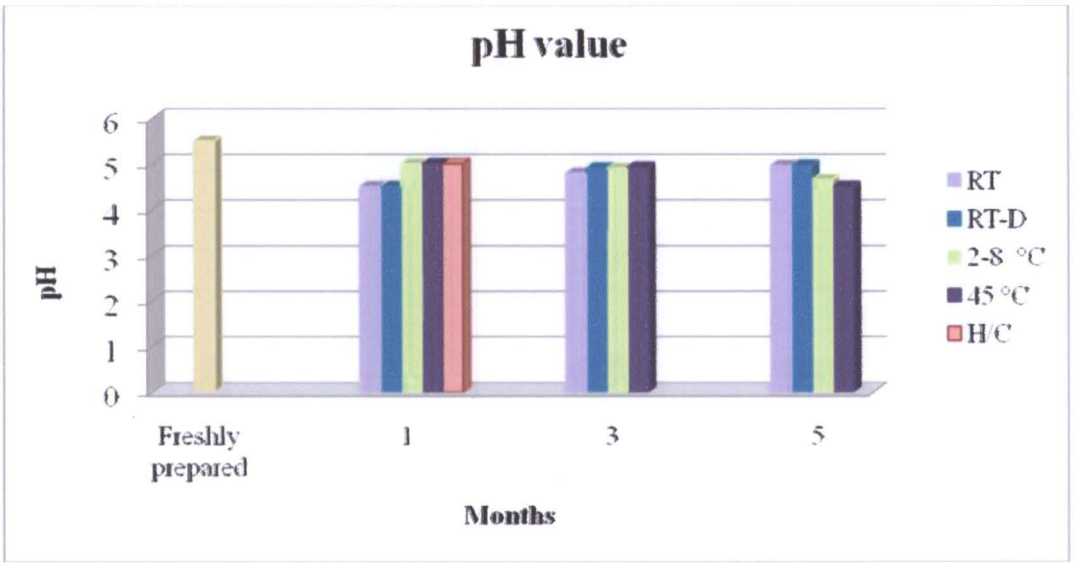


Figure 4.17 Comparing the pH value in W gel after the stability test

- Viscosity

The viscosity of W gel was determined and the results were showed in Table 4.11. After W gel passed the stability test for 1 month viscosity of it was increased but 3 and 5 months the viscosity was decreased showed no significant difference ($p>0.05$, pair t-test) from freshly prepared gel.

Table 4.11 Viscosity values of W gel freshly prepared and after the stability test

Conditions	Viscosity (Pascal)			
	Freshly prepared	1 month	3 month	5 month
RT	0.296	1.549	0.269	0.267
RT-D		1.375	0.288	0.257
2-8 °C		1.463	0.256	0.240
45 °C		1.563	0.917	0.521
H/C (6 cycles)		1.498	ND	ND

RT : Room temperature RT-D : Room temperature in the dark

H/C : Heating/Cooling cycling ND : No determined

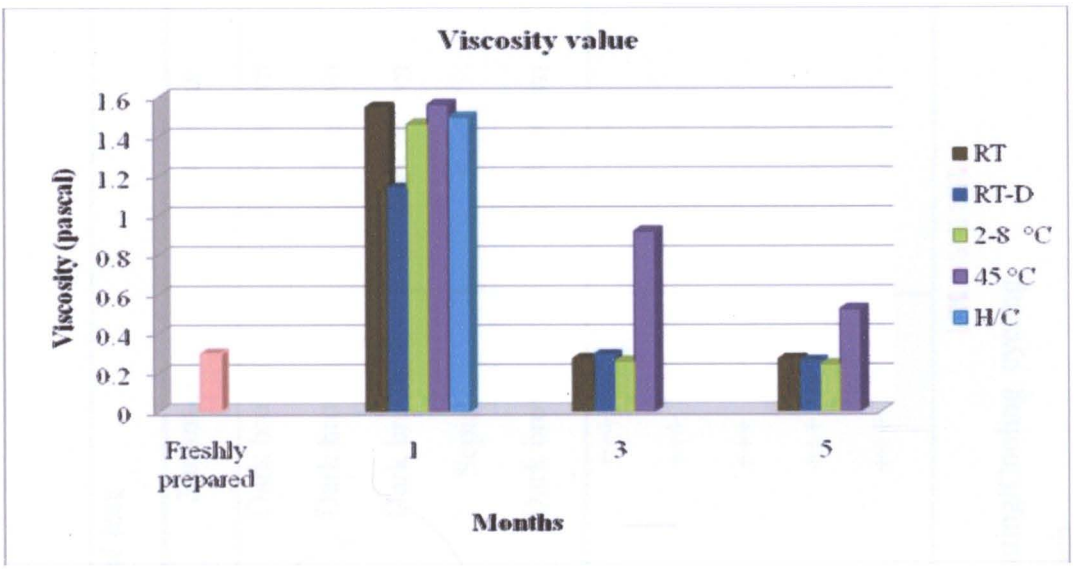


Figure 4.18 Comparing the viscosity value in W gel after the stability test

- Appearance of physical characteristics

After the stability test the W gel present good appearance with a homogeneous texure, color showed nearly no difference from freshly prepared as shown in Table 4.12. Even though at 45 °C, the color of the gel changed to a little darker after kept in 45°C for 5 months as Figure 4.19 which due to oxidation of substances in the formulation catalyzed by heat. The present study indicated that the light had no effect on the color change of the product because the W gel after storage in room temperature (dark) shown no difference from storage in room temperature (light).

Table 4.12 Appearance physical characteristics of W gel after the stability test

Characteristic	Conditions	Period of test			
		Freshly prepared	**1 month**	**3 months**	**5 months**
Color	**RT**	Dark brown	Dark brown	Dark brown	Dark brown
	RT-D		Dark brown	Dark brown	Dark brown
	2-8 °C		Dark brown	Dark brown	Dark brown
	45 °C		Sepia	Sepia	Sepia
	H/C (6 cycles)		Dark brown	Dark brown	Dark brown
Smoothness	**RT**	+++	+++	+++	+++
	RT-D		+++	+++	+++
	2-8 °C		+++	+++	+++
	45 °C		+++	+++	+++
	H/C (6 cycles)		+++	+++	+++

RT : Room temperature RT-D : Room temperature in the dark H/C : Heating/Cooling cycling

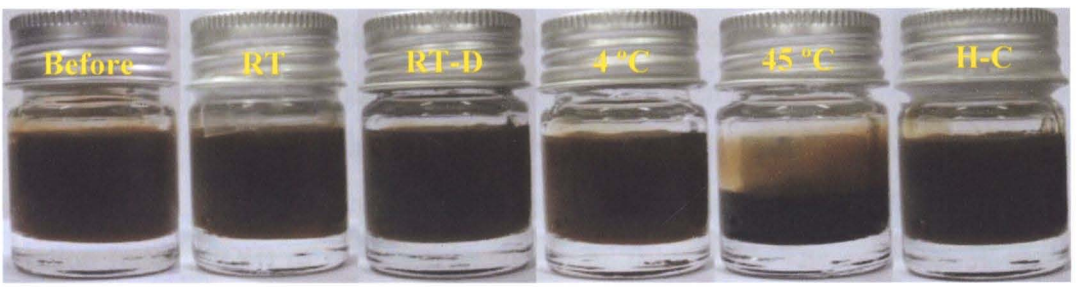
+ : Little ++ : Moderate +++ : Much

Table 4.12 (Continued) Appearance physical characteristics of W gel after the stability test

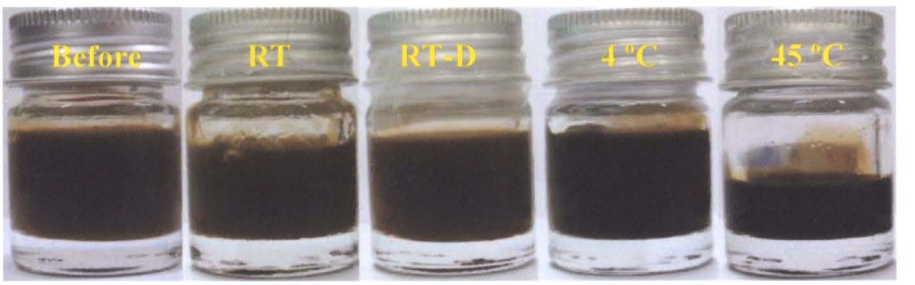
Characteristic	Conditions	Peroid of test			
		Freshly prepared	1 month	3 months	5 months
Separation - Precipitation	RT	X	X	X	X
	RT-D		X	X	X
	2-8 °C		X	X	X
	45 °C		X	X	X
	H/C (6 cycles)		X	X	X
Spreadability	RT	+++	+++	+++	+++
	RT-D		+++	+++	+++
	2-8 °C		+++	+++	+++
	45 °C		+++	+++	+++
	H/C (6 cycles)		+++	+++	+++

RT : Room temperature RT-D : Room temperature in the dark H/C : Heating/Cooling cycling

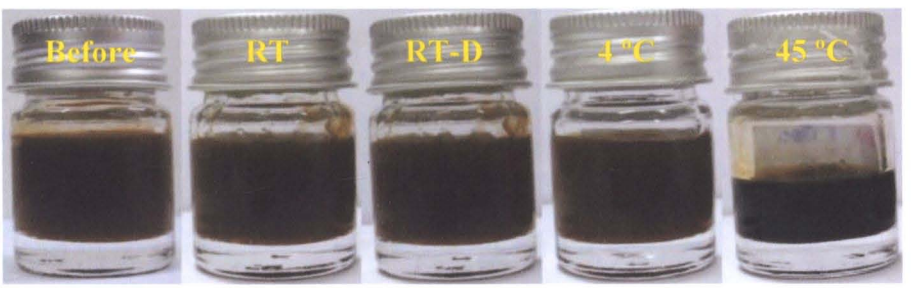
+ : Little ++ : Moderate +++ : Much X : Not Separation and Precipitation



1 month



3 months



5 months

Figure 4.19 Characteristics of W gel after stability test for 1 month, 3 months and 5 months in various conditions

4.7 Rabbit skin primary irritation test

The assessment of the skin irritation potential of chemicals and finished products is an essential part of the toxicological evaluation prior to manufacture, transport, or marketing. Thereby protecting the worker and consumer from adverse skin effects due to intended or accidental skin exposure. Traditionally, animal testing

procedures have provided the data needed to assess the more severe forms of skin toxicity, and current regulations may require animal test data before permission can be obtained to manufacture the products that contain them.

The gel base, W extract and W gel were assessed of skin irritation by modified Draize Rabbit Models as shown in Figure 4.20. The value of Primary Dermal Irritation Index (PDII) of these gel as shown in Table 4.13. The gel base, W extract and W gel exhibited no irritation (PDII Value < 0.5) as shown in Figure 4.21.

Figure 4.20 Skin irritation test of W extract, W gel, gel base and Gentamicin

Table 4.13 Primary irritation score of gel base, gentamicin, W extract and Wgel

Time (hr.)	The average score of erythema and eschar/edema formation [1]					
	Blank	**DI water**	**Gel base**	**Gentamicin**	**W extract**	**W gel**
1	0/0	0/0	0/0	0/0	0/0	0/0
24	0/0	0/0	0/0	0/0	0/0	0/0
48	0/0	0/0	0/0	0/0	0/0	0/0
72	0/0	0/0	0/0	0/0	0/0	0/0
PDI [2]	0	0	0	0	0	0
PDII [3]	0	0	0	0	0	0

PDII Value < 0.5 (non-irritation)

[1] Average all of rabbits.

[2] PDI is the average score of erythema and eschar/edema formation.

[3] The average score of erythema and eschar/edema formation at time 1, 24, 48 and 72 hours of the 3 rabbits, divided by the amount of time.

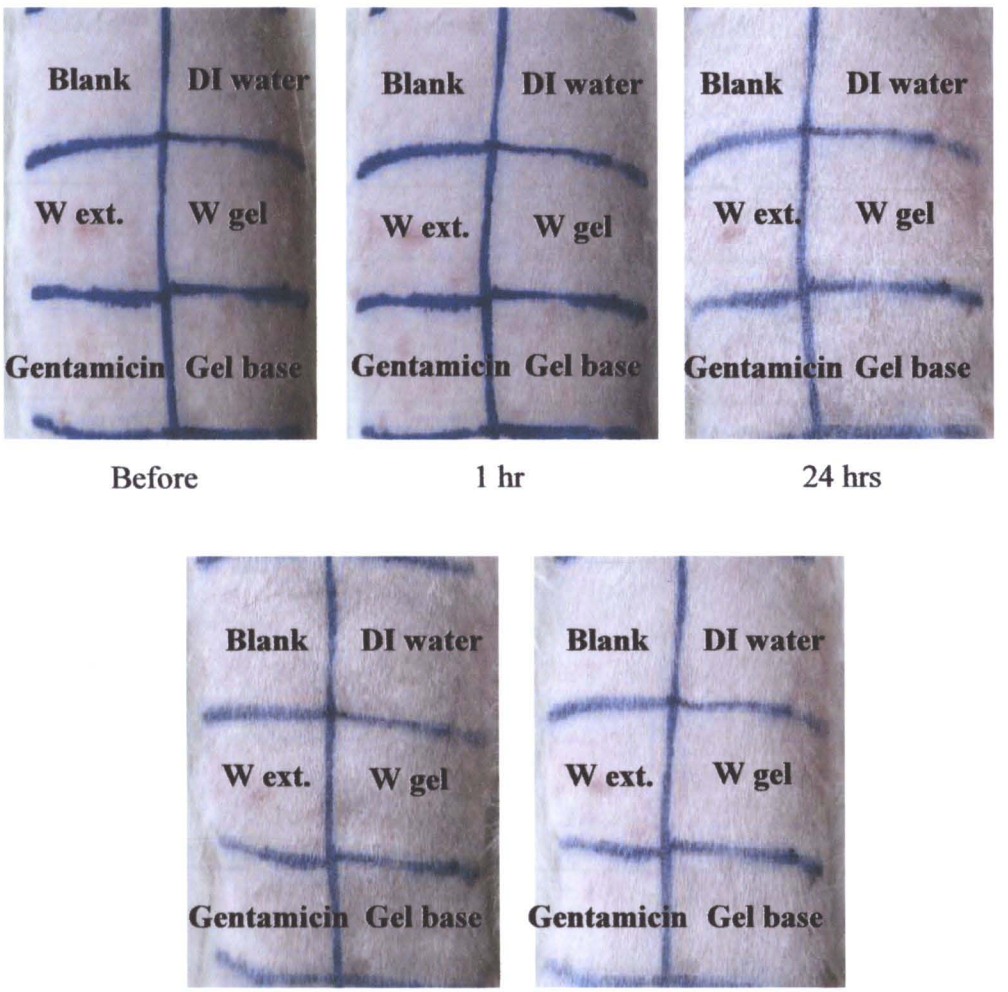


Figure 4.21 Skin irritation test in rabbits (assessment at 1, 24, 48, 72 hours after occlusion period)

4.8 Antibacterial activity of W gel after stability test

The comparison of antibacterial activity of W gel after the staying in various conditions for 1, 3 and 5 months against *S. aureus* ATCC 25923, MRSA, GAS and *P. aeruginosa* ATCC 27853 were determined by agar well diffusion method and comparing with W gel freshly prepared. The results revealed that the W gel could inhibit before and after stability test as shown in Table 4.14 and Figure 4.22 to Figure 4.28. Because effective concentrations of W extract in W gel preparation should not be less than 6.25%.

Table 4.14 Antibacterial activity test of W gel before and after the stability test for 1, 3 and 5 months

Time	Conditions	Inhibition zone ± SD (mm)			
		S. aureus **ATCC 25923**	**MRSA**	**GAS**	***P. aeruginosa*** **ATCC 27853**
Before the stability test		20.88 ± 1.26	21.66 ± 0.83	15.66 ± 1.52	20.88 ± 0.50
1 month	RT	23.77 ± 0.54	26.99 ± 1.44	27.99 ± 1.56	24.00 ± 1.00
	RT-D	22.33 ± 0.33	27.44 ± 1.26	29.32 ± 1.15	24.99 ± 1.20
	2-8 °C	23.22 ± 2.39	25.66 ± 1.15	28.99 ± 0.88	25.99 ± 0.45
	45 °C	25.66 ± 0.94	27.21 ± 1.26	26.33 ± 0.57	24.44 ± 1.26
	H/C (6 cycles)	14.44 ± 1.50	22.44 ± 1.34	25.77 ± 0.38	22.99 ± 1.20
3 months	RT	18.66 ± 0.57	19.66 ± 1.45	32.55 ± 0.37	31.99 ± 1.44
	RT-D	19.22 ± 1.35	16.66 ± 1.15	29.10 ± 1.26	31.33 ± 0.57
	2-8 °C	19.55 ± 1.50	17.66 ± 0.57	29.22 ± 1.35	31.33 ± 0.57
	45 °C	22.66 ± 0.57	19.66 ± 0.57	16.50 ± 0.70	36.55 ± 0.19
5 months	RT	19.66 ± 1.15	17.50 ± 0.50	26.99 ± 1.52	34.22 ± 0.69
	RT-D	18.66 ± 0.57	18.33 ± 1.15	25.33 ± 1.52	32.66 ± 1.15
	2-8 °C	20.66 ± 0.57	17.66 ± 1.52	26.88 ± 1.17	33.00 ± 0.00
	45 °C	23.00 ± 1.00	19.00 ± 1.00	19.66 ± 1.45	32.66 ± 0.57

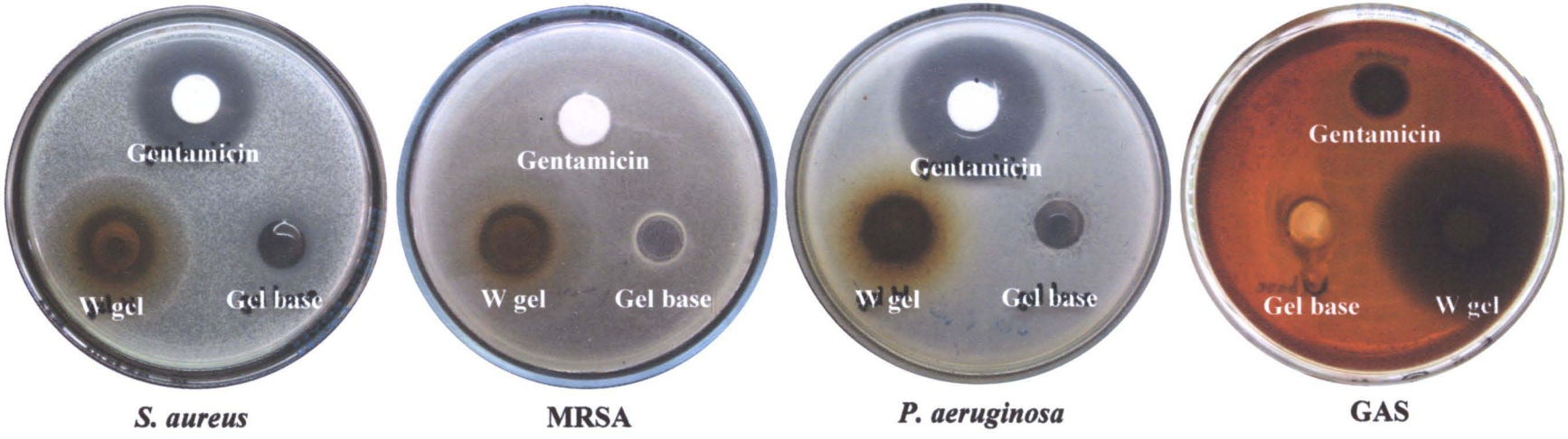
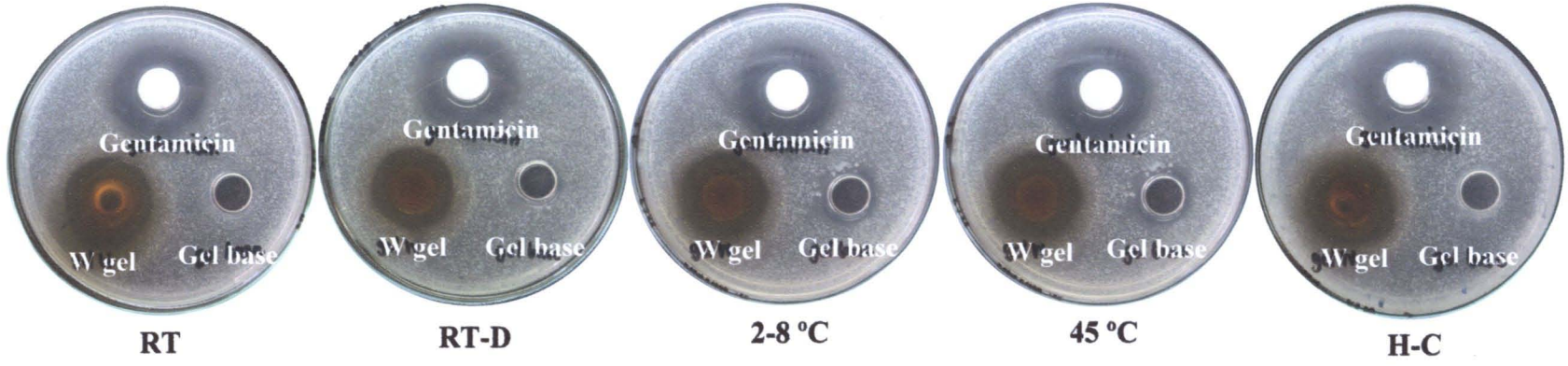


Figure 4.22 Antibacterial activity test of gel base, W gel and gentamicin before stability test



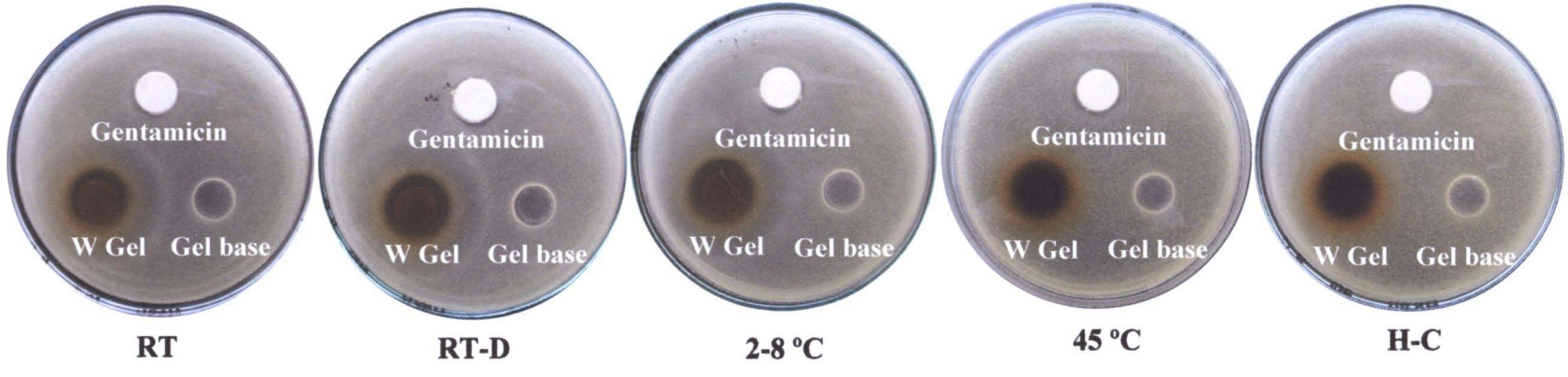


Figure 4.23 Antibacterial activity test of gel base, W gel and gentamicin against *S. aureus* and MRSA after the stability test 1 month

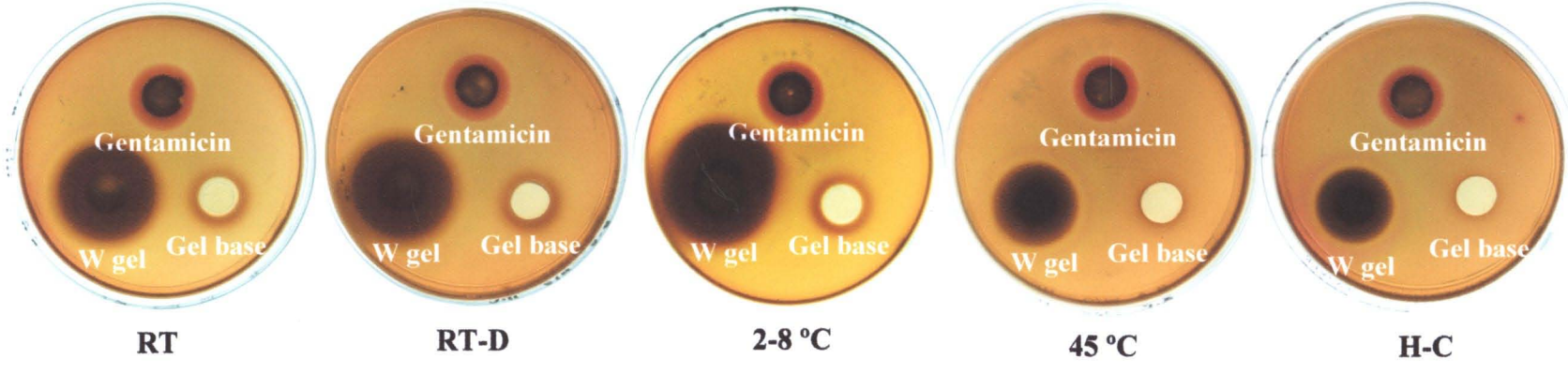


Figure 4.24 Antibacterial activity test of gel base, W gel and gentamicin against *P. aeruginosa* and GAS after the stability test 1 month

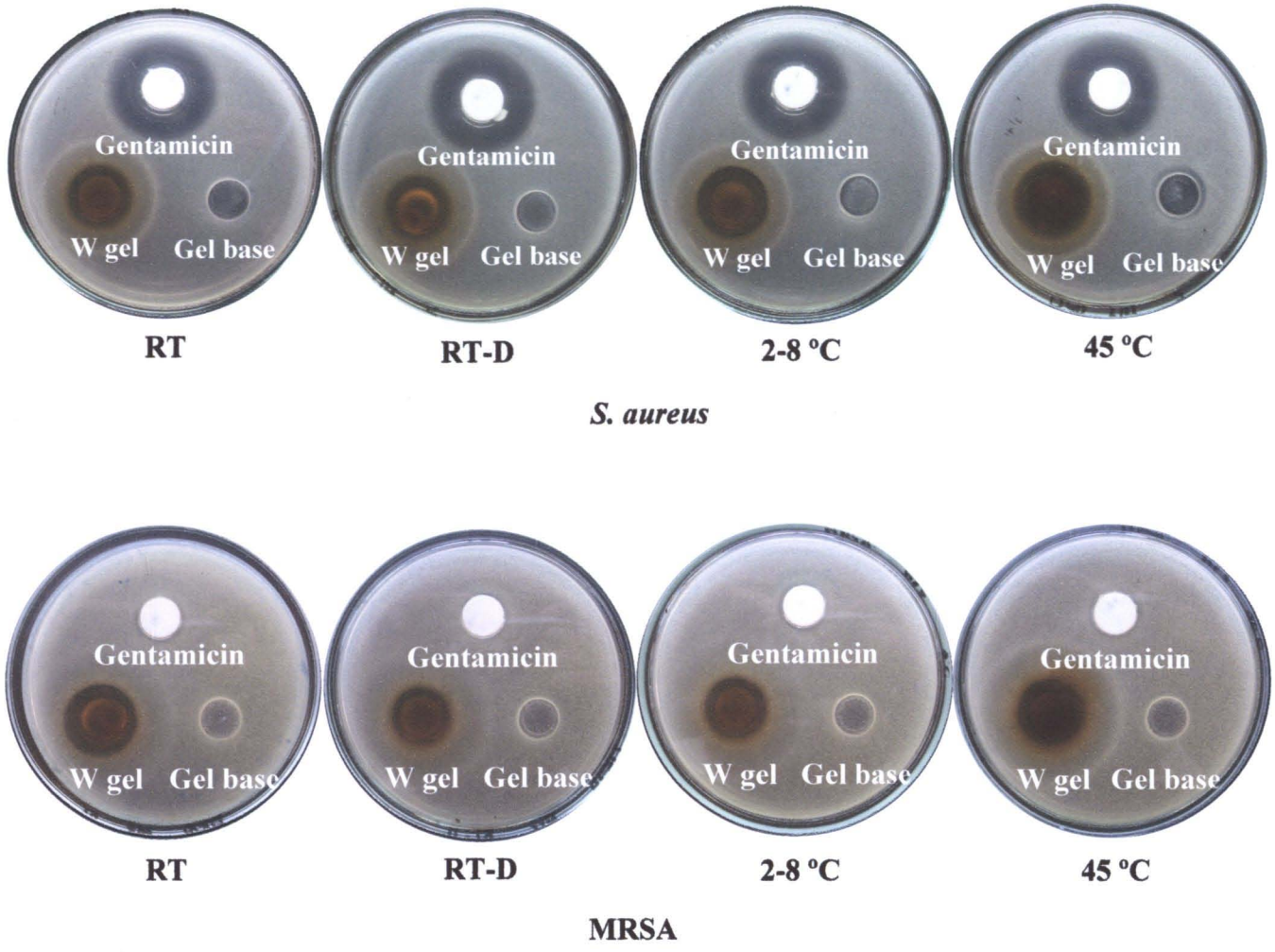


Figure 4.25 Antibacterial activity test of gel base, W gel and gentamicin against *S. aureus* and MRSA after the stability test 3 months

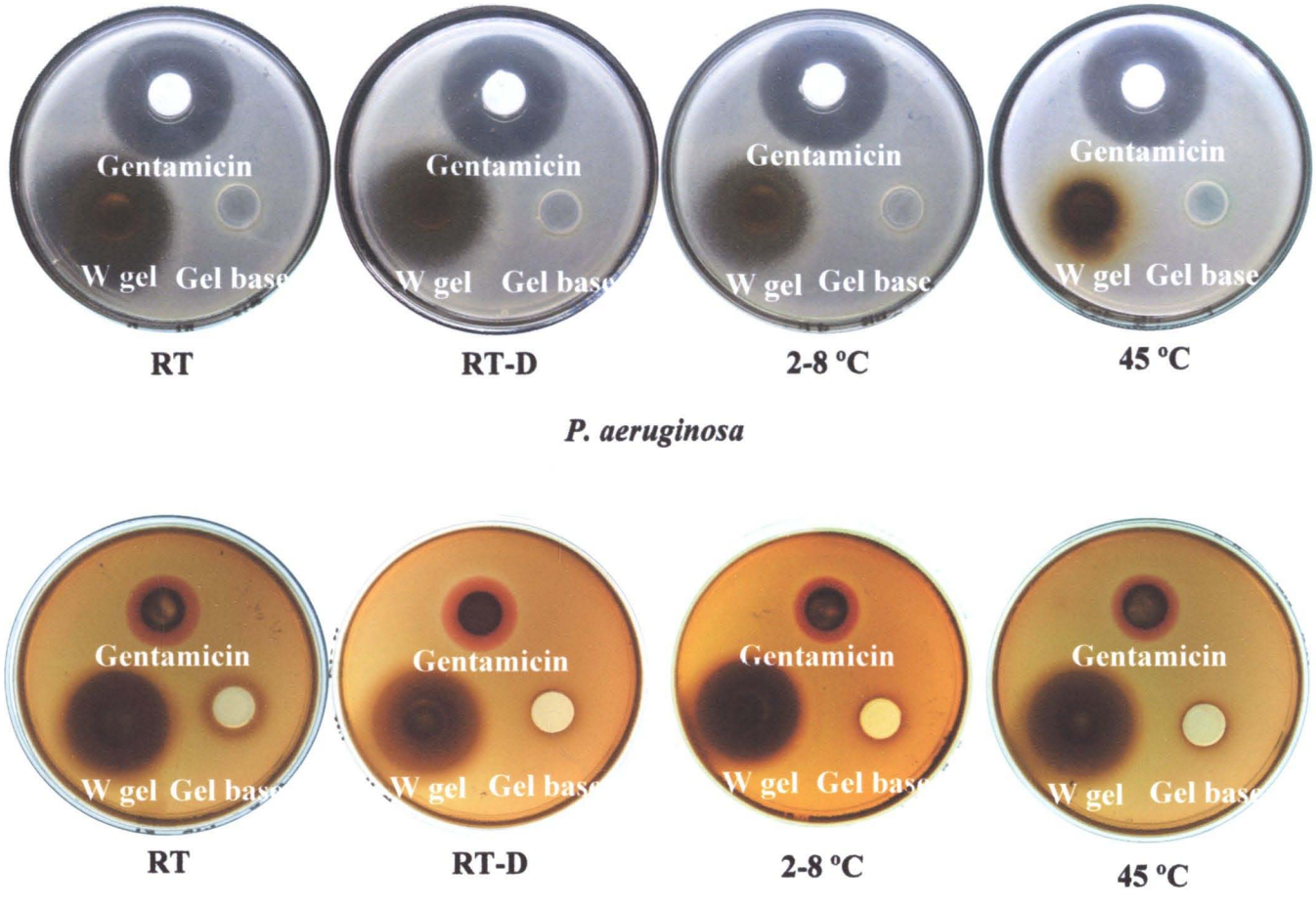


Figure 4.26 Antibacterial activity test of gel base, W gel and Gentamicin against *P. aeruginosa* and GAS after the stability test 3 months

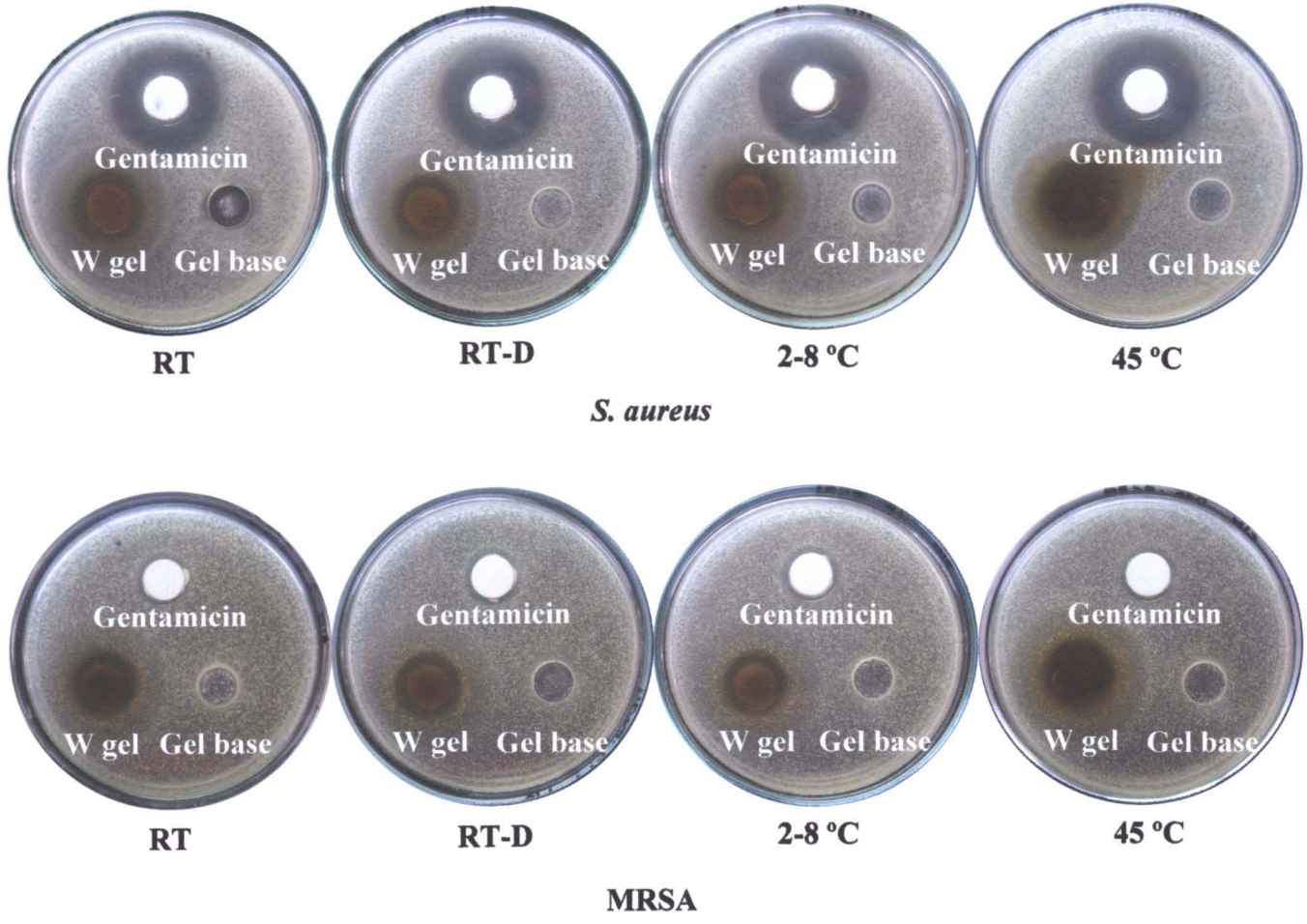


Figure 4.27 Antibacterial activity test of gel base, W gel and gentamicin against *S. aureus* and MRSA after the stability test 5 months

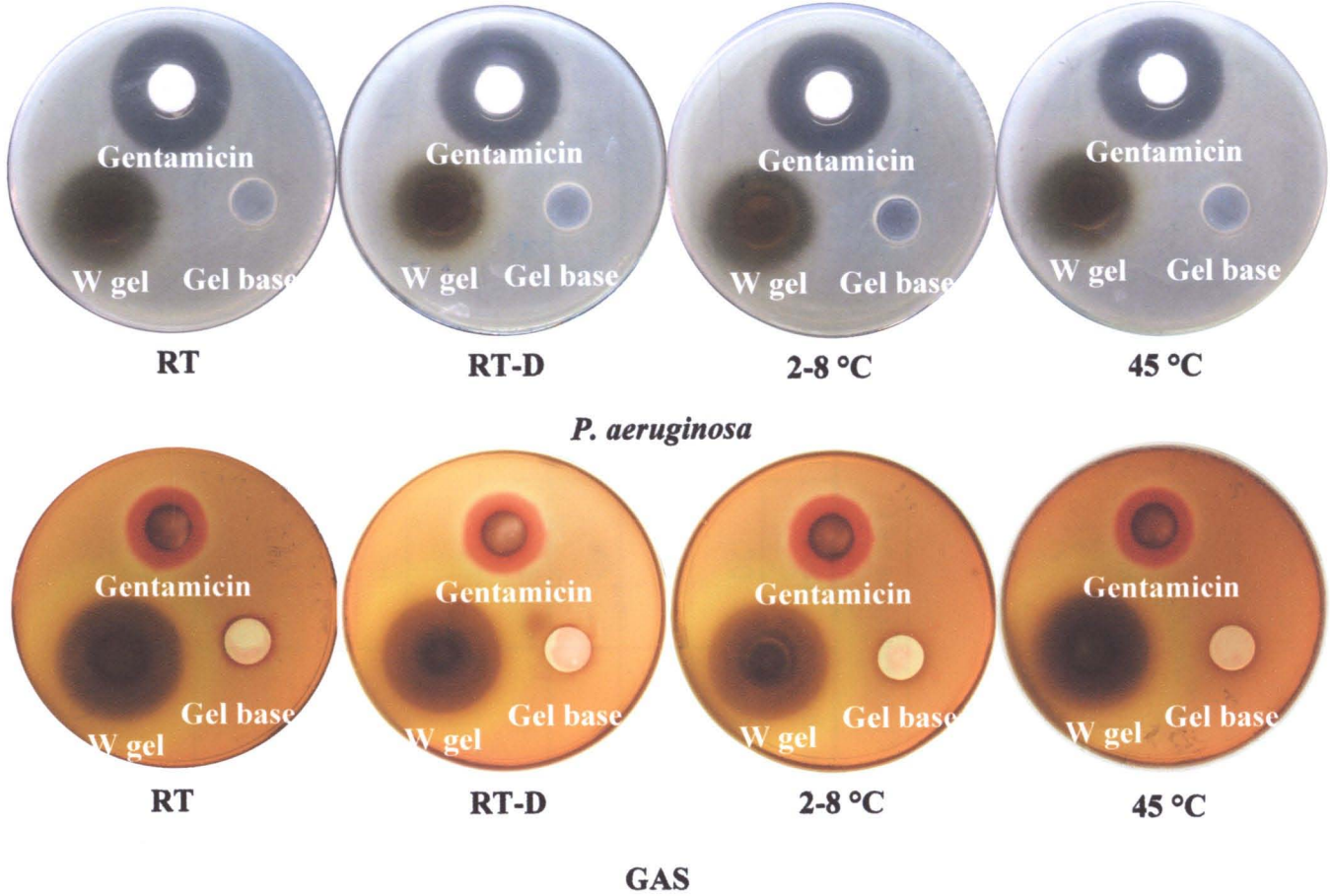


Figure 4.28 Antibacterial activity test of gel base, W gel and gentamicin against *P. aeruginosa* and GAS after the stability test 5 months

4.9 Satisfaction test of volunteers by questionnaire

The satisfaction of the volunteers of topical antibacterial gel containing beehive extract .The volunteers tries to use the product. After testing was finished, all volunteers were asked some question. To assess the 20 volunteers aged between 20 to 50 years. The satisfactory results were found ranged from "good" to "very good" showed more than 90% for appearance, separation-precipitation, viscosity, smoothness of gel and 80% for spread ability. The satisfaction in "improve" showed 5% for color and 15% for odor. In the overall satisfaction, W gel was high satisfaction (70% ranged from "good" to ''very good") as shown in Table 4.15.

Table 4.15 The percentage of satisfaction on W gel

Topic	The satisfaction (%)			
	Very good	**Good**	**Moderate**	**Improve**
Color	15	20	60	5
Odor	10	25	50	15
Appearance	30	65	5	-
Separation- Precipitation	55	45	-	-
Viscosity	35	55	10	-
Smoothness of gel	40	50	10	-
Spreadability	40	40	20	-
The overall satisfaction	15	55	30	-

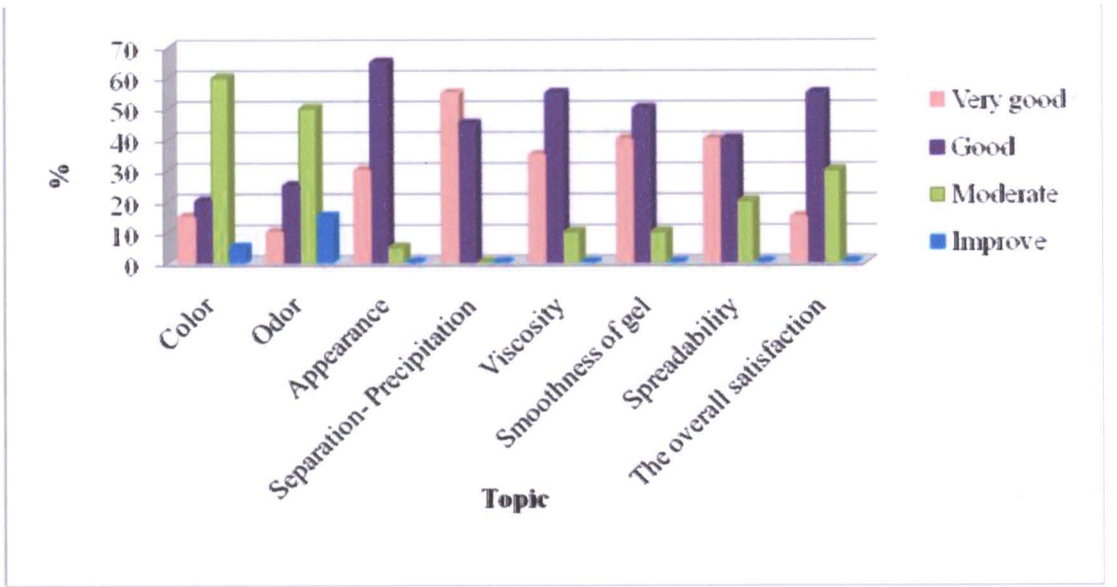


Figure 4.29 The percentage of satisfaction on W gel