

## APPENDIX A

### PREPARATION OF THE CHEMICAL REAGENTS

#### A.1 Fungal media preparation

##### (i) PDA (Potato Dextrose Agar)

39 grams of PDA powder was suspended into 1000 mL distilled water. The liquid medium was stirred until thoroughly mixed and sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

##### (ii) PDB (Potato Dextrose Broth)

26.5 grams of PDB powder medium were suspended into 1000 mL distilled water. The liquid medium was stirred until thoroughly mixed and sterilized at 121 °C 15 lbs pressure for 15 min.

#### A. 2 Preparation of Tween 20 solution for pour plate dilution series

5 mL of Tween 20 was dissolved into 100 mL distilled water. Nine mL of 5% v/v Tween 20 solution was pipetted into test tube. Each test tube was sterilized at 121 °C 15 lbs pressure for 15 min.

#### A. 3 Preparation fungal spore suspensions solution

Three-day old *A. flavus* spores from the culture grown in potato dextrose agar slant were aseptically transferred into screw-capped vials containing 10 mL of sterile 5%v/v of Tween 20. They were gradually mixed and count number spore by haemocytometer before used further.

#### A. 4 Reagent preparation for extraction ergosterol assay

##### (i) 25% alcoholic potassium hydroxide solution

25 grams of potassium hydroxide were dissolved into 35 mL distilled water and brought to 100 mL with absolute ethanol. It was stored in dark glass bottle at room temperature.



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## APPENDIX B

### FUNGAL COUNTING

#### B.1 Standard plate count

The standard plate count method consists of diluting a sample with 5% v/v Tween 20 diluent until the fungi are dilute enough to count accurately. That is, the final plates in the series should have between 30 and 300 colonies. Fewer than 30 colonies are not acceptable for statistical reasons (too few may not be representative of the sample), and more than 300 colonies on a plate are likely to produce colonies too closed to each other to be distinguished as distinct colony-forming units (CFUs). The assumption is that each viable fungal cell is separated from all others and will develop into a single discrete colony (CFU). Thus, the number of colonies should give the number of fungi that can grow under the incubation conditions employed. A wide series of dilutions (e.g.,  $10^{-4}$  to  $10^{-8}$ ) is normally plated because the exact number of fungi is usually unknown.

The procedures:

- (i) Label the bottom of six petri plates 1-15. Label five tubes of 5% v/v Tween 20  $10^{-4}$ ,  $10^{-5}$ ,  $10^{-6}$ ,  $10^{-7}$  and  $10^{-8}$ .
- (ii) Using aseptic technique, the initial dilution is made by transferring 1 mL of *A. flavus* spore solution sample to a 9 mL sterile 5% v/v Tween 20 blank (Figure B.1. This is a  $1/10$  or  $10^{-1}$  dilution).
- (iii) Immediately after the  $10^{-1}$  dilution has been shaken, uncap it and aseptically transfer 1ml to a second 9 mL 5% v/v Tween 20 blank. Since this is a  $10^{-1}$  dilution, this second blank represents a  $10^{-2}$  dilution of the original sample.
- (iv) Shake the  $10^{-2}$  dilution vigorously and transfer 1 mL to the third 9 mL blank. This third dilution represents a  $10^{-3}$  dilution of the original sample.
- (v) Repeat the Shake the  $10^{-3}$  dilution again and aseptically transfer 1.0 mL to one petri plate.
- (vi) Do the same for the  $10^{-5}$ ,  $10^{-6}$ ,  $10^{-7}$  and the  $10^{-8}$  dilutions.

- (vii) Remove one agar pour tube from the approximate 48 °C water bath. Carefully remove the cover from the 10<sup>-4</sup> petri plate and aseptically pour the agar into it.
- (viii) The agar and sample are immediately mixed by gently moving the plate as in a Figure B.1.
- (ix) Repeat this process for the remaining three plates.
- (x) After the pour plates have cooled and the agar has hardened, they are inverted and incubated at 28±2 °C for 2-3 days.
- (xi) At the end of the incubation period, select all of the petri plates containing between 30 and 300 colonies. Plates with more than 300 colonies cannot be counted and are designated too many to count. Plates with fewer than 30 colonies are designated too few to count.
- (xii) Count the colonies on each plate.
- (xiii) Calculate the number of bacteria (CFU) per milliliter or gram of sample by dividing the number of colonies by the dilution factor multiplied by the amount of specimen added to liquefied agar.

$$\frac{\text{Number of colonies (CFUs)}}{\text{Dilution X amount plated}} = \text{fungi/ml}$$

Example;

The number of fungi (CFU) per milliliter that were counted from the *A. flavus* fungal colonies were 54, 48, 52 colonies forming units by plating triplicated at 10<sup>-6</sup> dilution.

$$= \frac{54+56+52 \text{ CFUs}}{10^{-6} \times 3} = 54 \times 10^6 \text{ CFU/mL}$$

Therefore; The number of fungi from *A. flavus* culture was about 5.4×10<sup>7</sup> CFU/mL

- (xiv) Process once more to produce a 10<sup>-4</sup> dilution.

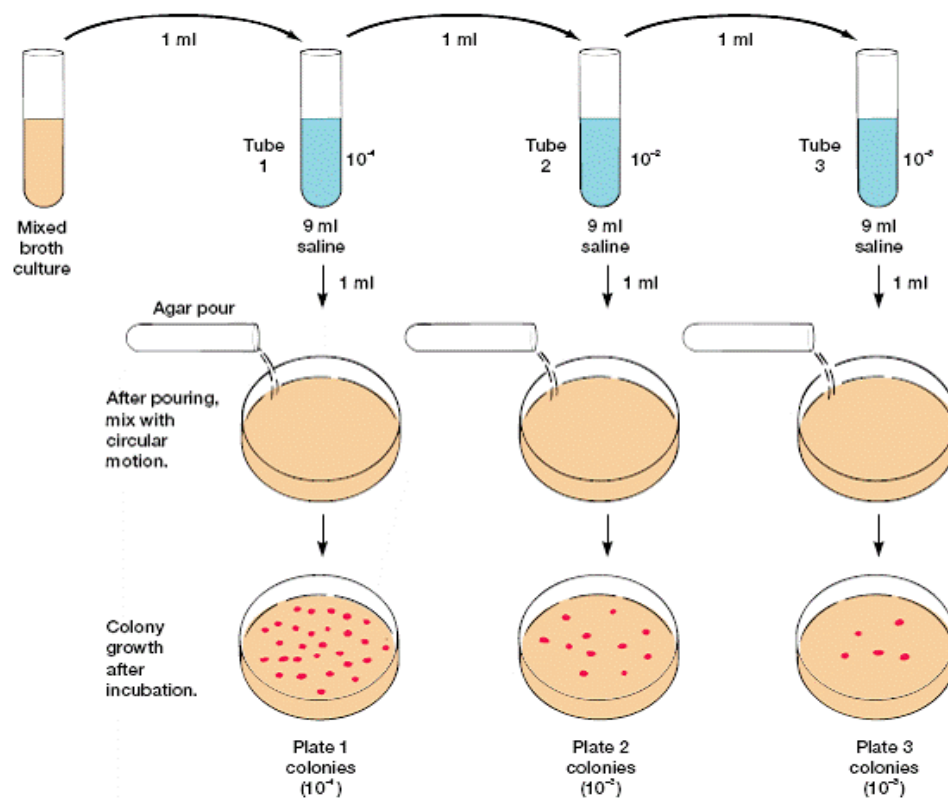


Figure B.1 Procedure of pour plate counting

([http://www.agro.kmutnb.ac.th/e-learning/521302/1\\_clip\\_image016.gif](http://www.agro.kmutnb.ac.th/e-learning/521302/1_clip_image016.gif))

(Date: November 18, 2014)

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## B.2 Direct microscope method

Petroff-Hausser counting chambers can be used as a direct method to determine the number of fungal cells in a culture or liquid medium. In this procedure, the number of cells in a given volume of culture liquid is counted directly in 10-20 microscope fields. The average number of cells per field is calculated and the number of fungal cells  $\text{mL}^{-1}$  of original sample can then be computed. A major advantage of direct counts is the speed at which results are obtained. The grid is divided into 9 large squares, each 1 mm x 1 mm, by triple lines. Each large square is divided into 25 medium squares, each 0.23 mm on a side, and each medium square is further divided into 16 small squares, each 0.05 mm on a side (Figure B.2).

The procedures:

- (i) Mix spore solution well.
- (ii) Add 10  $\mu\text{L}$  of spore solution to each side of the hemocytometer.
- (iii) Count number of spores in zones A, B, C and D on both sides of the hemocytometer, record them, and calculate the average of the two sides.

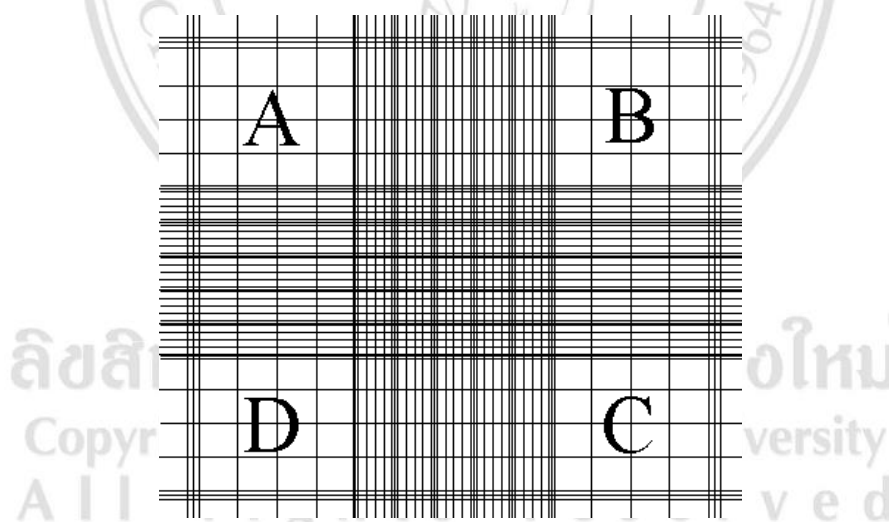


Figure B.2 Petroff-Hausser counting chamber

(<http://home.cc.umanitoba.ca/~adam/lab/images/Hemato.gif>)

(Date: November 18, 2014)

Where; A = Total number of cell or spore inside 9 large squares (cell)

B = Volume required of spore suspension ( $\mu\text{L}$ )

The surface in 1 small square =  $1/400 \text{ mm}^2$ , depth =  $1/10 \text{ mm}$

Therefore in 1 small square has a volume  $(1/400 \text{ mm}^2) \times (1/10 \text{ mm}) = 1/4000 \text{ mm}^3$

Since 16 small squares equal 1 large square

Therefore in 1 small square has a volume  $16 \times 1/4000 \text{ mm}^3 = 16/4000 \text{ mm}^3$

And 9 large squares have a volume  $= 9 \times (16/4000 \text{ mm}^3)$

Or referred that 9 large squares have a volume  $= 9 \times (16/4000) \mu\text{L}$

If in 9 large squares, the counted number of cells = A cells

Therefore in the volume of  $9 \times (16/4000) \mu\text{L}$  has cells = A cells

If the volume of spore suspension  $= B \mu\text{L}$

$$\text{Therefore in volume of } B \mu\text{L there are} = \frac{A \times B (\times \text{dilution})}{9 \times (16/4000)} \text{ cells}$$

Example;

Determination of spore suspension concentration of inoculated *A. flavus* was calculated as shown in Figure B. 3

7			2	3			6
4			5	4			5

Figure B.3 Spore numbers inside 4 zone squares of counting chamber in 2 times

Average of total spore number inside 4 zone squares as  $(18+18)/2 = 18$  cells

At dilution  $10^{-2}$ ; the volume of 1000  $\mu\text{L}$  of spore suspension has spores

$$= \frac{18 \times 1000 \times 10^{-2}}{9 \times (16/4000)} = 5 \times 10^3 \text{ cells}$$

Therefor; the concentration of spore suspension  $= 5 \times 10^3 \text{ cells/mL}$

## APPENDIX C

### Another *Zanthoxylum* species found in Thailand

#### C. *Zanthoxylum limonella* Alston

##### C. 1 Scientific classification

Kingdom: Plantae

Order: Sapindales

Family: Rutaceae

Genus: *Zanthoxylum*

Species: *Z. limonella* Alston

Common name: Makhan

Local name: Makhan (มะเขว่น, มะเข่น), Bakhan (บ่าเขว่น, บ่าเข่น)

##### C. 2 Botanical characteristics of plant

*Zanthoxylum limonella* Alston (Rutaceae), locally called “Makhan”, is an evergreen shrub distributed in the northern part of Thailand. *Z. limonella* Alston is a perennial plant 5-25 m high, spiny trunk, glossy leaves and red leaf stalk. The flowers are greenish white, in small corymbs and fruits are green and brown ripe with shiny black seeds. Characteristics of *Zanthoxylum limonella* Alston plant is shown in Figure C. 1.



**Figure C.1** Characteristics of *Zanthoxylum limonella* Alston: Stem (A), Flowers (B), Leaves (C), Fruits (D), Dry fruits (E)

([file:///C:/Users/Administrator/Downloads/Makwan\\_CS2.pdf](file:///C:/Users/Administrator/Downloads/Makwan_CS2.pdf)) (A-C)

(<http://www.pralanna.com/img/id/2dsc06044.jpg>) (D)

([http://4.bp.blogspot.com/24vFSw1fQ18/T7OJulAoNaI/AAAAAAAAALw/zCqal7\\_6UiU/s1600/Rutaceae.JPG](http://4.bp.blogspot.com/24vFSw1fQ18/T7OJulAoNaI/AAAAAAAAALw/zCqal7_6UiU/s1600/Rutaceae.JPG)) (E)

(Date: November 13, 2014)

### C. 3 Composition of essential oil

Itthipanichpong et al. (2002) reported that limonene (31.09%), terpin-4-ol (13.94%) and sabinene (9.13%) were major components of *Z. limonella* Alston dried fruit.

### C. 4 Benefits

*Z. limonella* Alston plants have been traditionally used in food; especially ripe fruits have been commercialized in local markets as a popular spice (Tangjitjaroenkun et al., 2012). Its roots, stem-barks, stems and fruits are used for treating stomachache and toothache (Tangjitjaroenkun et al., 2012). The essential oil from fruits affects the gastrointestinal system, exhibit stimulation effect on different smooth muscles by non-specific mechanism and antioxidant activity (Itthipanichpong et al., 2002; Tangjitjaroenkun et al., 2012). Charoenying et al., (2008) reported that the crude chloroform extract exhibited antimalarial activity against *Plasmodium falciparum* and antituberculous activity against *Mycobacterium tuberculosis* H37 Ra.

## APPENDIX D

### TABLES OF RAW DATA

Table D.1 Diameter (cm) of *Aspergillus flavus* colony grown in agar medium supplemented with the essential oil from *Anethum graveolens* L. at five different concentrations\*

Concentration		Day								
		1	2	3	4	5	6	7	8	9
0	1	1.23	2.33	3.76	4.46	5.90	6.94	7.70	8.36	8.44
	2	1.23	2.34	3.75	4.43	5.85	6.94	7.70	8.20	8.50
	3	1.23	2.35	3.77	4.54	5.88	6.94	7.67	8.10	8.50
	av	1.23±0.00	2.33±0.01	3.76±0.01	4.48±0.06	5.88±0.03	6.94±0.00	7.69±0.02	8.22±0.13	8.48±0.03
0.25	1	0.64	1.10	1.79	2.17	2.20	2.32	2.32	2.32	2.35
	2	0.42	0.76	1.35	1.74	2.31	2.42	2.43	2.45	2.45
	3	0.59	1.00	1.65	2.00	2.48	2.50	2.5	2.51	2.51
	av	0.55±0.12	0.95±0.17	1.60±0.22	1.97±0.22	2.33±0.14	2.41±0.09	2.42±0.09	2.43±0.01	2.45±0.08

Concentration		Day								
		1	2	3	4	5	6	7	8	9
0.50	1	0.40	0.64	1.15	1.42	1.82	1.90	1.99	2.01	2.08
	2	0.40	0.61	1.10	1.44	1.53	1.53	1.59	1.59	1.59
	3	0.40	0.83	1.45	1.95	2.44	2.50	2.58	2.58	2.60
	av	0.40±0.00	0.69±0.12	1.23±0.19	1.60±0.30	1.93±0.46	1.98±0.49	2.05±0.50	2.06±0.50	2.09±0.51
1.0	1	0.40	0.40	0.40	0.54	0.73	0.85	0.96	1.07	1.05
	2	0.40	0.40	0.40	0.57	0.67	0.82	0.94	1.00	1.08
	3	0.40	0.40	0.40	0.57	0.73	0.84	0.91	1.02	1.21
	av	0.40±0.00	0.40±0.00	0.40±0.00	0.56±0.02	0.71±0.03	0.84±0.02	0.94±0.03	1.03±0.03	1.11±0.09
1.5	1	0.40	0.40	0.40	0.40	0.40	0.40	0.55	0.66	0.71
	2	0.40	0.40	0.40	0.40	0.40	0.40	0.63	0.65	0.68
	3	0.40	0.40	0.40	0.40	0.40	0.40	0.53	0.58	0.64
	av	0.40±0.00	0.40±0.00	0.40±0.00	0.40±0.00	0.40±0.00	0.40±0.00	0.57±0.05	0.63±0.04	0.68±0.04
2.0	1	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
	2	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
	3	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
	av	0.40±0.00	0.40±0.00	0.40±0.00	0.40±0.00	0.40±0.00	0.40±0.00	0.40±0.00	0.40±0.00	0.40±0.00

\*Result presented in 3.3.1

Table D.2 Diameter (cm) of *Aspergillus flavus* colony grown in agar medium supplemented with the essential oil from *Zanthoxylum piperitum* at eight different concentrations\*

Concentration ( $\mu\text{L/mL}$ )		Day								
		1	2	3	4	5	6	7	8	9
0	1	1.23	2.33	3.76	4.46	5.90	6.94	7.70	8.36	8.44
	2	1.23	2.34	3.75	4.43	5.85	6.94	7.70	8.20	8.50
	3	1.23	2.35	3.77	4.54	5.88	6.94	7.67	8.10	8.50
	av	1.23 $\pm$ 0.00	2.33 $\pm$ 0.01	3.76 $\pm$ 0.01	4.48 $\pm$ 0.06	5.88 $\pm$ 0.03	6.94 $\pm$ 0.00	7.69 $\pm$ 0.02	8.22 $\pm$ 0.13	8.48 $\pm$ 0.03
1.0	1	0.40	0.85	1.44	1.83	2.39	3.0	3.65	3.90	4.45
	2	0.40	0.83	1.44	1.84	2.38	2.98	3.63	4.16	4.64
	3	0.40	0.83	1.45	1.85	2.48	3.03	3.60	4.26	4.55
	av	0.40 $\pm$ 0.00	0.84 $\pm$ 0.01	1.44 $\pm$ 0.01	1.84 $\pm$ 0.01	2.42 $\pm$ 0.06	3.00 $\pm$ 0.03	3.63 $\pm$ 0.03	4.11 $\pm$ 0.19	4.55 $\pm$ 0.01
1.5	1	0.40	0.60	0.89	1.00	1.60	2.04	2.65	3.00	3.36
	2	0.40	0.64	0.87	1.19	1.55	2.01	2.68	2.94	3.33
	3	0.40	0.57	0.82	1.07	1.40	1.95	2.44	2.54	2.89
	av	0.40 $\pm$ 0.00	0.60 $\pm$ 0.04	0.86 $\pm$ 0.04	1.15 $\pm$ 0.07	1.52 $\pm$ 0.10	2.00 $\pm$ 0.05	2.59 $\pm$ 0.13	2.83 $\pm$ 0.25	3.18 $\pm$ 0.26

Concentration ( $\mu\text{L/mL}$ )		Day								
		1	2	3	4	5	6	7	8	9
2.0	1	0.40	0.40	0.51	0.75	0.95	1.18	1.53	1.82	1.87
	2	0.40	0.40	0.54	0.71	0.82	0.99	1.38	1.51	1.74
	3	0.40	0.40	0.60	0.78	1.00	1.23	1.61	1.82	2.02
	av	0.40 $\pm$ 0.00	0.40 $\pm$ 0.00	0.55 $\pm$ 0.04	0.75 $\pm$ 0.05	0.92 $\pm$ 0.09	1.13 $\pm$ 0.13	1.51 $\pm$ 0.12	1.72 $\pm$ 0.18	1.88 $\pm$ 0.14
2.5	1	0.40	0.40	0.40	0.68	0.71	0.83	1.07	1.13	1.32
	2	0.40	0.40	0.40	0.67	0.80	0.91	1.17	1.30	1.52
	3	0.40	0.40	0.40	0.68	0.82	1.02	1.24	1.36	1.53
	av	0.40 $\pm$ 0.00	0.40 $\pm$ 0.00	0.40 $\pm$ 0.00	0.68 $\pm$ 0.01	0.78 $\pm$ 0.06	0.92 $\pm$ 0.10	1.16 $\pm$ 0.09	1.26 $\pm$ 0.12	1.46 $\pm$ 0.12
3.0	1	0.40	0.40	0.40	0.40	0.40	0.76	0.89	0.98	1.09
	2	0.40	0.40	0.40	0.40	0.40	0.77	0.81	1.06	1.21
	3	0.40	0.40	0.40	0.40	0.40	0.71	0.88	0.98	1.14
	av	0.40 $\pm$ 0.00	0.40 $\pm$ 0.00	0.40 $\pm$ 0.00	0.40 $\pm$ 0.00	0.40 $\pm$ 0.00	0.75 $\pm$ 0.03	0.89 $\pm$ 0.02	1.01 $\pm$ 0.05	1.15 $\pm$ 0.06
3.5	1	0.40	0.40	0.48	0.56	0.59	0.60	0.62	0.63	0.73
	2	0.40	0.40	0.42	0.54	0.63	0.70	0.71	0.86	1.05
	3	0.40	0.40	0.44	0.56	0.60	0.60	0.64	0.89	1.02
	av	0.40 $\pm$ 0.00	0.40 $\pm$ 0.00	0.45 $\pm$ 0.03	0.55 $\pm$ 0.01	0.62 $\pm$ 0.02	0.65 $\pm$ 0.05	0.68 $\pm$ 0.04	0.73 $\pm$ 0.12	0.93 $\pm$ 0.18

Concentration ( $\mu\text{L/mL}$ )		Day								
		1	2	3	4	5	6	7	8	9
4.0	1	0.40	0.40	0.42	0.52	0.53	0.55	0.55	0.64	0.69
	2	0.40	0.40	0.44	0.53	0.54	0.60	0.60	0.71	0.82
	3	0.40	0.40	0.41	0.53	0.57	0.62	0.62	0.66	0.77
	av	0.40 $\pm$ 0.00	0.40 $\pm$ 0.00	0.42 $\pm$ 0.02	0.53 $\pm$ 0.01	0.55 $\pm$ 0.02	0.59 $\pm$ 0.04	0.59 $\pm$ 0.03	0.67 $\pm$ 0.04	0.76 $\pm$ 0.07
4.5	1	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
	2	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
	3	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
	av	0.40 $\pm$ 0.00	0.40 $\pm$ 0.00	0.40 $\pm$ 0.00	0.40 $\pm$ 0.00	0.40 $\pm$ 0.00	0.40 $\pm$ 0.00	0.40 $\pm$ 0.00	0.40 $\pm$ 0.00	0.40 $\pm$ 0.00

\*Result presented in 3.3.2

Table D.3 Effect of concentrations of *Anethum graveolens* L. at 0.25  $\mu\text{L/mL}$  and *Zanthoxylum piperitum* at 1  $\mu\text{L/mL}$  on ergosterol content in plasma membrane of *A.flavus* in PDB for 4 days

Sample	Filter paper	Filter paper + Pellet	wet	A <sub>230</sub>	A <sub>282</sub> **	% Ergosterol content
Control 1	0.5571	10.0781	9.5210	1.0414	1.0250	0.25 $\pm$ 0.03 <sup>a</sup>
Control 2	0.5613	11.0498	10.4885	1.0631	1.0562	
Control 3	0.5562	9.2227	8.6665	0.9056	1.0315	
<i>A. graveolens</i> 1	0.5818	5.3261	4.7443	0.5776	0.6304	0.17 $\pm$ 0.01 <sup>b</sup>
<i>A. graveolens</i> 2	0.5534	4.3283	3.7749	0.5730	0.5102	
<i>A. graveolens</i> 3	0.5488	5.1947	4.6459	0.5734	0.6346	
<i>Z. piperitum</i> 1	0.5504	4.6113	4.0609	0.4705	0.4775	0.18 $\pm$ 0.02 <sup>b</sup>
<i>Z. piperitum</i> 2	0.5691	4.1480	3.5789	0.6119	0.5122	
<i>Z. piperitum</i> 3	0.5521	4.0624	3.5103	0.5223	0.5131	

\*Result presented in 3.6.1

\*\*Control dilution =  $10^6$ , *A. graveolens* and *Z. piperitum* dilution =  $10^4$

Values are mean (n = 3)  $\pm$  standard deviations. Values followed by the same letter in each column are not significantly different in ANOVA and Duncan Multiple Range Test (p < 0.05).

Table D.4 Efficacy of essential oil in the conservation of Bird Chilli, a model product after 9 days of incubation\*

Sample	Number Spore				% Inhibition
	1	2	3	av	
Control	37	21	17	$2.50 \times 10^{6a}$	-
<i>A. graveolens</i> MIC	4	6	2	$4.00 \times 10^{5b}$	84.00
<i>A. graveolens</i> 2XMIC	2	1	2	$1.67 \times 10^{5b}$	93.32
<i>Z. piperitum</i> MIC	10	10	19	$1.30 \times 10^{6a}$	48.00
<i>Z. piperitum</i> 2XMIC	4	2	2	$2.67 \times 10^{5b}$	89.32

\*Result presented in 3.7.1

Values are mean (n = 3)  $\pm$  standard deviations. Values followed by the same letter in each column are not significantly different in ANOVA and Duncan Multiple Range Test (p < 0.05).

## APPENDIX E

### Mass spectra and the structures of main components of the essential oils

The mass spectra of pure compounds are presented in order to be compared with the result in section 3.2. Their structures are also documented [W8N05ST (Wiley ver.8.0)].

#### E 1. The major component of *Anethum graveolens* L. essential oil

##### 1.1 (-)-Limonene

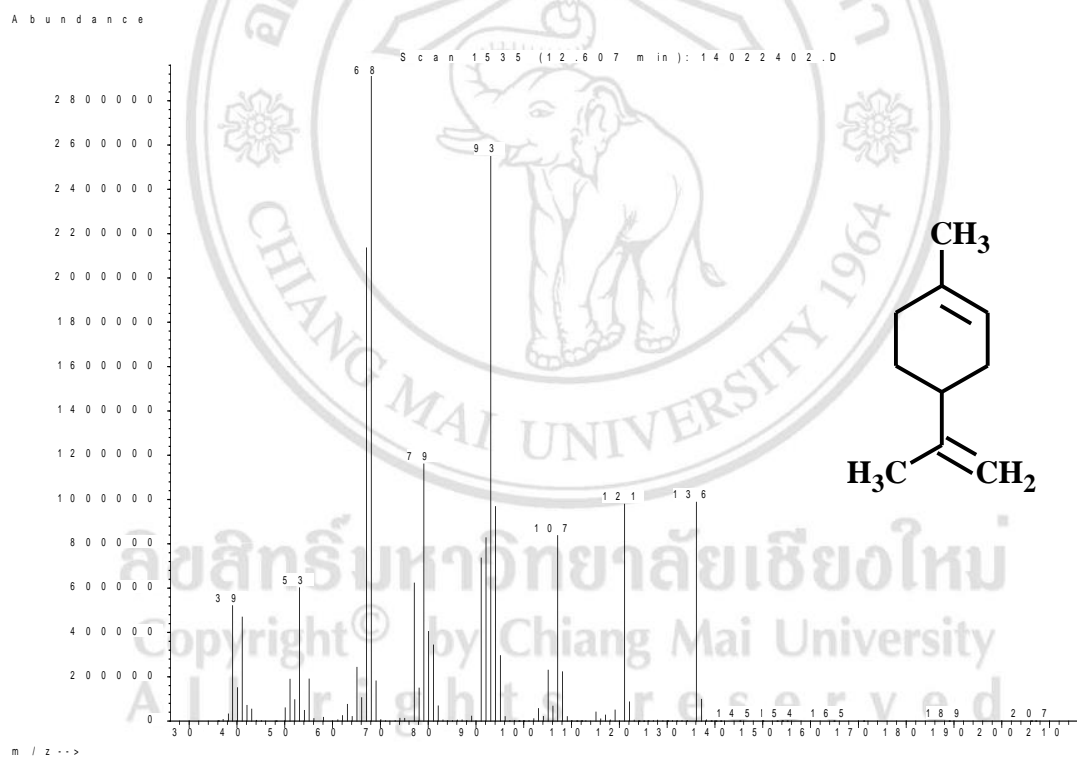


Figure E.1.1 The mass spectrum and structure of (-)-limonene

## 1.2 Dihydrocarvone

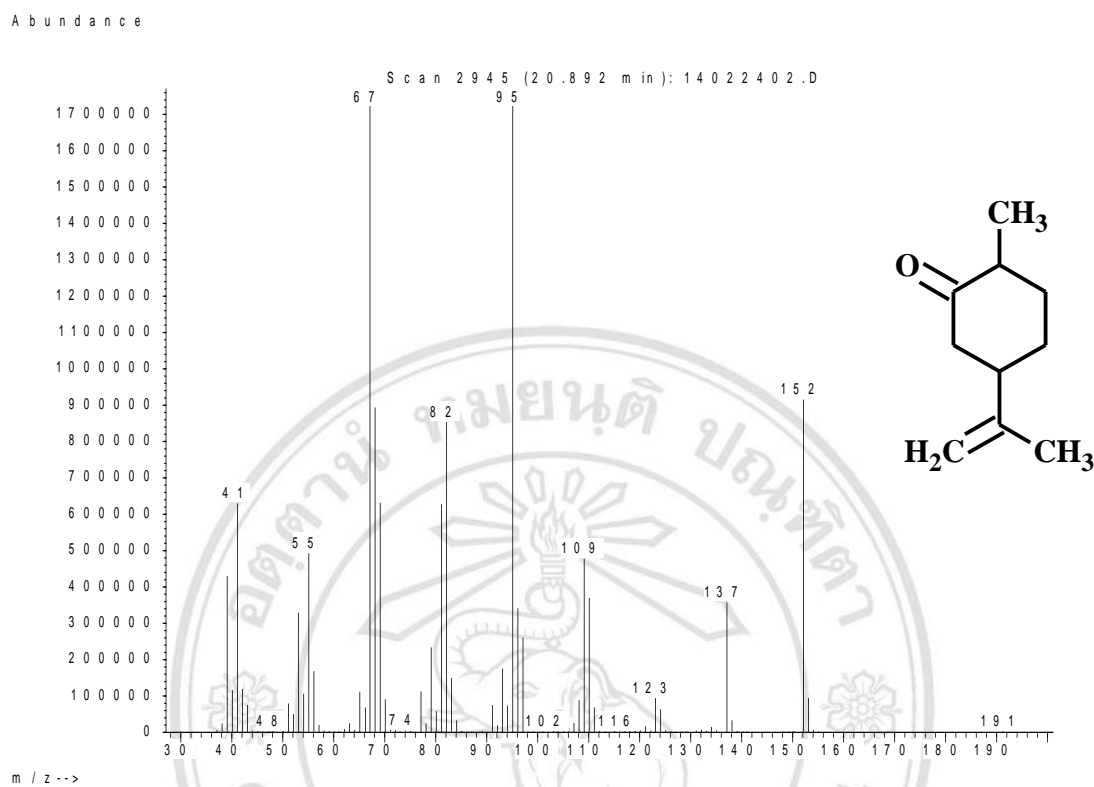
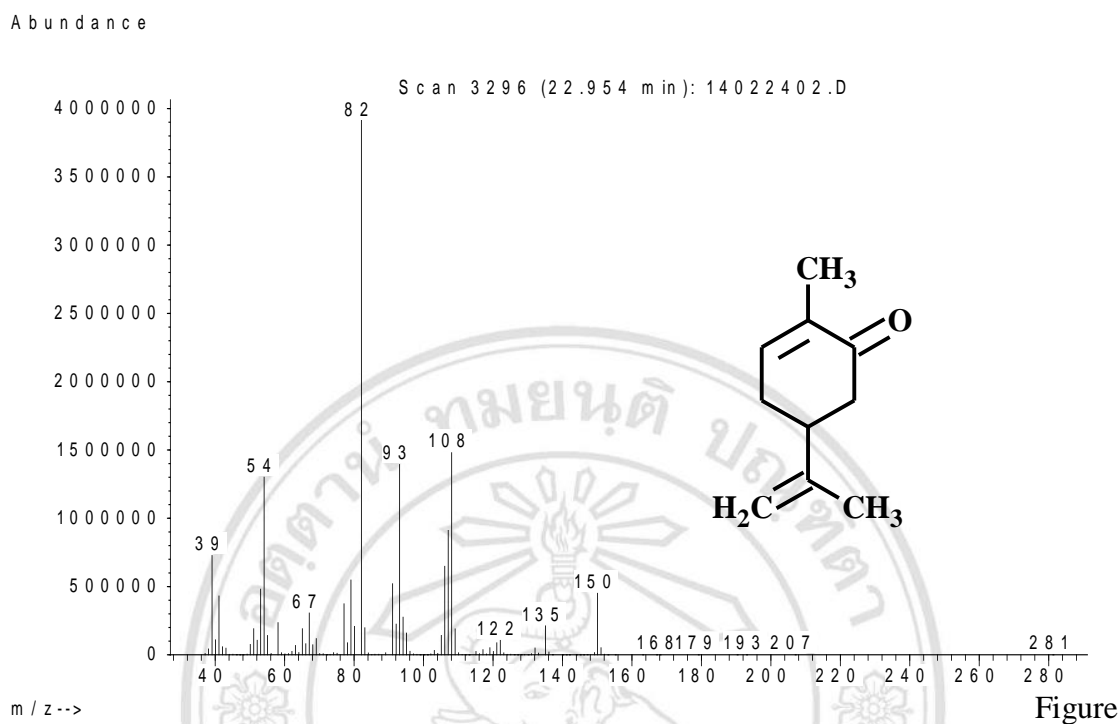


Figure E.1.2 The mass spectrum and structure of dihydrocarvone

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### 1.3 (+)-Carvone



E.1.3 The mass spectrum and structure of (+)-carvone

#### 1.4 *trans*-Isodillapiole

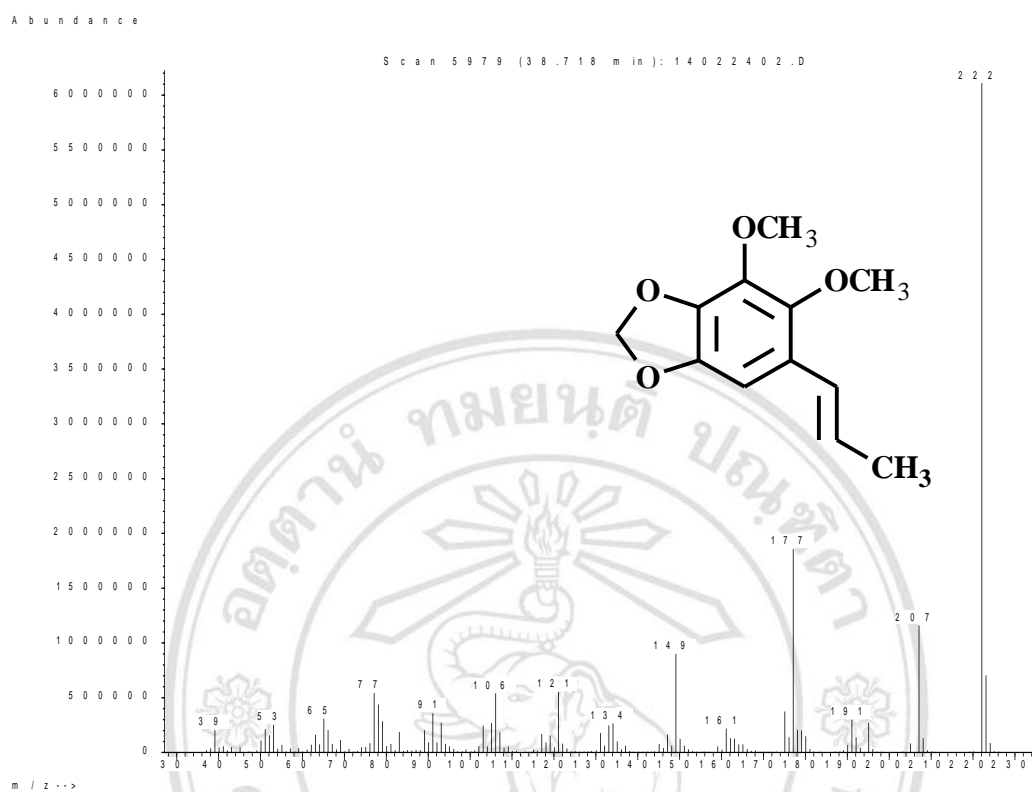
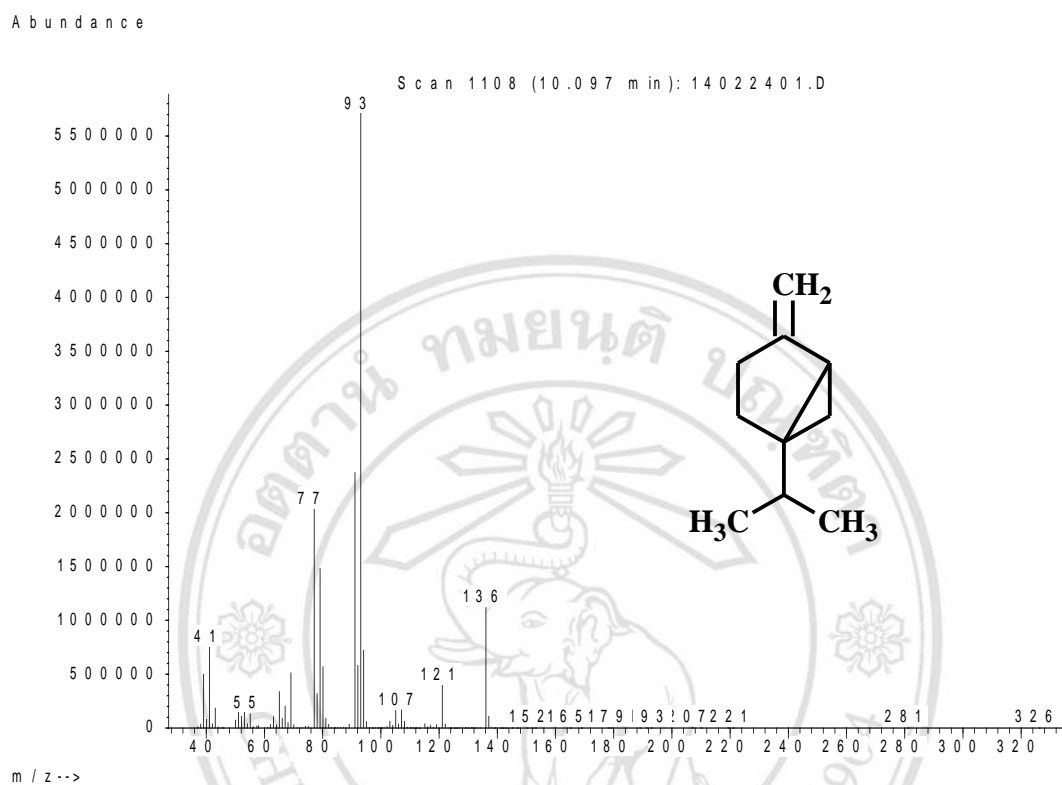


Figure E.1.4 The mass spectrum and structure of *trans*-Isodillapiole

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## E 2. The major component of *Zanthoxylum piperitum* essential oil

### 2.1 (+)-Sabinene



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## 2.2 $\beta$ -Phellandrene

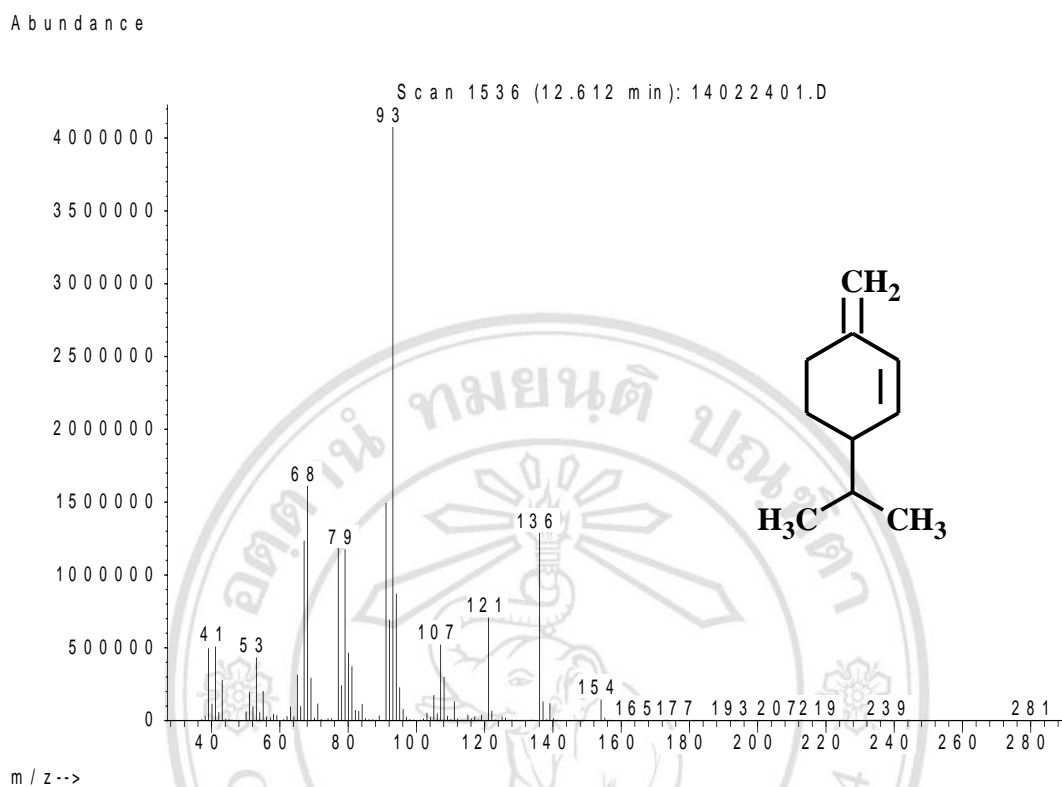


Figure E. 2.2 The mass spectrum and structure of  $\beta$ -phellandrene

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## 2.3 Brevifolin

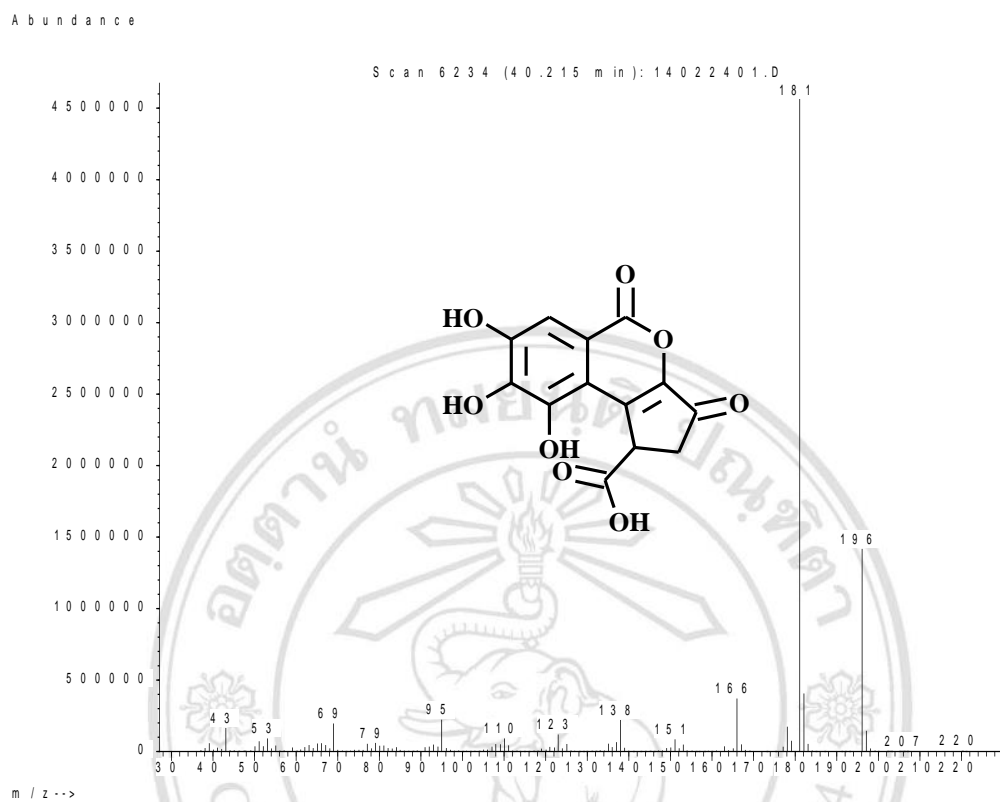
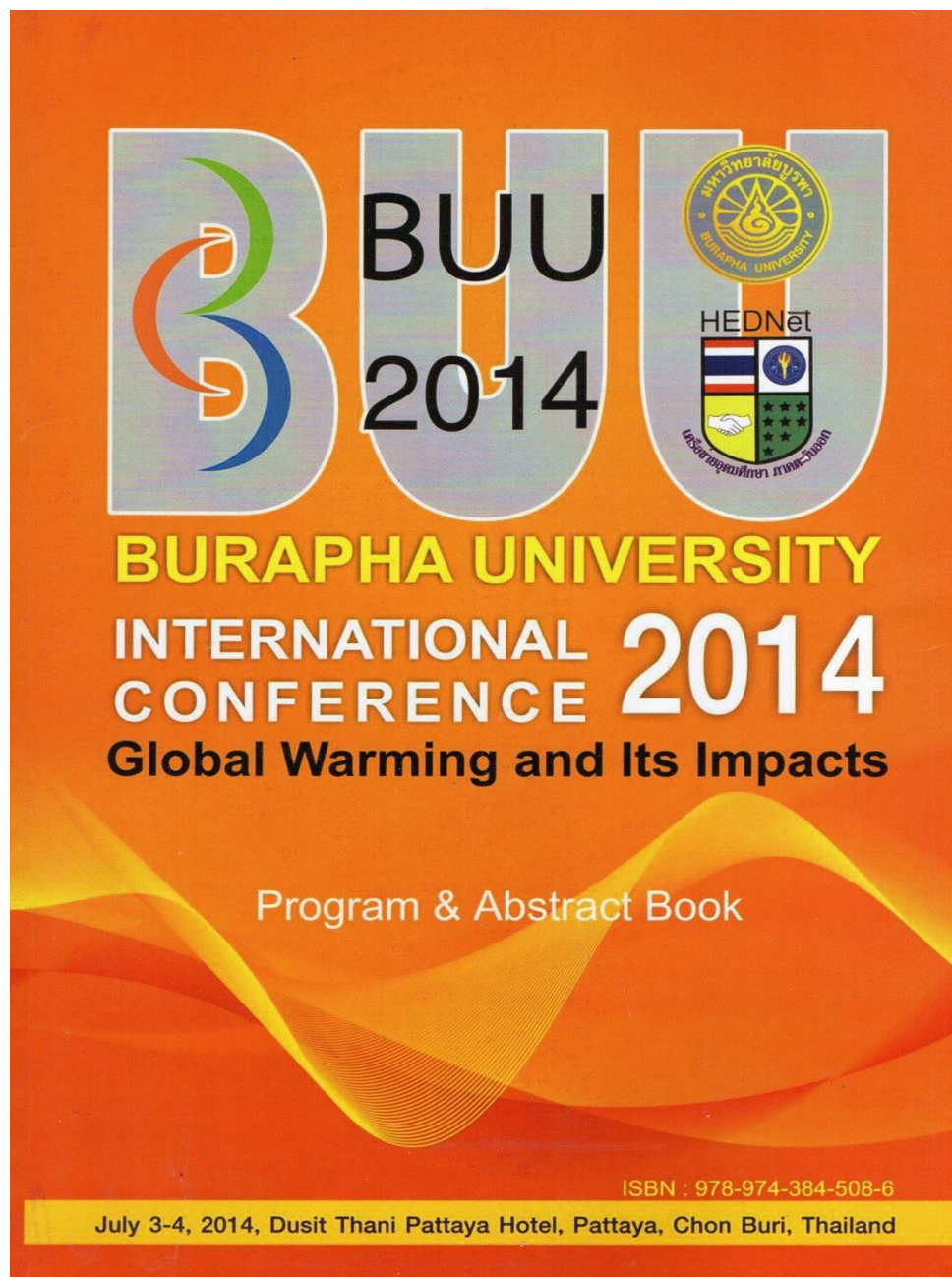


Figure E. 2.3 The mass spectrum and structure of brevifolin

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## PUBLICATION

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STP652-16

## Antifungal Activity of Essential oils from Some Spices Against *Aspergillus flavus*

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### ABSTRACT

The antifungal activities of essential oil from four spices, *Anethum graveolens* L. (Dill), *Foeniculum vulgare* Mill. (Fennel), *Coriandrum sativum* (Coriander), *Zanthoxylum* spp. (Prikhom), against *Aspergillus flavus* isolated from bird chili powder were investigated. The preliminary study on antifungal activity was evaluated at a concentration of 1 µl/ml by poisoned food technique in solid culture. *A. graveolens* L. oil exhibited highly effective antifungal activity, thus *A. graveolens* L. was selected to determine the minimal inhibitory concentration (MIC) using the concentrations of the essential oil between 0.25 and 2 µl/ml. The wet and dry mycelial weights of the treated fungus were also determined in a liquid culture. The minimum inhibitory concentration of *A. graveolens* L. for the tested fungus was found to be 2.0 µl/ml on 9th day of the mycelial growth. Thus, the essential oil of *A. graveolens* L. could be potentially used as an alternative food preservative against spoilage caused by the fungus.

**Keywords:** *Anethum graveolens* L., Antifungal, Essential oil, Minimal Inhibitory Concentration (MIC), Spices

### INTRODUCTION

Contamination of spoilage microorganism in food has been a serious problem in food industries and consumers. Food is easily attacked by various microorganisms such as *Aspergillus* species leading to significant economic loss. Some species introduce a very serious risk for consumers because of the dangerous secondary metabolites they produce in crops (Tian et al., 2012). In particular, *Aspergillus flavus* is mainly responsible for spoilage in many foods and production of aflatoxins (Beyki et al., 2014). Aflatoxins are widely known for carcinogenic, teratogenic, hepatotoxic, mutagenic, and immunosuppressive properties, and can inhibit several metabolic systems (Joseph et al., 2005). High moisture content and high temperature are the major factors for the growth of pathogenic fungi and the production of the toxin in many countries, especially in tropical and subtropical regions (Chun et al., 2006). The use of synthetic preservatives or antimicrobial agents to control fungal spoilage of food has led to a number of environmental and health problems. Consumers and food processors concern about using synthetic preservative and require new self-preservative that are biodegradable and environmental friendly. Various plant products have been recognized and used for food preservation and in medicine because of their antifungal properties. Spices are the tropical



Burapha University International Conference 2014 (BUU2014)  
July 3-4, 2014, Dusit Thani Pattaya, Pattaya, Thailand

## ACCEPTANCE LETTER

*June 3, 2014*

Dear Chorpaka Phuangsi,

On behalf of the organizing committee, I am glad to inform that your manuscript entitled "**Antifungal Activity of Essential oils from Some Spices Against *Aspergillus flavus***" has been accepted for **poster** presentation at the Burapha University International Conference held in Dusit Thani Pattaya, Pattaya, Thailand, on July 3-4, 2014. Your manuscript will be published in peer-reviewed proceedings. Acceptance of your contribution carries with it the obligation for at least one of the authors to actually present it at the meeting. Details on registration form, methods of payment, author guidelines, important dates and other practical issues can be found on the conference website [www.buu2014.buu.ac.th](http://www.buu2014.buu.ac.th).

You must access the <http://www.buu2014.buu.ac.th/?q=node/27> and attach the "Scanned Bank Transfer Slip" together as soon as possible. Please note that if you did not attach the slip at the same time as you fill in the registration form, please fill out the new registration form again.

Make sure to register for the conference as soon as possible to confirm your participation. Registration must be completed before June 10, 2014.

Your contribution will be of great value to the success of this event. We are looking forward to seeing you at the conference.

Kind regards,

A handwritten signature in black ink, appearing to read 'Somtawin'.

Assist. Prof. Somtawin Jaritkhuan, Ph.D.  
General Co-chair  
Email: [buu2014@buu.ac.th](mailto:buu2014@buu.ac.th)



Burapha University International Conference  
"Global Warming and Its Impacts"  
July 3-4, 2014, Pattaya, Thailand

## Best Poster Award

to

**Chorpaka Phuangsrri**

for the paper entitled

**Antifungal Activity of Essential oils  
from Some Spices Against *Aspergillus flavus***

Professor Sompol Pongthai, FRTCOG, MPH, LLB

Acting President of Burapha University

Chairman of the Universities in Higher Education Development Eastern Network



บัณฑิตวิทยาลัย มหาวิทยาลัยเชียงใหม่

เกียรติบัตรฉบับนี้ให้ไว้เพื่อแสดงว่า

นางสาวช่อผกา พวงศรี

สหสาขาวิชาเทคโนโลยีชีวภาพ

เป็นผู้สร้างชื่อเสียงและเป็นแบบอย่างที่ดีงามแก่นักศึกษาสหสาขาวิชา ประจำปีการศึกษา ๒๕๕๕-๒๕๕๖

ขอให้ประสบความสำเร็จทุกด้านตลอดไป

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(รองศาสตราจารย์ ดร.เอกชัย แสงอินทร์)

คณบดีบัณฑิตวิทยาลัย มหาวิทยาลัยเชียงใหม่

## CURRICULUM VITAE

**Name** Miss Chorpaka Phuangsri

**Date of Birth** February 14, 1990

### Education Background

2012 Bachelor of Science (Biotechnology)  
Silpakorn University

2014 Master of Science (Biotechnology)  
Chiang Mai University

### Poster Presentations

Phuangsri C., Nuntawan N., Niamsup H. Antifungal Activity of Essential Oils from Some Spices Against *Aspergillus flavus*. *Burapha University International Conference 2014*. July 3-4, 2014; Pattaya, Thailand

### Academic Performance

I received best poster presentation award in Burapha University International Conference 2014.

