CHAPTER IV
RESULTS AND DISCUSSION

4.1 Extraction of essential oils

The amount of oil extracted from each plant was calculated and presented as percent yield (% v/w) based on fresh plant at 30ºC. The results as shown in Table 4.1 demonstrate that each plant used in this study gave different quantities of oil. It was found that among the studied plant samples, the peel of *C. maxima* (Burm.) Merr. gave the highest yield of 0.759 %, followed by the rhizome of *Z. cassumunar* Roxb., the leaves of *C. aurantifolia* Swing., the stem of *C. citratus* Stapf., and the peel of *C. aurantifolia* Swing. with percent yield of 0.477%, 0.341%, 0.275% and 0.271%, respectively. *C. asiatica* Urban. and the leaf of *C. amboinicus* Lour. gave the lowest yield of 0.005%. Tg Kamazeri1 et al. reported that the yield of essential oil extracted by hydrodistillation from rhizome of *Z. cassumunar* was 0.30% w/w [135], less than that obtained in this study. Nakamura et al extracted the essential oil from of the rhizome *O. gratissimum* and found that the oil of 0.21% less than the yield of this oil obtained from the present study [136]. Kelly et al. reported that place and time of plant collection played an important role on essential oil yield [137, 138]. Singh et al reported that the essential oil extracted from C. maxima leaves was 0.730% v/w whereas in this study when the fruit peel of *C. maxima* was used instead of the leaves, the yield was 0.769%, higher than that previously reported. The different of essential oil yield was considered to be due to the different parts of plants [139, 140].

Table 4.1 The amount oil extracted as percent yield (% v/w) relative to fresh weight

No.	Scientific name	Local Name	Part of plants	% yield (v/w)
1	*Apium graveolens* Linn.	คื่นไช่	Whole Plant	0.018
2	*Anethum graveolens* Linn.	ผักชีลาว	Whole Plant	0.023
3	*Centella asiatica* Urban.	บัวบก	Whole Plant	0.005
4	*Coriandrum sativum* Linn.	ผักชี	Whole Plant	0.088
5	*Eryngium foetidum* Linn.	ผักชีฝรั่ง	Whole Plant	0.023
6	*Polyscias fruticosa* Harms.	เล็บครุฑ	Leaf	0.113
7	*Eupatorium odoratum* Linn.	สาบเสือ	Whole Plant	0.010
8	*Spilanthes acmella* Murr.	ผักคราดหัวแหวน	Whole Plant	0.012
9	*Cymbopogon citratus* Stapf.	ตะไคร้	Stem	0.275
10	*Coleus amboinicus* Lour.	เนียมหูเสือ	Leaf	0.005
11	*Melissa officinalis* Linn.	สะระแหน่	Leaf	0.027
12	*Ocimum basilicum* Linn.	โหระพา	Leaf	0.182
13	*Ocimum canum* Sims.	แมงลัก	Stem and leaf	0.049
14	*Ocimum gratissimum* Linn.	ยี่หร่า	Leaf	0.052
15	*Ocimum sanctum* Linn.	กะเพรา	Leaf	0.175
16	*Cinnamomum bejolghota* Sweet.	อบเชย	Leaf	0.064

Table 4.1 (continued)

No.	Scientific name	Local Name	Part of plants	% yield (v/w)
17	*Sesamum indicum* Linn.	งา	Seed	-
18	*Piper sarmentosum* Roxb.	ชะพลู	Leaf	0.020
19	*Polygonum odoratum* Lour.	ผักแพรว	Stem and leaf	0.038
20	*Citrus aurantifolia* Swing.	มะนาว	Leaf	0.341
21	*Citrus aurantifolia* Swing.	มะนาว	Peel	0.271
22	*Citrus maxima* (Burm.) Merr.	ส้มโอ	Leaf	0.120
23	*Citrus maxima* (Burm.) Merr.	ส้มโอ	Peel	0.759
24	*Houttuynia cordata* Thunb.	พลูคาว	Leaf	0.018
25	*Boesenbergia pandurata* Roxb.	กระชาย	Rhizome	0.196
26	*Curcuma longa* Linn.	ขมิ้นชัน	Rhizome	0.221
27	*Curcuma zedoaria* (Berg.) Roscoe.	ขมิ้นอ้อย	Rhizome	0.236
28	*Zingiber cassumunar* Roxb.	ไพล	Rhizome	0.477
29	*Zingiber officinale* Roscoe.	ขิง	Rhizome	0.154

4.2 Study of outer appearance and density of oil

The physical appearance of the oil used in this study was shown in Figure 4.1. It was found that all of the oils showed outer appearance as clear liquid with low viscosity. Most of the oils presented the pale yellow color and aromatic odor. However, the oils of *Z.officinale* Roscoe., *B.pandurata* Roxb., *O.canum* Sims., *P.odoratum* Lour., *P. fruticosa* Harms., *A. graveolens* Linn., and *E. foetidum* Linn. showed intense strong yellow color whereas the oil of *P.sarmentosum* Roxb. was dark in color. The different species of plants color was considered to be due to the difference in plant species which possess different genotype.

A B C D E F G H I J K L M

N O P Q R S T U V W X Y Z AA BB CC

Figure 4.1 The physical appearance of the oil used in this study. The explanation of the letter in this figure is appeared in table 4.2

Table 4.2 The explanation of the letter in figure 4.1

Letter	List of oil
A	*Sesamum indicum* Linn.
B	*Zingiber officinale* Roscoe.
C	*Cinnamomum bejolghota* Sweet.
D	*Zingiber cassumunar* Roxb.
E	*Cymbopogon citratus* Stapf.
F	*Boesenbergia pandurata* Roxb.
G	*Ocimum gratissimum* Linn.
H	*Curcuma zedoaria* (Berg) Roscoe.
I	*Curcuma longa* Linn.
J	*Citrus maxima* (Burm.) Merr. (Leaf)
K	*Citrus aurantifolia* Swing. (Leaf)
L	*Citrus maxima* (Burm.) Merr. (Peel)
M	*Citrus aurantifolia* Swing. (Peel)
N	*Ocimum basilicum* Linn.
O	*Ocimum canum* Sims.
P	*Ocimum sanctum* Linn.
Q	*Piper sarmentosum* Roxb.
R	*Polygonum odoratum* Lour.
S	*Polyscias fruticosa* Harms.
T	*Spilanthes acmella* Murr.
U	*Anethum graveolens* Linn.

Table 4.2 (Continued)

Letter	List of oil
V	*Apium graveolens* Linn
W	*Centella asiatica* Urban
X	*Coleus amboinicus* Lour
Y	*Coriandrum sativum* Linn
Z	*Eryngium foetidum* Linn
AA	*Eupatorium odoratum* Linn

Table 4.3 The density of oil used in this experiment

No.	Type of oil	Density (g/ml)	SD
1	*Apium graveolens* Linn.	0.91	0.02
2	*Anethum graveolens* Linn.	0.94	0.04
3	*Centella asiatica* Urban.	0.92	0.02
4	*Coriandrum sativum* Linn.	0.78	0.02
5	*Eryngium foetidum* Linn.	0.76	0.01
6	*Polyscias fruticosa* Harms.	0.81	0.01
7	*Eupatorium odoratum* Linn.	0.90	0.02
8	*Spilanthes acmella* Murr.	0.80	0.01
9	*Cymbopogon citratus* Stapf.	0.89	0.02
10	*Coleus amboinicus* Lour.	0.67	0.03
11	*Melissa officinalis* Linn.	0.88	0.02
12	*Ocimum basilicum* Linn.	0.91	0.01
13	*Ocimum canum* Sims.	0.81	0.01
14	*Ocimum gratissimum* Linn.	0.85	0.01
15	*Ocimum sanctum* Linn.	0.94	0.01
16	*Cinnamomum bejolghota* Sweet.	0.80	0.02
17	*Sesamum indicum* Linn.	0.87	0.01
18	*Piper sarmentosum* Roxb.	0.83	0.02
19	*Polygonum odoratum* Lour.	0.76	0.03
20	*Citrus aurantifolia* Swing. (Peel)	0.81	0.01
21	*Citrus aurantifolia* Swing. (Leaf)	0.84	0.01

Table 4.3 (Continued)

No.	Type of oil	Density (g/ml)	SD
22	*Citrus maxima* (Burm.) Merr. (Peel)	0.82	0.02
23	*Citrus maxima* (Burm.) Merr. (Leaf)	0.84	0.004
24	*Houttuynia cordata* Thun.b	0.92	0.03
25	*Boesenbergia pandurata* Roxb.	0.85	0.01
26	*Curcuma longa* Linn.	0.88	0.02
27	*Curcuma zedoaria* (Berg.) Roscoe.	0.79	0.002
28	*Zingiber cassumunar* Roxb.	0.90	0.01
29	*Zingiber officinale* Roscoe.	0.86	0.01

The density of the oils was shown in Table 4.3. All of the oils used in this study showed the density value ranged from 0.67 to 0.94 g/mL. It was noted that both essential and fixed oils possess the density less than 1 g/mL. The oil of *O.sanctum* Linn. showed the highest a density of 0.94 g/mL. The lowest density was found in the oil of *C. amboinicus* Lour. with a density value of 0.67 g/mL. Sukatta et al. study the oil of *Z.cassumunar* and reported the density of this oil was 0.93 g/mL [141]. The density of *Z.cassumunar* extracted in the present study was 0.90 g/mL. The result was in good agreement with the previous results.

4.3 Study of essential oil components by gas chromatography / mass spectrometer

The twenty nine essential oils from Thai medicinal plants were analyzed for their compositions by Gas Chromatography and Mass Spectrometry technique (GC-MS). The results of the chromatogram were shown in Figures 4.2 to 4.29.

The identified chemical components of volatile oils analysis by GC-MS together with their retention time were shown in Tables 4.4 to 4.21.

4.3.1 *Apium graveolens* Linn.

GC/MS analysis resulted in the identification of 12 components but 3 components cannot be identified (Figure 4.2 and Table 4.4). Among these, limonene was found to be the main component with the amount of 62.12%.

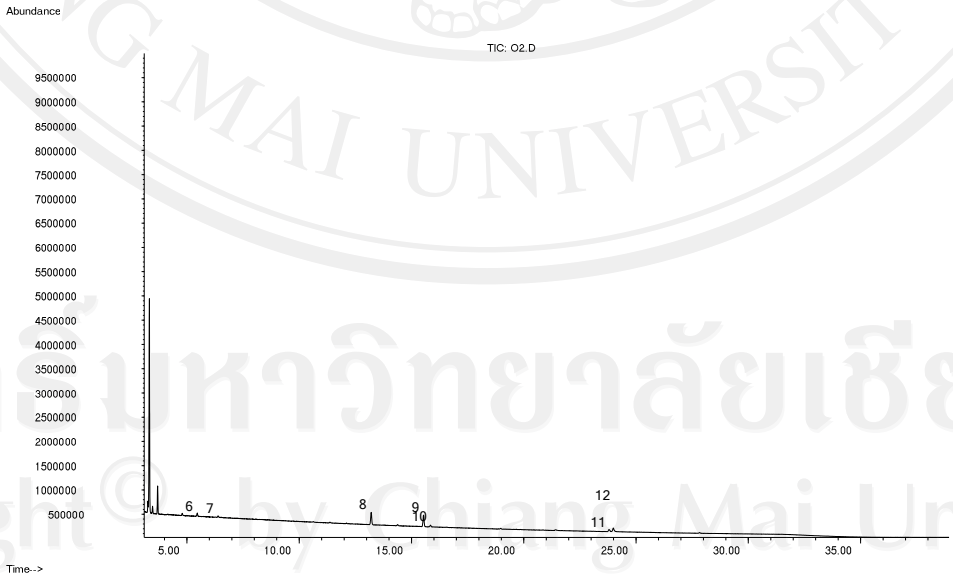


Figure 4.2 GC chromatogram of essential oil from *Apium graveolens* Linn.

Table 4.4 Chemical components of essential oil from *Apium graveolens* Linn.

Peak No.	RT (min)	Compounds	% Area	% QA
1	3.27	ortho-cymene	3.08	95
2	3.33	limonene	66.12	98
3	3.48	trans-beta-ocimene	2.04	98
4	3.69	gamma-terpinene	8.12	97
5	4.76	unidentified	0.82	-
6	5.46	unidentified	1.37	-
7	6.39	estragole	0.70	98
8	13.25	caryophyllene	6.53	99
9	15.54	beta-selinene	6.16	99
10	15.85	alpha-selinene	1.02	99
11	23.80	unidentified	1.42	-
12	23.99	3-amino-4-pyrazolecarbonitrile	2.61	80

4.3.2 *Anethum graveolens Linn.*

GC/MS analysis resulted in the identification of 15 components but 5 components cannot be identified (Figure 4.3 and Table 4.5). Among these, calarene (38.6%), dil ether (26.9%) limonene (11.91%) and orthi-cymene (11.46%) were found to be the main components.

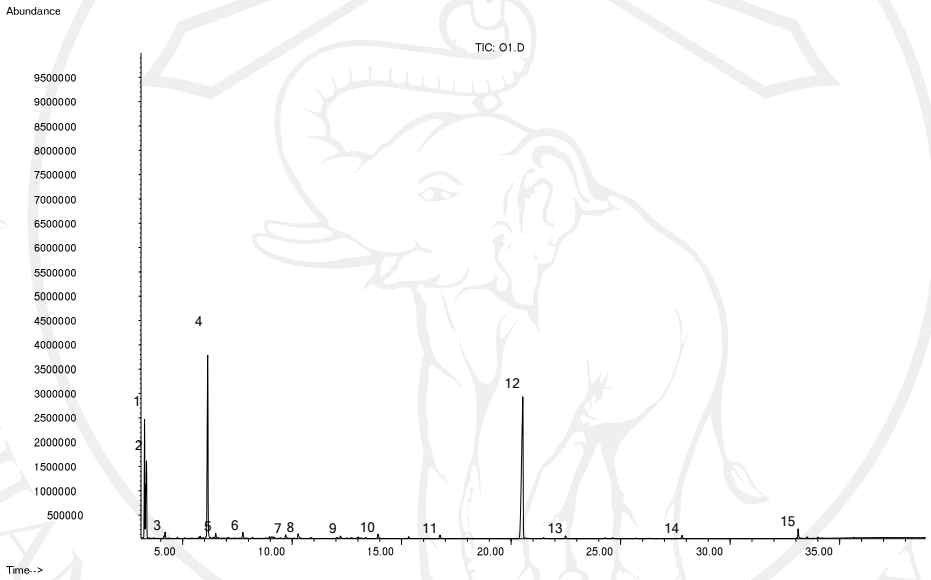


Figure 4.3 GC chromatogram of essential oil from *Anethum graveolens* Linn.

Table 4.5 Chemical components of essential oil from *Anethum graveolens* Linn.

Peak No.	RT (min)	Compounds	% Area	% QA
1	3.27	ortho-cymene	11.46	95
2	3.33	limonene	11.91	98
3	4.21	n-undecane	0.68	92
4	6.14	dill ether	26.91	95
5	6.51	alpha-phellandrene epoxide	0.65	89

Table 4.5 (Continued)

Peak No.	RT (min)	Compounds	% Area	% QA
6	7.75	unidentified	1.01	-
7	9.71	unidentified	0.60	-
8	10.28	unidentified	1.00	-
9	12.21	unidentified	0.51	-
10	13.90	aromadendrene	0.94	99
11	16.76	myristicin	0.70	98
12	20.53	calarene	38.60	93
13	22.48	apiole	0.52	99
14	27.81	neophytadiene	0.63	99
15	33.14	unidentified	1.34	-

4.3.3 *Centella asiatica* Urban.

GC/MS analysis resulted in the identification of 24 components but a component cannot be identified (Figure 4.4 and Table 4.6). Among these, Alpho-humulend (28.05%), caryophyllene (25.48%) aeta-elemene (11.63%) and alpha-copaene (11.35%) were found to be the main components.

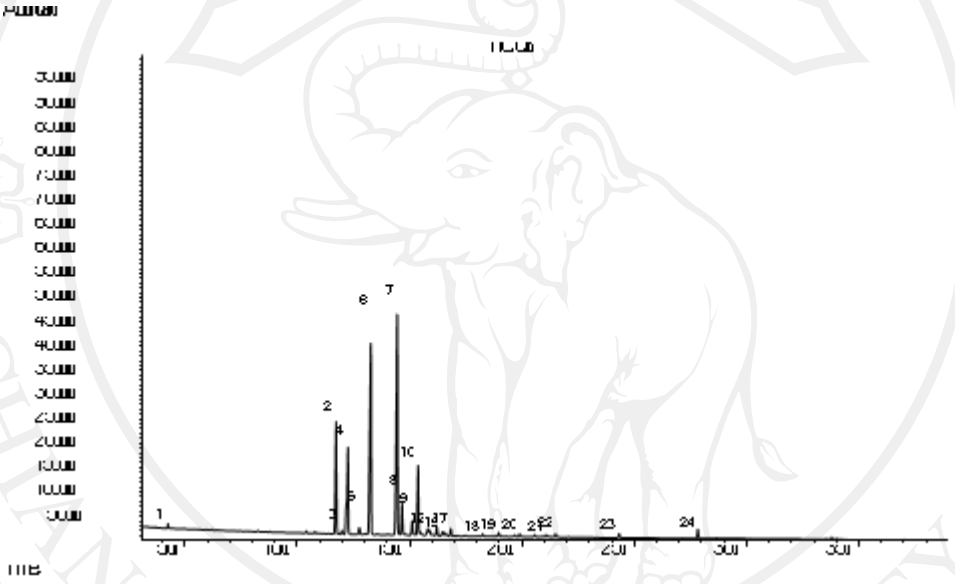


Figure 4.4 GC chromatogram of essential oil from *Centella asiatica* Urban.

Table 4.6 Chemical components of essential oil from *Centella asiatica* Urban.

Peak No.	RT (min)	Compounds	% Area	% QA
1	4.26	linalool	0.36	97
2	11.72	alpha-copaene	11.35	99
3	11.99	unidentified	0.39	-
4	12.25	aeta-elemene	11.63	99
5	12.77	decyl acetate	0.86	91

Table 4.6 (Continued)

Peak No.	RT (min)	Compounds	% Area	% QA
6	13.25	caryophyllene	25.48	99
7	14.41	alpha-humulene	28.05	99
8	14.65	allo-aromadendrene	3.15	99
9	15.12	di-epi-alpha-cedrene I	1.73	90
10	15.37	germacrene D	7.54	99
11	15.45	beta-farnesene	0.40	99
12	15.83	2-tridecanone	1.12	96
13	15.93	pentadecane	0.58	99
14	16.21	germacrene A	1.01	91
15	16.46	allo-ocimene	0.47	87
16	16.54	alpha-amorphene	0.39	98
17	16.84	delta-cadinene	0.84	99
18	18.26	trans-nerolidol	0.31	95
19	18.97	caryophyllene oxide	0.54	95
20	19.90	beta-oplopenone	0.33	98
21	21.07	alpha-ylangene	0.31	83
22	21.50	trans-muurolol	0.36	98
23	24.33	mintsulfide	0.49	94
24	27.81	neophytadiene	0.96	99

4.3.4 *Coriandrum sativum* Linn.

GC/MS analysis resulted in the identification of 11 components. (Figure 4.5 and Table 4.7). Among these, n-decanal (28.25%), 3-dodecen-1-al (19.03%) and n-decanol (11.63%) were the main component.

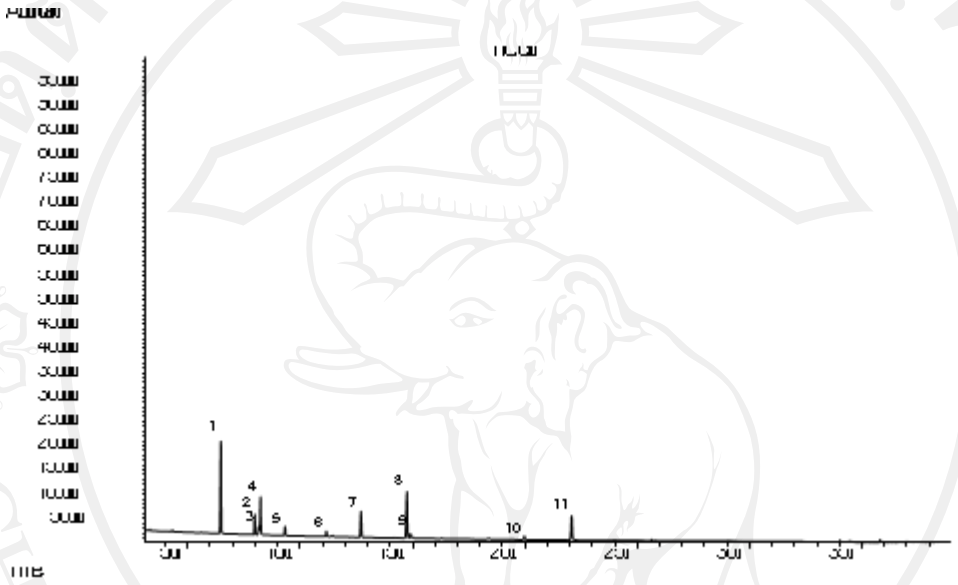


Figure 4.5 GC chromatogram of essential oil from *Coriandrum sativum* Linn.

Table 4.7 Chemical components of essential oil from *Coriandrum sativum* Linn.

Peak No.	RT (min)	Compounds	% Area	% QA
1	6.46	n-decanal	28.25	91
2	7.98	(E)-2-decenal	6.41	90
3	8.16	trans-2-nonen-1-ol	2.70	72
4	8.23	n-decanol	12.59	91
5	9.33	undecanal	3.11	91
6	11.17	trans-2-undecenal	2.01	90
7	12.72	dodecanal	9.54	91

Table 4.7 (Continued)

Peak No.	RT (min)	Compounds	% Area	% QA
8	14.84	3-dodecen-1-al	19.03	97
9	14.92	trans-2-undecen-1-ol	1.47	87
10	19.98	tetradecanal	1.55	94
11	22.09	nor-copaanone	10.98	97

4.3.5 *Eryngium foetidum* Linn.

GC/MS analysis resulted in the identification of 13 components but 2 components cannot be identified (Figure 4.6 and Table 4.8). Among these, 3-dodecen-1-al (67.25%), dodecanal (7.49%) and nor-copaanone (7.13%) were the main component.

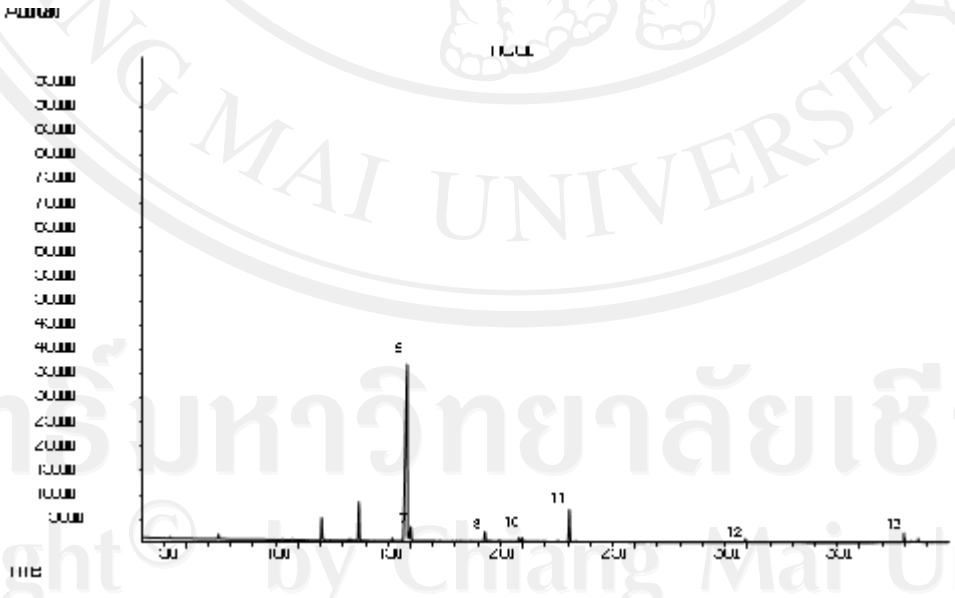


Figure 4.6 GC chromatogram of essential oil from *Eryngium foetidum* Linn.

Table 4.8 Chemical components of essential oil from *Eryngium foetidum* Linn.

Peak No.	RT (min)	Compounds	% Area	% QA
1	6.46	n-decanal	0.76	91
2	11.05	eugenol	4.30	94
3	12.72	dodecanal	7.49	91
4	14.19	unidentified	0.64	-
5	14.84	3-dodecen-1-al	67.25	97
6	14.92	trans-2-undecen-1-ol	1.55	87
7	15.01	cyclododecane	2.88	96
8	18.31	unidentified	2.64	-
9	19.84	dodecanoic acid	1.23	72
10	19.98	tetradecanal	0.99	94
11	22.09	nor-copaanone	7.13	97
12	29.89	nonadecane	0.77	99
13	36.96	n-stenol = octadecanol	0.75	81

4.3.6 *Polyscias fruticosa* Harms.

GC/MS analysis resulted in the identification of 19 components but 7 components cannot be identified (Figure 4.7 and Table 4.9). Among these, 2-tridecanone (32.93%), germacrene B (8.18%) and germacrene D (7.07%) were the main component.

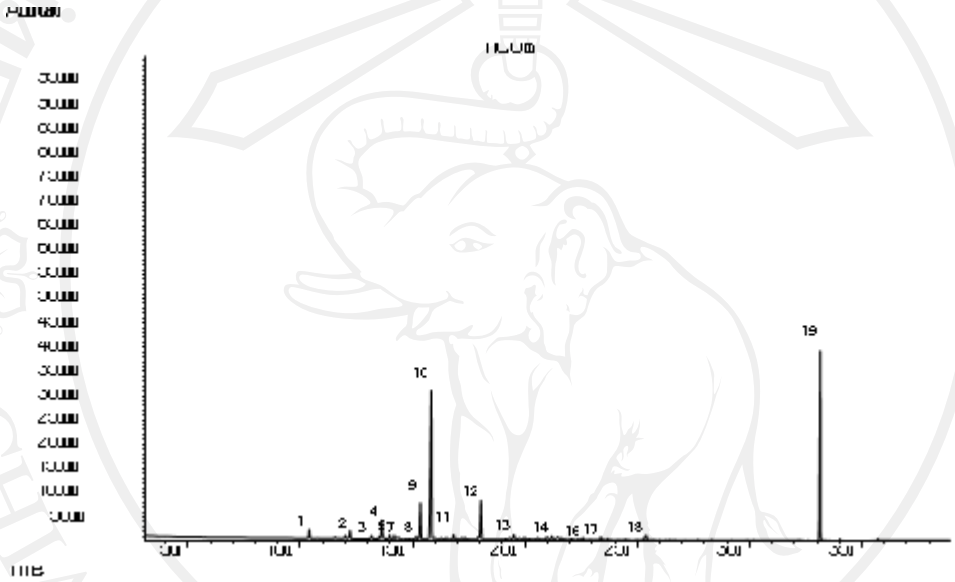


Figure 4.7 GC chromatogram of essential oil from *Polyscias fruticosa* Harms.

Table 4.9 Chemical components of essential oil from *Polyscias fruticosa* Harms.

Peak No.	RT (min)	Compounds	% Area	% QA
1	10.42	delta-elemene	1.54	98
2	12.25	beta-elemene	1.61	99
3	13.18	unidentified	0.79	-
4	13.66	gamma-elemene	3.96	99
5	13.98	3,7-guaiadiene	0.84	87
6	14.19	unidentified	0.74	-

Table 4.9 (Continued)

Peak No.	RT (min)	Compounds	% Area	% QA
7	14.41	alpha-humulene	0.53	99
8	15.19	alpha-amorphene	0.53	98
9	15.37	germacrene D	7.07	99
10	15.83	2-tridecanone	32.93	93
11	16.84	delta-cadinene	0.96	99
12	18.06	germacrene B	8.18	99
13	19.48	unidentified	1.19	-
14	21.25	alpha-copaene	1.03	97
15	21.50	trans-muurolol	1.30	98
16	22.59	unidentified	0.51	-
17	23.37	unidentified	0.74	-
18	25.45	unidentified	1.25	-
19	33.14	unidentified	25.44	-

4.3.7 *Eupatorium odoratum* Linn.

GC/MS analysis resulted in the identification of 27 components but 2 components cannot be identified (Figure 4.8 and Table 4.10). Among these, germacrene D (19.34%), geyrene B (17.30%) and caryophyllene (11.66%) were the main component.

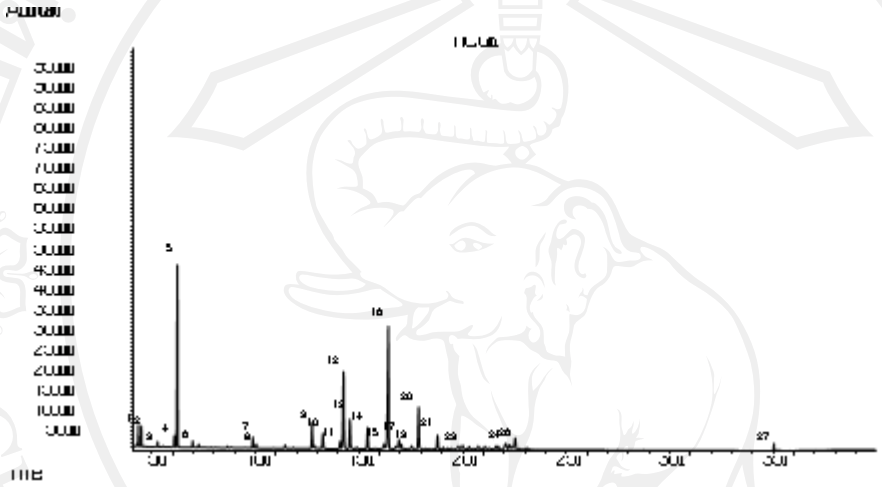


Figure 4.8 GC chromatogram of essential oil from *Eupatorium odoratum* Linn.

Table 4.10 Chemical components of essential oil from *Eupatorium odoratum* Linn.

Peak No.	RT (min)	Compounds	% Area	% QA
1	3.33	limonene	1.59	98
2	3.48	Trans-beta-ocimene	1.51	98
3	4.26	linalool	0.43	97
4	5.07	unidentified	1.06	-
5	5.22	geyrene	17.30	91
6	5.94	terpinen-4-ol	0.67	97
7	8.87	Sec-butyl ethyl-benzene	1.35	90

Table 4.10 (Continued)

Peak No.	RT (min)	Compounds	% Area	% QA
1	3.33	limonene	1.59	98
8	9.03	pregeijerene	0.54	94
9	11.72	alpha-copaene	3.54	99
10	12.25	beta-elemene	2.23	99
11	13.09	unidentified	1.16	-
12	13.25	caryophyllene	11.66	99
13	13.52	*	4.36	98
14	14.41	alpha-humulene	3.07	99
15	15.19	alpha-amorphene	1.08	98
16	15.37	germacrene D	19.34	99
17	15.93	bicyclogermacrene	1.98	99
18	16.04	alpha-muurolene	0.76	99
19	16.54	alpha-amorphene	0.75	98
20	16.84	delta-cadinene	6.48	99
21	17.75	elemol	2.24	91
22	18.80	spathulenol	1.01	99
23	18.97	caryophyllene oxide	1.11	95
24	21.10	alpha-cadinol	1.84	86
25	21.25	alpha-copaene	1.02	97
26	21.54	valencene	2.35	96
27	34.02	neophytadiene	0.62	96

* : is 1H-Cyclopenta[1,3]cyclopropa[1,2]benzene, octahydro-7-methyl-3-methylene-4-(1-methylethyl)-, [3aS-(3a.alpha.,3b.beta.,4.beta.,7.alpha.,7aS*)]

4.3.8 *Spilanthes acmella* Murr.

GC/MS analysis resulted in the identification of 23 components but a component cannot be identified (Figure 4.9 and Table 4.11). Among these, beta-farnesene (54.38%), caryophyllene (14.58%) and beta-elemene (4.53%) were the main component.

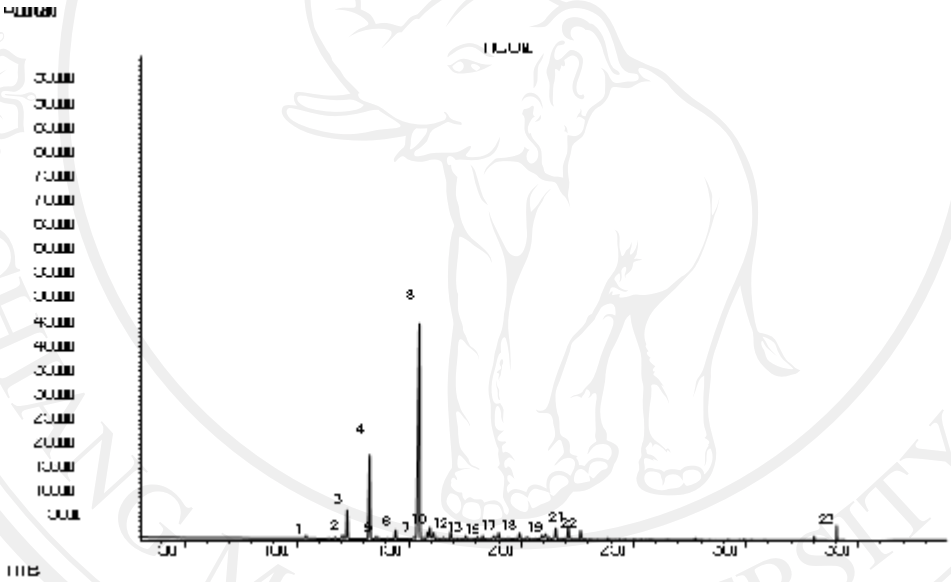


Figure 4.9 GC chromatogram of essential oil from *Spilanthes acmella* Murr.

Table 4.11 Chemical components of essential oil from *Spilanthes acmella* Murr.

Peak No.	RT (min)	Compounds	% Area	% QA
1	10.42	delta-elemene	0.46	98
2	12.02	beta-bourbonene	0.72	99
3	12.25	beta-elemene	4.53	99
4	13.25	caryophyllene	14.58	99

Table 4.11 (Continued)

Peak No.	RT (min)	Compounds	% Area	% QA
5	13.52	*	0.43	98
6	14.41	alpha-humulene	1.53	99
7	15.19	alpha-amorphene	0.46	98
8	15.45	beta-farnesene	54.38	99
9	15.83	2-tridecanone	1.25	93
10	15.93	bicyclogermacrene	2.15	99
11	16.10	trans-alpha-bisabolene	1.34	98
12	16.84	delta-cadinene	1.09	99
13	17.51	cis-alpha-bisabolene	0.60	98
14	18.06	germacrene B	0.43	99
15	18.26	trans-nerolidol	0.56	95
16	18.80	spathulenol	0.64	99
17	18.97	caryophyllene oxide	1.23	95
18	19.92	beta-oplopenone	1.02	91
19	21.10	alpha-cadinol	1.57	86
20	21.54	valencene	2.14	96
21	22.09	nor-copaanone	2.44	97
22	22.65	unidentified	1.66	-
23	34.02	neophytadiene	1.21	96

* : 1H-Cyclopenta[1,3]cyclopropa[1,2]benzene, octahydro-7-methyl-3-methylene-4-(1-methylethyl)-, [3aS-(3a.alpha.,3b.beta.,4.beta.,7.alpha.,7aS*)]

4.3.9 *Cymbopogon citratus* Stapf.

GC/MS analysis resulted in the identification of 16 components but 4 components cannot be identified (Figure 4.10 and Table 4.12). Among these, geranial (42.01%), z-citral (32.07%) and geraniol (5.21%) were the main component.

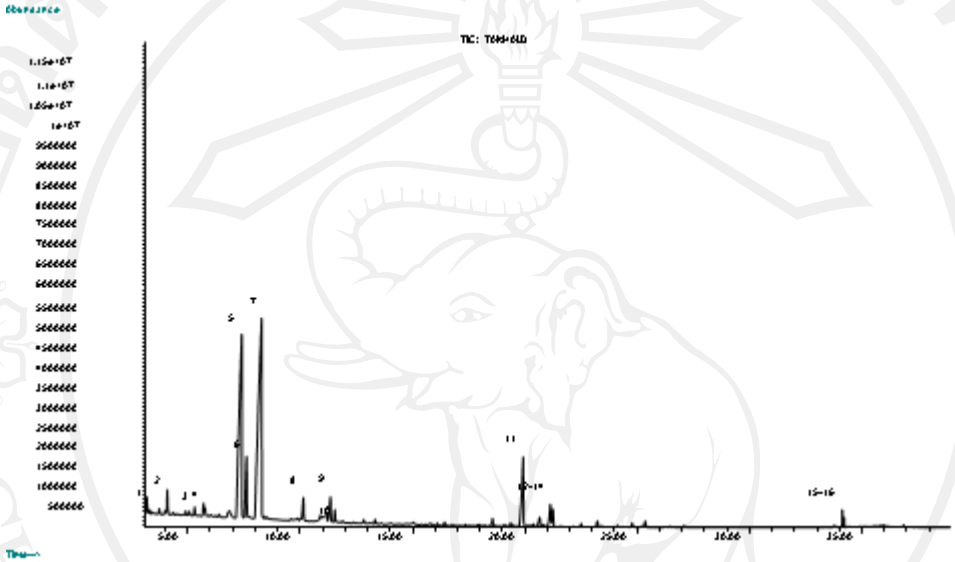


Figure 4.10 GC chromatogram of essential oil from *Cymbopogon citratus* Stapf.

Table 4.12 Chemical components of essential oil from *Cymbopogon citratus* Stapf.

Peak No.	RT (min)	Compounds	% Area	% QA
1	3.18	trans-beta-ocimene	0.58	95
2	4.08	n-undecene	1.40	96
3	5.31	D-camphor	0.49	98
4	5.70	(+)-borneol	0.35	99
5	7.40	z-citral = neral	32.07	96
6	7.61	geraniol	5.21	95
7	8.28	geranial	42.01	95

Table 4.12 (Continued)

Peak No.	RT (min)	Compounds	% Area	% QA
8	10.14	unidentified	1.82	-
9	11.35	piperitenone oxide	2.25	98
10	11.54	decaoic acid	0.79	91
11	19.93	unidentified	7.86	-
12	20.66	beta-maaliene	0.94	90
13	21.14	alpha-cadinol	2.04	74
14	21.25	alpha-copaene	1.40	89
15	34.13	unidentified	0.51	-
16	34.21	unidentified	0.29	-

4.3.10 *Coleus amboinicus* Lour.

GC/MS analysis resulted in the identification of 16 components but 4 components cannot be identified (Figure 4.11 and Table 4.13). Among these, thymol (63.83%), caryophyllene (21.05%) and alpha-humulene (5.82%) were the main component.

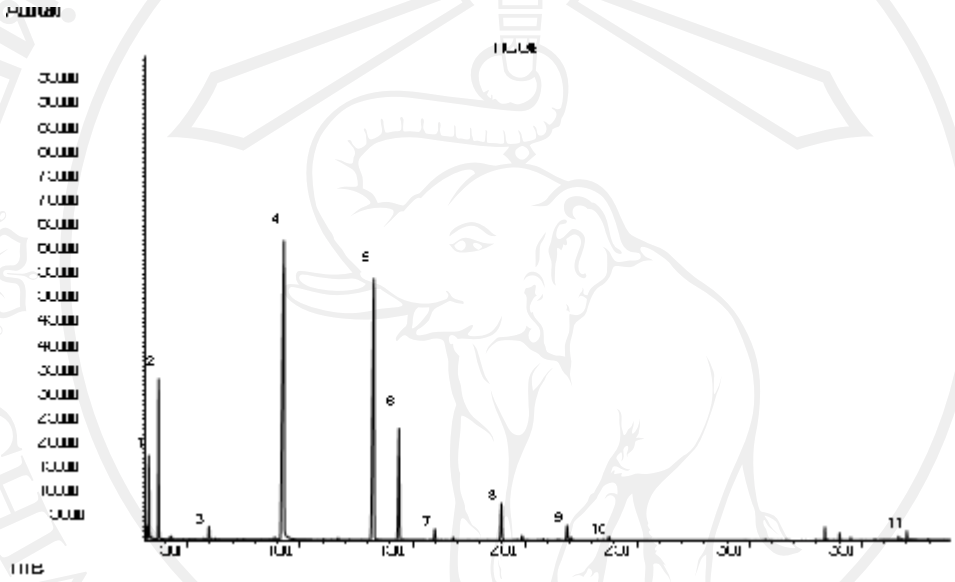


Figure 4.11 GC chromatogram of essential oil from *Coleus amboinicus* Lour.

Table 4.13 Chemical components of essential oil from *Coleus amboinicus* Lour.

Peak No.	RT (min)	Compounds	% Area	% QA
1	3.27	ortho-cymene	1.72	95
2	3.69	gamma-terpinene	1.32	97
3	5.94	terpinen-4-ol	0.89	97
4	9.25	thymol	63.83	91
5	13.25	caryophyllene	21.05	99
6	14.41	alpha-humulene	5.82	99

Table 4.13 (Continued)

Peak No.	RT (min)	Compounds	% Area	% QA
1	3.27	ortho-cymene	1.72	95
7	16.04	alpha-muurolene	0.48	99
8	18.97	caryophyllene oxide	2.67	95
9	21.91	unidentified	0.94	-
10	23.80	unidentified	0.48	-
11	37.01	unidentified	0.60	-

4.3.11 *Melissa officinalis* Linn.

GC/MS analysis resulted in the identification of 26 components but 3 components cannot be identified (Figure 4.12 and Table 4.14). Among these, piperitenone oxide (10.74%), germacrene D (10.06%) and caryophyllene (8.50%) were the main component.

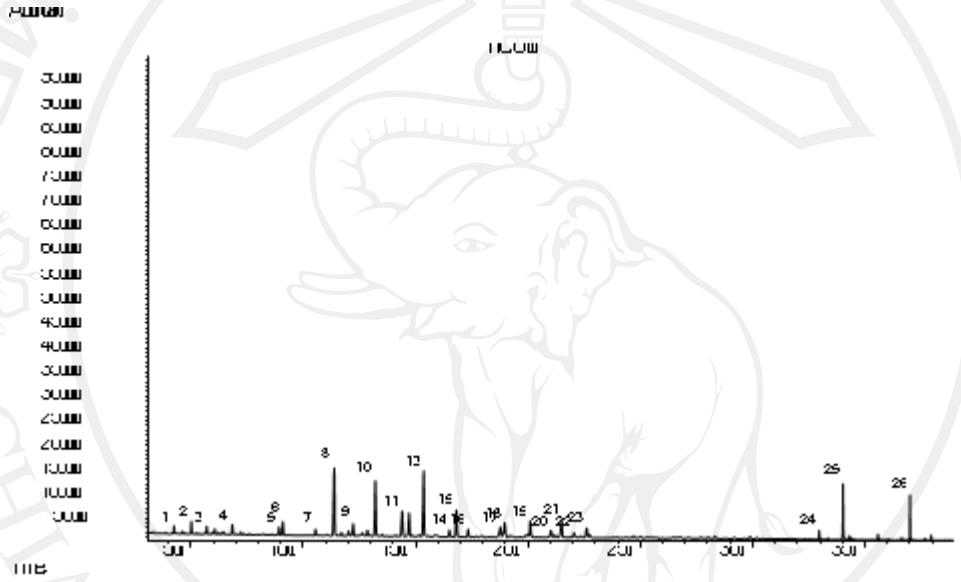


Figure 4.12 GC chromatogram of essential oil from *Melissa officinalis* Linn.

Table 4.14 Chemical components of essential oil from *Melissa officinalis* Linn.

Peak No.	RT (min)	Compounds	% Area	% QA
1	4.26	linalool	0.79	97
2	5.04	unidentified	1.35	-
3	5.71	borneol L	1.01	97
4	6.85	unidentified	1.27	-
5	8.97	2-undecanone	1.18	95

Table 4.14 (Continued)

Peak No.	RT (min)	Compounds	% Area	% QA
6	9.09	dihydro edulan I	1.75	96
7	10.55	piperitenone	0.93	97
8	11.38	piperitenone oxide	10.74	99
9	12.25	beta-elemene	1.83	99
10	13.25	caryophyllene	8.50	99
11	14.41	alpha-humulene	4.72	99
12	14.71	epi-icyclosesquiphellandrene	3.83	97
13	15.37	germacrene D	10.06	99
14	16.54	alpha-amorphene	1.17	98
15	16.82	cis-calamenene	4.48	97
16	17.35	aromadendrene	1.23	91
17	18.80	spathulenol	2.32	99
18	18.97	caryophyllene oxide	2.63	95
19	20.11	naphthalene,1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)	2.56	90
20	21.02	alpha-cadinol	1.65	91
21	21.50	trans-muurolol	3.71	98
22	22.06	unidentified	1.17	-
23	22.63	alpha-longipinene	2.04	91
24	33.02	1,6,10,14-hexadecatetraen-3-ol	0.82	94

Table 4.14 (Continued)

Peak No.	RT (min)	Compounds	% Area	% QA
25	34.02	neophytadiene	4.17	96
26	37.01	unidentified	3.07	-

4.3.12 *Ocimum basilicum* Linn.

GC/MS analysis resulted in the identification of 14 components (Figure 4.13 and Table 4.15). Among these, decanal (82.64%), alpha-trans-bergamotene (3.29%) and 1,8-cineole (3013%) were the main component.

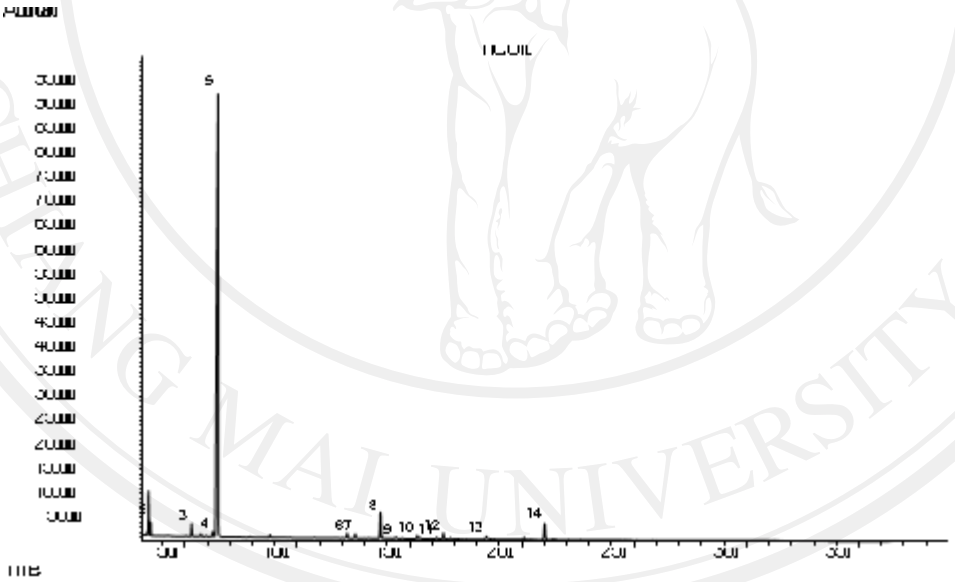


Figure 4.13 GC chromatogram of essential oil from *Ocimum basilicum* Linn.

Table 4.15 Chemical components of essential oil from *Ocimum basilicum* Linn.

Peak No.	RT (min)	Compounds	% Area	% QA
1	3.39	1,8-cineole	3.13	99
2	3.48	trans-beta-ocimene	0.96	98
3	5.30	camphor	1.23	98

Table 4.15 (Continue)

Peak No.	RT (min)	Compounds	% Area	% QA
4	6.24	alpha-terpineol	0.72	91
5	6.46	n-decanal	82.64	91
6	12.25	beta-elemene	0.70	99
7	12.59	methyl eugenol	0.59	98
8	13.71	alpha-trans-bergamotene	3.29	86
9	14.41	alpha-humulene	0.30	99
10	15.37	germacrene D	0.45	99
11	16.21	germacrene A	0.33	91
12	16.54	alpha-amorphene	0.89	98
13	18.42	4-methoxycinnamaldehyde	0.69	97
14	21.02	alpha-cadinol	2.38	91

4.3.13 *Ocimum canum* Sims.

GC/MS analysis resulted in the identification of 14 components but a component cannot be identified (Figure 4.14 and Table 4.16). Among these, geranial (35.08%), neral (27.35%) and linalool (5.51%) were the main component.

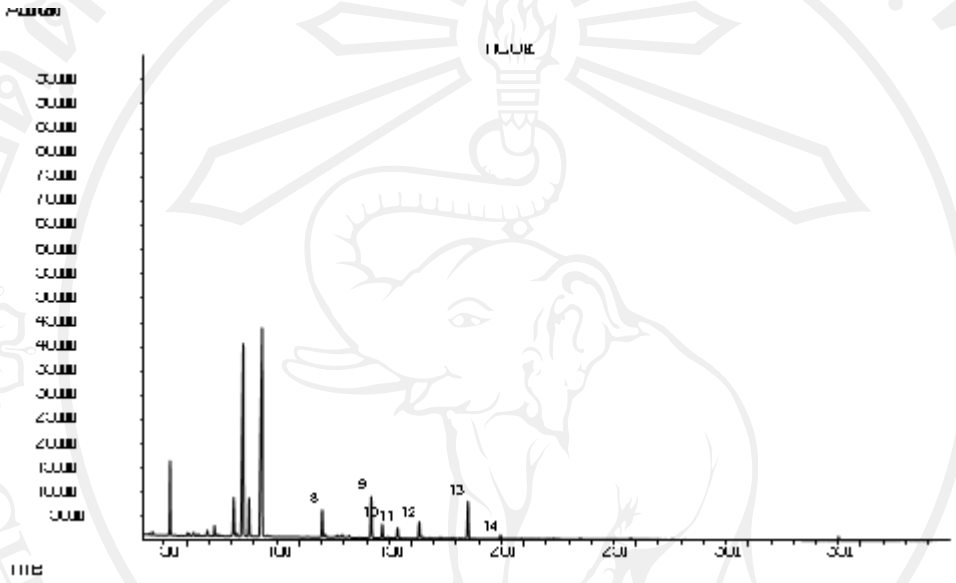


Figure 4.14 GC chromatogram of essential oil from *Ocimum canum* Sims.

Table 4.16 Chemical components of essential oil from *Ocimum canum* Sims.

Peak No.	RT (min)	Compounds	% Area	% QA
1	4.26	linalool	5.51	97
2	5.92	unidentified	0.42	-
3	6.24	alpha-terpineol	0.94	91
4	7.09	nerol	4.16	86
5	7.51	neral	27.35	97
6	7.77	geraniol	3.69	97

Table 4.16 (Continued)

Peak No.	RT (min)	Compounds	% Area	% QA
7	8.35	geranial	35.08	95
8	11.05	eugenol	3.09	94
9	13.25	caryophyllene	4.87	99
10	13.71	alpha-trans-bergamotene	1.57	86
11	14.41	alpha-humulene	1.29	99
12	15.37	germacrene D	1.92	99
13	17.51	cis-alpha-bisabolene	4.58	98
14	18.97	caryophyllene oxide	0.64	95

4.3.14 *Ocimum gratissimum* Linn.

GC/MS analysis resulted in the identification of 23 components but 4 components cannot be identified (Figure 4.15 and Table 4.17). Among these, eugenol (23.70%), diepi-alpha-cedren I (12.47%) and trans-alpha-bergamotene (9.04%) were the main component.

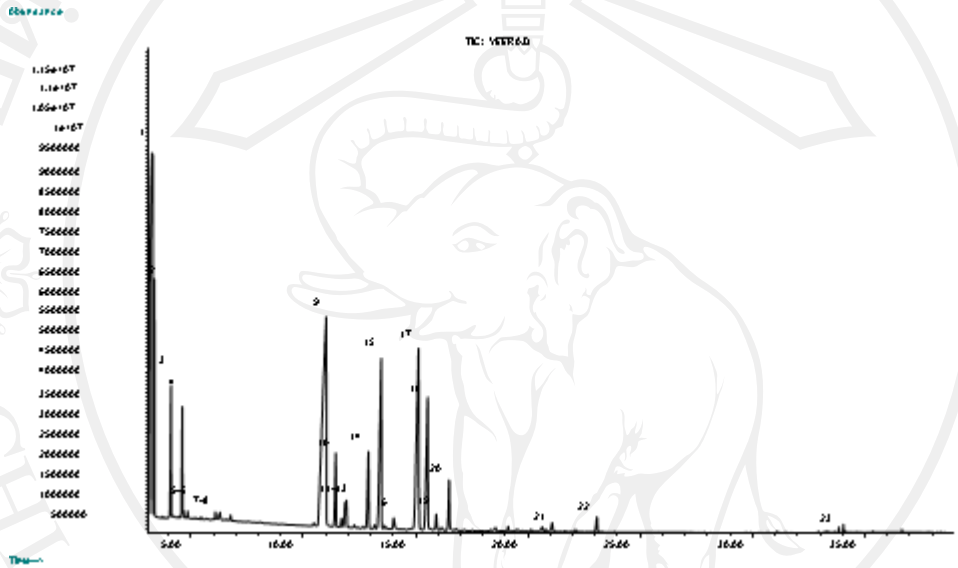


Figure 4.15 GC chromatogram of essential oil from *Ocimum gratissimum* Linn.

Table 4.17 Chemical components of essential oil from *Ocimum gratissimum* Linn.

Peak No.	RT (min)	Compounds	% Area	% QA
1	3.29	ortho-cymene	26.75	97
2	3.38	1,8-Cineole	3.64	98
3	4.12	trans-linaool oxide	2.96	97
4	4.62	neo-allo-ocimene	2.21	98
5	4.67	p-mentha-1,5,8-triene	0.14	97

Table 4.17 Chemical components of essential oil from *Ocimum gratissimum* Linn.

Peak No.	RT (min)	Compounds	% Area	% QA
6	4.86	unidentified	0.14	-
7	6.09	para-cymen-8-ol	0.18	90
8	6.30	dodecane	0.19	87
9	11.02	eugenol	23.70	97
10	11.45	1-undecanol	2.48	99
11	11.71	alpha-copaene	0.30	96
12	11.87	geranyl acetate	0.81	99
13	11.93	unidentified	0.72	-
14	12.91	alpha-gurjunene	3.02	99
15	13.48	trans-alpha-bergamotene	9.04	90
16	14.03	unidentified	0.51	-
17	15.14	diepi-alpha-cedren I	12.47	99
18	15.54	beta-selinene	6.45	91
19	15.92	bicyclogermacrene	0.58	93
20	16.50	alpha-amorphene	1.82	98
21	21.09	alpha-cadinol	0.42	86
22	23.08	unidentified	0.60	-
23	33.84	neophytadiene	0.10	90

4.3.15 *Ocimum sanctum* Linn.

GC/MS analysis resulted in the identification of 16 components but a component cannot be identified (Figure 4.16 and Table 4.18). Among these, caryophyllene (55.19%), beta-elemene (20.43%) and eugenol (10.80%) were the main component.

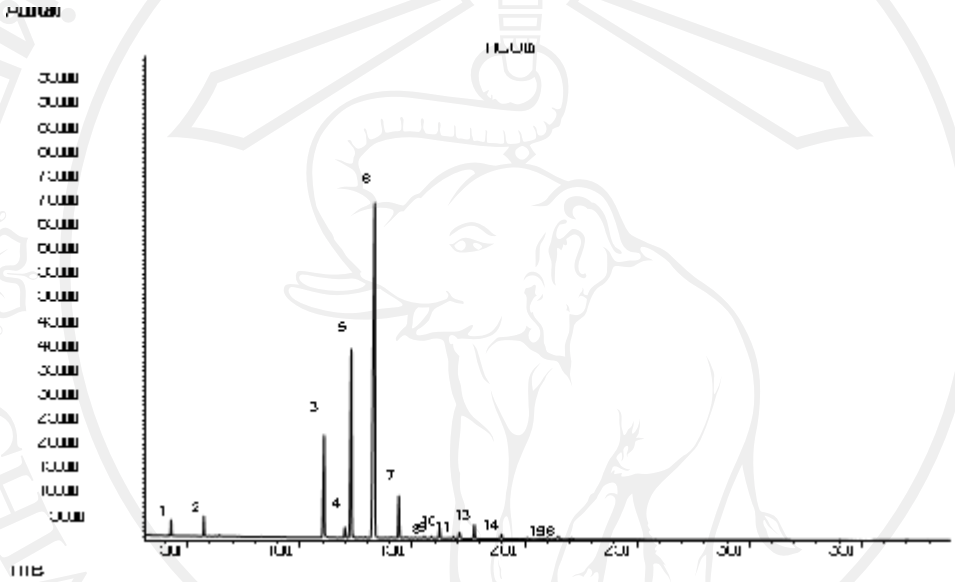


Figure 4.16 GC chromatogram of essential oil from *Ocimum sanctum* Linn.

Table 4.18 Chemical components of essential oil from *Ocimum sanctum* Linn.

Peak No.	RT (min)	Compounds	% Area	% QA
1	4.26	linalool	0.86	97
2	5.71	borneol L	1.34	97
3	11.05	eugenol	10.80	94
4	11.99	unidentified	1.02	-
5	12.25	beta-elemene	20.43	99
6	13.25	caryophyllene	55.19	99

Table 4.18 (Continued)

Peak No.	RT (min)	Compounds	% Area	% QA
7	14.41	alpha-humulene	4.09	99
8	15.54	beta-selinene	0.22	99
9	15.85	alpha-selinene	0.24	99
10	16.21	germacrene A	1.12	91
11	16.84	delta-cadinene	0.26	99
12	17.10	tricyclo[4.1.0.0(2,4)] heptane	0.85	90
13	17.75	elemol	1.46	91
14	18.97	caryophyllene oxide	0.61	95
15	21.02	alpha-cadinol	0.25	91
16	21.50	trans-muurolol	0.49	98

4.3.16 *Cinnamomum bejolghota* Sweet.

GC/MS analysis resulted in the identification of 22 components but a component cannot be identified (Figure 4.17 and Table 4.19). Among these, linalool (35.56%), alpha-cadinol (5.86%) and delta-cadinene (4.36%) were the main component.

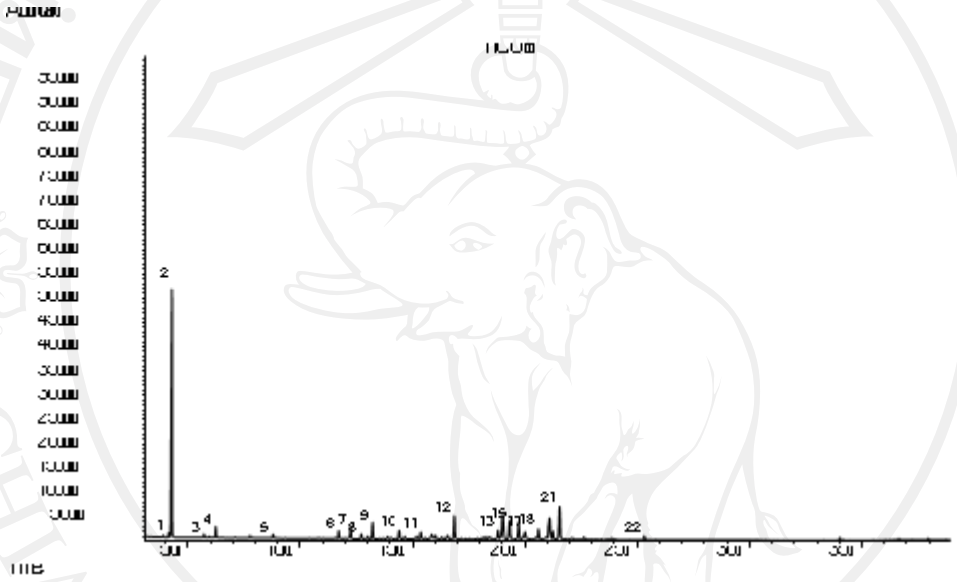


Figure 4.17 GC chromatogram of essential oil from *Cinnamomum bejolghota* Sweet.

Table 4.19 Chemical components of essential oil from *Cinnamomum bejolghota* Sweet.

Peak No.	RT (min)	Compounds	% Area	% QA
1	4.15	trans-linaool oxide	0.51	91
2	4.26	linalool	35.56	97
3	5.71	borneol L	0.54	97
4	6.24	alpha-terpineol	1.45	91
5	8.80	bornyl acetate	0.57	99

Table 4.19 (Continued)

Peak No.	RT (min)	Compounds	% Area	% QA
6	11.72	alpha-copaene	1.27	99
7	12.25	beta-lemene	2.24	99
8	12.72	dodecanal	0.80	91
9	13.25	caryophyllene	2.83	99
10	14.41	alpha-humulene	1.57	99
11	15.37	germacrene D	1.33	99
12	16.84	delta-cadinene	4.36	99
13	18.80	spathulenol	1.98	99
14	18.97	caryophyllene oxide	6.61	95
15	19.32	viridiflorol	3.87	99
16	19.75	unidentified	3.58	-
17	19.98	tetradecanal	1.34	94
18	20.61	naphthalene,1,2,3,4,4a,7-hexahydro-1,6…	2.09	95
19	21.10	alpha-cadinol	5.86	86
20	21.25	alpha-copaene	1.62	97
21	21.55	trans-cadinol	6.65	93
22	25.31	benzyl benzoate	0.86	98

4.3.17 *Piper sarmentosum* Roxb.

GC/MS analysis resulted in the identification of 20 components but 2 components cannot be identified (Figure 4.18 and Table 4.20). Among these, caryophyllene (42.62%), alpha-selinene (9.92%), beta-selinene (7.50%) and beta-elemene (6.43%) were the main component.

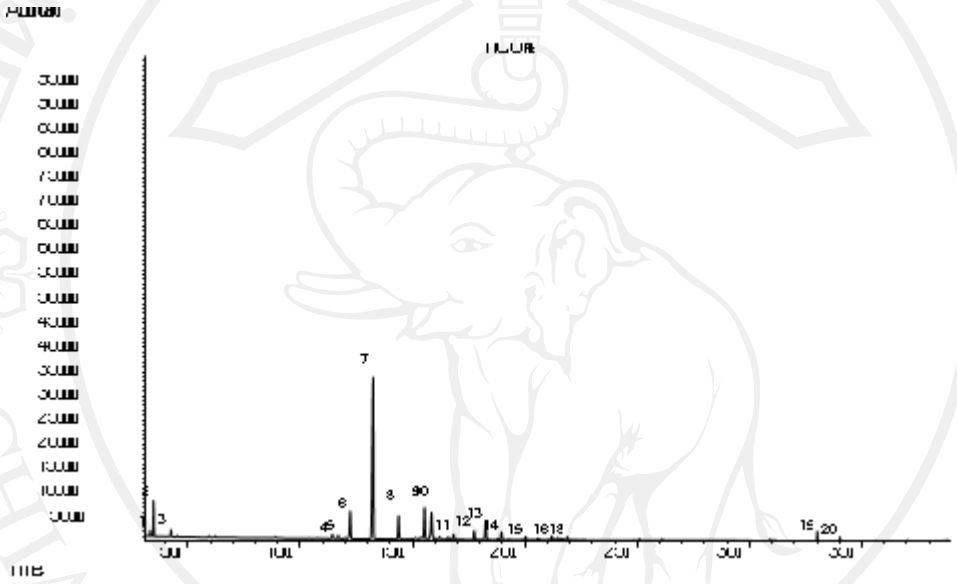


Figure 4.18 GC chromatogram of essential oil from *Piper sarmentosum* Roxb.

Table 4.20 Chemical components of essential oil from *Piper sarmentosum* Roxb.

Peak No.	RT (min)	Compounds	% Area	% QA
1	3.33	limonene	0.70	98
2	3.48	trans-beta-ocimene	4.36	98
3	4.26	linalool	0.93	97
4	11.43	phenyl propyl acetate	1.02	90
5	11.72	alpha-copaene	0.75	99
6	12.25	beta-elemene	6.43	99

Table 4.20 (Continued)

Peak No.	RT (min)	Compounds	% Area	% QA
7	13.25	caryophyllene	42.62	99
8	14.41	alpha-humulene	5.35	99
9	15.54	beta-selinene	7.50	99
10	15.85	alpha-selinene	7.92	99
11	16.84	delta-cadinene	1.26	99
12	17.75	elemol	2.03	91
13	18.26	trans-nerolidol	4.38	95
14	18.97	caryophyllene oxide	2.15	95
15	20.07	unidentified	0.85	-
16	21.25	alpha-copaene	1.01	97
17	21.50	trans-muurolol	0.88	98
18	21.91	unidentified	0.91	-
19	33.02	1,6,10,14-hexadecatetraen-3-ol	1.18	94
20	34.02	neophytadiene	0.58	96

4.3.18 *Polygonum odoratum* Lour.

GC/MS analysis resulted in the identification of 20 components (Figure 4.19 and Table 4.21). Among these, dodecanal (45.79%), n-decanal (11.70%) and caryophyllene (10.41%) were the main component.

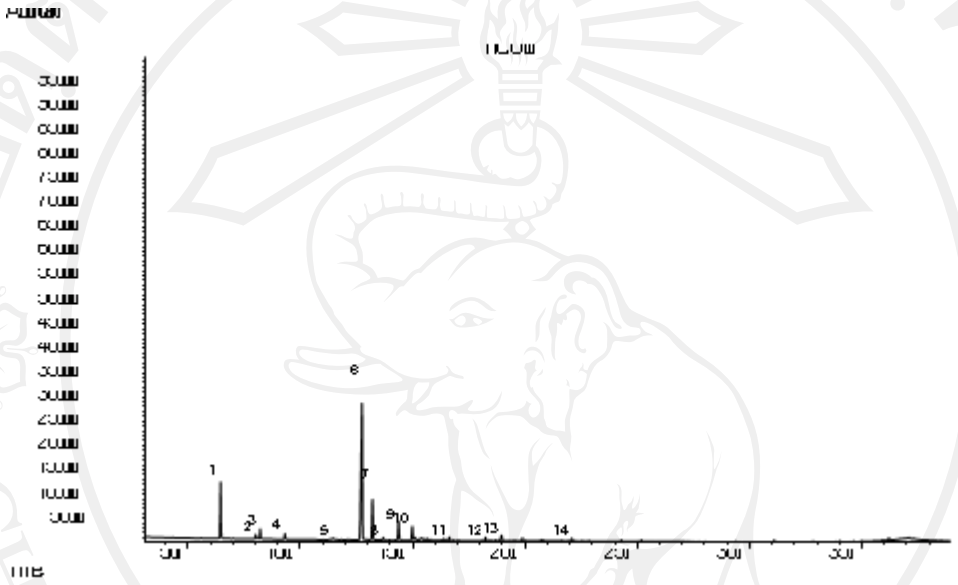


Figure 4.19 GC chromatogram of essential oil from *Polygonum odoratum* Lour.

Table 4.21 Chemical components of essential oil from *Polygonum odoratum* Lour.

Peak No.	RT (min)	Compounds	% Area	% QA
1	6.46	n-decanal	11.70	91
2	8.02	4-bromo-2-chlorophenol	1.41	99
3	8.23	1-decanol	2.30	91
4	9.33	undecanal	1.34	91
5	11.45	n-undecanol	0.83	87
6	12.72	dodecanal	45.79	91
7	13.25	caryophyllene	10.41	99

Table 4.21 (Continued)

Peak No.	RT (min)	Compounds	% Area	% QA
8	13.71	alpha-trans-bergamotene	0.74	86
9	14.41	alpha-humulene	5.45	99
10	14.99	1-dodecanol	3.81	95
11	16.64	7-epi-alpha-selinene	1.00	98
12	18.26	trans-nerolidol	1.01	95
13	18.97	caryophyllene oxide	1.91	95
14	22.09	nor-copaanone	1.26	97

4.3.19 *Citrus aurantifolia* Swing. (Leaf)

GC/MS analysis resulted in the identification of 24 components (Figure 4.20 and Table 4.22). Among these, ortho-cymene (26.43%), L-camphor (19.89%), and citronellol (8.47%) were the main component.

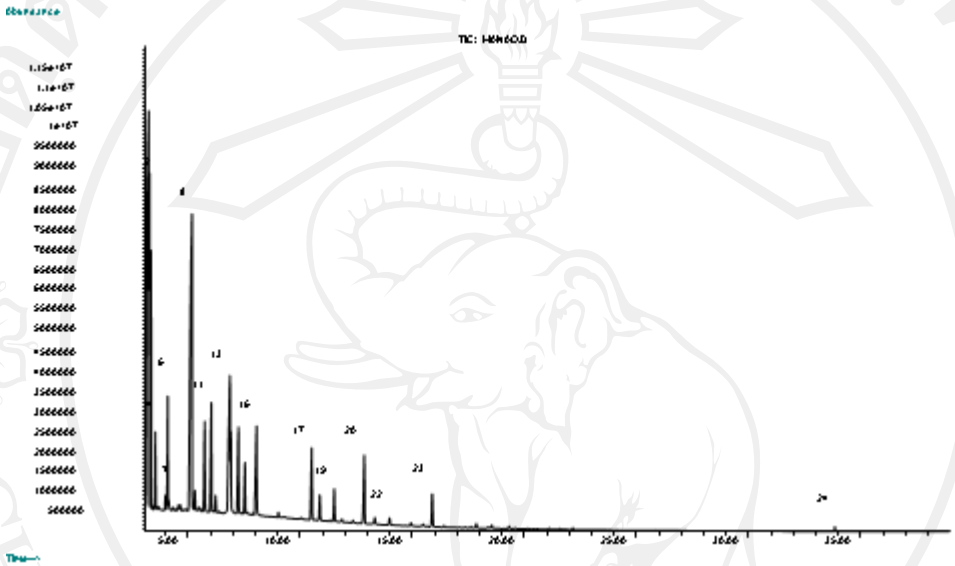


Figure 4.20 GC chromatogram of essential oil from *Citrus aurantifolia* Swing. (Leaf)

Table 4.22 Chemical components of essential oil from *Citrus aurantifolia* Swing. (Leaf)

Peak No.	RT (min)	Compounds	% Area	% QA
1	3.26	ortho-cymene	26.43	98
2	3.29	limonene	5.76	99
3	3.37	1,8-cineole	5.26	98
4	3.56	gamma-terpinene	1.42	97
5	3.99	alpha-terpinolene	0.34	98
6	4.11	n-undecene	2.99	97

Table 4.22 (Continued)

Peak No.	RT (min)	Compounds	% Area	% QA
7	4.15	trans-linaool oxide	0.27	91
8	5.18	L-camphor	19.89	98
9	5.32	D-camphor	0.56	97
10	5.75	borneol	2.98	97
11	6.05	para-cymen-8-ol	3.70	91
12	6.23	alpha-terpineol	0.49	91
13	6.88	citronellol	8.47	98
14	7.25	z-citral = neral	3.24	96
15	7.55	geraniol	1.70	94
16	8.07	geranial	4.22	96
17	10.52	gitronellyl acetate	2.75	95
18	10.88	neryl acetate	0.90	97
19	11.53	decaoic acid	1.18	91
20	12.87	alpha-gurjunene	2.83	99
21	13.33	tranns-beta-caryophyllene	0.30	91
22	13.99	3,7-guaiadiene	0.30	97
23	15.89	bicyclogermacrene	1.34	91
24	33.84	neophytadiene	0.09	96

4.3.20 *Citrus aurantifolia* Swing. (Peel)

GC/MS analysis resulted in the identification of 24 components (Figure 4.21 and Table 4.23). Among these, ortho-cymene (43.09%), para-cymen-8-ol (8.66%), and gamma-terpinene (7.08%) were the main component.

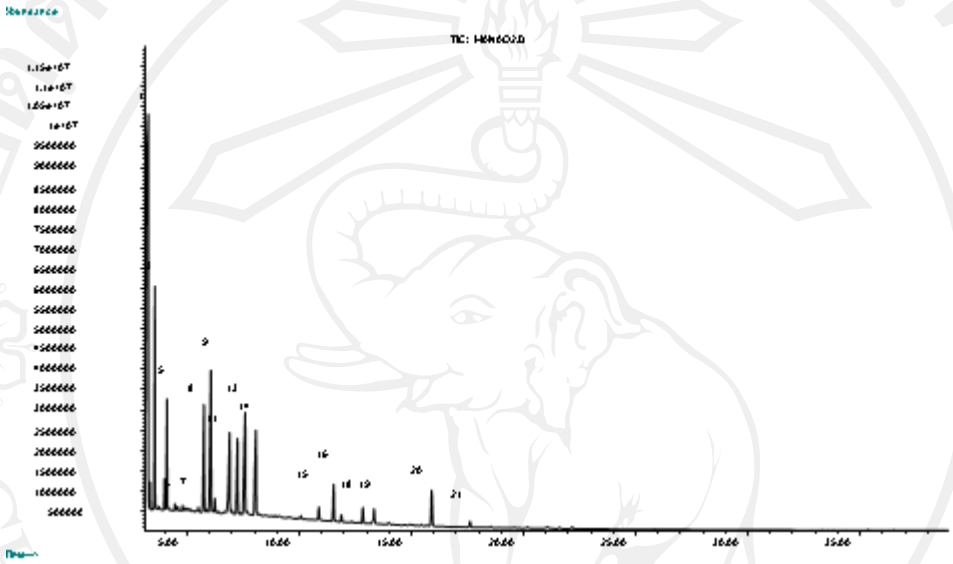


Figure 4.21 GC chromatogram of essential oil from *Citrus aurantifolia* Swing. (Peel)

Table 4.23 Chemical components of essential oil from *Citrus aurantifolia* Swing. (Peel)

Peak No.	RT (min)	Compounds	% Area	% QA
1	3.26	ortho-cymene	43.09	98
2	3.34	limonene	0.61	97
3	3.56	gamma-terpinene	7.08	97
4	3.99	alpha-terpinolene	1.01	98
5	4.10	n-undecene	3.83	96
6	4.46	alpha-fenchyl alcohol	0.24	98

Table 4.23 (Continued)

Peak No.	RT (min)	Compounds	% Area	% QA
1	3.26	ortho-cymene	43.09	98
7	5.49	(-)-borneol	0.35	90
8	5.75	(+)-borneol	5.45	98
9	6.06	para-cymen-8-ol	8.66	91
10	6.24	alpha-terpineol	0.60	91
11	6.89	citronellol	4.97	94
12	7.24	z-citral= neral	4.07	97
13	7.59	geraniol	6.95	94
14	8.06	geranial	5.44	95
15	10.87	neryl acetate	0.71	87
16	11.53	decaoic acid	1.92	91
17	11.87	geranyl acetate	0.35	91
18	12.83	alpha-gurjunene	0.86	99
19	13.34	trans-alpha- caryophyllene	0.98	91
20	15.90	bicyclogermacrene	2.26	91
21	17.61	germacrene B	0.34	98

4.3.21 *Citrus maxima (Burm.)* Merr. (Leaf)

GC/MS analysis resulted in the identification of 24 components but a component cannot be identified (Figure 4.22 and Table 4.24). Among these, alpha-terpinene (16.24%), geranial (13.49%), trans-linaool oxide (12.98%), trans-geraniol (11.71%) and z-citral (10.67%) were the main component.

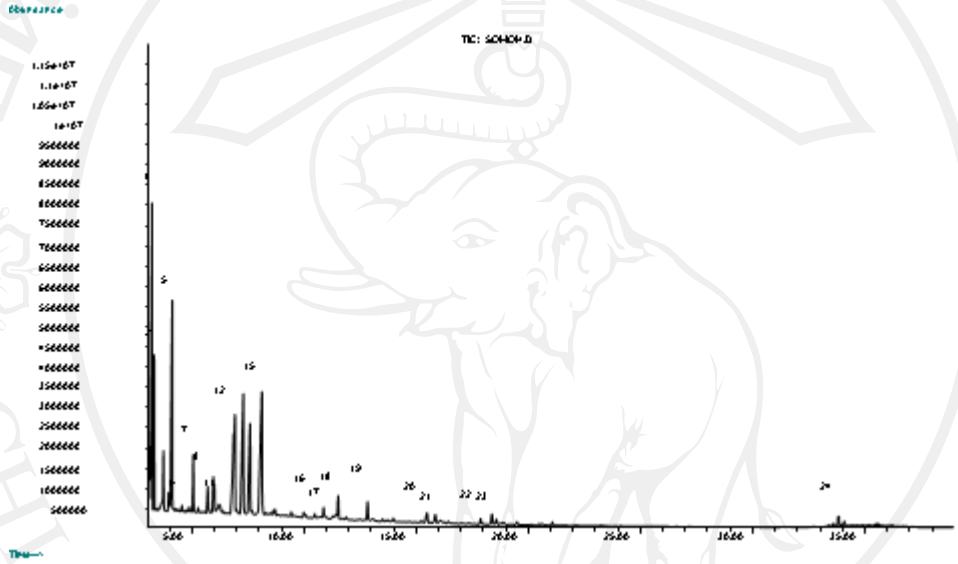


Figure 4.22 GC chromatogram of essential oil from *Citrus maxima* (Burm.) Merr.(Leaf)

Table 4.24 Chemical components of essential oil from *Citrus maxima* (Burm.) Merr.(Leaf)

Peak No.	RT (min)	Compounds	% Area	% QA
1	3.23	alpha-terpinene	16.24	94
2	3.33	limonene	4.68	98
3	3.73	gamma-terpinene	3.39	91
4	3.97	alpha-terpinolene	0.99	87
5	4.13	trans-linaool oxide	12.98	97

Table 4.24 (Continued)

Peak No.	RT (min)	Compounds	% Area	% QA
6	4.56	neo-allo-ocimene	0.30	90
7	5.07	unidentified	2.59	-
8	5.71	(+)-borneol	1.65	97
9	5.97	terpinen-4-ol	2.07	96
10	6.01	para-cymen-8-ol	1.12	91
11	6.22	alpha-terpineol	1.36	99
12	6.92	trans-geraniol	11.71	91
13	7.30	z-citral= neral	10.67	96
14	7.60	geraniol	7.38	93
15	8.13	geranial	13.49	95
16	10.49	citronellyl acetate	0.24	91
17	10.88	neryl acetate	0.70	91
18	11.53	decaoic acid	1.47	90
19	12.84	alpha-gurjunene	1.20	99
20	15.49	germacrene-D	0.96	95
21	15.86	bicyclogermacrene	0.70	95
22	17.87	delta-nerolidol	0.34	91
23	18.38	(+)-spathulenol	0.78	99
24	33.85	neophytadiene	0.31	95

4.3.22 *Citrus maxima (Burm.)* Merr. (Peel)

GC/MS analysis resulted in the identification of 11 components but a component cannot be identified (Figure 4.23 and Table 4.25). Among these, oetho-cymene (94.55%) was the main component.

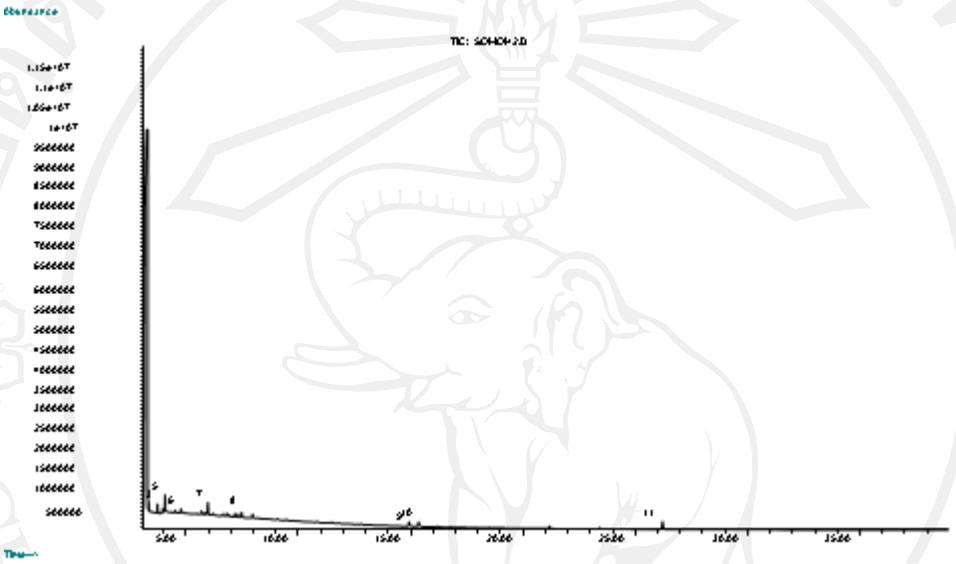


Figure 4.23 GC chromatogram of essential oil from *Citrus maxima* (Burm.) Merr.(Peel)

Table 4.25 Chemical components of essential oil from *Citrus maxima* (Burm.) Merr.(peel)

Peak No.	RT (min)	Compounds	% Area	% QA
1	3.26	ortho-cymene	94.55	98
2	3.35	limonene	0.32	98
3	3.74	gamma-terpinene	0.45	91
4	3.97	alpha-terpinolene	0.16	98
5	4.07	n-undecene	0.91	96
6	4.78	unidentified	0.27	-

Table 4.25 (Continued)

Peak No.	RT (min)	Compounds	% Area	% QA
7	5.99	para-cymen-8-ol	1.02	90
8	7.49	z-citral = neral	0.45	89
9	14.94	1-dodecanol	0.52	99
10	15.36	germacrene D	0.49	99
11	26.22	nootkatone	0.85	99

4.3.23 *Houttuynia cordata* Thunb.

GC/MS analysis resulted in the identification of 17 components but 3 components cannot be identified (Figure 4.24 and Table 4.26). Among these, 2-undecanone (48.08%), caryophyllene (10.69%), alpha-selinene (9.27%) and decanoic acid (6.97%) were the main component.

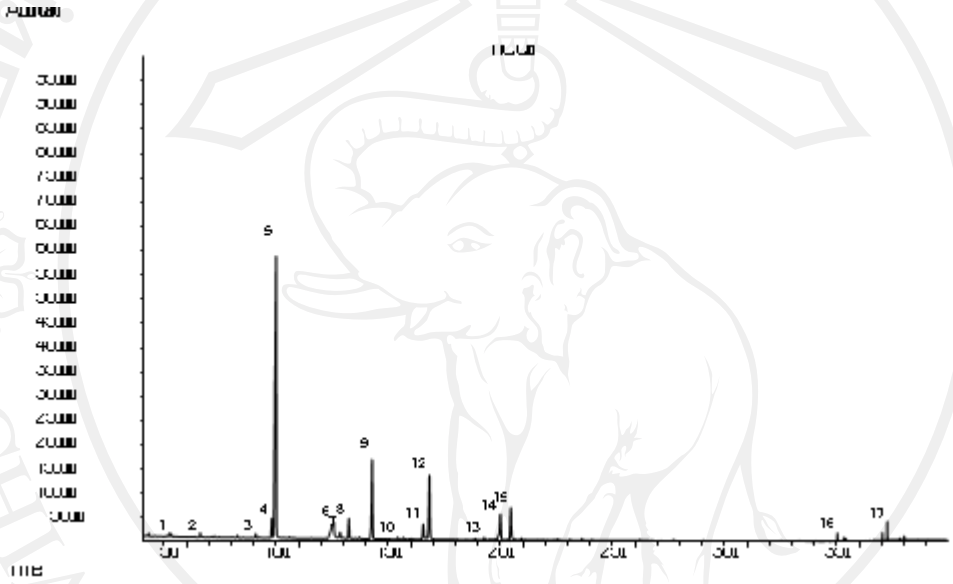


Figure 4.24 GC chromatogram of essential oil from *Houttuynia cordata* Thunb.

Table 4.26 Chemical components of essential oil from *Houttuynia cordata* Thunb.

Peak No.	RT (min)	Compounds	% Area	% QA
1	4.26	linalool	0.34	97
2	5.63	nonyl alcohol	0.36	91
3	8.10	unidentified	0.45	-
4	8.80	bornyl acetate	2.35	99
5	8.97	2-undecanone	48.08	95
6	11.58	decanoic acid	6.97	97

Table 4.26 (Continued)

Peak No.	RT (min)	Compounds	% Area	% QA
7	11.84	geranyl acetate	1.17	99
8	12.25	beta-elemene	2.60	99
9	13.25	caryophyllene	10.69	99
10	14.41	alpha-humulene	0.31	99
11	15.54	beta-selinene	2.37	99
12	15.85	alpha-selinene	9.27	99
13	18.26	trans-nerolidol	0.37	95
14	18.97	caryophyllene oxide	4.39	95
15	19.48	unidentified	4.74	-
16	34.02	neophytadiene	0.59	96
17	36.26	unidentified	0.92	-

4.3.24 *Boesenbergia pandurata* (Roxb.) Schltr.

GC/MS analysis resulted in the identification of 16 components but 2 components cannot be identified (Figure 4.25 and Table 4.27). Among these, geraniol (30.35%), 1, 8-cineole (20.79%), L-camphor (20.40%) and ortho-cymene (15.74%) were the main component.

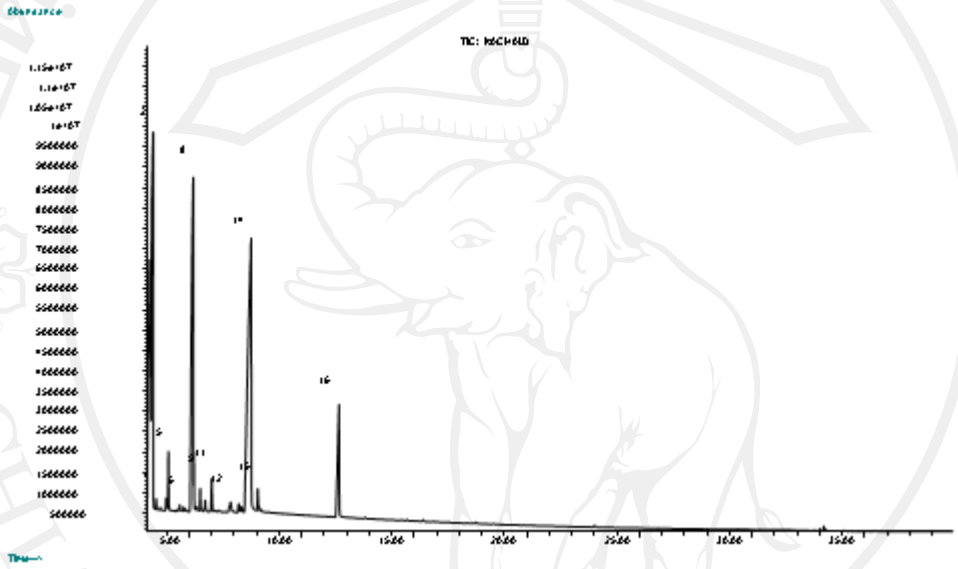


Figure 4.25 GC chromatogram of essential oil from *Boesenbergia pandurata* (Roxb.) Schltr.

Table 4.27 Chemical components of essential oil from *Boesenbergia pandurata* (Roxb.) Schltr.

Peak No.	RT (min)	Compounds	% Area	% QA
1	3.27	ortho-cymene	15.74	99
2	3.40	1,8-cineole	20.79	97
3	3.54	gamma-terpinene	0.19	97
4	3.98	alpha-terpinolene	0.26	98
5	4.09	n-undecene	1.39	97

Table 4.27 (Continued)

Peak No.	RT (min)	Compounds	% Area	% QA
6	4.59	neo-allo-ocimene	0.19	98
7	4.76	unidentified	0.11	-
8	5.18	L-camphor	20.40	98
9	5.50	(-)-borneol	0.64	95
10	5.72	(+)-borneol	0.39	97
11	6.00	para-cymen-8-ol	0.90	91
12	6.84	unidentified	0.60	-
13	7.21	cis-3-hexenyl valerate	0.37	93
14	7.76	geraniol	30.35	94
15	8.05	geranial	0.86	97
16	11.66	methyl cinnamate	6.01	97

4.3.25 *Curcuma longa* Linn.

GC/MS analysis resulted in the identification of 32 components but 7 components cannot be identified (Figure 4.26 and Table 4.28). Among these, beta-bisabolene (48.59%), and alpha-tumerone (13.21%) were the main component.

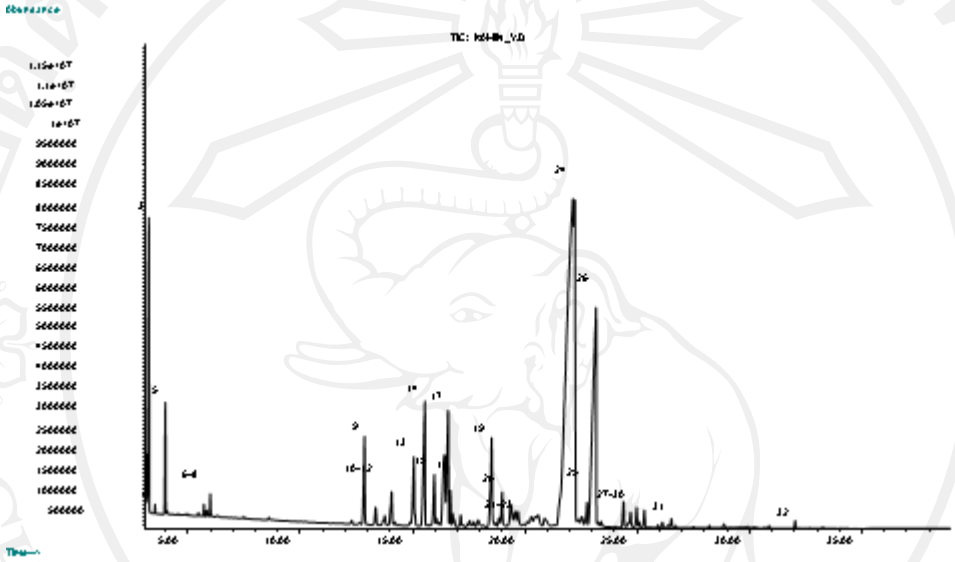


Figure 4.26 GC chromatogram of essential oil from *Curcuma longa* Linn.

Table 4.28 Chemical components of essential oil from *Curcuma longa* Linn.

Peak No.	RT (min)	Compounds	% Area	% QA
1	3.13	para-cymene	0.72	95
2	3.19	alpha-terpinene	0.23	98
3	3.27	ortho-cymene	4.87	99
4	3.54	gamma-terpinene	0.10	97
5	4.00	alpha-terpinolene	1.37	98
6	5.71	(+)-borneol	0.18	97
7	5.84	terpinen-4-ol	0.10	91

Table 4.28 (Continued)

Peak No.	RT (min)	Compounds	% Area	% QA
8	6.00	para-cymen-8-ol	0.33	90
9	12.86	alpha-gurjunene	1.97	96
10	13.36	tranns-beta-caryophyllene	0.44	98
11	13.77	beta-santalol	0.24	93
12	14.07	unidentified	0.88	-
13	15.06	gamma-gurjunene	1.93	99
14	15.55	beta-selinene	3.79	95
15	15.97	alpha-muurolene	1.29	98
16	16.43	unidentified	3.30	-
17	16.59	beta-sesquiphellandrene	3.36	99
18	16.68	unidentified	0.67	-
19	18.52	unidentified	2.72	-
20	18.99	caryophyllene oxide	0.85	98
21	19.39	unidentified	0.94	-
22	19.58	unidentified	0.60	-
23	19.71	unidentified	0.49	-
24	22.23	beta-bisabolene	48.59	91
25	22.77	germacrone	0.53	94
26	23.17	alpha-tumerone	13.21	95

Table 4.28 (Continued)

Peak No.	RT (min)	Compounds	% Area	% QA
27	24.40	(6R,1'R)-6-(1',5'-dimethylhex-4'-enyl)-3- methylcyclohex-2-enone	0.61	93
28	24.71	unidentified	0.37	-
29	24.98	(2-nitro-2-propenyl)-cyclohexane	0.46	80
30	25.32	benzyl benzoate	0.38	87
31	26.52	unidentified	0.22	-
32	32.03	unidentified	0.15	-

4.3.26 *Curcuma zedoaria* (Berg.) Roscoe.

GC/MS analysis resulted in the identification of 16 components but 7 components cannot be identified (Figure 4.27and Table 4.29). Among these, limonene (37.68%), alpha-terpinene (9.88%) and alpha-gurjunene (8.00%) were the main component.

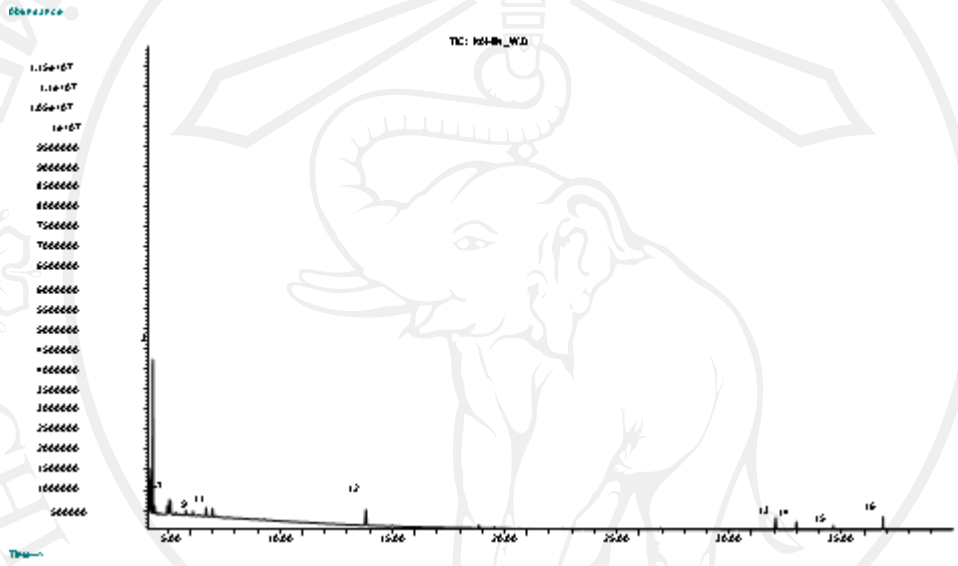


Figure 4.27 GC chromatogram of essential oil from *Curcuma zedoaria* (Berg.) Roscoe.

Table 4.29 Chemical components of essential oil from *Curcuma zedoaria* (Berg.) Roscoe.

Peak No.	RT (min)	Compounds	% Area	% QA
1	3.19	alpha-terpinene	9.88	87
2	3.25	ortho-cymene	7.64	98
3	3.34	limonene	37.68	97
4	3.43	unidentified	1.99	-
5	3.98	alpha-terpinolene	2.34	98

Table 4.29 (Continued)

Peak No.	RT (min)	Compounds	% Area	% QA
6	4.08	n-undecene	4.33	92
7	4.13	trans-linaool oxide	3.91	91
8	4.82	unidentified	1.91	-
9	5.14	unidentified	1.96	-
10	5.71	(+)-borneol	3.17	96
11	6.00	para-cymen-8-ol	3.03	91
12	12.83	alpha-gurjunene	8.00	99
13	31.07	unidentified	6.74	-
14	31.99	unidentified	3.62	-
15	33.63	unidentified	1.07	-
16	35.84	unidentified	2.74	-

4.3.27 *Zingiber cassumunar* Roxb.

GC/MS analysis resulted in the identification of 16 components but 2 components cannot be identified (Figure 4.28and Table 4.30). Among these, terpinen-4-ol (55.88%), and gamma-terpinene (11.05%) were the main component.

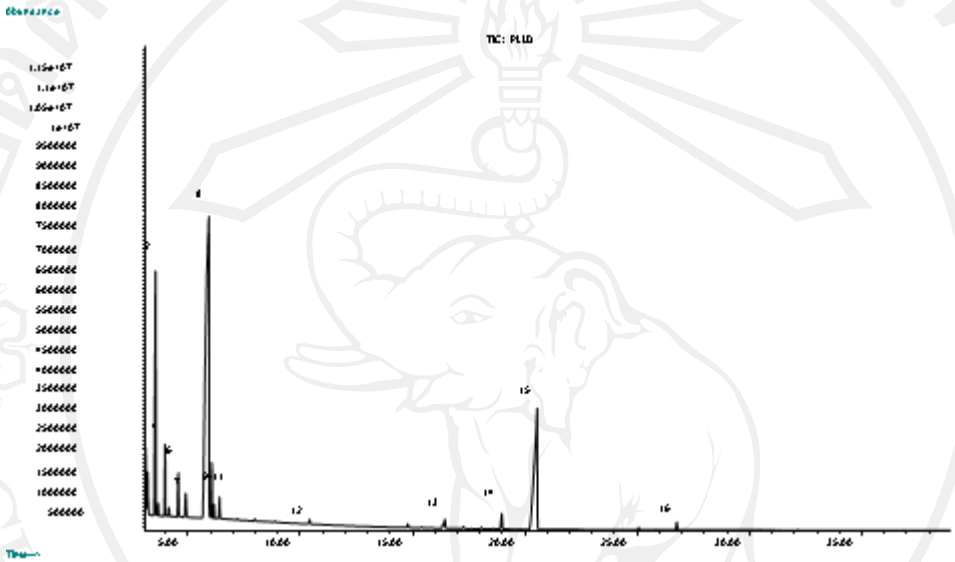


Figure 4.28 GC chromatogram of essential oil from *Zingiber cassumunar* Roxb.

Table 4.30 Chemical components of essential oil from *Zingiber cassumunar* Roxb.

Peak No.	RT (min)	Compounds	% Area	% QA
1	3.21	alpha-terpinene	0.33	94
2	3.56	gamma-terpinene	11.05	97
3	3.68	Cis-sabinenehydrate	0.45	98
4	3.99	alpha-terpinolene	2.53	98
5	4.16	trans-linaool oxide	0.39	95
6	4.58	neo-allo-ocimene	2.10	89
7	4.90	gamma-terpinene	1.47	96

Table 4.30 (Continued)

Peak No.	RT (min)	Compounds	% Area	% QA
8	5.93	terpinen-4-ol	55.88	98
9	6.07	para-cymen-8-ol	1.97	91
10	6.16	dill ether	0.49	89
11	6.41	Cis-piperitol	0.86	95
12	10.42	delta-elemene	0.26	83
13	16.44	allo-ocimene	0.57	99
14	18.98	caryophyllene oxide	0.99	90
15	20.55	unidentified	20.59	-
16	26.77	unidentified	0.50	-

4.3.28 *Zingiber officinale* Roscoe.

GC/MS analysis resulted in the identification of 20 components but 2 components cannot be identified (Figure 4.29 and Table 4.31). Among these, geranial (22.63%), z-citral (17.26%), ortho-cymene (13.42%), and beta-selinene (11.40%) were the main component.

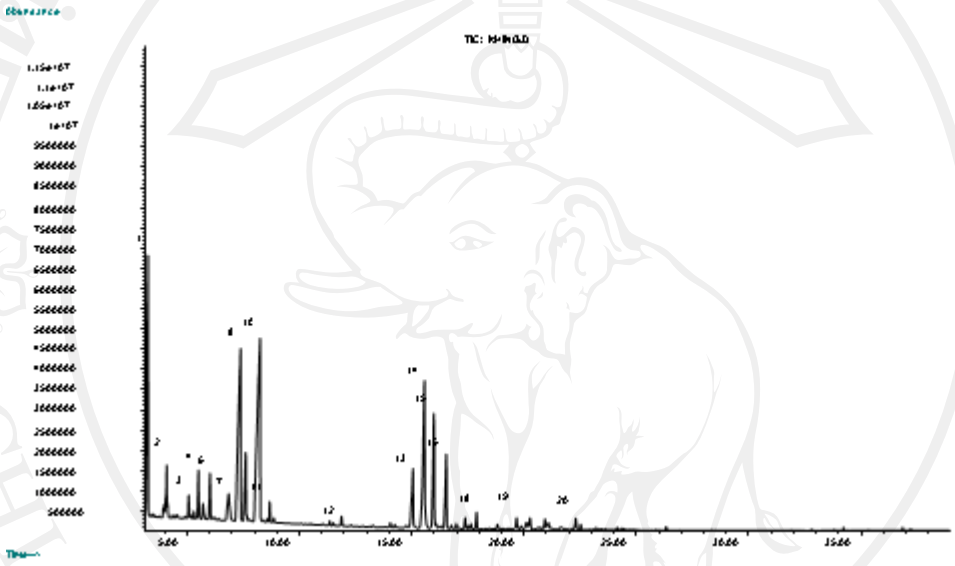


Figure 4.29 GC chromatogram of essential oil from *Zingiber officinale* Roscoe.

Table 4.31 Chemical components of essential oil from *Zingiber officinale* Roscoe.

Peak No.	RT (min)	Compounds	% Area	% QA
1	3.26	ortho-cymene	13.42	99
2	4.10	n-undecene	2.56	97
3	5.07	unidentified	0.85	-
4	5.51	(-)-borneol	2.06	91
5	5.71	(+)-borneol	0.90	95
6	6.02	terpinen-4-ol	2.06	91

Table 4.31 (Continued)

Peak No.	RT (min)	Compounds	% Area	% QA
7	6.85	unidentified	2.67	-
8	7.37	z-citral = neral	17.26	96
9	7.61	geraniol	3.91	94
10	8.25	geranial	22.63	95
11	8.68	2-undecanone	0.71	94
12	11.87	geranyl acetate	0.41	96
13	15.05	gamma-gurjunene	4.16	99
14	15.57	beta-selinene	11.40	94
15	15.99	alpha-muurolene	6.39	94
16	16.54	alpha-amorphene	4.27	99
17	17.38	alpha-gurjunene	0.54	91
18	17.89	d-nerolidol	0.78	91
19	19.67	zingiberene	0.67	90
20	22.30	unidentified	0.61	-

4.4 The study of the biological activities of the oils

4.4.1 Antioxidant activity

4.4.1.1 Ferric reducing antioxidant power (FRAP)

FRAP assay, which depends on the reduction of ferric-tripyridyltriazine (Fe(III)-TPTZ) complex to the ferroustripyridyltriazine (Fe(II)-TPTZ) by a reductant at low pH, (Fe(II)-TPTZ has a dark blue colour and can be monitored at 595 nm [142]. The reducing power property indicates that the oil sample are electron donors and can reduce the oxidized intermediates of lipid peroxidation process, so that they can acts as primary and secondary antioxidants [143].

The standard curve was ploted as concentration of standard ferrous sulfate ($FeSO_4 \cdot 7H_2O$) solution and the absorbance at 595 nm. The representative regression coefficient (r^2) was 0.99 and the linear regression equation was y = 0.0154x – 0.0419 0419 (y= Absorbance and x= concentration of $FeSO_4.7H_2O$). The meaning of equivalent concentration (EC) was defined as the concentration of antioxidant having a ferric-TPTZ reducing ability equivalent to that of 1 mM $FeSO_4 \cdot 7H_2O$.

The antioxidant activity of the oils ranged widely from 0.012 to 3.743 mM/mg sample. The oil of *O. gratissimum* Linn. showed the highest antioxidant activity with a EC1 value of 3.743 ±0.016 mM/mg , followed by those of *O.sanctum Linn.*, *M.officinalis* Linn. and *Z.cassumunar* Roxb. with EC values of 3.257 ± 0.093, 0.909 ± 0.054 and 0.514 ± 0.069 mM/mg , respectively (Table 4.32). The oil of *S.acmella* Murr. showed the lowest antioxidant activity among the oil samples included in this study with the EC value of 0.012 ± 0.009 mM/mg.

Table 4.32 Ferric reducing power (EC) of oils

No.	Type of oil	EC1 (mM/mg)
1	*Ocimum gratissimum* Linn.	3.743 ± 0.016
2	*Ocimum sanctum* Linn.	3.257 ± 0.093
3	*Melissa officinalis* Linn.	0.909 ± 0.054
4	*Zingiber cassumunar* Roxb.	0.514 ± 0.069
5	*Piper sarmentosum* Roxb.	0.450 ± 0.014
6	*Polyscias fruticosa* Harms.	0.428 ± 0.006
7	*Curcuma longa* Linn.	0.401 ± 0.014
8	*Citrus aurantifolia* Swing. (Leaf)	0.380 ± 0.018
9	*Citrus maxima* (Burm.) Merr. (Peel)	0.370 ± 0.017
10	*Ocimum basilicum* Linn.	0.370 ± 0.026
11	*Cinnamomum bejolghota* Sweet.	0.363 ± 0.041
12	*Sesamum indicum* Linn.	0.362 ± 0.014
13	*Citrus aurantifolia* Swing. (Peel)	0.346 ± 0.008
14	*Anethum graveolens* Linn.	0.335 ± 0.026
15	*Polygonum odoratum* Lour.	0.332 ± 0.020
16	*Citrus maxima* (Burm.) Merr. (Leaf)	0.306 ± 0.005
17	*Curcuma zedoaria* (Berg.) Roscoe.	0.299 ± 0.002
18	*Zingiber officinale* Roscoe.	0.285 ± 0.033
19	*Eryngium foetidum* Linn.	0.276 ± 0.011
20	*Coriandrum sativum* Linn.	0.272 ± 0.019
21	*Apium graveolens* Linn.	0.245 ± 0.003

Table 4.32 (Continued)

No.	Type of oil	EC1 (mM/mg)
22	*Boesenbergia pandurata* Roxb.	0.199 ± 0.037
23	*Cymbopogon citratus* Stapf.	0.170 ± 0.022
24	*Ocimum canum* Sims.	0.135 ± 0.022
25	*Coleus amboinicus* Lour.	0.032 ± 0.004
26	*Houttuynia cordata* Thunb.	0.016 ± 0.003
27	*Centella asiatica* Urban.	0.015 ± 0.007
28	*Eupatorium odoratum* Linn.	0.013 ± 0.001
29	*Spilanthes acmella* Murr.	0.012 ± 0.009

4.4.1.2 ABTS Method

ABTS radical cation assays, expressed as TEAC value, was used for evaluation of free radical-scavenging properties of oil sample. The percentage inhibition with absorbance at 740 nm is calculated and plotted as concentration of standard Trolox solution. The representative regression coefficient (r^2) was 0.99 and the linear regression equation was y= 2.0088x + 0.2942. The definition of TEAC is mg of standard Trolox with the equivalent antioxidant capacity to 1 mg of sample. The results of investigation are shown in Table 4.33. A total of 29 oil samples evaluated as their TEAC values indicated high variation in antioxidant activity. Total antioxidant activity, measured by the ABTS method, ranged from less than 0.001 to 1.059 mM/mg trolox equivalents.

The oil of *O.gratissimum* Linn.showed the highest antioxidant activity with a TEAC value of 1.059 ± 0.008 mM trolox equivalents/mg sample, followed by those

of *O.sanctum* Linn., *M.officinalis* Linn. and *C.amboinicus* Lour. with TEAC values of 1.055 ± 0.013, 0.281 ± 0.001 and 0.270 ± 0.006 mM trolox equivalents/mg sample, respectively.

Adeola et al [144] reported the antioxidant of *O. gratissimum* volatile oil extracted by hydrodistillation. They found that the volatile oil of *O. gratissimum* showed good antioxidant activity when used in scavenging DPPH radicals. This work confirmed that high antioxidant activity of the essential oil of *O. gratissimum* found in the present study. For *O. sanctum* many researches also reported about its antioxidant activity [145, 146]. However, the comparison of antioxidant activity between these two oils has not been reported anywhere. This present study is the first report to compare the antioxidant activity of these oils. The results indicated that both *O. gratissimum* oil and *O. sanctum* oil have high antioxidant activity and *O. gratissimum* oil showed significantly higher activity than *O. sanctum* oil. Eileen et al reported that the antioxidant of these two plants was due to the phenolic components existing in each cultivars [147]. The eugenol which found in GC-MS chromatogram of *O. gratissimum* and *O. sanctum* was also reported as the strong antioxidant [148, 149].

Table 4.33 Trolox Equivalent Antioxidant Capacity (TEAC) values of oils

No.	Type of oil	TEAC (mM/mg)
1	*Ocimum gratissimum* Linn.	1.059 ± 0.008
2	*Ocimum sanctum* Linn.	1.055 ± 0.013
3	*Melissa officinalis* Linn.	0.281 ± 0.001
4	*Coleus amboinicus* Lour.	0.270 ± 0.006
5	*Curcuma longa* Linn.	0.234 ± 0.003
6	*Polyscias fruticosa* Harms.	0.200 ± 0.017
7	*Zingiber cassumunar* Roxb.	0.190 ± 0.011
8	*Cinnamomum bejolghota* Sweet.	0.176 ± 0.002
9	*Citrus aurantifolia* Swing. (Leaf)	0.103 ± 0.006
10	*Zingiber officinale* Roscoe.	0.065 ± 0.006
11	*Ocimum canum* Sims.	0.045 ± 0.020
12	*Piper sarmentosum* Roxb.	0.041 ± 0.004
13	*Ocimum basilicum* Linn.	0.032 ± 0.017
14	*Citrus aurantifolia* Swing. (Peel)	0.021 ± 0.006
15	*Citrus maxima* (Burm.) Merr. (Leaf)	0.020 ± 0.010
16	*Sesamum indicum* Linn.	0.018 ± 0.008
17	*Polygonum odoratum* Lour.	0.018 ± 0.009
18	*Eryngium foetidum* Linn.	0.017 ± 0.016
19	*Cymbopogon citratus* Stapf.	0.013 ± 0.006
20	*Spilanthes acmella* Murr.	0.012 ± 0.012
21	*Boesenbergia pandurata* Roxb.	0.010 ± 0.010

Table 4.33 (Continued)

No.	Type of oil	TEAC (mM/mg)
22	*Citrus maxima* (Burm.) Merr. *(Peel)*	0.008 ± 0.006
23	*Eupatorium odoratum* Linn.	0.003 ± 0.014
24	*Curcuma zedoaria* (Berg.) Roscoe.	0.003 ± 0.018
25	*Apium graveolens* Linn.	0.000 ± 0.016
26	*Anethum graveolens* Linn.	0.000 ± 0.003
27	*Centella asiatica* Urban.	0.000 ± 0.024
28	*Coriandrum sativum* Linn.	0.000 ± 0.011
29	*Houttuynia cordata* Thunb.	0.000 ± 0.015

4.4.2 Tyrosinase inhibition activity

Tyrosinase is the rate limiting enzyme. It catalyzed pigment formation from oxidation of caffeic acid and 4-methylcatechol in human body at slow rate. It is mainly involved in two distinct reactions of melanin synthesis; firstly, the hydroxylation of a monophenol and secondly, the conversion of an o-diphenol to the corresponding o-quinone. o-Quinone undergoes several reactions to eventually form melanin [150, 151]. L-DOPA is an intermediate product during oxidation of L-tyrosine and is used commonly as an enzyme substrate of tyrosinase.

The tyrosinase inhibition activity of the oils ranged widely from 0 to 74.86 % . The oil of *H. cordata* Thunb. showed the highest tyrosinase inhibition activity with a inhibition value of 74.86±2.95%, followed closely by the oil of *C.citratus* Stapf. with the inhibition value of 73.89±2.11%. The essential oils of *A.graveolens* Linn. and *O.canum* Sims.showed the moderate activity with inhibition

values of 69.71±3.07 and 66.34±8.38% , respectively (Table 4.34). Among the oil samples included in this study, the oil of *C.maxima* (Burm.) Merr. peel showed the lowest tyrosinase inhibition activity.

Table 4.34 Tyrosinase inhibition activity of oils

No.	Type of oil	% Inhibition
1	*Houttuynia cordata* Thunb.	74.86±2.95
2	*Cymbopogon citratus* Stapf.	73.89±2.11
3	*Apium graveolens* Linn.	69.71±3.07
4	*Ocimum canum* Sims.	66.34±8.38
5	*Zingiber officinale* Roscoe.	65.01±7.84
6	*Citrus maxima* (Burm.) Merr. (Leaf)	62.50±0.74
7	*Citrus aurantifolia* Swing. (Leaf)	60.37±2.56
8	*Ocimum basilicum* Linn.	53.97±3.16
9	*Citrus aurantifolia* Swing. (Peel)	50.12±4.11
10	*Boesenbergia pandurata* Roxb.	45.88±4.84
11	*Curcuma zedoaria* (Berg.) Roscoe.	42.90±5.32
12	*Zingiber cassumunar* Roxb.	36.93±3.22
13	*Polygonum odoratum* Lour.	31.97±5.67
14	*Melissa officinalis* Linn.	28.07±4.52
15	*Ocimum sanctum* Linn.	28.01±9.54
16	*Ocimum gratissimum* Linn.	22.02±8.86
17	*Anethum graveolens* Linn.	21.16±3.84

Table 4.34 (Continued)

No.	Type of oil	% Inhibition
18	*Spilanthes acmella* Murr.	14.77±9.60
19	*Coleus amboinicus* Lour.	10.51±3.38
20	*Centella asiatica* Urban.	7.78±5.65
21	*Eupatorium odoratum* Linn.	4.12±4.61
22	*Eryngium foetidum* Linn.	4.12±3.07
23	*Curcuma longa* Linn.	2.41±1.18
24	*Piper sarmentosum* Roxb.	2.41±6.31
25	*Coriandrum sativum* Linn.	0.28±6.76
26	*Polyscias fruticosa* Harms.	0.00±0.02
27	*Sesamum indicum* Linn.	0.00±0.04
28	*Cinnamomum bejolghota* Sweet.	0.00±0.04
29	*Citrus maxima* (Burm.) Merr. (Peel)	0.00±0.01

In order to select the most potential oils for further study, the biolological activity as well as some characteristics of oils such as color and odor are one of the important criteria beside the yield value of the oil extraction. As mentioned above, the oil of *O.gratissimum* Linn. and of *O. sanctum* Linn. showed the highest antioxidant activity, however, the oils showed very less of antityrosinase activity. Moreover, the yield value of these oils was too low. Similar to the oil of *H. cordata* Thunb that showed the highest antityrosinase activity but no antioxidant activity and the yield value of the oil was also too low. Moreover, the oil possesses a bad smell. The essential oil of *C. citratus* was selected for further study because it gave moderate

antioxidant and high antityrosinase activity with good smell and the yield value of this oil was high enough. It was also reported that this oil was easy to be extracted in a large amount [152]. In addition, some reports were also revealed that lemongrass oil showed the antimicrobial activities. It was ideal to be used as drug carriers for the antimicrocial drug. Taweechaisupapong et al reported that among the tested essential oils, lemongrass oil exhibited the most effective killing activity and possessed the strongest inhibitory effect on Candida biofilm formation [153]. The results of Aiemsaard et al demonstrate that *S. agalactiae* and *B. cereus* are more susceptible to lemongrass oil, citral and geraniol than *S. aureus* and *E. coli*. The lemongrass oil appears to have multiple targets in the bacterial cell, depending on concentration used as well as the amount of its components [154].

Sesame oil was also selected for further study because of its characteristic of fixed oil and having an antioxidant activity and widely used in the industry [155, 156]. This oil is available in the market, so it is not difficult to get the large amount of oil.

4.5 Solubility test of the oil

The results of solubility study of sesame oil and lemongrass oil in various pharmaceutical solvents were shown in Table 4.35. It was found that sesame oil was highly dissolved in non-polar solvent such as mineral oil and hexane. Whereas lemongrass oil was well soluble in moderate polar and non-polar solvent such as ethanol, PEG400, mineral oil and hexane. It was noted that both oils could dissolve well in isopropyl myristate which is a non-polar oily liquid widely used in topical cosmetic product [157].

Table 4.35 The solubility of sesame oil and lemongrass oil in various solvents

Solvent	Quantity of solvent per a part of oil	
	Sesame Oil	**Lemongrass Oil**
Water	>10000	>10000
Propylene glycol	>10000	500
Dimethyl sulfoxide	>10000	10
Isopropyl myristate	10	300
Methanol	>10000	10
PEG400	>10000	600
Ethanol	>10000	10
Hexane	10	10
Mineral oil	10	10

4.6 Refractive index and surface tension of oil

The refractive index is a dimensionless number that describes how light or any other radiation propagates through that medium or liquid. The value of refractive index hence is an identical value of a substance like essential oil and could be used for quality control of oils. In this study, the refractive index of both oils was measured and the results were shown in Table 4.36. The refractive index of lemongrass oil was a little higher than that of sesame oil. These values were significant different (P-value = 0.001). The refractive index of pure water is generally known as 1.333. The results in this study indicated that the refractive index of the oils were significantly higher than that of pure water.

Table 4.36 The refractive index of sesame oil and lemongrass oil

Substance	Refractive index
Sesame oil	1.483±0.0006
Lemongrass oil	1.491±0.0002

Surface tension is a contractive tendency of the surface of a liquid that allows it to resist an external force. High polar liquids demonstrate high surface free energy and high surface tension [158]. Therefore, the surface tension value of a liquid could indentify its polarity. In this study, two oils were determined for their surface tension in comparison with those of some pure solvents. The results were shown in Table 4.37. It was found that surface tension values of both oils were similar, however much lower than water and higher than hexane. It was noted that surface tention values of

the oils were similar to those of intermediate polar solvents. Therefore, the results of this study indicated that the polarity of the oils is of a moderate level.

Table 4.37 Surface tension of sesame oil, lemongrass oil and various solvents

Substance	Surface Tension (dyne/cm)
Sesame oil	31.4
Lemongrass oil	30.6
Water	71.1
Dimethyl sulfoxide	43.6
PEG400	37.6
Propylene glycol	36.0
Isopropyl myristate	28.5
Methanol	23.1
Mineral oil	22.9
Ethanol	22.6
Hexane	18.3

4.7 Cytotoxicity tests

The cell viability of human peripheral blood mononuclear cells (PBMCs) could indicate the safety of the oil samples. In general, cell viability greater than 85% after exposure to test samples is recognized as safe for human use [159]. Figure 4.30 displays cell viability after contact with different concentrations of the oils. The results demonstrate that after 48 hr incubation with *C.citratus* and *S.indicum* oils, the

cell viability was more than 90%. More importantly, it is noted that the cell viability after *C.citratus* oil exposure was constantly near 100% whereas that of *S.indicum* oils was slightly decreased with higher oil concentration. This result indicates *C.citratus* oil *S.indicum* oil is nontoxic to human cells.

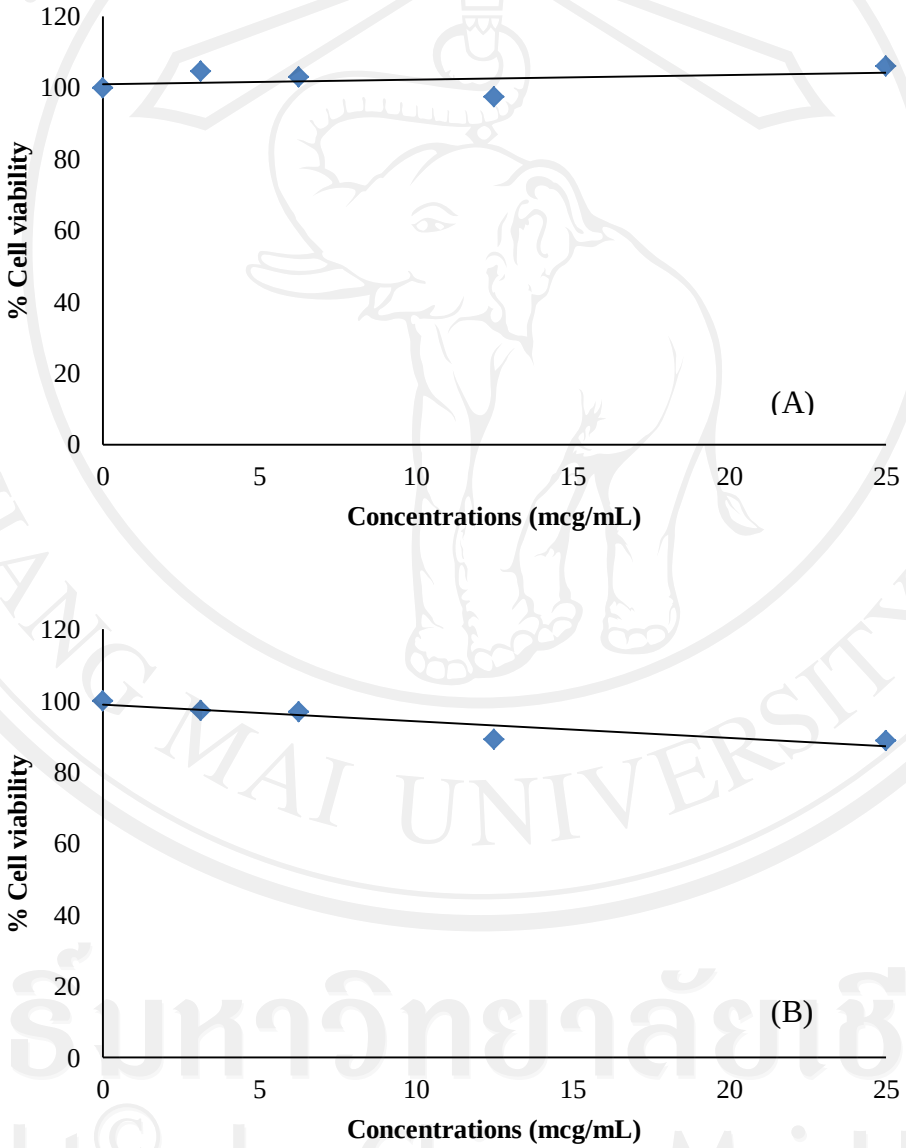


Figure 4.30 Cytotoxicity of the oil of *C.citratus* (lemongrass, A) and *S.indicum* (sesame, B) on normal human PBMCs.

4.8 Study of phase diagram

4.8.1 Preliminary study for phase diagram construction

In this research, preliminary experiment was conducted to study the trend of plant essential oil microemulsion formation from the components existing as well as to compare the efficiency of two common surfactants used. Tween 20 and Tween 80 were used as major surfactants whereas an absolute ethanol acted as a cosurfactant. With various ratios of surfactant to cosurfactant, many systems composed of the surfactant mixture, water and oil were obtained as shown in Table 4.38 to Table 4.45. The appearance of each system was different as shown in Table 4.46 to Table 4.53 and Figure 4.31 and Figure 4.32 respectively.

The results showed that the ratio of surfactant to cosurfactant caused some effects to the systems. As seen in Figures 4.31 to 4.32, some systems gave clear liquid expecting a microemulsion occurred whereas some systems were opaque expecting a coarse or conventional emulsion occurred, in Tween 20 containing systems, the surfactant and cosurfactant ratios of 1:1, 2:1, 3:1 gave the microemulsion with oil amount of 2%, but the ratio of 9:1 showed no microemulsion. In Tween 80 containing systems, all ratios of the surfactant and cosurfactant gave the microemulsion with oil amount of 2-7%. The results showed that both Tween 20 and Tween 80 with ethanol had capacity to form the micremulsion. However, the amount of cosurfactant was the point that should be considered. It was found that when higher amount of the cosurfactant was mixed in the surfactant system, the amount of surfactant needed was decreased.

Tween 20 and Tween 80 are non-ionic liquid surfactant. The outer appearances of these two surfactants are quite different. The viscosity of Tween 80 is

higher than Tween 20 causing more difficult to transfer. Moreover, the color of Tween 80 was intense yellow while that of system of Tween 20 was bright pale yellow which leads Tween 20 to look better appearance than Tween 80. The increasing of surfactant: cosurfactant ratio does not affect the systems on the clearness or opacity.

The results of this preliminary experiment could be concluded that the type of surfactant and the proportion of ethanol played an important role on the capability of oil dissolving in the system. It was also found that the system of Tween 20 containing systems looked better appearance and taste than the systems containing Tween 80. Furthermore, Amr.E et al reported that Tween 20 showed superior solubilization power with lemongrass oil over Tween 80 [160]. Therefore Tween 20 was selected for further experiments.

Table 4.38 % w/w of Tween20, ethanol, oil and water (Tween 20: ethanol = 1:1)

System	Formula	Volume (% w/w)				
		Tween20	**EtOH**	**Oil**	**Water**	**Total**
1	T(1)E(1)-1	48.75	48.93	2.32	0.00	100.00
2	T(1)E(1)-2	47.72	47.73	2.25	2.31	100.00
3	T(1)E(1)-3	47.03	46.84	2.04	4.08	100.00
4	T(1)E(1)-4	39.00	38.88	1.71	20.41	100.00
5	T(1)E(1)-5	32.77	32.59	1.52	33.12	100.00
6	T(1)E(1)-6	24.79	24.59	1.06	49.55	100.00
7	T(1)E(1)-7	19.77	19.69	0.99	59.54	100.00
8	T(1)E(1)-8	16.54	16.55	0.72	66.19	100.00
9	T(1)E(1)-9	14.11	14.41	0.76	70.72	100.00
10	T(1)E(1)-10	12.46	12.46	0.53	74.55	100.00
11	T(1)E(1)-11	11.13	11.14	0.45	77.28	100.00
12	T(1)E(1)-12	10.01	10.02	0.43	79.55	100.00
13	T(1)E(1)-13	48.90	48.91	2.14	0.05	100.00

Table 4.39 % w/w of Tween20, ethanol, oil and water (Tween 20: ethanol = 2:1)

System	Formula	Volume (% w/w)				
		Tween20	**EtOH**	**Oil**	**Water**	**Total**
1	T(2)E(1)-1	65.10	32.76	2.14	0.00	100.00
2	T(2)E(1)-2	64.07	32.02	2.06	1.85	100.00
3	T(2)E(1) -3	62.17	30.95	1.98	4.90	100.00
4	T(2)E(1)-4	52.14	26.08	1.81	19.97	100.00
5	T(2)E(1)-5	43.41	21.74	1.56	33.29	100.00
6	T(2)E(1)-6	37.55	18.72	1.20	42.53	100.00
7	T(2)E(1)-7	32.86	16.63	1.03	49.49	100.00
8	T(2)E(1)-8	26.36	13.19	0.92	59.54	100.00
9	T(2)E(1)-9	22.00	10.97	0.74	66.30	100.00
10	T(2)E(1)-10	18.89	9.38	0.68	71.05	100.00
11	T(2)E(1)-11	16.56	8.32	0.59	74.53	100.00
12	T(2)E(1)-12	14.76	7.40	0.52	77.32	100.00
13	T(2)E(1)-13	13.31	6.67	0.44	79.58	100.00
14	T(2)E(1)-14	12.09	6.05	0.41	81.44	100.00

Table 4.40 % w/w of Tween20, ethanol, oil and water (Tween 20: ethanol = 3:1)

System	Formula	Volume (% w/w)				
		Tween20	**EtOH**	**Oil**	**Water**	**Total**
1	T(3)E(1)-1	73.52	24.51	1.97	0.00	100.00
2	T(3)E(1)-2	71.82	24.14	2.12	1.92	100.00
3	T(3)E(1)-3	57.96	19.78	1.82	20.44	100.00
4	T(3)E(1)-4	48.23	16.08	1.47	34.22	100.00
5	T(3)E(1)-5	37.04	12.43	1.20	49.33	100.00
6	T(3)E(1)-6	29.73	9.96	0.89	59.41	100.00
7	T(3)E(1)-7	24.87	8.26	0.81	66.07	100.00
8	T(3)E(1)-8	21.29	7.23	0.61	70.87	100.00
9	T(3)E(1)-9	18.65	6.26	0.54	74.54	100.00
10	T(3)E(1)-10	16.57	5.59	0.48	77.36	100.00
11	T(3)E(1)-11	14.94	4.98	0.45	79.63	100.00
12	T(3)E(1)-12	13.61	4.53	0.41	81.45	100.00

Table 4.41 % w/w of Tween20, ethanol, oil and water (Tween 20: ethanol = 9:1)

System	Formula	Volume (% w/w)				
		Tween20	**EtOH**	**Oil**	**Water**	**Total**
1	T(9)E(1)-1	87.85	9.79	2.37	0.00	100.00
2	T(9)E(1)-2	86.14	9.87	2.23	1.76	100.00
3	T(9)E(1)-3	84.17	9.45	2.12	4.26	100.00
4	T(9)E(1)-4	70.03	7.89	1.79	20.28	100.00
5	T(9)E(1)-5	58.65	6.62	1.47	33.26	100.00
6	T(9)E(1)-6	44.39	5.02	1.11	49.47	100.00
7	T(9)E(1)-7	35.67	4.08	0.87	59.39	100.00
8	T(9)E(1)-8	29.75	3.50	0.73	66.01	100.00
9	T(9)E(1)-9	25.50	3.03	0.67	70.80	100.00
10	T(9)E(1)-10	22.37	2.54	0.62	74.47	100.00
11	T(9)E(1)-11	19.94	2.25	0.48	77.33	100.00
12	T(9)E(1)-12	15.04	18.06	0.34	66.55	100.00
13	T(9)E(1)-13	16.37	1.83	0.40	81.39	100.00

Table 4.42 % w/w of Tween80, ethanol, oil and water (Tween 80: ethanol = 1:1)

System	Formula	Quantity (% w/w)				
		Tween80	**EtOH**	**Oil**	**Water**	**Total**
1	T80(1)E(1)-1	48.80	48.92	2.28	0.00	100.00
2	T80(1)E(1)-2	48.11	48.04	3.85	0.00	100.00
3	T80(1)E(1)-3	46.53	46.74	3.51	3.23	100.00
4	T80(1)E(1)-4	47.69	47.89	2.43	1.99	100.00
5	T80(1)E(1)-5	46.65	46.68	3.50	3.16	100.00
6	T80(1)E(1)-6	47.04	47.02	2.70	3.24	100.00
7	T80(1)E(1)-7	38.70	38.57	3.38	19.35	100.00
8	T80(1)E(1)-8	46.23	46.03	3.15	4.58	100.00
9	T80(1)E(1)-9	32.46	32.45	2.48	32.61	100.00
10	T80(1)E(1)-10	24.42	24.63	2.00	48.95	100.00
11	T80(1)E(1)-11	14.11	14.16	1.11	70.62	100.00
12	T80(1)E(1)-12	11.04	11.02	0.70	77.23	100.00
13	T80(1)E(1)-14	38.24	38.06	2.96	20.75	100.00

Table 4.43 % w/w of Tween80, ethanol, oil and water (Tween 80: ethanol = 2:1)

System	Formula	Quantity (% w/w)				
		Tween80	**EtOH**	**Oil**	**Water**	**Total**
1	T80(2)E(1)-1	64.80	32.42	2.78	0.00	100.00
2	T80(2)E(1)-2	63.95	31.99	4.06	0.00	100.00
3	T80(2)E(1)-3	62.80	31.20	3.45	2.55	100.00
4	T80(2)E(1)-4	61.75	31.16	3.26	3.83	100.00
5	T80(2)E(1)-5	60.58	30.23	3.53	5.66	100.00
6	T80(2)E(1)-6	60.66	30.54	3.98	4.82	100.00
7	T80(2)E(1)-7	51.65	25.69	3.29	19.36	100.00
8	T80(2)E(1)-8	42.03	52.11	2.56	3.30	100.00
9	T80(2)E(1)-9	42.98	21.91	2.79	32.32	100.00
10	T80(2)E(1)-10	32.50	16.30	2.16	49.04	100.00
11	T80(2)E(1)-11	18.75	9.47	1.18	70.60	100.00
12	T80(2)E(1)-12	14.70	7.36	0.97	76.96	100.00
13	T80(2)E(1)-13	61.34	30.68	4.18	3.80	100.00

Table 4.44 % w/w of Tween80, ethanol, oil and water (Tween 80: ethanol = 3:1)

System	Formula	Quantity (% w/w)				
		Tween80	**EtOH**	**Oil**	**Water**	**Total**
1	T80(3)E(1)-1	71.38	23.95	4.67	0.00	100.00
2	T80(3)E(1)-2	69.88	23.42	4.34	2.36	100.00
3	T80(3)E(1)-3	68.02	23.09	4.07	4.82	100.00
4	T80(3)E(1)-4	68.76	23.51	4.29	3.43	100.00
5	T80(3)E(1)-5	69.27	23.39	4.50	2.84	100.00
6	T80(3)E(1)-6	68.04	22.72	4.15	5.09	100.00
7	T80(3)E(1)-7	57.42	19.28	3.69	19.61	100.00
8	T80(3)E(1)-9	48.26	16.24	3.08	32.42	100.00
9	T80(3)E(1)-10	36.31	12.06	2.31	49.32	100.00
10	T80(3)E(1)-11	21.14	7.05	1.40	70.42	100.00
11	T80(3)E(1)-12	16.51	5.56	1.09	76.84	100.00

Table 4.45 % w/w of Tween80, ethanol, oil and water (Tween 80: ethanol = 9:1)

System	Formula	Quantity (% w/w)				
		Tween80	**EtOH**	**Oil**	**Water**	**Total**
1	T80(9)E(1)-1	83.28	9.38	7.34	0.00	100.00
2	T80(9)E(1)-2	82.82	9.21	7.97	0.00	100.00
3	T80(9)E(1)-3	80.42	9.03	8.03	2.52	100.00
4	T80(9)E(1)-4	80.97	9.03	8.45	1.55	100.00
5	T80(9)E(1)-5	81.26	9.21	7.24	2.29	100.00
6	T80(9)E(1)-6	79.90	9.07	7.25	3.78	100.00
7	T80(9)E(1)-7	67.36	7.58	6.16	18.90	100.00
8	T80(9)E(1)-8	78.78	8.94	7.24	5.04	100.00
9	T80(9)E(1)-9	56.86	6.38	5.18	31.58	100.00
10	T80(9)E(1)-10	43.16	4.97	3.92	47.95	100.00
11	T80(9)E(1)-11	25.19	2.88	2.31	69.63	100.00
12	T80(9)E(1)-12	19.71	2.22	1.78	76.30	100.00
13	T80(9)E(1)-13	67.51	7.55	6.05	18.90	100.00

Table 4.46 Physical appearance of formulation of Tween 20, ethanol, oil and water (Tween 20: ethanol = 1:1)

Formulation	Appearance (color/ clarity/ turbidity/ separation)
T(1)E(1)-1	Light yellow, Clarity
T(1)E(1)-2	Light yellow, Clarity
T(1)E(1)-3	Light yellow, Seperated
T(1)E(1)-4	Light yellow, Turbid
T(1)E(1)-5	Light yellow, Turbid
T(1)E(1)-6	Light yellow, Seperated
T(1)E(1)-7	Light yellow, Seperated
T(1)E(1)-8	Light yellow, Seperated
T(1)E(1)-9	Light yellow, Seperated
T(1)E(1)-10	Light yellow, Seperated
T(1)E(1)-11	Light yellow, Seperated
T(1)E(1)-12	Light yellow, Seperated
T(1)E(1)-13	Light yellow, Seperated

Table 4.47 Physical appearance of formulation of Tween 20, ethanol, oil and water (Tween 20: ethanol = 2:1)

Formulation	Appearance (color/ clarity/ turbidity/ separation)
T(2)E(1)-1	Light yellow, Clarity
T(2)E(1)-2	Light yellow, Clarity
T(2)E(1) -3	Light yellow, Seperated
T(2)E(1)-4	Light yellow, Turbid
T(2)E(1)-5	Light yellow, Turbid, Seperated
T(2)E(1) -6	Light yellow, Turbid, Seperated
T(2)E(1)-7	Light yellow, Turbid, Seperated
T(2)E(1)-8	Light yellow, Seperated
T(2)E(1)-9	Light yellow, Seperated
T(2)E(1)-10	Light yellow, Seperated
T(2)E(1)-11	Light yellow, Seperated
T(2)E(1)-12	Light yellow, Seperated
T(2)E(1)-13	Light yellow, Seperated
T(2)E(1)-14	Light yellow, Seperated

Table 4.48 Physical appearance of formulation of Tween 20, ethanol, oil and water (Tween 20: ethanol = 3:1)

Formulation	Appearance (color/ clarity/ turbidity/ separation)
T(3)E(1)-1	Light yellow, Clarity
T(3)E(1)-2	Light yellow, Clarity
T(3)E(1)-3	Light yellow, Turbid
T(3)E(1)-4	Light yellow, Turbid, Seperated
T(3)E(1)-5	Light yellow, Seperated
T(3)E(1)-6	Light yellow, Seperated
T(3)E(1)-7	Light yellow, Seperated
T(3)E(1)-8	Light yellow, Seperated
T(3)E(1)-9	Light yellow, Seperated
T(3)E(1)-10	Light yellow, Seperated
T(3)E(1)-11	Light yellow, Seperated
T(3)E(1)-12	Light yellow, Seperated

Table 4.49 Physical appearance of formulation of Tween 20, ethanol, oil and water (Tween 20: ethanol = 9:1)

Formulation	Appearance (color/ clarity/ turbidity/ separation)
T(9)E(1)-1	Light yellow, Clarity
T(9)E(1)-2	Light yellow, Turbid
T(9)E(1)-3	Light yellow, Turbid
T(9)E(1)-4	Light yellow, Turbid
T(9)E(1)-5	Light yellow, Turbid
T(9)E(1)-6	Light yellow, Turbid
T(9)E(1)-7	Light yellow, Turbid, Seperated
T(9)E(1)-8	Light yellow, Seperated
T(9)E(1)-9	Light yellow, Seperated
T(9)E(1)-10	Light yellow, Seperated
T(9)E(1)-11	Light yellow, Seperated
T(9)E(1)-12	Light yellow, Seperated
T(9)E(1)-13	Light yellow, Turbid, Seperated

Table 4.50 Physical appearance of formulation of Tween 80, ethanol, oil and water (Tween 80: ethanol = 1:1)

Formulation	Appearance (color/ clarity/ turbidity/ separation)
T80(1)E(1)-1	Yellow, Clarity
T80(1)E(1)-2	Yellow, Clarity
T80(1)E(1)-3	Yellow, Clarity
T80(1)E(1)-4	Yellow, Clarity
T80(1)E(1)-5	Yellow, Turbid
T80(1)E(1)-6	Yellow, Clarity
T80(1)E(1)-7	Yellow, Clarity
T80(1)E(1)-8	Yellow, Turbid
T80(1)E(1)-9	Yellow, Turbid
T80(1)E(1)-10	Yellow, Turbid
T80(1)E(1)-11	Yellow, Turbid
T80(1)E(1)-12	White, Turbid
T80(1)E(1)-13	White, Turbid
T80(1)E(1)-14	Yellow, Turbid

Table 4.51 Physical appearance of formulation of Tween 80, ethanol, oil and water (Tween 80: ethanol = 2:1)

Formulation	Appearance (color/ clarity/ turbidity/ separation)
T80(2)E(1)-1	Yellow, Clarity
T80(2)E(1)-2	Yellow, Clarity
T80(2)E(1)-3	Yellow, Clarity
T80(2)E(1)-4	Yellow, Turbid
T80(2)E(1)-5	Yellow, Turbid
T80(2)E(1)-6	Yellow, Turbid
T80(2)E(1)-7	Yellow, Turbid
T80(2)E(1)-8	Yellow, Turbid
T80(2)E(1)-9	Yellow, Turbid
T80(2)E(1)-10	Yellow, Turbid
T80(2)E(1)-11	White, Turbid
T80(2)E(1)-12	White, Turbid
T80(2)E(1)-13	Yellow, Turbid

Table 4.52 Physical appearance of formulation of Tween 80, ethanol, oil and water (Tween 80: Ethanol = 3:1)

Formulation	Appearance (color/ clarity/ turbidity/ separation)
T80(3)E(1)-1	Yellow, Clarity
T80(3)E(1)-2	Yellow, Clarity
T80(3)E(1)-3	Yellow, Seperated
T80(3)E(1)-4	Yellow, Seperated
T80(3)E(1)-5	Yellow, Seperated
T80(3)E(1)-6	Yellow, Seperated
T80(3)E(1)-7	Yellow, Turbid
T80(3)E(1)-8	Yellow, Turbid
T80(3)E(1)-9	Yellow, Turbid
T80(3)E(1)-10	Yellow, Turbid, Seperated
T80(3)E(1)-11	Light yellow, Seperated

Table 4.53: Physical appearance of formulation of Tween 80, ethanol, oil and water (Tween 80 : Ethanol = 9:1)

Formulation	Appearance (color/ clarity/ turbidity/ separation)
T80(9)E(1)-1	Yellow, Clarity
T80(9)E(1)-2	Yellow, Clarity
T80(9)E(1)-3	Yellow, Seperated
T80(9)E(1)-4	Yellow, Seperated
T80(9)E(1)-5	Yellow, Seperated
T80(9)E(1)-6	Yellow, Seperated
T80(9)E(1)-7	Yellow, Clarity
T80(9)E(1)-8	Yellow, Seperated
T80(9)E(1)-9	Yellow, Turbid
T80(9)E(1)-10	White, Turbid
T80(9)E(1)-11	Yellow, Seperated
T80(9)E(1)-12	Yellow, Seperated
T80(9)E(1)-13	Yellow, Clarity
T80(2)E(1)-14	Yellow, Clarity

(A)

(B)

(C)

(D)

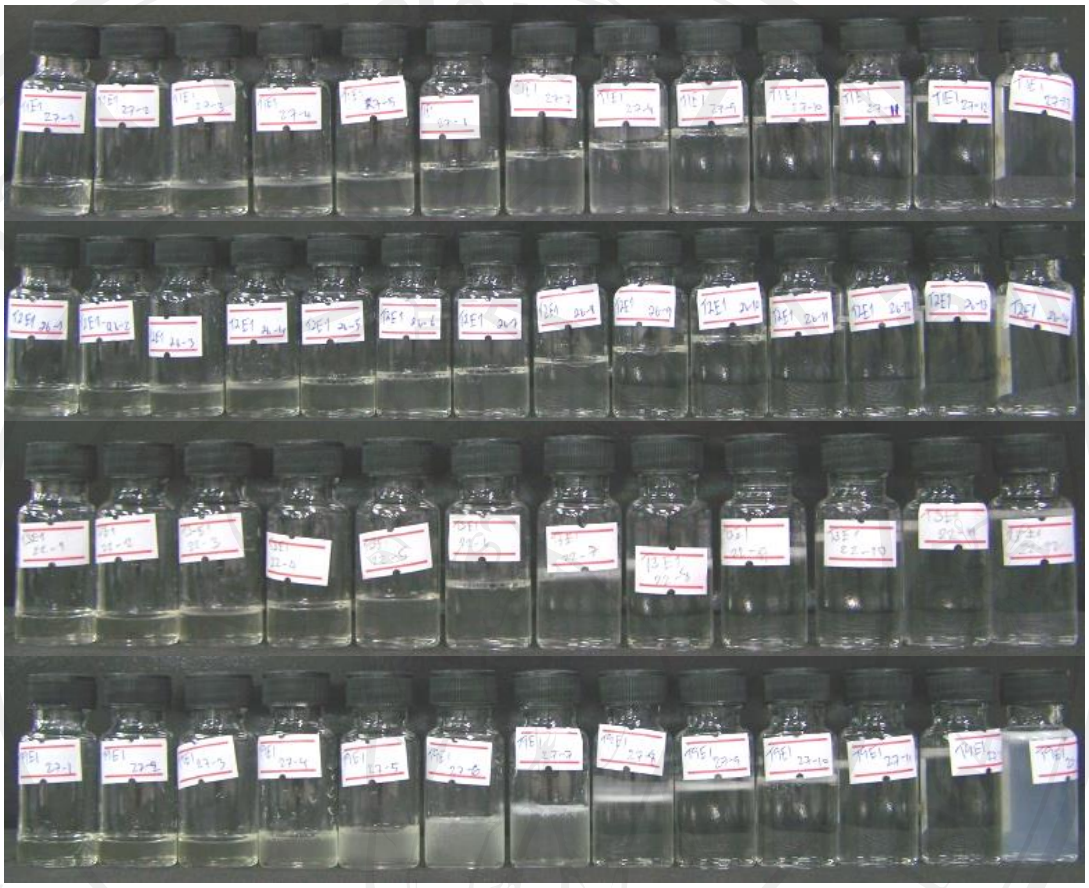


Figure 4.31 Effect of Tween 20 and ethanol with water and lemongrass oil (*C. citratus* Stapf.) The ratio of Tween 20 and ethanol are (A)=1:1, (B)=2:1, (C)=3:1, (D)=9:1

(A)

(B)

(C)

(D)

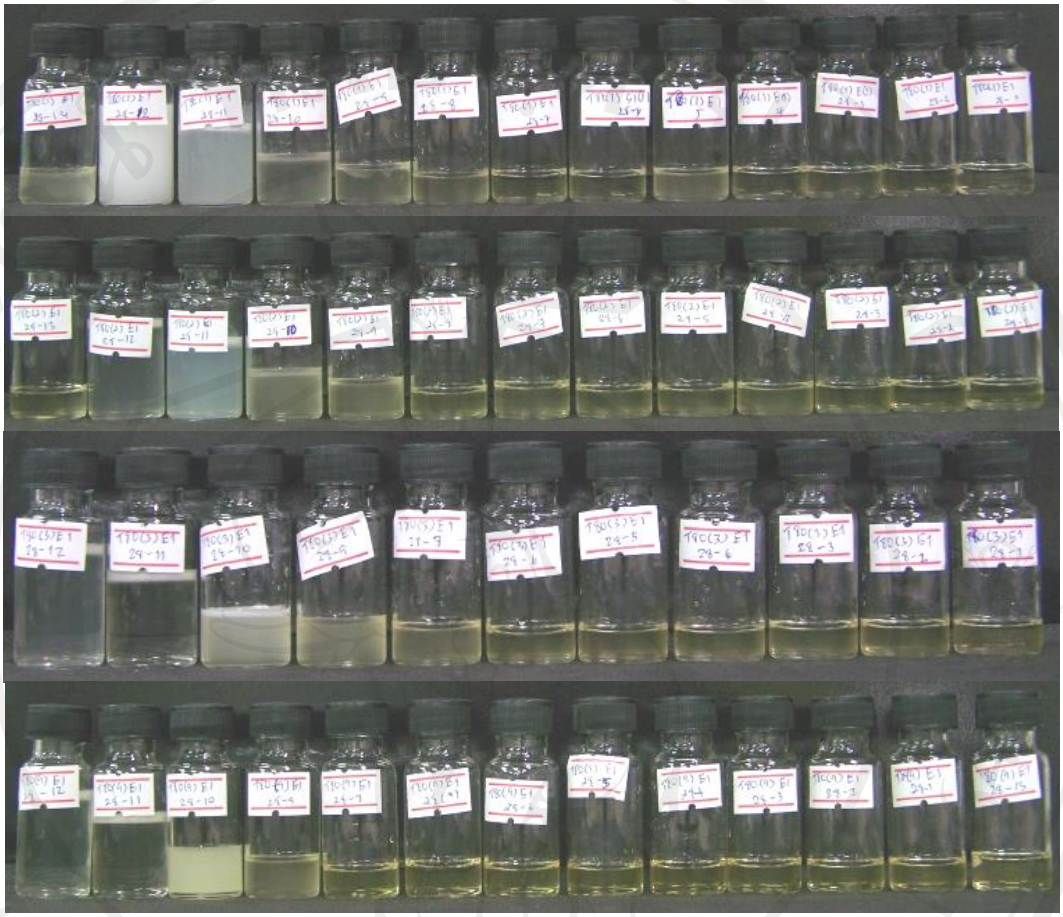


Figure 4.32 Effect of Tween 80 and ethanol with water and lemongrass oil (*C. citratus* Stapf.) The ratio of Tween 80 and ethanol are (A)=1:1, (B)=2:1, (C)=3:1, (D)=9:1

4.8.2 Phase diagram construction

Ternary phase diagram of three components was constructed for investigating of the microemulsion region. The phase diagram showed ratios of water phase, oil phase and surfactant phase. In case of a cosurfactant is used, the surfactant phase meant a system of a main surfactant in combination with a cosurfactant in a certain ratio. The ternary phase diagram in this case was then a so-called pseudoternary phase diagram. The desired microemulsion represented the clear thermodynamically stable, isotropic liquid mixtures of oil, water and surfactant mixture.

Tween 20, Triton X 114, Brij 97 and Tween 85 were comparatively evaluated for their potential as a main surfactant most suitable for lemongrass oil and sesame oil microemulsion development. According to these, the effects of various factors on the microemulsion phase diagram of both oils were studied.

4.8.2.1 Effect of surfactant type

Titration method was used to investigate microemulsion area in phase diagram because this method gave more details in number ratio of oil: surfactant: water. In order to study the effect of surfactant, four types of surfactants, Brij 97 or polyoxyethylene (10) oleoyl ether with HLB = 12.4, Triton X 114 or polyoxyethylene (8) isooctylphenyl ether with HLB = 12.4, Tween 20 or polyoxyethylene (20) sorbitan monolaurate with HLB = 16.7, Tween 85 or polyoxyethylene (20) sorbitan trioleate with HLB = 11.0, were selected to use because of their nonionic property and suitable HLB values for o/w microemulsion. The most suitable surfactant for lemongrass oil or sesame oil was also investigated.

The results showed that the most suitable surfactant for both oils was different. Tween 85 exhibited to be the most suitable for sesame oil while Tween 20, Triton X

1 1 4 and Brij 9 7 gave the high microemulsion area for lemongrass oil, as shown in Figure 4.33 to 4.36. Considering of HLB value, the results revealed that lemongrass oil required a system with higher HLB than sesame oil. The study of Orafidiya et al showed that droplet size of eucalyptus, lippia and peppermint oils was decresed when the HLB of surfactant was increased. However, they reported that the most suitable HLB to yield the smallest size was about 12 [161]. Zhang et al studied the fixed oil with brine residue emulsion and found that the HLB needed for the smallest diameter of droplet was about HLB=10.7 [162]. These reports explored the effect of HLB of the surfactant on the size of the internal droplet phase of the emulsions and indicated that only suitable HLB value was needed for the oil to make the emulsion of the required droplet size. The result of our study was in correspondence with these studies.

4.8.2.2 Effect of type and proportion of cosurfactant

The system which contained only surfactant gave the narrow microemulsion area as shown in Figure 4.37. Cosurfactants usually used in the formulation of microemulsion are moderate carbon chain (C2-C8) of alcohol. Consideration of suitable cosurfactant depended on their ability to assist a main surfactant like Tween 20 or Tween 80 or other polysorbates to reduce the surface tension of ther internal droplet liquid. In the present study, five cosurfactants having different carbon atom in their molecule as shown in Table 4.54 were selected in the development of lemongrass oil and sesame oil microemulsions. The results found that five cosurfactants in any tested proportion gave the different microemulsion area. The results indicated that among five cosutfactant used, ethanol expressed the widest area of microemulsion. It was also found that the greater the number of carbon in

cosurfactant molecule, the less area of the microemulsion in the phase decreased obtained as shown in Figure 4.37 to 4.43.

Ratios of surfactant to cosurfactant used in this experiment were 1:2, 1:1, and 2:1. The results found that 2:1 ratio gave the largest microemulsion area on the phase diagram of lemongrass oil and sesame oil, followed by 1:1 and 1:2 ratio, respectively as shown in Figure 4.32 to 4.42. These results led to the conclusion that ethanol was the most suitable cosurfactant for the interested system of lemongrass oil and sesame oil. The result of the present experiment was in accordance with the results of Klossek et al which found that the short chain alcohol adding as cosolvent extended the homogeneous single phase area in ternary or pseudo-ternary systems [163]. Ethanol has less toxic without cause of irritation for skin [164-166]. Some previous reports expressed that the use of ethanol as cosurfactant in microemulsion of ethyloleate gave small droplet size in both o/w and w/o system [167].

The results of this study demonstrated that the ratio of surfactant to cosurfactant plyed an important role on microemulsion area obtained. According to these results, 2:1 ratio of surfactant: co-surfactant was selected used in the next experiment.

The effects of the molecular structure of cosurfactant would be studied. The comparison between isopropanol and n-propanol, which is a molecular straight chain and branch chain, were added into the system of 2: 1 ratio of surfactant: co-surfactant. The results revealed that n-propanol, which is a straight chain alcohol, causing the increase of microemulsion area more than isopropanol, branch chain alcohol. The result was shown in Figure 4.50. The higher potential of a straight chain alcohol on increasing the microemulsion found in this study was considered to be due to the

solubility enhancing effect of the short straight chain alcohol. Alcohols with higher degree of branched chain or carbon chain length were found to decrease the aqueous solubility of the oils [168].

Table 4.54: Cosurfactants used in this study

Common Name	**Synonyms**	**Molecular formula / Structure formula**
Ethyl alcohol	ethanol	CH_3CH_2OH
Propyl alcohol	*n*-propanol or propan-1-ol	$CH_3CH_2CH_2OH$
Butyl alcohol	*n*-butanol or butan-1-ol	$CH_3CH_2CH_2CH_2OH$
Amyl alcohol	*n*-pentanol or pentan-1-ol	$CH_3CH_2CH_2CH_2CH_2OH$
Octyl alcohol	*n*-octanol or octan-1-ol	$CH_3(CH_2)_7OH$

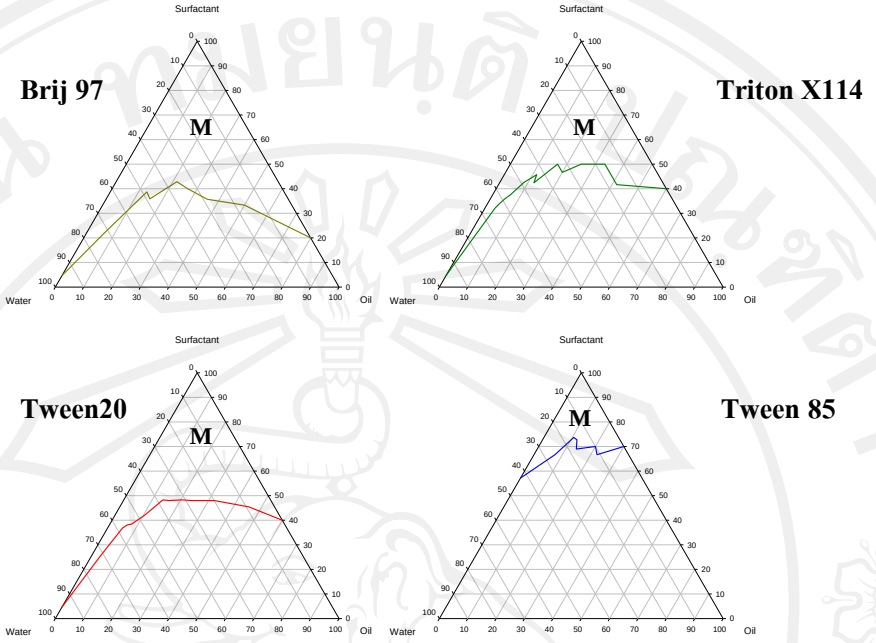


Figure 4.33 Microemulsion area (M) of lemongrass oil in various surfactant systems with ethanol (1 : 1)

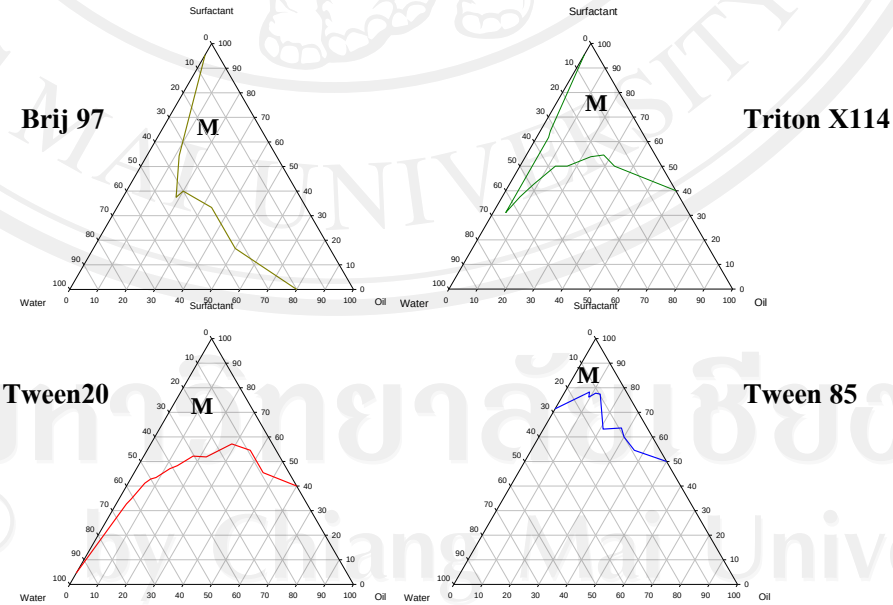


Figure 4.34 Microemulsion area (M) of lemongrass oil in various surfactant systems with ethanol (1 : 2)

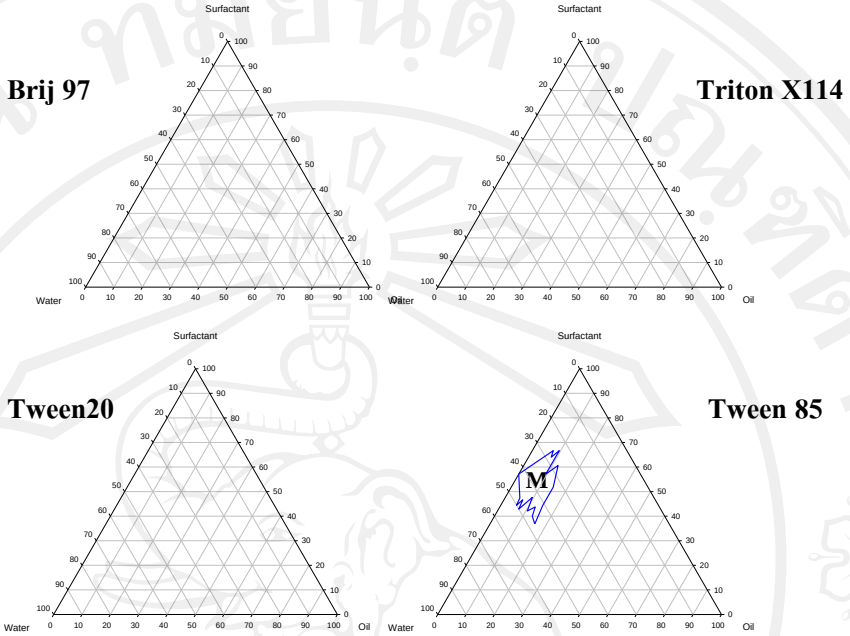


Figure 4.35 Microemulsion area (M) of sesame oil in various surfactant systems with ethanol (1 : 1)

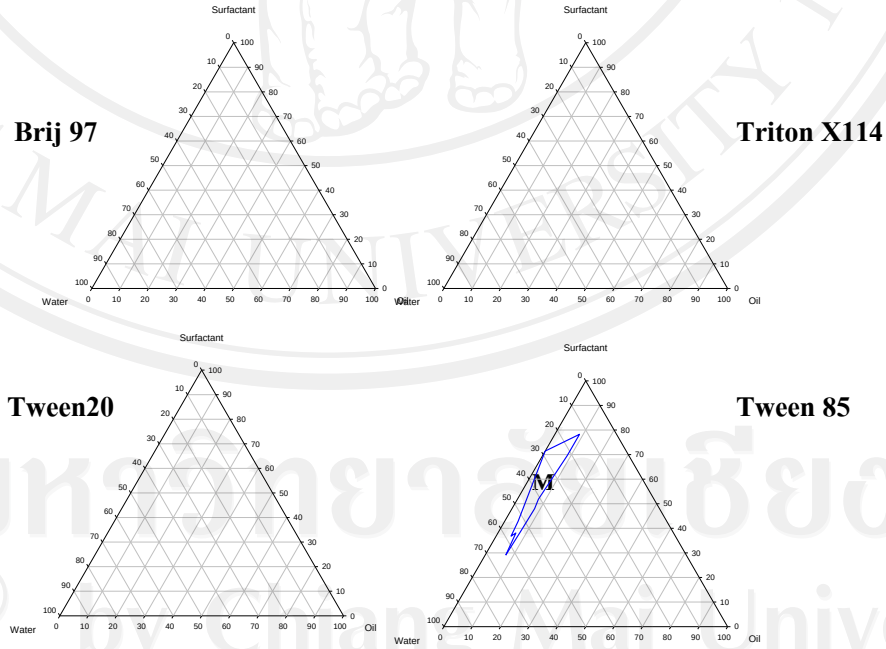


Figure 4.36 Microemulsion area (M) of sesame oil in various surfactant systems with ethanol (1 : 2)

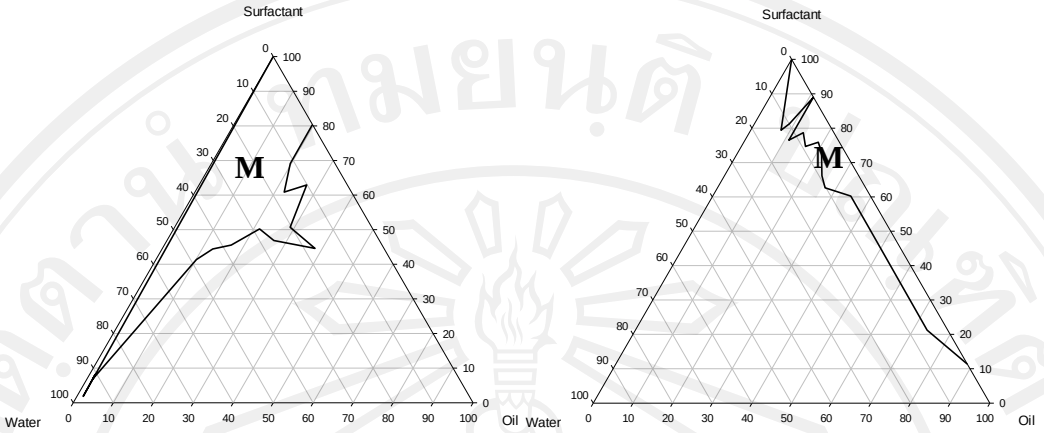


Figure 4.37 Microemulsion area (M) of lemongrass oil in Tween 20 and sesame oil in Tween 85 without cosurfactant

4.8.2.3 Effect of type and concentration of electrolyte

When electrolyte was added to the microemulsion, it usually affects the ionic strength of the system [169]. After adding sodium chloride and calcium chloride to the aqueous system, the results revealed that both types of electrolyte had no significant effect on microemulsion area. The microemulsion area did not clearly show the changing of microemulsion area. The result was shown in Figure 4.46 – 4.49. In general, the ionic strength would affect space of the surfactant head decreased. The molecules of surfactant would move together and the droplet size would decreased, the area of the microemulsion decreased. The results according to Mcclements et al study of ionic strength of emulsion with non-ionic surfactant was not attected the droplet size of the internal phase [170]. However, as nonionic surfactants were used in the systems, hence it was considered that it might be because of the characteristics of the nonionic surfactant that was not easily changed by the

type of ionic strength of the systems. Hence, the ionic strength changed by both types of electrolytes showed no effect on microemulsion area of the microemulsions developed in this study.

4.8.2.4 Effect of pH

The effect of pH was studied in the range pH 4.0 - 8.0. The results revealed that pH 4.0 - 6.0 did not affect the area of the microemulsions of lemongrass oil but the microemulsion area was decreased at pH 8.0. The result was shown in Figure 4.51. In case of sesame oil the microemulsion was not changed at pH 4.0 and 6.0. However the microemulsion area tended to decrease at pH 8.0 as that of lemongrass as shown in Figure 4.52. It was concluded that pH 8.0 could decrease the microemulsion area but not significantly.

Nevertheless, the previous study showed that pH had no effect on microemulsion area with nonionic surfactant [171].

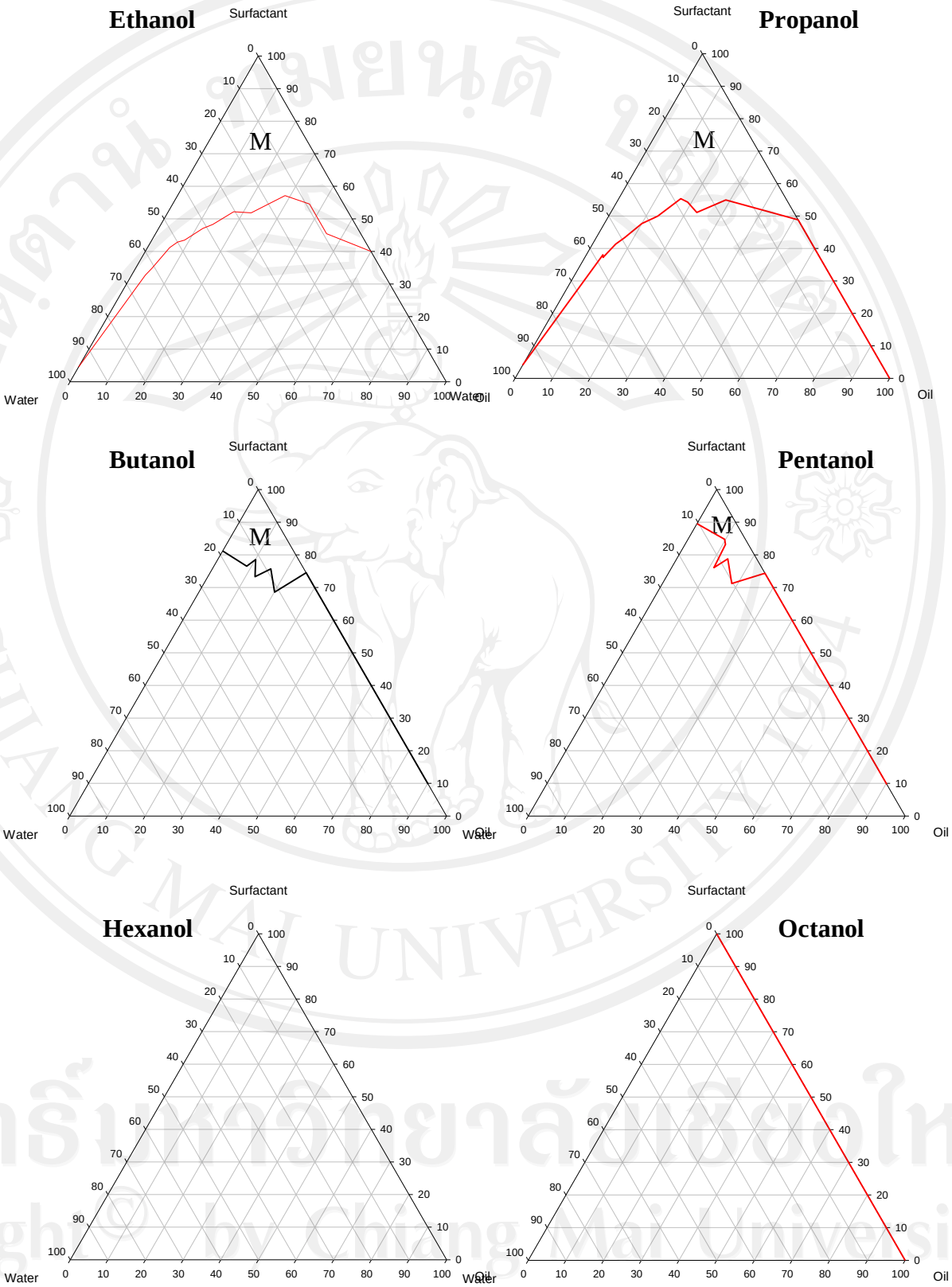


Figure 4.38 Effect of carbon atom in co-surfactant molecules on the phase diagram of lemongrass oil (the ratio of surfactant: co-surfactant is 1:2)

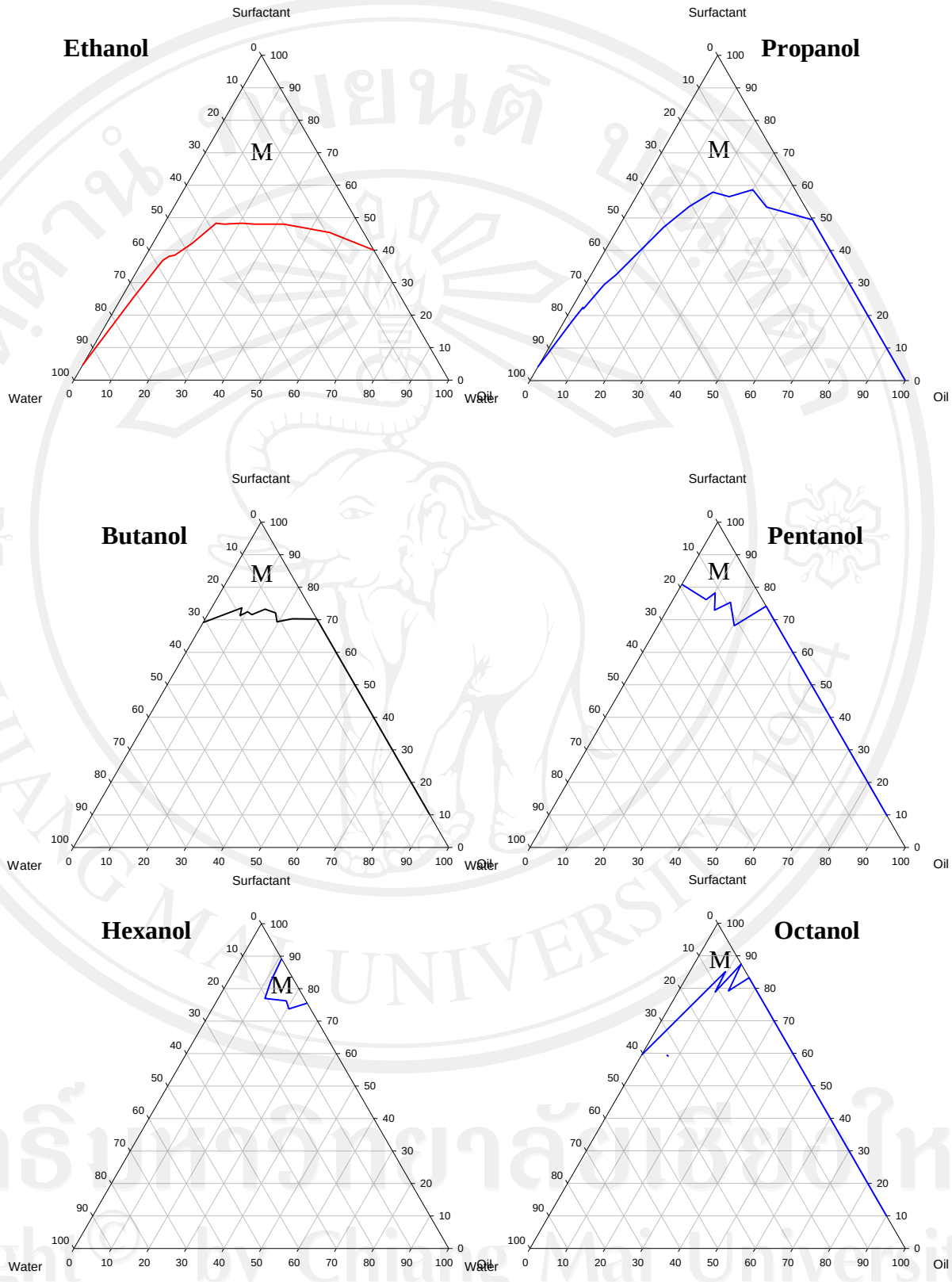


Figure 4.39 Effect of carbon atom in co-surfactant molecules on the phase diagram of lemongrass oil (the ratio of surfactant: co-surfactant is 1:1)

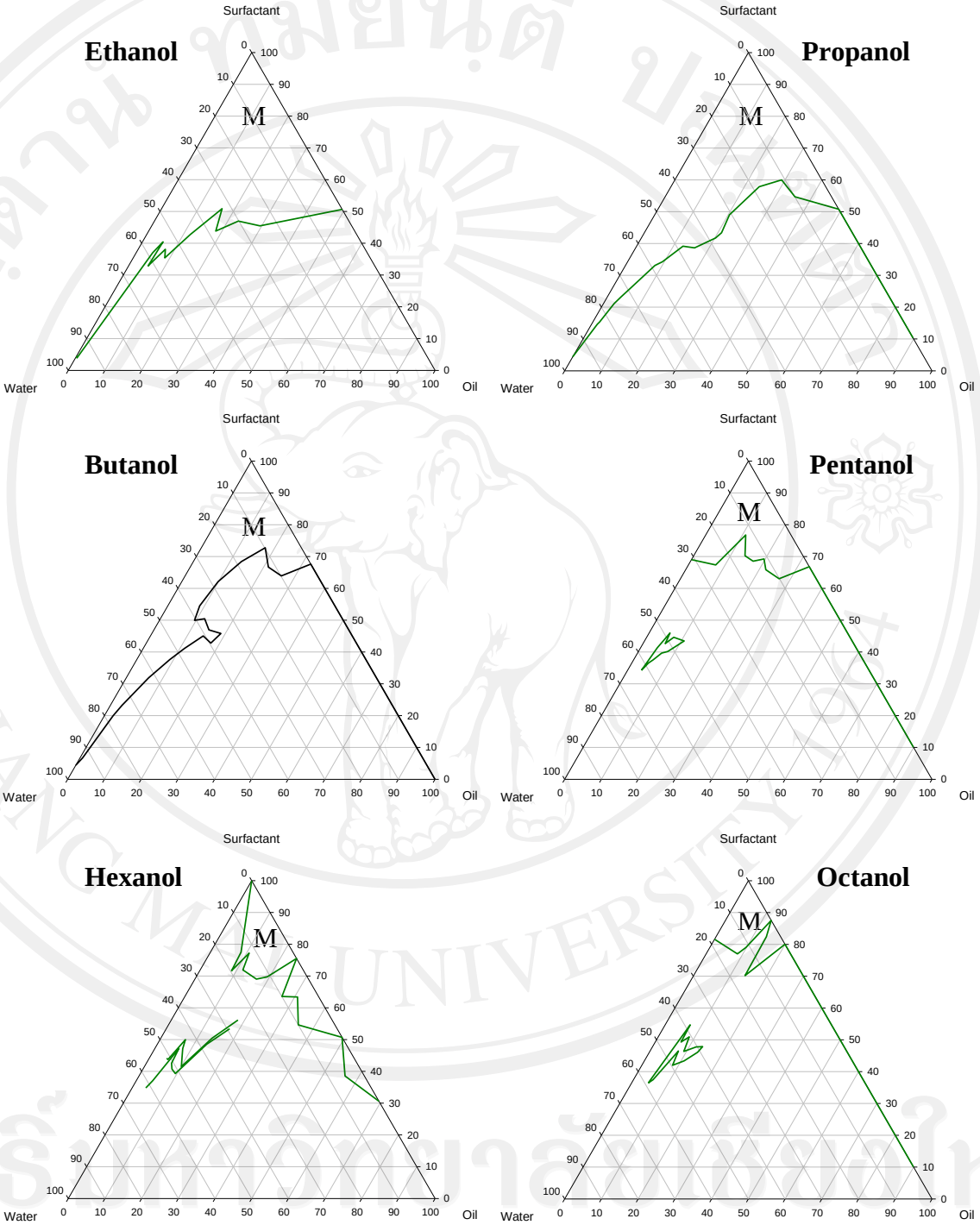


Figure 4.40 Effect of carbon atom in co-surfactant molecules on the phase diagram of lemongrass oil (the ratio of surfactant: co-surfactant is 2:1)

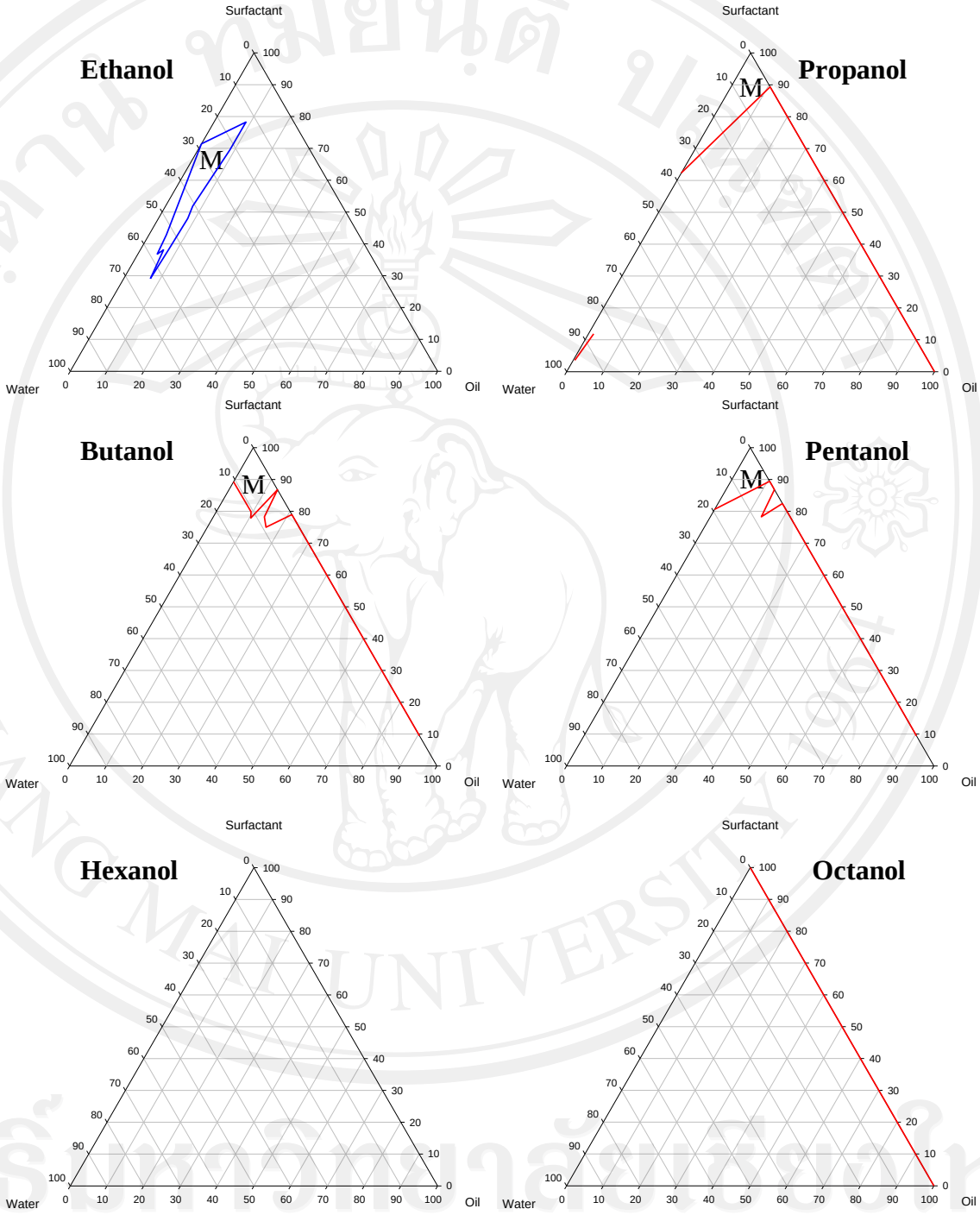


Figure 4.41 Effect of carbon atom in co-surfactant molecules on the phase diagram of sesame oil (the ratio of surfactant: co-surfactant is 1:2)

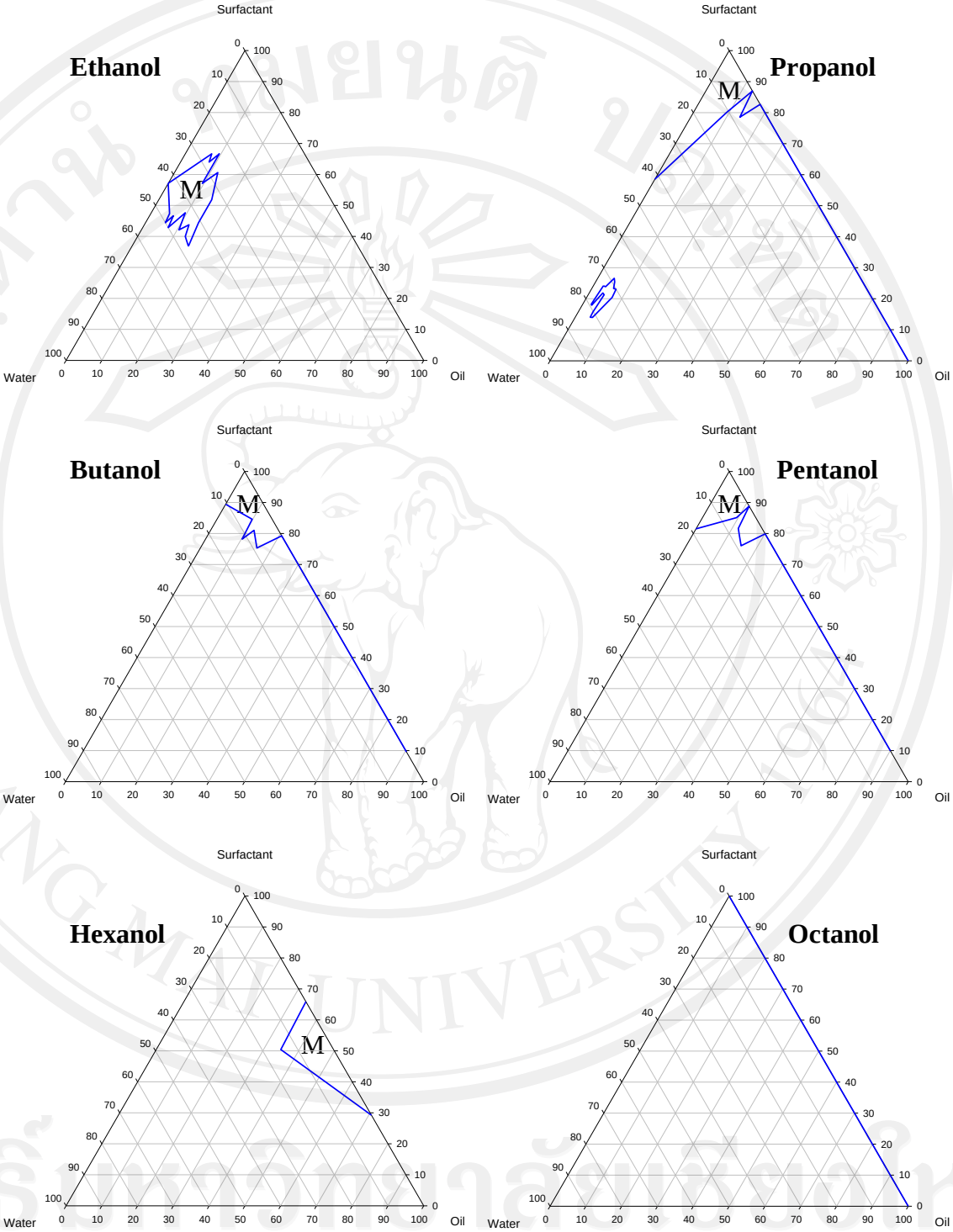


Figure 4.42 Effect of carbon atom in co-surfactant molecules on the phase diagram of sesame oil (the ratio of surfactant: co-surfactant is 1:1)

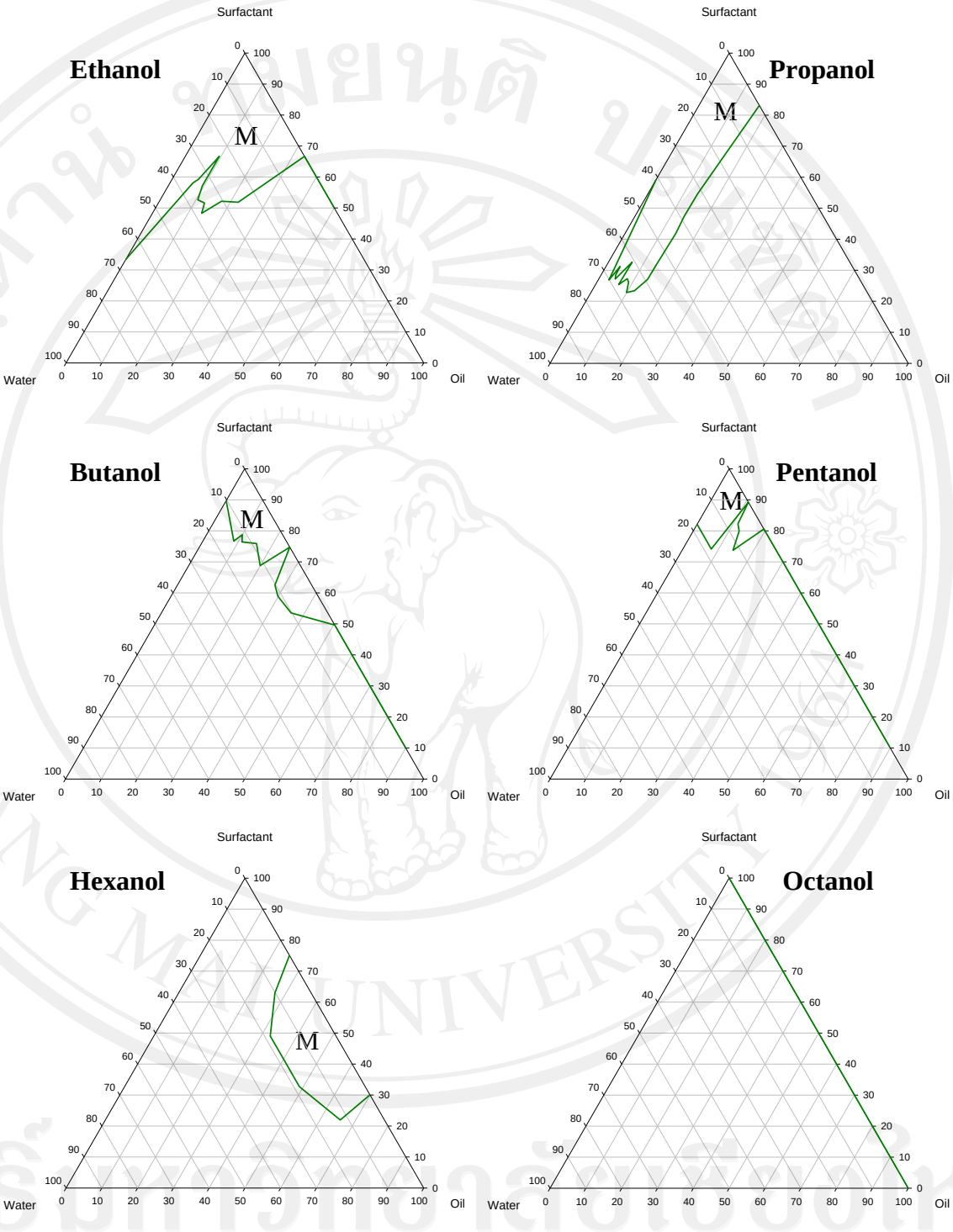


Figure 4.43 Effect of carbon atom in co-surfactant molecules on the phase diagram of sesame oil (the ratio of surfactant: co-surfactant is 2:1)

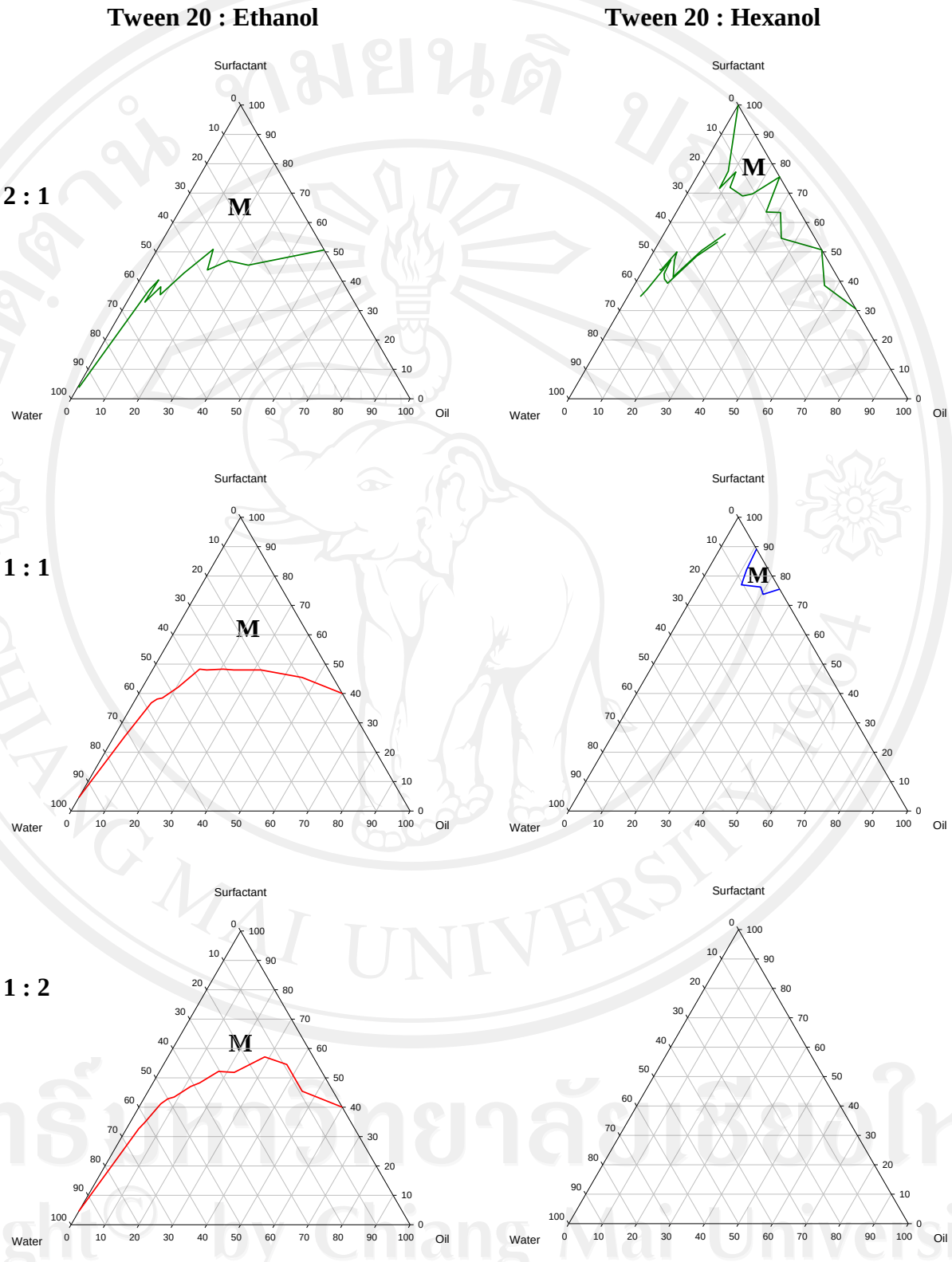


Figure 4.44 Effect of surfactant : co-surfactant on the microemulsion area (M) of lemongrass oil

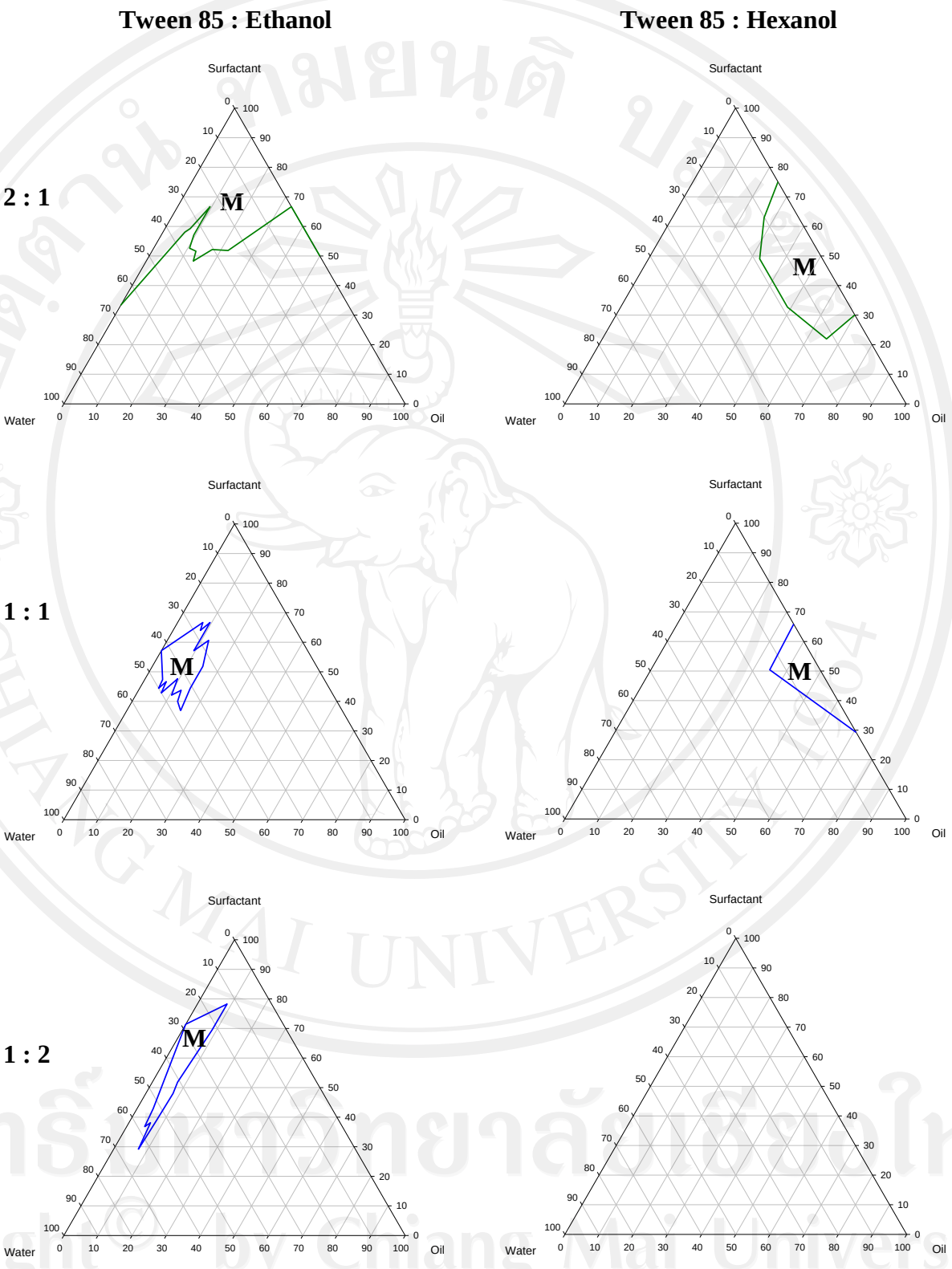


Figure 4.45 Effect of surfactant : co-surfactant on the microemulsion area (M) of sesame oil

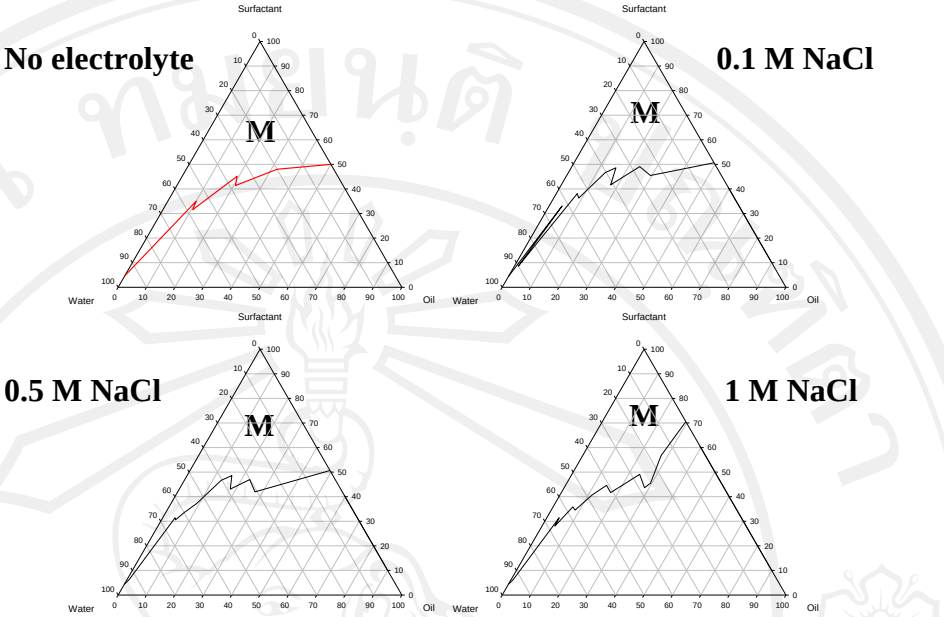


Figure 4.46 Effect of NaCl on the microemulsion area (M) of lemongrass oil (surfactant system is Tween 20 : Ethanol = 2:1)

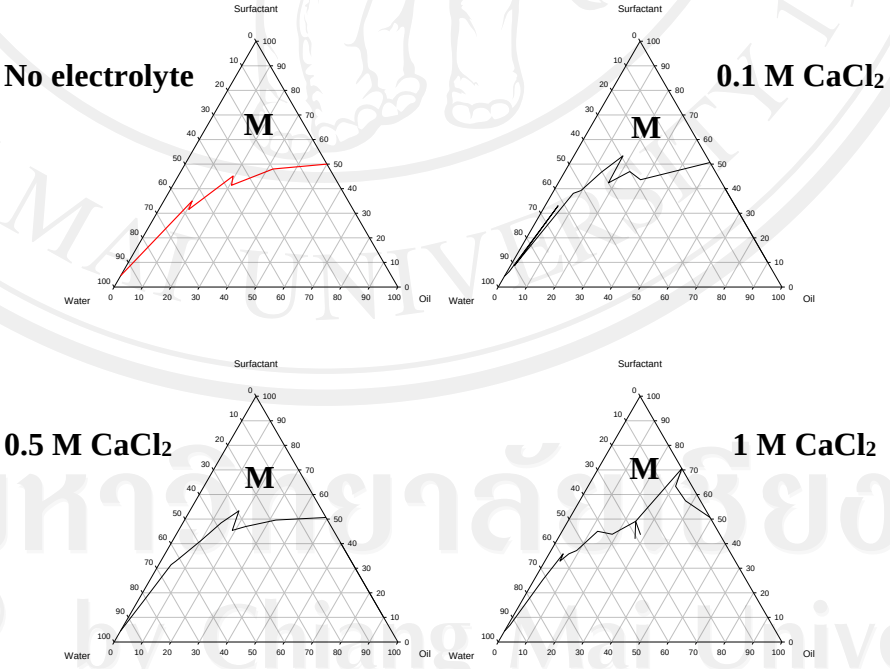


Figure 4.47 Effect of $CaCl_2$ on the microemulsion area (M) of lemongrass oil (surfactant system is Tween 20 : Ethanol = 2:1)

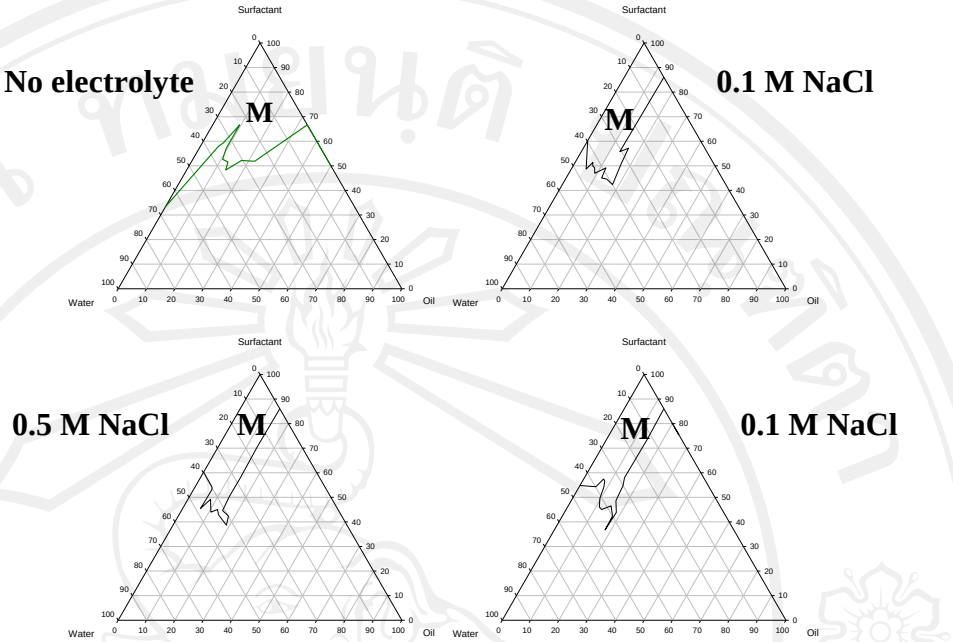


Figure 4.48 Effect of NaCl on the microemulsion area (M) of sesame oil (surfactant system is Tween 85 : Ethanol = 2:1)

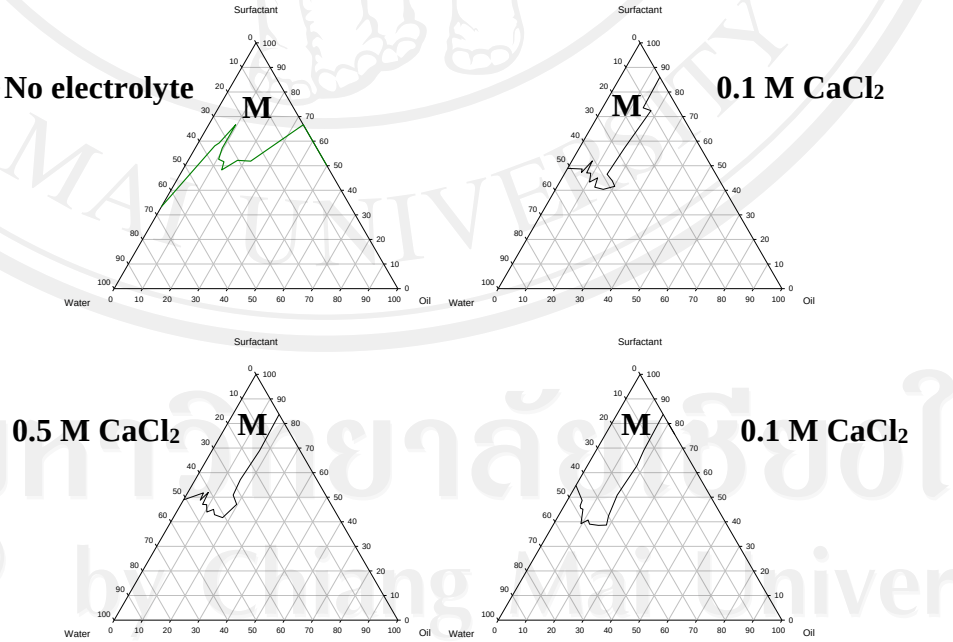


Figure 4.49 Effect of $CaCl_2$ on the microemulsion area (M) of sesame oil (surfactant system is Tween 85 : Ethanol = 2:1)

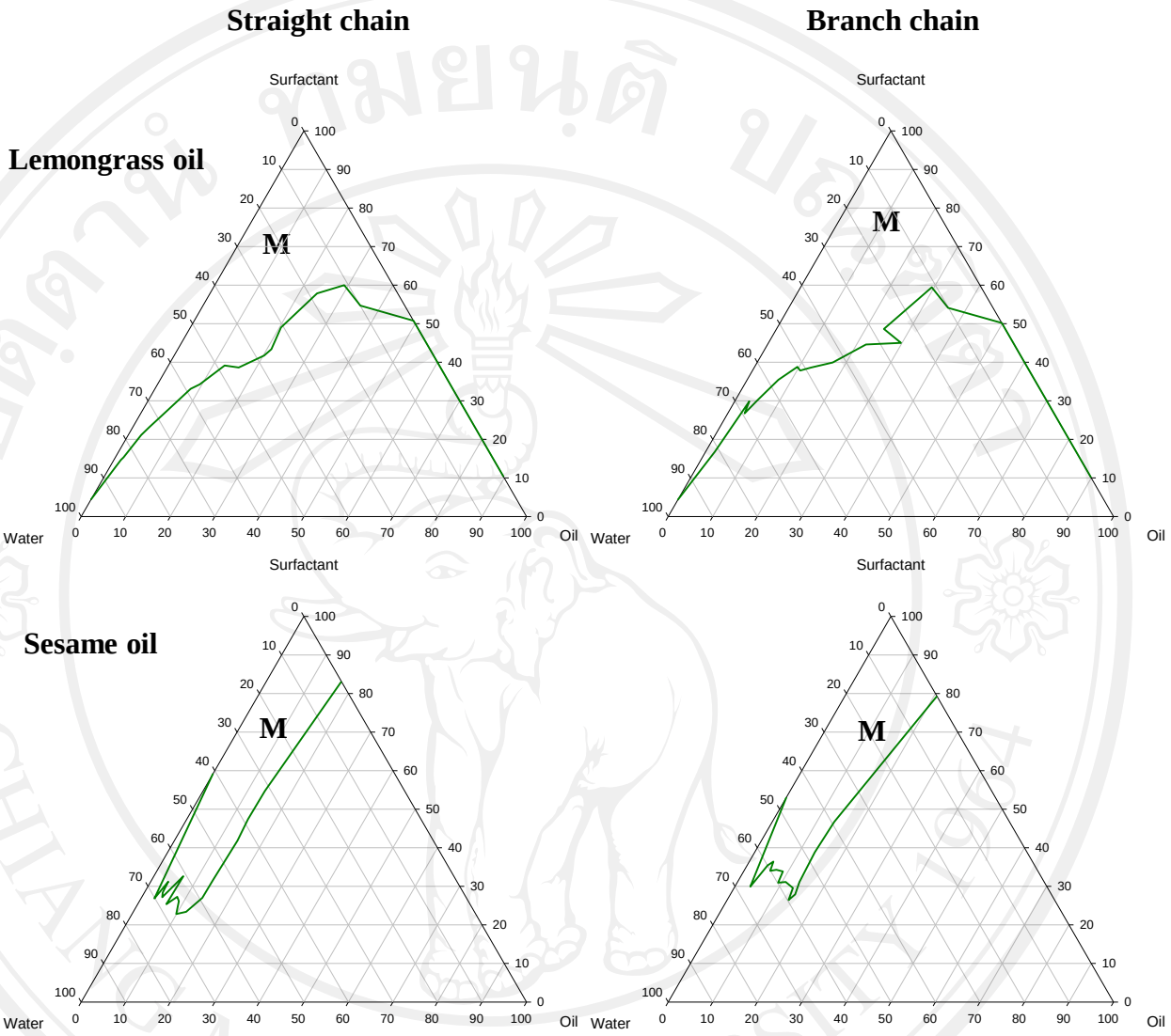


Figure 4.50 Effect of chemical structure on the microemulsion area (M) of lemongrass oil (surfactant system is Tween 20 : Ethanol = 2:1) and sesame oil (surfactant system is Tween 85 : Ethanol = 2:1)

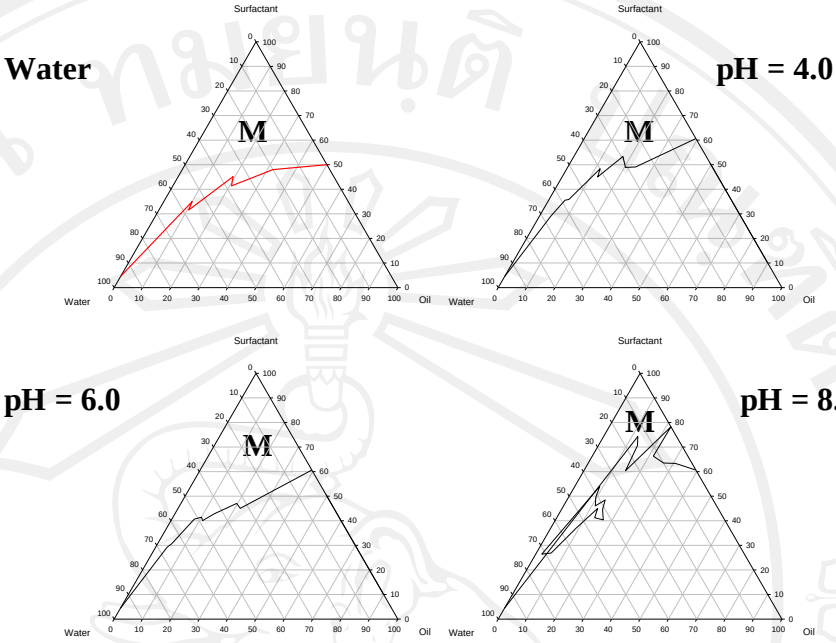


Figure 4.51 Effect of pH on the microemulsion area (M) of lemongrass oil (surfactant system is Tween 20 : Ethanol = 2:1)

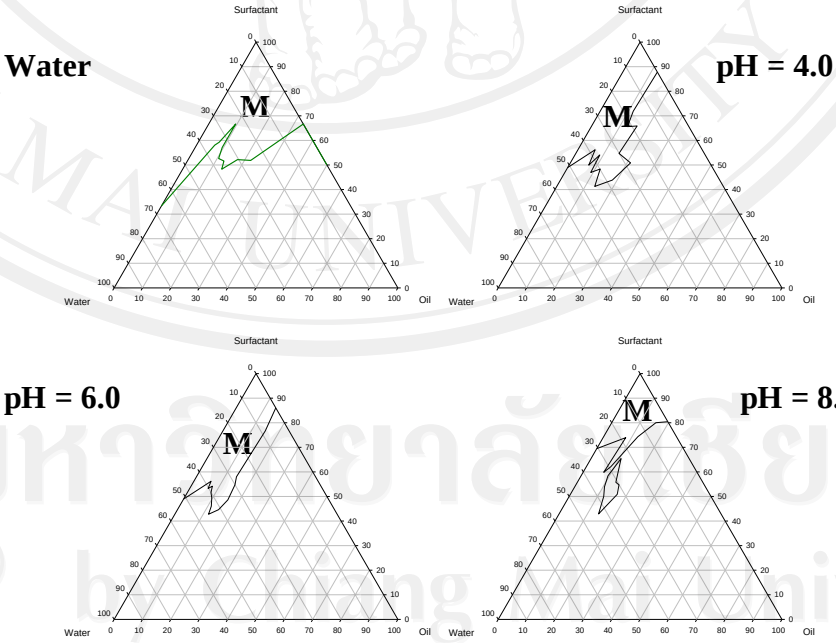


Figure 4.52 Effect of pH on the microemulsion area (M) of sesame oil (surfactant system is Tween 85 : Ethanol = 2:1)

4.9 Microemulsion base and drug loaded microemulsion preparation

The best formulations of microemulsions of both lemongrass oil and sesame oil were formulated by selecting the most suitable composition from the phase diagrams of both oils. These microemulsions were comparatively studied with other type of emulsions having the same concentration of oil (10%) as shown in Table 4.55. Formula-1 was prepared as a representative conventional emulsion using the energy from the high pressure homogenizer. Formula-2 to Formula-5 were the microemulsion formulations prepared without supplying any energy except gently mixed. The difference among these four formulations was that Formula-2 and Formula-3 had less amount of surfactant than Formula-4 and Formula-5. Formula-2 and Formula-4 were the microemulsions without cosurfactant wheras the other two microemulsions were composed of ethanol as the cosurfactants. After loading of 1% of clotrimazole, a water-insoluble drug, there were 20 formulations including those without drug. The emulsion preparations of all 20 formulations were shown in Table 4.55 and the characteristics of each preparation was shown in Figure 4.53 to 4.56.

The results showed that the characteristic of the sesame oil formulation looked dark yellow, while lemongrass formulations showed the light yellow in color. Conventional emulsions of the two oils were opaque and phase separation occurred within a short period of storage at room temperature. The precipitation of clotrimazole in these two formulations was seen in an hour of standing after preparation indicating that the preparations could not load fully amount of the incorporated drug.

Several studies showed the advantage of microemulsions on higher potential of drug loading than the conventional emulsions [172-174]. In the present study, the

microemulsion formulations of lemongrass oil which contained a main surfactant and a cosurfactant showed the characteristics of clear liquid, homogeneous, and good appearance. The lemongrass oil formulations without cosurfactant also provided the good miscibility of the oil and water. All lemongrass oil microemulsions showed upload fully amount of the incorporated clotrimazole without any precipitation of drug and the clear appearance of the product did not change after storage. This result indicated that the microemulsions of lemongrass oil could be prepared very easily whether or not containing a cosurfactant. The essential oil showed better solubility in several solvents than sesame oil, even non-polar solvent and polar solvent (Table 4.35). This property led to the ease of microemulsion forming with or without a cosurfactant. However, it was found that the cosurfactant was still needed for the stability of the microemulsions. Changez et al [175] and Singla et al [176] studied the microemulsions formulated by using only the main surfactant and by using the mixture of a surfactant and a cosufactant. They noted that microemulsions could be formed by both the single surfactant alone as well as by the mixture of a surfactant and a cosurfactant. However, they reported that the cosurfactant made the microemulsions more stable.

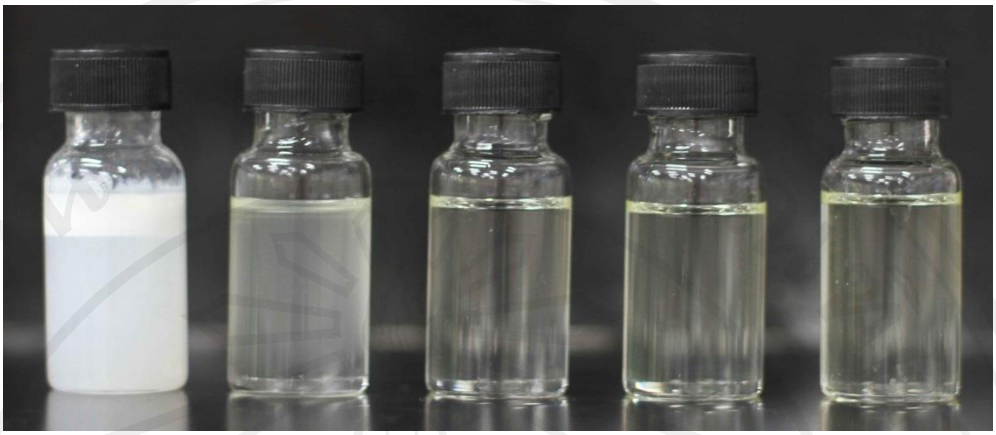
Sesame oil formulations of Formula-2 and Formula-4 could not be characterized as microemulsion because of their opaque look even no phase separation nor drug precipitation occurred. Formula-3 and Formula-5 of sesame oil formulations showed the characteristics of the micromulsion which was clear. No drug precipitation was observed from these two formulations. This result revealed that sesame oil needed both surfactant and cosurfactant to form the microemulsions. Several studies on the fixed oil microemulsion development reported the similar

results that good achievement on microemulsion forming was done by using the combination of a surfactant and a cosurfactant. For example, the development of diclofenac loaded microemulsion of soybean oil [177], valdecoxib loaded microemulsion of oleic acid [178] and voriconazole loaded microemulsion of paraffin and jojoba oil [179]. These studies showed that the lipophilic drugs were loaded into to the fixed oil microemulsion by using the mixture of surfactant and cosurfactant. The quantity of surfactant and cosurfactant mixture was various until 70%. However, the surfactant system for microemussion used in this thesis was 60%.

Formula-5 of both oils showed no drug precipitation and demonstrated high performance property of the microemulsion system. It was found that the surfactant system composed of 40% of surfactant and 20% of cosurfactant was the most suitable condition for forming of lemongrass oil and sesame oil microemulsion. The results showed that mixing of the surfactant and the cosurfactant of these respective concentrations could well stabilize themicroemulsions of the selected oils with and without drug loading. The quantity of surfactant system found to be the most suitable concentration of the microemulsions in this experiment was in good agreement with those previous studies which used about 30% surfactant to stabilize their developed microemulsions. The result was also corresponding to the study of Zhang et al who used 30% of surfactant system in the development of nimodipine loaded microemulsion [180] and Vicentini et al who formulated quercetin loaded microemulsion by using 47.5% of surfactant system [181].

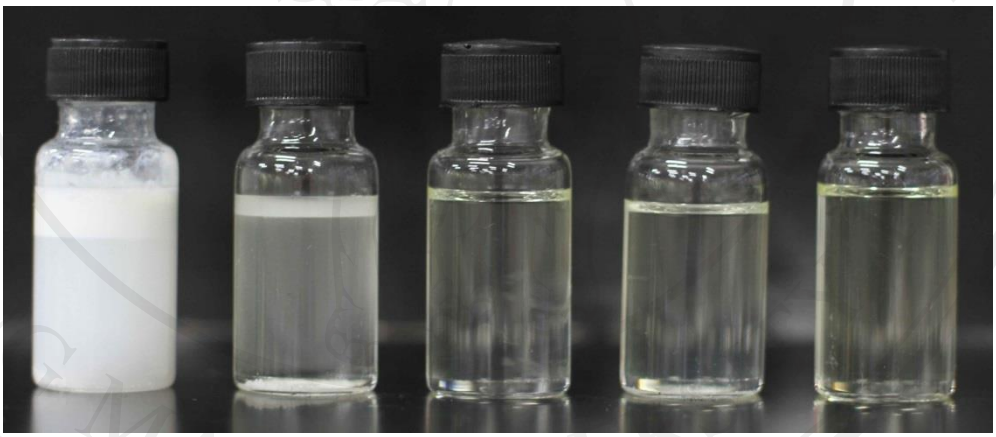
Table 4.55 The formulation of microemulsion and conventional emulsion used in this study

Formula	Name	Quantity (% w/w)				Preparation technic
		Oil	Surfactant	Cosurfactant	Water	
(Lemongrass oil = LM)						
1	LM-1	10	10	0	80	Conventional emulsion
2	LM-2	10	33	0	57	Microemulsion
3	LM-3	10	33	17	40	Microemulsion
4	LM-4	10	40	0	50	Microemulsion
5	LM-5	10	40	20	30	Microemulsion
(Sesame oil = SE)						
1	SE-1	10	10	0	80	Conventional emulsion
2	SE-2	10	33	0	57	Microemulsion
3	SE-3	10	33	17	40	Microemulsion
4	SE-4	10	40	0	50	Microemulsion
5	SE-5	10	40	20	30	Microemulsion



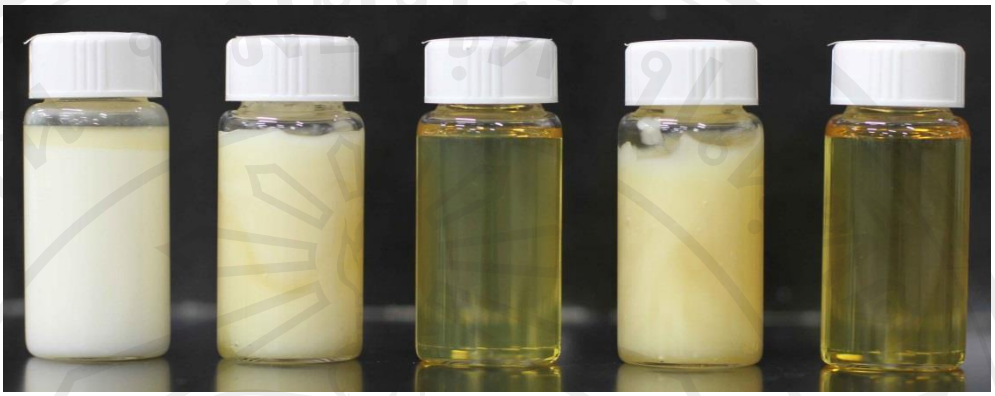
(LM-1) **(LM-2)** **(LM-3)** **(LM-4)** **(LM-5)**

Figure 4.53 Appearance of the lemongrass oil microemulsion without drug



(LM-1) **(LM-2)** **(LM-3)** **(LM-4)** **(LM-5)**

Figure 4.54 Appearance of the lemongrass oil microemulsion with drug



(SE-1) **(SE-2)** **(SE-3)** **(SE-4)** **(SE-5)**

Figure 4.55 Appearance of the sesame oil microemulsion without drug

(SE-1) **(SE-2)** **(SE-3)** **(SE-4)** **(SE-5)**

Figure 4.56 Appearance of the sesame oil microemulsion with drug

The results of size and size distribution study were shown in Table 4.56 and Table 4.57. The results showed that the droplet size of conventional emulsion was significantly bigger than those of other formulations and it was noted that the size displayed quite high distribution value indicating high size distribution. Interestingly, the microemulsions of both selected oils showed small size droplets and narrow size distribution.

It is noted that the internal droplet of the microemulsions of both oils was different depending on the type and amount of the surfactant and the cosurfactant used. This result was similar to that studied by Zhong et al who investigated the formation and characterisation of mint oil microemulsions with various surfactants and cosurfactants [182]. The results of this study also found that some formulations showed a slightly opaque gel-like characteristic. It was considered that the internal structure is bicontinuous structure [183] and therefore could not be measured with the PCS. Experimental results on comparison between the formulations contained drugs and those without drug found that the droplet size of the microemulsions containing drug was larger than those without drug.

Electrical conductivity is the property which describes the structure of a system consisting of water, oil and surfactants [184, 185]. If the conductivity of the emulsion system is greater than 50 μS/cm, the system is an o/w type which easily compatible with water. If the conductivity is lower than this value, the system is classified as a w/o type which incompatible with water [186]. The results of the present study as shown in Table 4.56 and Table 4.57 who investigated that the conductivity of the 20 formulations was greater than 100 μS/cm. Therefore, it was ensured that all systems were o/w type.

Table 4 .56 Droplet size and size distribution of internal phase and conductivity of microemulsion systems without drug

Formula	size (nm)	Polydispersion Index	Conductivity (µS)
(Lemongrass oil = LM)			
LM-1	292.77 ± 79.11	0.78 ± 0.06	11440.00 ± 88.89
LM-2	ND	ND	3960.00 ± 10.00
LM-3	24.54 ± 0.33	0.38 ± 0.02	1353.00 ± 44.71
LM-4	52.64 ± 1.20	0.35 ± 0.01	2623.33 ± 76.38
LM-5	28.77 ± 2.62	0.22 ± 0.01	654.33 9.07
(Sesame oil = SE)			
SE-1	529.23 ± 19.50	0.98 ± 0.02	6103.33 ± 55.08
SE-2	ND	ND	403.33 ± 3.21
SE-3	76.38 ± 0.26	0.46 ± 0.01	1741.67 ± 35.73
SE-4	ND	ND	157.30 ± 39.09
SE-5	74.58 ± 0.51	0.55 ± 0.01	938.67 ± 24.58

*ND = Cannot be measured with an instrument due to a semi-rigid system

Table 4 .57 Droplet size and size distribution of internal phase and conductivity of microemulsion systems with drug

Formula	size (nm)	Polydispersion Index	Conductivity (µS)
(Lemongrass oil = LM)			
LM-1	1023.80 ± 39.63	1.00 ± 0.00	10400.00 ± 173.49
LM-2	ND	ND	4683.33 ± 20.82
LM-3	27.00 ± 0.51	0.29 ± 0.00	1477.67 ± 15.04
LM-4	58.44 ± 3.06	0.27 ± 0.03	3106.67 ± 28.87
LM-5	54.16 ± 1.02	0.17 ± 0.01	801.00 ± 18.00
(Sesame oil = SE)			
SE-1	812.17 ± 47.07	0.85 ± 0.03	4790.00 ± 121.66
SE-2	ND	ND	477.00 ± 2.65
SE-3	97.23 ± 1.89	0.35 ± 0.04	1739.67 ± 41.43
SE-4	ND	ND	191.33 ± 18.06
SE-5	76.44 ± 0.30	0.33 ± 0.01	902.00 ± 6.08

*ND = Cannot be measured with an instrument due to a semi-rigid system

4.10 Stability study of microemulsion

The stability study of lemongrass oil microemulsions and sesame oil microemulsions demonstrated that different types and concentrations of compositions existing in the microemulsions played an important role on chemical and physical stability of the microemulsions. The details were as followings.

4.11.1 Stability of the formulations without drug

The outer appearance of the lemongrass oil formulation without clotrimazole was shown in Figure 4.59. The results found that formula-1 (the conventional emulsion) showed phase separation after kept in a short period in all conditions. Fotmula-2, formula-3, formula-4 and formula-5 were still clear and looked stable. Lemongrass oil showed higher advantage than sesame oil that its microemulsions could be prepared in various ratios of surfactant systems. In case of sesame oil, only formula-3 and formula-5 showed the characteristics of microemulsions, as shown in Figure 4.60. They looked clear and stable in every condition even their yellow colors were intense. In contrast to formula-2 and formular-4 which were not clear and not stable when kept at high temperature at 45ºC and in heating and cooling cycle conditions..

4.11.2 Stability of clotrimazole loaded formulations

Consider to the outter appearance as shown in Figures 4.60 and 4.62, formula-1 of both oils stored at 45°C showed phase separation whereas the others which were microemulsions showed no phase separation. This result demonstrated that the conventional emulsions had less capacity for oil loading than the microemulsions. The experiment also found that the drug is chemically stable when the microemulsions

were kept in cold temperature of 4°C. The products of formula-2 to formula-5 which were kept at room temperature of 30°C were stable as similar as the products which were stored at 4°C. This result demonstrated the advantages of the microemulsion products that showed high stability even they were not kept in the refrigerator.

In the comparison of clarity between formula-3 and formula-5, the results showed found that lemongrass oil and sesame oil systems were similar to each other. The appearance of formula-5 was clearer than that of formula-3. Concerning the compositions existing in each preparation, it was found that formula-5 contained higher amount of surfactant mixture than formula-3. It was considered that the high amount of surfactant in the formulation could play a role on stabilization of the oil droplets of the product along the standing for a period of at least 5 months. The results of the present study confirmed that the amount of surfactant system was important to protect the formulations from phase separation. Even there was no phase separation of formula-2, formula-3, formular-4, and formula-5, it was noted that there was some color change but different degree between the two oils. Seseme oil microemulsion showed intense yellow while keeping during a period of 5 months whereas lemongrass oil microemulsions expressed light yellow. The color change of these formulations was shown in Figure 4.63 to 4.66.

Formula-5 of both oils were found to be the most stable formulation among all microemulsions developed in this study when stored at 4°C as shown in Figure 4.60 to Figure 4.65.

Formula-2 to formula-5 of lemongrass oil microemulsion systems showed complete property of desirable microemulsion which appeared as a low viscous clear

monophasic liquids. During storage, formula-3 and formula-5 had shown to be the best microemulsion with proper ratio of compositon existing. They showed no phase separation, no drug precipitation during storage at different temperatures studied. Formula-2 and formula-4 showed slightly drug precipitation.

Only Formula-3 and Formula-5 of sesame oil microemulsion systems showed were completely characteristics of microemulsions with clear monophasic appearance. For drug loaded microemulsions of sesame oil, it was also found that the appearance of formula-3 and formula-5 was beautiful clear monophasic liquid without any precipitation of the drug. As the surfactant system of these preparations contained both major surfactant and cosurfactant, therefore this result supported that cosurfactant was the necessary component for the microemulsion.

Chemical analysis of the drug during storage indicated that the amount of clotrimazole significantly reduced when the products were kept in a high temperature (45ºC) condition, especially the drug existing in formula-1 (the conventional emulsion). It was found that clotrimazole existing in formula-1 decreased very fast when compared to other formulas which had the same type of oil as shown in Figures 4.57 to 4.58 and Table 4.59 to 4.63. Clotrimazole was more stable in formula-2 to formula-5 of both oils when kept in room temperature and 4ºC. The results showed that during storage of 5 months in these conditions, more than 80% of clotrimazole still remained in these formulations. These results suggested that the microemulsions could be kept in both room temperature and in low temperature. However, clotrimazole was decomposed in the high temperature in first order kinetic. As these formulations were of microemulsion type, the results of this study confirmed that microemulsion was the high performance system to prevent drug decomposition. This

result was corresponding with Al-Adham et al who studied the antimicrobial drug loading microemulsions and found that the drug content was still high in microemulsion during a long period of keeping [187]. Lv et al studied the stability enhancement of chloramphenicol in the microemulsion formulations; they found that the microemulsions could prolonge adherence time and a delayed release of the drug [188]. Hejazi et al studied the physicochemical properties of clindamycin loaded microemulsions and found that the microemulsions could prolong the shelf life of the preparations [189]. The results in the present studied were in good agreement with these previous reports that the developed microemulsions of both oils could protect the stability of clotrimazole.

In conclusion, formula-3 and formula-5 were the suitable vehicles for clotrimazole due to their high stability, small size and good appearance. However, the formula-5 contained more surfactant and showed higher benefit.

Table 4.58 The amount of drug remaining in formula-1 of lemongrass oil and sesame oil emulsion system

Formula	Time (month)	Drug quantity (%)		
		High Temperature (45°C)	**Room Temperature (30°C)**	**Cold Temperature (4°C)**
LM-1	0	100.64 ± 4.72	100.27 ± 6.14	100.24 ± 0.31
	1	70.04 ± 1.81	92.92 ± 1.03	97.13 ± 2.27
	2	58.75 ± 2.74	88.52 ± 3.95	94.74 ± 4.60
	3	52.43 ± 4.16	85.21 ± 2.29	90.63 ± 3.46
	5	47.72 ± 0.41	80.16 ± 0.98	86.94 ± 2.39
SE-1	0	100.01 ± 2.23	100.01 ± 2.23	100.01 ± 2.23
	1	69.32 ± 5.07	77.26 ± 2.14	90.50 ± 2.17
	2	55.03 ± 0.63	66.19 ± 1.46	85.01 ± 1.85
	3	41.12 ± 2.00	54.56 ± 1.86	80.96 ± 4.08
	5	27.91 ± 0.85	47.16 ± 3.40	79.83 ± 5.96

Table 4.59 The amount of drug remaining in formula-2 of lemongrass oil and sesame oil microemulsion system

Formula	Time (month)	Drug quantity (%)		
		High Temperature (45°C)	**Room Temperature (30°C)**	**Cold Temperature (4°C)**
LM-2	0	100.27 ± 6.14	100.64 ± 4.2	100.48 ± 1.50
	1	71.59 ± 1.54	92.35 ± 3.95	95.03 ± 4.98
	2	61.62 ± 3.09	89.25 ± 1.34	92.97 ± 1.64
	3	56.80 ± 6.21	87.65 ± 2.73	91.55 ± 4.39
	5	50.16 ± 0.51	84.91 ± 1.45	91.19 ± 5.72
SE-2	0	100.35 ± 2.88	100.35 ± 2.88	100.35 ± 2.88
	1	78.18 ± 4.70	91.46 ± 2.14	94.07 ± 2.68
	2	68.57 ± 4.08	85.65 ± 1.99	88.54 ± 3.04
	3	63.81 ± 2.40	83.93 ± 1.96	86.64 ± 0.44
	5	57.58 ± 2.20	75.64 ± 1.65	84.56 ± 2.27

Table 4.60 The amount of drug remaining in formula-3 of lemongrass oil and sesame oil microemulsion system

Formula	Time (month)	Drug quantity (%)		
		High Temperature (45°C)	**Room Temperature (30°C)**	**Cold Temperature (4°C)**
LM-3	0	100.48 ± 1.50	100.55 ± 6.65	100.27 ± 6.14
	1	80.44 ± 4.22	99.03 ± 0.56	97.29 ± 1.16
	2	74.76 ± 2.66	95.55 ± 2.89	96.55 ± 1.30
	3	68.38 ± 1.10	94.18 ± 1.77	96.50 ± 1.88
	5	66.96 ± 0.86	92.65 ± 7.41	94.07 ± 4.00
SE-3	0	100.23 ± 1.70	100.23 ± 1.70	100.23 ± 1.70
	1	82.50 ± 3.22	95.89 ± 5.88	97.63 ± 3.66
	2	76.99 ± 7.39	91.67 ± 2.50	94.44 ± 1.06
	3	72.12 ± 3.52	88.42 ± 2.59	92.35 ± 2.61
	5	68.87 ± 2.30	86.59 ± 3.90	90.57 ± 0.71

Table 4.61 The amount of drug remaining in formula-4 of lemongrass oil and sesame oil microemulsion system

Formula	Time (month)	Drug quantity (%)		
		High Temperature (45°C)	**Room Temperature (30°C)**	**Cold Temperature (4°C)**
LM-4	0	100.55 ± 5.65	100.48 ± 1.50	100.64 ± 4.72
	1	81.06 ± 6.68	97.08 ± 2.72	98.74 ± 0.91
	2	74.00 ± 1.68	95.15 ± 8.03	98.58 ± 1.58
	3	67.60 ± 3.83	92.24 ± 1.81	96.03 ± 2.06
	5	63.64 ± 1.37	89.69 ± 3.85	95.23 ± 1.44
SE-4	0	100.19 ± 3.84	100.23 ± 1.70	100.16 ± 1.72
	1	75.81 ± 0.57	92.63 ± 3.51	94.26 ± 0.69
	2	68.59 ± 0.89	88.41 ± 2.27	93.85 ± 1.30
	3	64.00 ± 4.09	87.61 ± 6.91	91.74 ± 3.31
	5	60.70 ± 1.87	82.72 ± 2.53	90.07 ± 0.62

Table 4.62 The amount of drug remaining in formula-5 of lemongrass oil and sesame oil microemulsion system

Formula	Time (month)	Drug quantity (%)		
		High Temperature (45°C)	**Room Temperature (30°C)**	**Cold Temperature (4°C)**
LM-5	0	100.24 ± 0.31	100.24 ± 0.31	100.55 ± 5.65
	1	91.39 ± 2.48	98.04 ± 3.11	99.65 ± 2.90
	2	88.61 ± 4.26	95.89 ± 2.25	99.59 ± 7.24
	3	83.73 ± 2.98	93.95 ± 2.33	98.82 ± 6.09
	5	76.53 ± 3.18	91.41 ± 3.27	97.64 ± 2.61
SE-5	0	100.16 ± 1.72	100.19 ± 3.84	100.16 ± 1.72
	1	88.28 ± 4.09	95.67 ± 2.97	99.10 ± 1.12
	2	79.76 ± 4.47	92.26 ± 0.69	98.51 ± 2.25
	3	74.50 ± 5.52	91.37 ± 2.83	97.34 ± 2.83
	5	68.48 ± 3.95	89.26 ± 4.62	93.43 ± 4.82

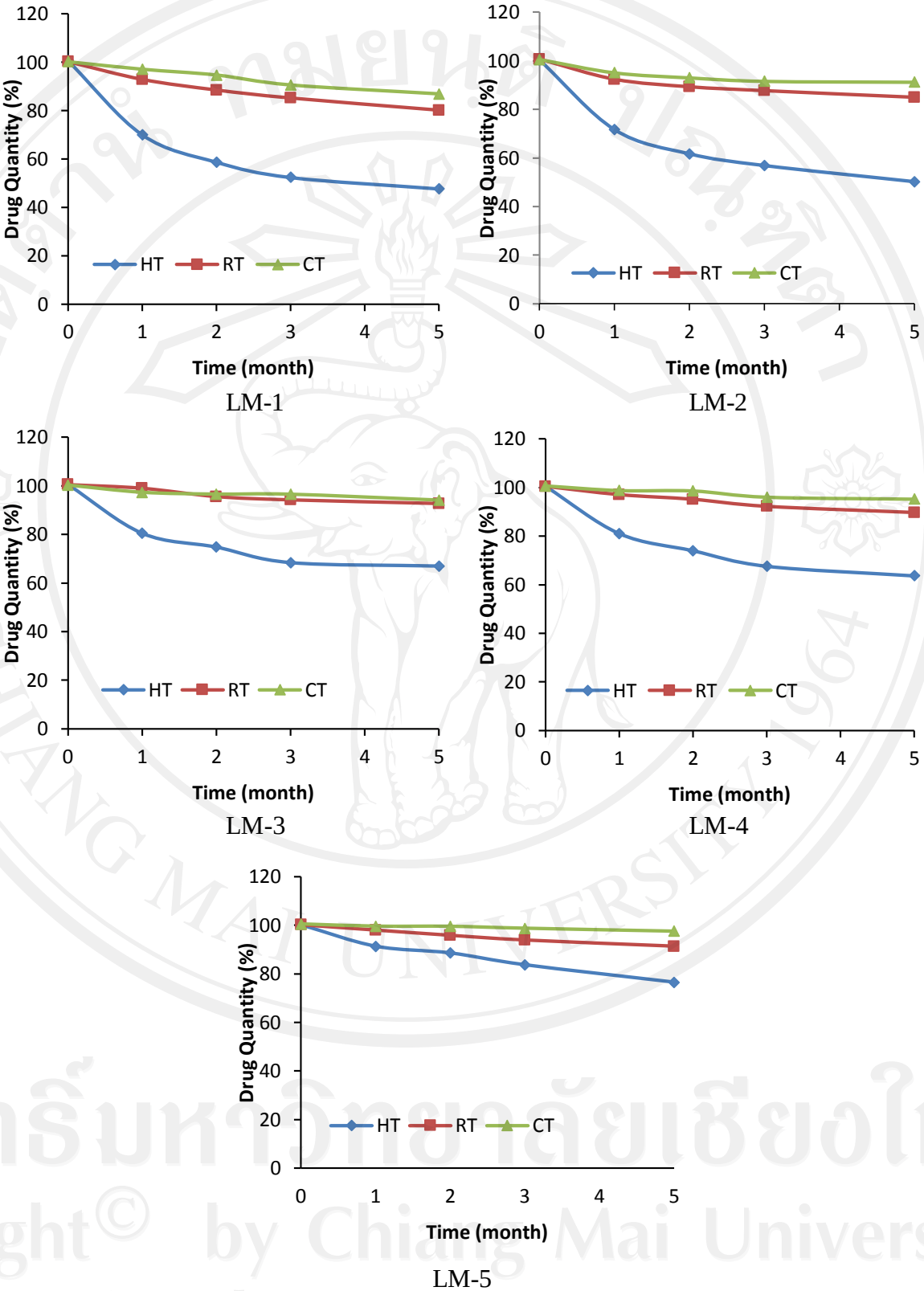


Figure 4.57 The amount of drug remaining in lemongrass oil microemulsion system

(HT = 45°C, RT = Room temperature about 30°C, LT = 4°C)

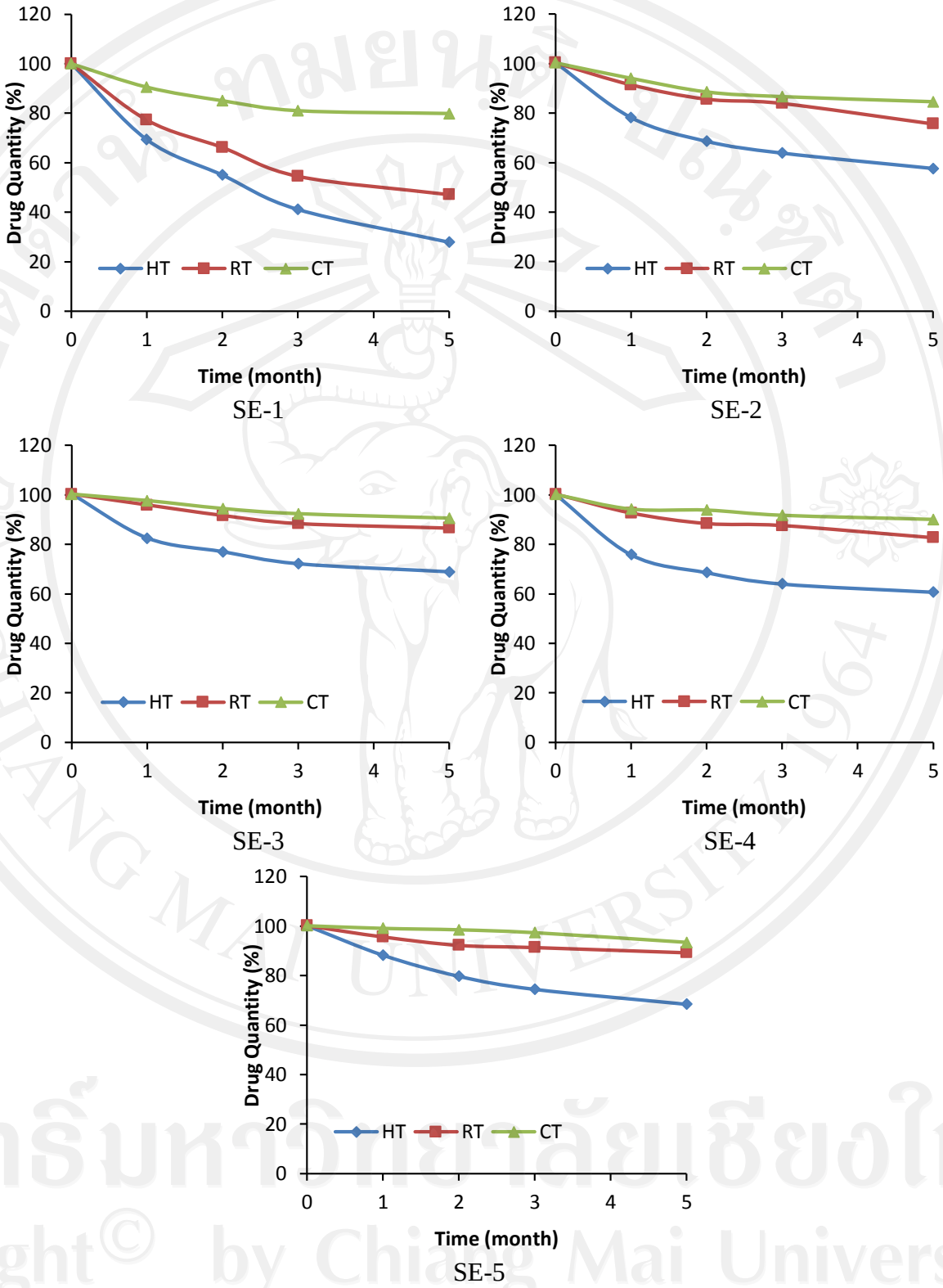


Figure 4.58 The amount of drug remaining in sesame oil microemulsion system (HT = 45°C, RT = Room temperature about 30°C, LT = 4°C)

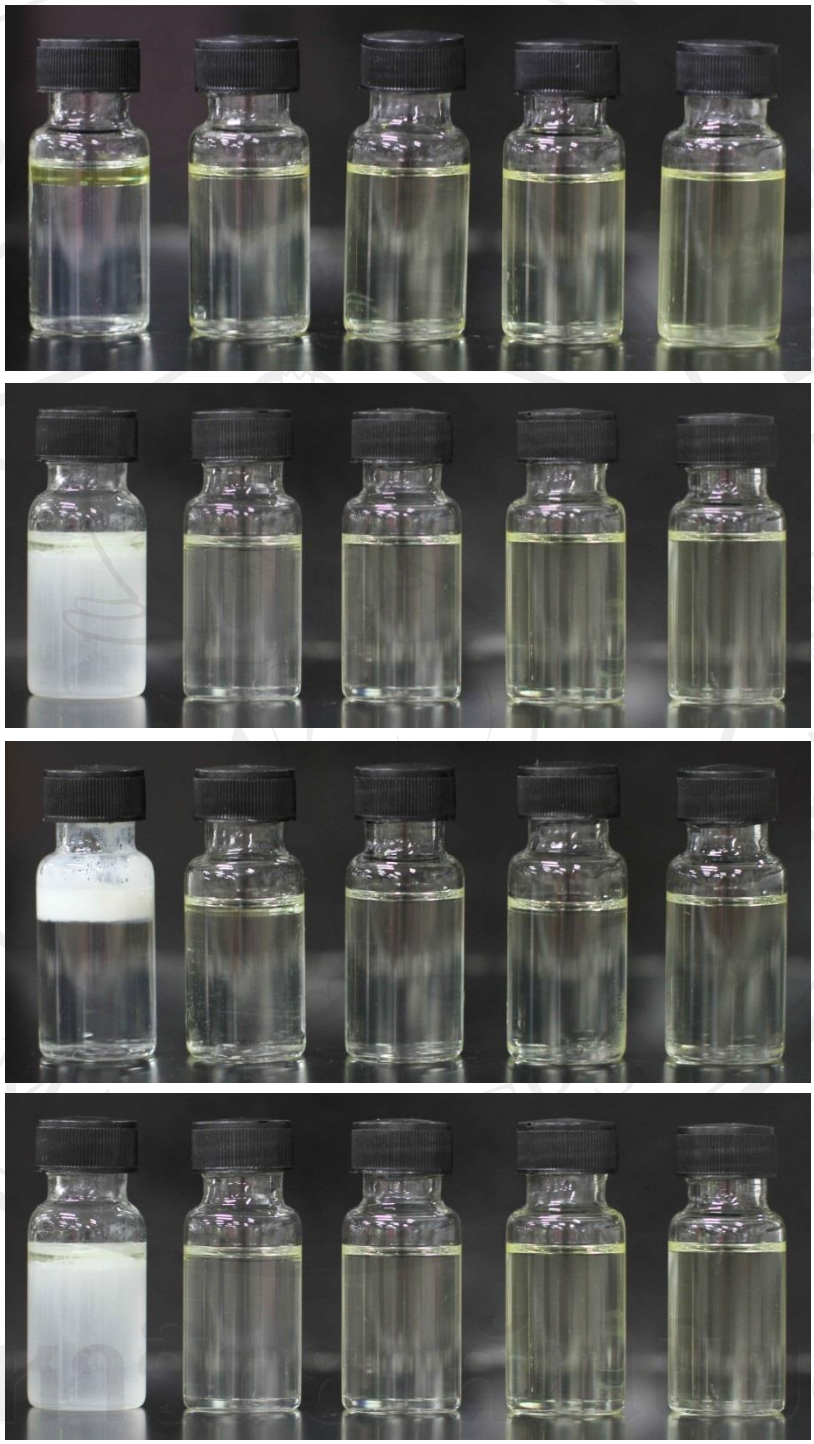


Figure 4.59 The outter appearance of lemongrass oil microemulsion systems without drug which were kept in various conditions. (HT = 45°C, RT = Room temperature about 30°C, LT = 4°C, HCC = heating and cooling cycle)

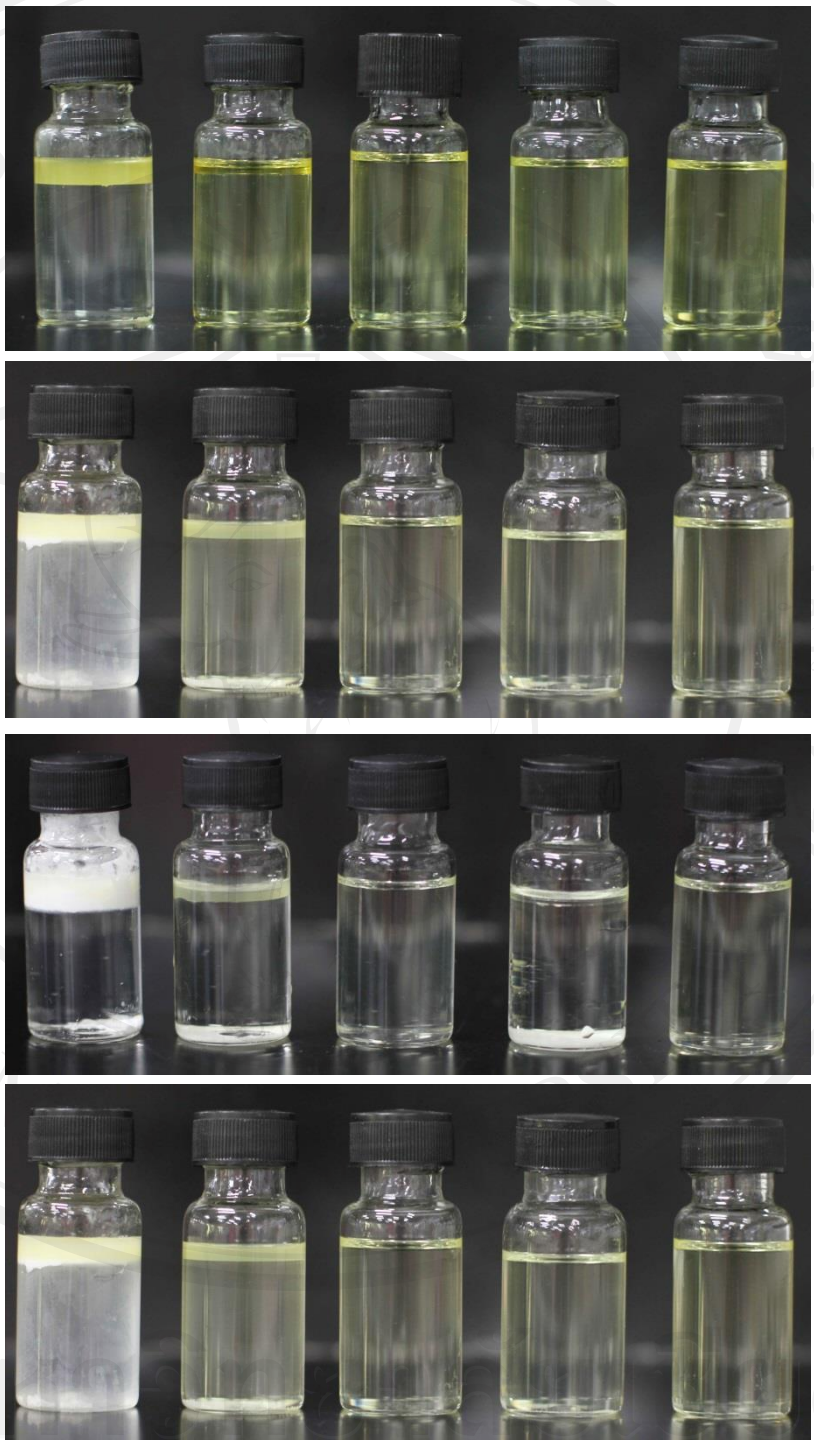


Figure 4.60 The outter appearance of lemongrass oil microemulsion systems with drug which were kept in various conditions. (HT = 45°C, RT = Room temperature about 30 C, LT = 4°C, HCC = heating and cooling cycle)

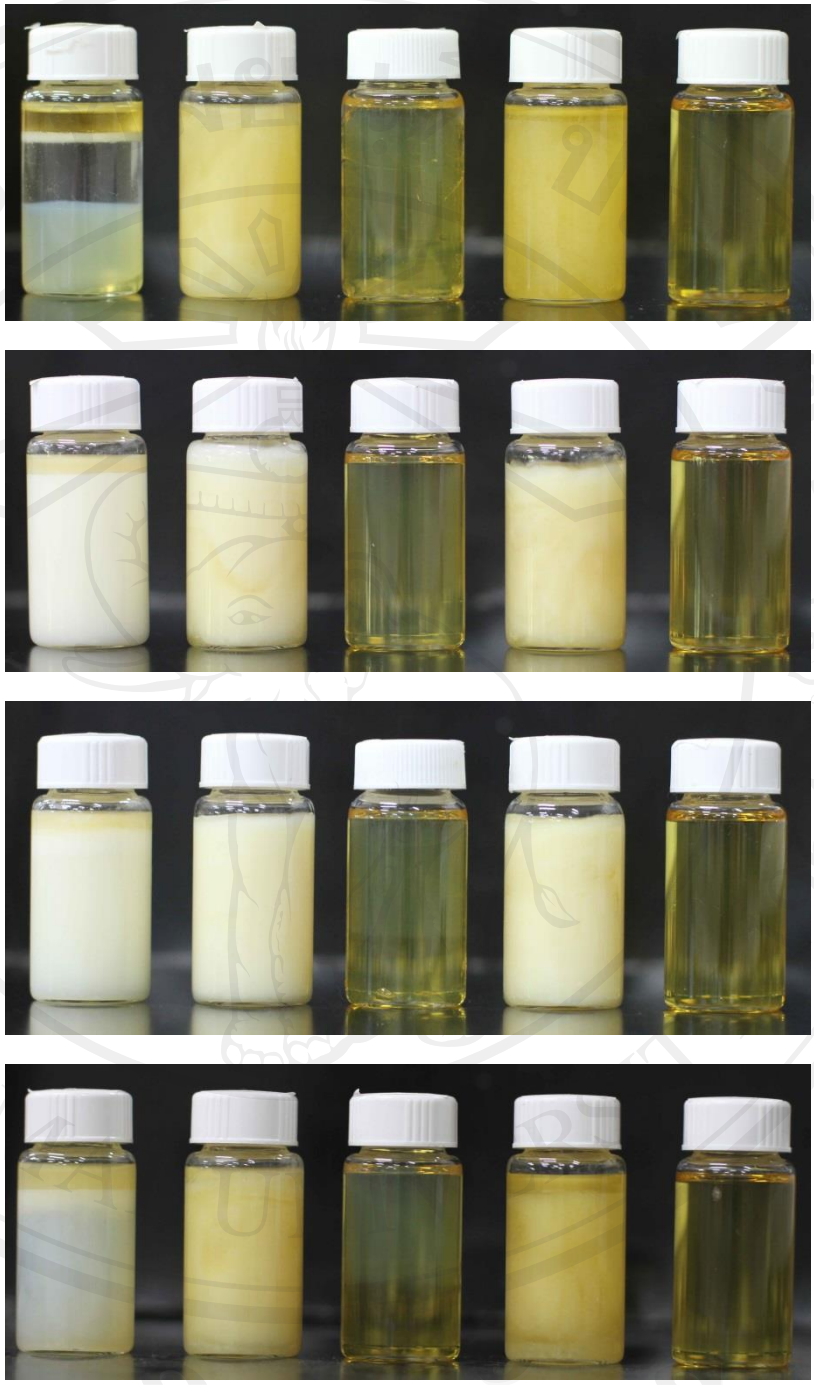


Figure 4.61 The outter appearance of sesame oil microemulsion systems without drug which were kept in various conditions. (HT = 45 °C, RT = Room temperature about 30 C, LT = 4°C, HCC = heating and cooling cycle)

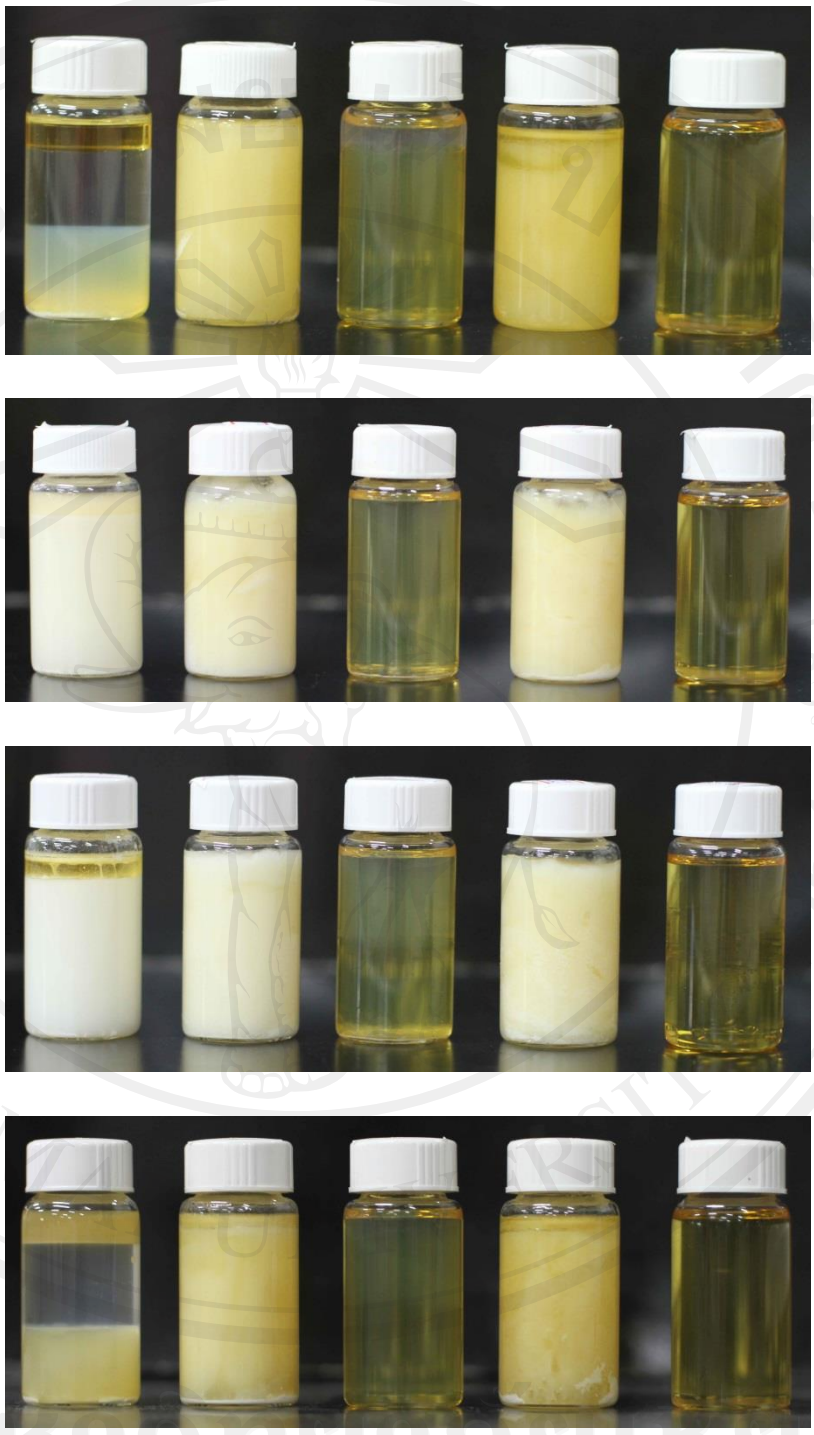


Figure 4.62 The outter appearance of sesame oil microemulsion systems with drug which were kept in various conditions. (HT = 45°C, RT = Room temperature about 30°C, LT = 4°C, HCC = heating and cooling cycle)

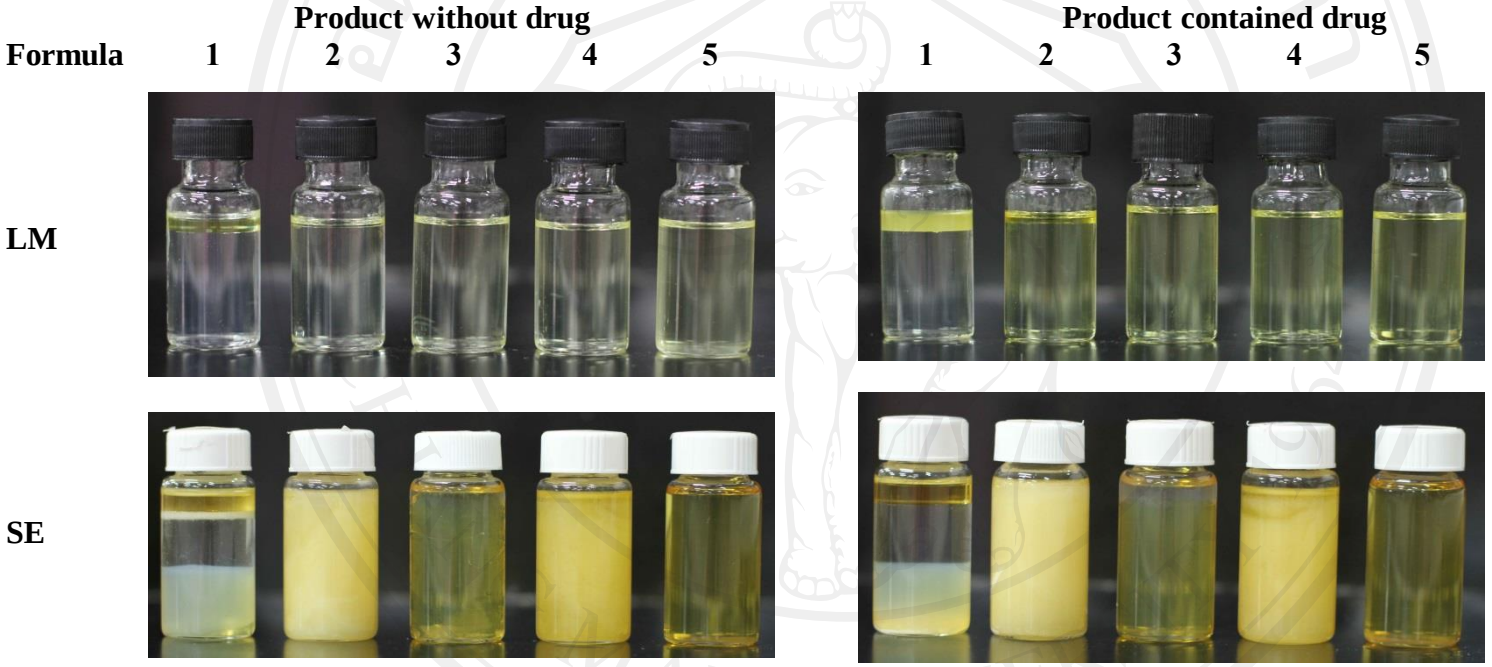


Figure 4.63 Comparison of characteristic appearance of various formulations stored at 45°C

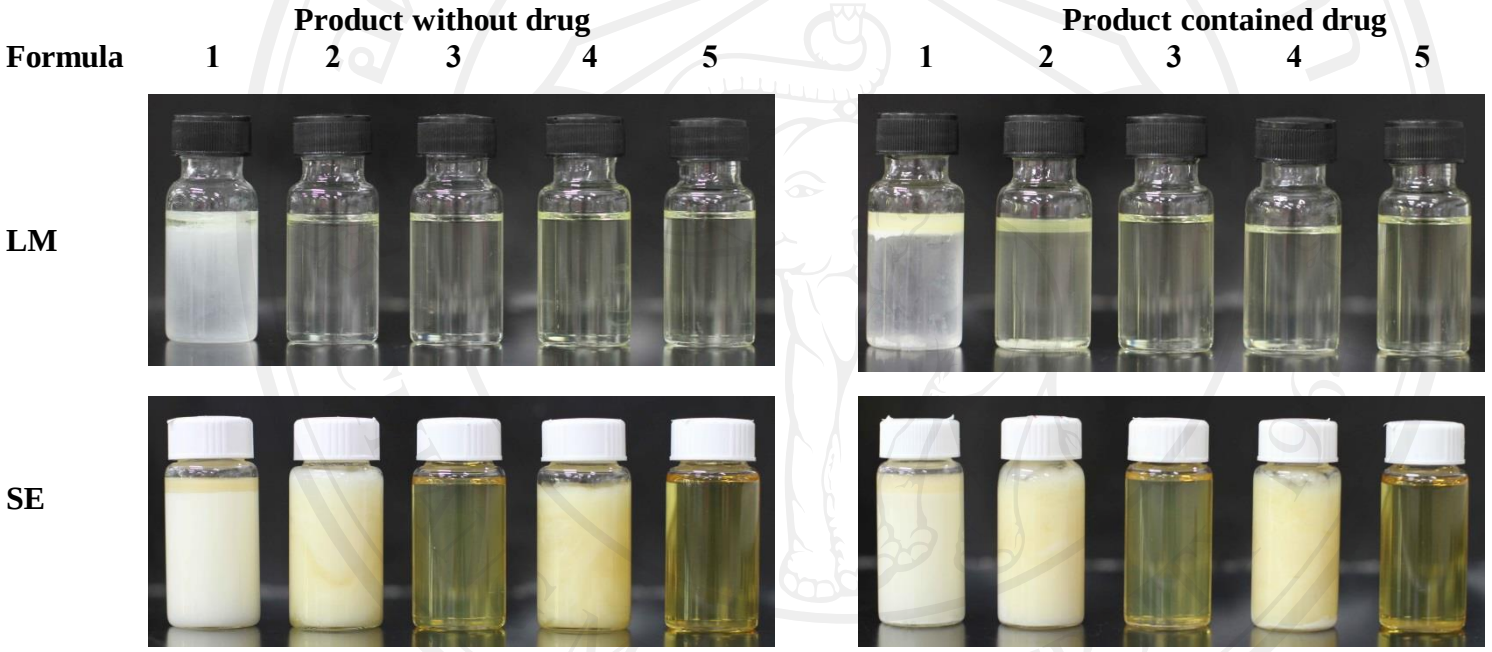


Figure 4.64 Comparison of characteristic appearance of various formulations stored at 30°C (Room Temperature)

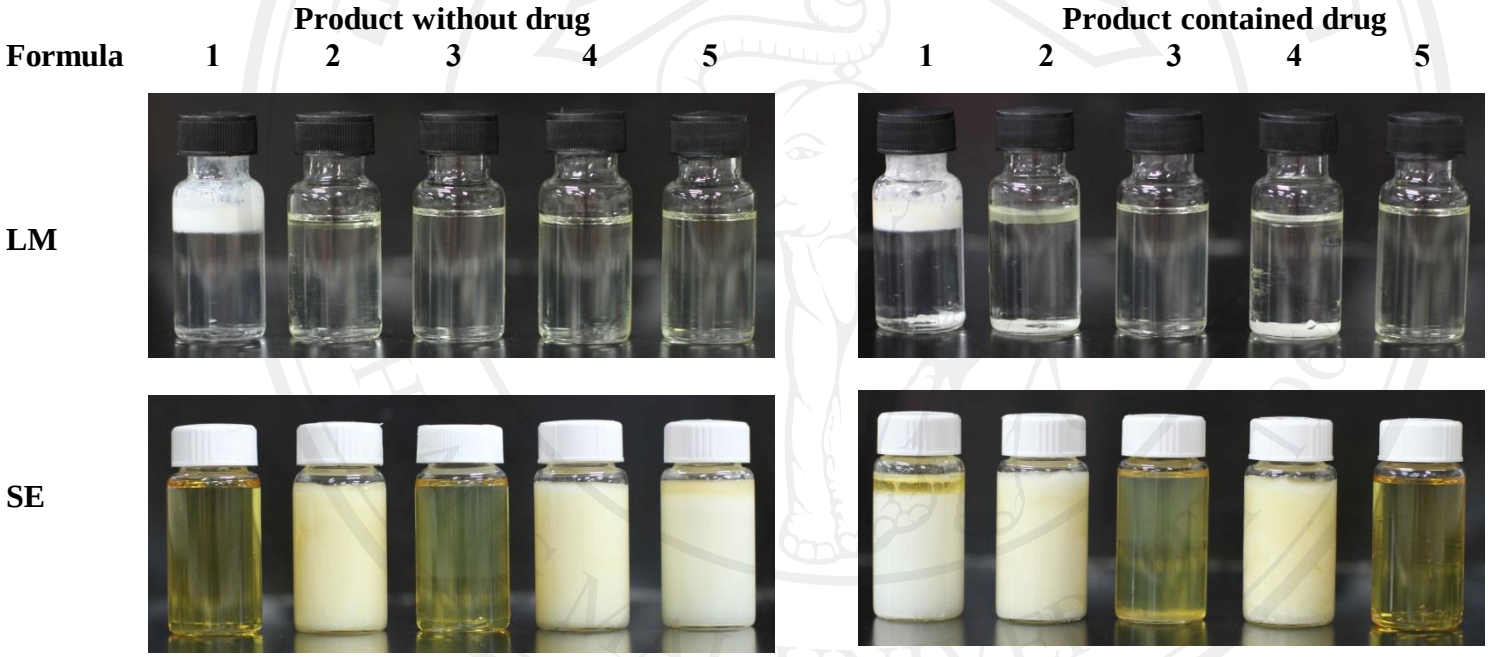


Figure 4.65 Comparison of characteristic appearance of various formulations stored at 4°C

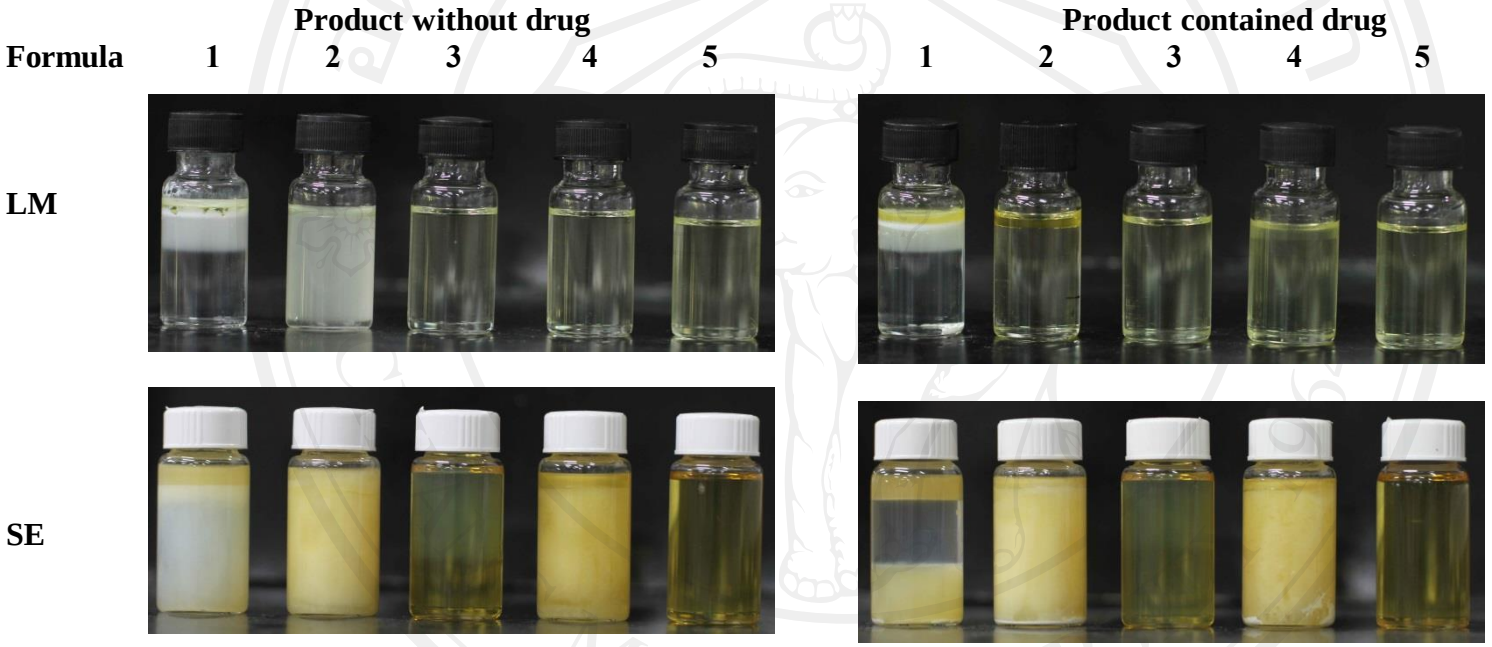


Figure 4.66 Comparison of characteristic appearance of various formulations stored in heating-cooling cycle

Figure 4.67 The product of miroemulsion of sesame oil and lemongrass oil

4.11 Drug release study

As mentioned above that the stability study found that the formula-1 of both oils showed instability with phase separation within 1 hr after prepared. The period of drug release study performance was up to 10 hr so the tested preparations should be stable throughout the study period. Therefore, in the study of drug release, formula-1 of both oils was omitted and only formula-2 to formula-5 of sesame oil and lemongrass oil were used. The release of clotrimazole from Defungal®, the commercial product used as a positive control was compared with that released from the developed microemulsions. To compare the means of all release data and to assess statistical significance between them, one-way analysis of variance (ANOVA) was carried out. The results found that the releasing rate of all formulations were significance different with p-value of 0.001.

The results demonstrated that the four systems of lemongrass oil could release the drug faster than Defungal®, as shown in Table 4.63 and Figure 4.68. Formula-3 showed the fastest drug release rate with the rate of 0.1247%/min followed by

formula-5, formular-2 and formula-4 with the rate of 0.1242, 0.1149 and 0.1079%/min respectively as shown in Table 4.65. These results revealed the potential of surfactant mixture composed of 33% surfactant and 17% cosurfactant on drug release property of microemulson. It was found that the size of internal droplet was the important factor for releasing the drug from the microemulsions. As mentioned that the size of the internal droplet of formula-3 was smallest, therefore, it could be concluded that the microemulsion with smallest droplet size could give the highest release. Drug release property of microemulsions of sesame oil was similar to those of lemongrass oil. Sesame oil microemulsions of formula-3 could release the drug faster than the commercial products. Sesame oil formula-5 showed similar release property as the commercial product as shown in Table 4.64 and Figure 4.69. The release rate of formula-3 and formula-5 were 0.0684 and 0.0508%/min respectively. The release rate of formula-2 and formula-4 of sesame oil were slower than formula-3 and formula-5 with the release rate of 0.0394 and 0.0371%/min. It was found that the drug release from sesame oil microemulsions was slower than that from lemongrass oil microemulsions. This was considered to be due to the droplet size of sesame oil microemulsioins that was slightly bigger than the droplet size of lemongrass microemulsions. Furthermore, the bigger droplet size of formula-2 and formula-4 expressed the lower releasing of the drug.

From these experiments, the results suggested that the microemulsion with the surfactant and cosurfactant gave faster drug release than those with surfactant alone without cosurfactant. Furthermore, lemongrass oil microemulsions which had the smaller droplet size showed better drug release than sesame oil microemulsions which had a larger droplet size. Subramanian et al studied celecoxib loaded microemulsions

and showed the influence of proportion of surfactant and cosurfactant on decreasing the droplet size of the internal phase and permeation property through the skin as well as their anti-inflamatory activity of the microemulsions [190]. Singh et al revealed that the different types of oil and cosurfactants affected the drug release [191]. Therefore, the release results of the microemulsions developed in the present study was in accordance with these previous reports. Ethanol and surfactant were also reported as the drug penetration enhancer through the skin [192]. Hence, it is an advantage of the desirable microemulsion formula of both oils to have an ethanol as a cosurfactant in the surfactant system.

Table 4.63 Amount of drug released from lemongrass oil microemulsion systems

Time (min)	Cumulative amount of drug released (%)				
	LM-2	**LM-3**	**LM-4**	**LM-5**	**DF®**
10	6.38 ± 0.13	4.93 ± 0.14	4.95 ± 0.04	5.13 ± 0.17	4.73 ± 0.09
20	6.24 ± 0.14	5.77 ± 0.29	5.46 ± 0.19	6.67 ± 1.02	5.34 ± 0.04
30	7.01 ± 0.28	6.57 ± 0.34	6.04 ± 0.36	7.38 ± 1.44	6.00 ± 0.13
45	7.84 ± 0.24	7.81 ± 0.49	7.04 ± 0.55	8.89 ± 1.04	7.00 ± 0.31
60	8.87 ± 0.10	9.22 ± 0.54	8.30 ± 1.04	10.47 ± 1.21	8.06 ± 0.41
90	12.21 ± 1.82	11.92 ± 0.82	10.67 ± 1.45	13.43 ± 1.42	10.03 ± 0.88
120	15.69 ± 1.03	15.36 ± 1.58	14.64 ± 1.28	16.16 ± 2.49	12.09 ± 1.01
180	21.08 ± 0.72	22.85 ± 2.33	19.72 ± 2.73	24.78 ± 4.83	15.25 ± 1.50
240	28.95 ± 0.16	30.77 ± 2.79	25.56 ± 4.78	33.25 ± 5.44	18.53 ± 1.71
300	36.97 ± 0.21	38.90 ± 2.72	32.17 ± 3.93	41.09 ± 7.08	21.47 ± 2.30
360	44.15 ± 0.33	46.81 ± 3.42	39.40 ± 3.37	46.88 ± 8.58	24.16 ± 2.48
420	51.74 ± 0.21	55.06 ± 2.54	47.23 ± 2.43	55.74 ± 7.97	26.70 ± 2.61
480	57.54 ± 0.25	62.21 ± 3.48	53.71 ± 1.18	64.01 ± 11.27	29.16 ± 2.68
540	65.31 ± 2.53	69.58 ± 1.68	60.73 ± 0.39	70.58 ± 11.02	31.45 ± 2.92
600	72.53 ± 2.67	77.16 ± 2.47	68.95 ± 3.37	75.73 ± 11.69	33.83 ± 3.10

* DF® = The commercial pharmaceutical product of 1% Clotrimazole

Table 4.64 Amount of drug released from sesame oil microemulsion systems

Time (min)	Cumulative amount of drug released (%)				
	SE-2	SE-3	SE-4	SE-5	DF®
10	4.70 ± 0.09	5.01 ± 0.20	4.65 ± 0.09	4.79 ± 0.06	4.73 ± 0.09
20	4.98 ± 0.04	5.38 ± 0.05	4.92 ± 0.06	5.40 ± 0.28	5.34 ± 0.04
30	5.48 ± 0.12	6.03 ± 0.04	5.45 ± 0.01	5.95 ± 0.30	6.00 ± 0.13
45	6.09 ± 0.20	7.11 ± 0.13	5.92 ± 0.07	6.70 ± 0.33	7.00 ± 0.31
60	6.84 ± 0.42	8.26 ± 0.22	6.38 ± 0.08	7.55 ± 0.22	8.06 ± 0.41
90	7.71 ± 0.23	10.39 ± 0.43	7.25 ± 0.27	9.09 ± 0.25	10.03 ± 0.88
120	8.92 ± 0.35	12.73 ± 0.53	8.33 ± 0.44	11.06 ± 0.49	12.09 ± 1.01
180	11.53 ± 1.07	16.64 ± 0.77	10.19 ± 0.74	13.72 ± 0.56	15.25 ± 1.50
240	13.76 ± 1.06	20.57 ± 1.17	12.42 ± 0.98	16.66 ± 0.58	18.53 ± 1.71
300	15.96 ± 1.46	24.54 ± 1.34	15.46 ± 1.81	19.77 ± 0.73	21.47 ± 2.30
360	18.61 ± 1.46	28.62 ± 1.78	17.61 ± 1.32	22.97 ± 0.85	24.16 ± 2.48
420	21.14 ± 1.55	32.16 ± 2.34	20.48 ± 1.19	25.52 ± 1.22	26.70 ± 2.61
480	23.08 ± 1.69	37.15 ± 3.13	21.99 ± 0.35	29.27 ± 3.13	29.16 ± 2.68
540	25.54 ± 1.74	40.94 ± 4.15	23.78 ± 0.07	31.53 ± 4.15	31.45 ± 2.92
600	27.79 ± 2.12	46.80 ± 6.85	26.26 ± 0.88	34.97 ± 6.85	33.83 ± 3.10

* DF® = The commercial pharmaceutical product of 1% Clotrimazole

Table 4.65 Release rate of clotrimazole from the microemulsion systems and their respective correlation coefficient of the linear equations obtained from the plot between the amount of drug release vs. time

Formulation	Release rate (%/min)	R^2
DF®	0.0500±0.0050	0.9923
LM-2	0.1149±0.0105	0.9975
LM-3	0.1247±0.0037	0.9984
LM-4	0.1079±0.0012	0.996
LM-5	0.1242±0.0193	0.9985
SE-2	0.0394±0.0033	0.9997
SE-3	0.0684±0.0068	0.9989
SE-4	0.0371±0.0060	0.9978
SE-5	0.0508±0.0030	0.9995

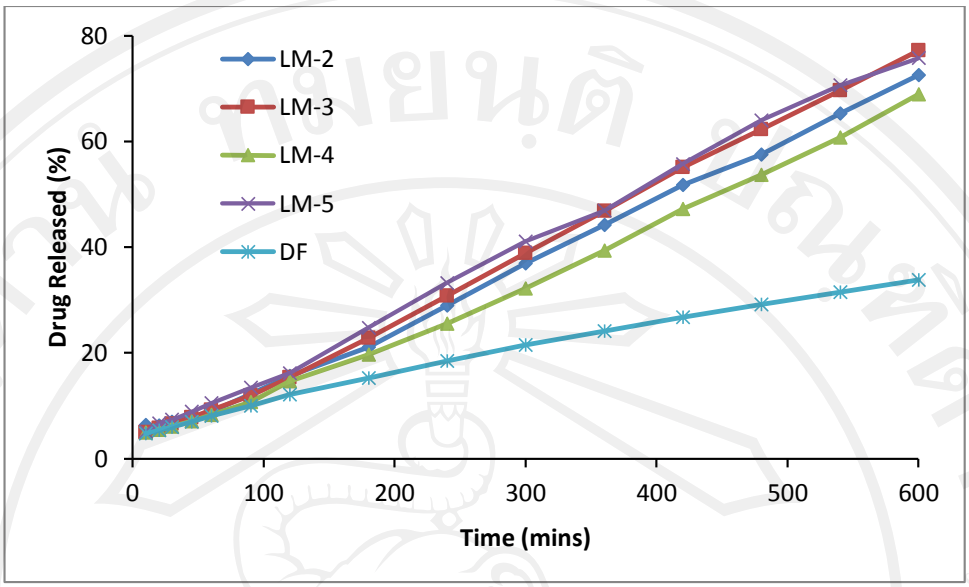


Figure 4.68 Release profile of drug from lemongrass oil microemulsion systems

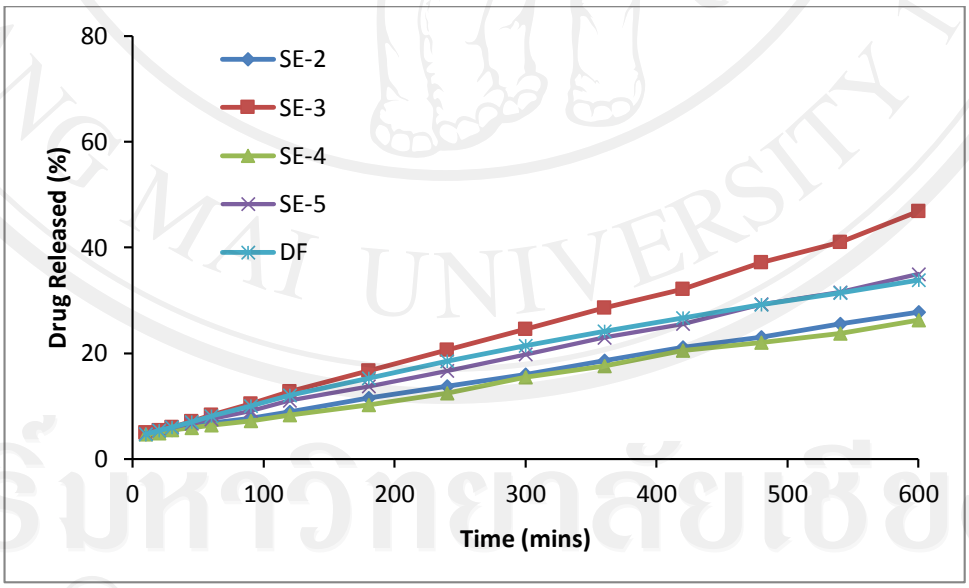


Figure 4.69 Release profile of drug from sesame oil microemulsion systems