CHAPTER 3

RESULTS

1. Plant extracts

From fifteen plant species, 17 dried materials derived from different parts were selected to extracting with steam distillation and solvent (ethanol/hexane) maceration. In most cases, only one part of each plant was used whereas two parts of *Aegle marmelos* (leaf, fruit) and *Zingiber zerumbet* (rhizome, flower) were separately extracted. Several plant products with different physical characteristics such as appearance, color, and odor were obtained from this extraction. Isolation of essential oils by steam distillation discovered that only two plant materials, *Saussurea lappa* root and *A. marmelos* leaf, provided the liquid oils with a yield of 0.32 and 1.50% (v/w) based on dry weight, respectively. These essential oils were less dense than water, and their physical properties are presented in Table 3.1. The distillate oil of *S. lappa* root was pale yellow with pungent odor. For *A. marmelos*, the light yellow with aromatic odor oil was derived from leaf where as its fruit yielded no oil. None of the remaining plant samples offered the essential oils.

In the solvent extraction, it was revealed that ethanolic and hexane plant extracts presented in Table 3.2 and Table 3.3, respectively, demonstrated differences in yield, appearance, color, and odor. While extractions with ethanol yielded from 5.12 to 65.00% (w/w), the hexane extractions provided yields ranged from 0.66 to 15.98% (w/w). The maximum yield of ethanolic extracts was obtained from *Rheum palmatum* rhizome (65.00%), followed by *Acacia concinna* pods (27.16%), and *Zingiber zerumbet* rhizome (26.74%), where as that of hexane extracts was received from *Ocimum americanum* seed (15.98%), followed by *Clitoria ternatea* seed (9.05%), and *Ligusticum sinense* rhizome (5.20%). The minimum yields of ethanolic and hexane extracts were derived from *Z. zerumbet* flower (5.12%) and *R. palmatum* root (0.66%), respectively.

Table 3.1 Physical characteristics and percentage yields (% Yield) of essential oils derived from steam distillation of 17 samples of fifteen plant species

Plants & Part used	**Appearance**	**Color**	**Odor**	**% Yield (V/W)**
O. basilicum (Leaf)	-	-	-	-
O. americanum (Seed)	-	-	-	-
C. odoratum (Stem & leaf)	-	-	-	-
S. lappa (Root)	Liquid	Pale yellow	Pungent	0.32
B. orellana (Seed)	-	-	-	-
C. ternatea (Seed)	-	-	-	-
A. concinna (Pods)	-	-	-	-
V. zizanioides (Rhizome & Root)	-	-	-	-
R. palmatum (Root)	-	-	-	-
A. marmelos				
Leaf	Liquid	Light yellow	Aromatic	1.50
Fruit	-	-	-	-
H. cordata (Leaf)	-	-	-	-
L. sinense (Rhizome)	-	-	-	-
A. dahurica (Root)	-	-	-	-
L. camara (Flower)	-	-	-	-
Z. zerumbet				
Flower	-	-	-	-
Rhizome	-	-	-	-

Table 3.2 Physical characteristics and percentage yields of ethanolic extracts derived from 17 samples of fifteen plant species

Plants & Part used	Appearance	Color	Odor	% Yield (W/W)
O. basilicum (Leaf)	Solid	Olive-green	Strong	7.42
O. americanum (Seed)	Semi-solid	Pale-yellow	Slightly aromatic	12.72
C. odoratum (Stem & Leaf)	Solid	Olive-green	Pungent aromatic	9.35
S. lappa (Root)	Solid	Light-brown	Characteristic penetrating	20.47
B. orellana (Seed)	Solid	Red-orange	Sour smelling	9.07
C. ternatea (Seed)	Solid	Light-brown	Pungent	15.00
A. concinna (Pods)	Viscous	Brown	Sweet smelling	27.16
V. zizanioides (Rhizome & Root)	Solid	Dark-brown	Aromatic	14.98
R. palmatum (Root)	Solid	Brown	Characteristic	65.00
A. marmelos				
Leaf	Solid	Olive-green	Peculiar aromatic	5.36
Fruit	Solid	Light-brown	Aromatic	9.97
H. cordata (Leaf)	Solid	Olive-green	Peculiar aromatic	15.77
L. sinense (Rhizome)	Semi-solid	Brown	Aromatic	25.1
A. dahurica (Root)	Solid	Brown	Caramel-like	7.70
L. camara (Flower)	Solid	Light-brown	Distinctive pungent	22.80
Z. zerumbet				
Flower	Viscous	Light-brown	Spicy smelling	5.12
Rhizome	Solid	Yellow	Spicy smelling	26.74

Table 3.3 Physical characteristics and percentage yields of hexane extracts derived from 17 samples of fifteen plant species

Plants & Part used	Appearance	Color	Odor	% Yield (W/W)
O. basilicum (Leaf)	Viscous	Olive-green	Strong	3.34
O. americanum (Seed)	Viscous	White opaque	Slightly aromatic	15.98
C. odoratum (Stem & leaf)	Solid	Olive-green	Pungent aromatic	3.75
S. lappa (Root)	Viscous	Light-brown	Characteristic penetrating	3.48
B. orellana (Seed)	Viscous	Orange	Sour smelling	2.25
C. ternatea (Seed)	Solid	Yellow	Pungent	9.05
A. concinna (Pods)	Solid	Light-brown	Sweet smelling	0.73
V. zizanioides (Rhizome & Root)	Solid	Brown	Aromatic	0.83
R. palmatum (Root)	Semi-solid	Yellow	Characteristic	0.66
A. marmelos				
Leaf	Solid	Olive-green	Peculiar aromatic	2.09
Fruit	Solid	Yellow	Aromatic	1.25
H. cordata (Leaf)	Viscous	Olive-green	Peculiar aromatic	4.74
L. sinense (Rhizome)	Viscous	Light-brown	Aromatic	5.20
A. dahurica (Root)	Viscous	Light-brown	Caramel-like	1.35
L. camara (Flower)	Solid	Orange	Distinctive pungent	3.74
Z. zerumbet				
Flower	Viscous	Light-brown	Spicy smelling	2.57
Rhizome	Solid	Gold yellow	Spicy smelling	3.26

2. Repellent activity of plant extracts against *Ae. aegypti* mosquito

Topical application of 25% DEET and plant products, including essential oils, ethanolic extracts, and hexane extracts provided the effectiveness for protection against *Ae. aegypti* mosquitoes with varying degrees of repellency (Table 3.4). While the root oil of *S. lappa* exerted repellent potential against *Ae. aegypti* with the median complete-protection time of 2.75 hr, no repellency was observed from *A. marmelos* leaf oil. Repellency determination of solvent extracts revealed that both ethanolic and hexane extracts from *O. americanum* seed, *Chromolaena odoratum* (stem & leaf), *A. concinna* pods, *R. palmatum* root, *A. marmelos* fruit, *Lantana camara* flower, and *Z. zerumbet* flower were ineffective in repelling mosquitoes. Only five ethanolic extracts, including *Ocimum basilicum* leaf, *Houttuynia cordata* leaf, *Angelica dahurica* root, *A. marmelos* leaf, and *L. sinense* rhizome possesses repellency with median complete-protection times of 0.25, 0.5, 0.5, 0.75, and 5.0 hr, respectively. The other twelve ethanolic extracts appeared to be inefficient.

Repellent activities were observed in most hexane extracts, including *S. lappa* root, *Bixa orellana* seed, *C. ternatea* seed, *Vetiveria zizanioides* (rhizome & root), *A. marmelos* leaf, *H. cordata* leaf, *L. sinense* rhizome, and *Z. zerumbet* rhizome, with the median complete-protection times ranging from 0.25-6.5 hr. The hexane extract of *L. sinense* rhizome afforded the greatest repellent efficacy, with a median complete-protection time of 6.5 (5.0-8.0) hr, which was comparable to that of DEET (6.25, 5.0-6.5 hr). No local skin reaction such as rash, swelling, irritation, or other allergic responses was observed during the study period.

Table 3.4 Repellency of 25% DEET and plant products, including essential oils, ethanolic extracts, and hexane extracts against female *Ae. aegypti* mosquitoes

Plants & Part used	Thai name	Median complete-protection time (Range, hr)		
		Essential oil	Ethanolic extract	Hexane extract
O. basilicum (Leaf)	โหระพา	ND	0.25 (0.0-0.5)	0.0 (0.0-0.5)
O. americanum (Seed)	แมงลัก	ND	0.0 (0.0)	0.0 (0.0)
C. odoratum (Stem & Leaf)	สาบเสือ	ND	0.0 (0.0)	0.0 (0.0-0.5)
S. lappa (Root)	โกฐกระดูก	2.75 (2.5-3.0)	0.0 (0.0)	2.0 (1.0-3.5)
B. orellana (Seed)	คำแสด	ND	0.0 (0.0)	0.25 (0.0-0.5)
C. ternatea (Seed)	อัญชัน	ND	0.0 (0.0)	0.5 (0.0-1.0)
A. concinna (Pods)	ส้มป่อย	ND	0.0 (0.0)	0.0 (0.0)
V. zizanioides (Rhizome & Root)	แฝกหอม	ND	0.0 (0.0)	0.5 (0.0-1.5)
R. palmatum (Root)	โกฐน้ำเต้า	ND	0.0 (0.0)	0.0 (0.0)
A. marmelos				
Leaf	มะตูม	0.0 (0.0)	0.75 (0.5-1.5)	2.25 (1.5-3.0)
Fruit	มะตูม	ND	0.0 (0.0)	0.0 (0.0)
H. cordata (Leaf)	คาวพลู	ND	0.5 (0.5-1.0)	0.75 (0.0-1.0)
L. sinense (Rhizome)	โกฐหัวบัว	ND	5.0 (4.0-5.5)	6.5 (5.0-8.0)
A. dahurica (Root)	โกฐสอ	ND	0.5 (0.0-0.5)	0.0 (0.0)
L. camara (Flower)	ผกากรอง	ND	0.0 (0.0)	0.0 (0.0)
Z. zerumbet				
Flower	กระทือ	ND	0.0 (0.0)	1.5 (0.5-2.0)
Rhizome	กระทือ	ND	0.0 (0.0)	0.0 (0.0-0.5)
25% DEET		6.25 (5.0-6.5)		

ND: not determined; as no essential oil was obtained from this plant species.

3. Repellent activity of *L. sinense* hexane extract against two target mosquitoes, *Ae. aegypti* and *An. minimus*

The most effective plant sample, 25% ethanolic solutions of *L. sinense* hexane extract (LHE) established from the repellent screening tests was formulated with and without 5% vanillin and evaluated for repellency in comparison to 25% DEET solutions, with and without 5% vanillin, against *Ae. aegypti* and *An. minimus*. According to the results demonstrated in Table 3.5, it appeared that ethanol preparations of LHE and DEET, with and without 5% vanillin added, were effective in repelling both *Ae. aegypti* and *An. minimus*. While 25% LHE alone provided median complete-protection times of 6.5 (5.5-9.5) and 11.5 (9.0-14.0) hr against *Ae. aegypti* and *An. minimus*, respectively; incorporation of 5% vanillin increased repellency of LHE against *Ae. aegypti* and *An. minimus* with the prolonged median complete-protection times of 11.0 (7.0-13.5) and 12.5 (9.0-16.0) hr, respectively. Correspondingly, vanillin also extended the protection times of 25% DEET against *Ae. aegypti* and *An. minimus* from 8.0 (5.0-9.5) hr to 8.75 (7.5-11.0) hr and from 11.5 (10.5-15.0) hr to 14.25 (11.0-18.0) hr, respectively. There was no repellency against these mosquito species of the control solution (5% vanillin in ethanol). No skin irritation, rashes, swelling, or other allergic responses were observed during the study period. The best repellent samples in each group, 25% LHEv and 25% DEETv were selected for further repellent study under field conditions.

Table 3.5 Repellent activity of *L. sinense* hexane extract (LHE) and DEET, with and without 5% vanillin, against *Ae. aegypti* and *An. minimus* mosquitoes

Repellent sample	**Median complete-protection time (Range, hr)***	
	Ae. aegypti	***An. minimus***
25% LHE	6.5 (5.5-9.5)	11.5 (9.0-14.0)
25% LHE + 5% vanillin (25% LHEv)	11.0 (7.0-13.5)	12.5 (9.0-16.0)
25% DEET	8.0 (5.0-9.5)	11.5 (10.5-15.0)
25% DEET + 5% vanillin (25% DEETv)	8.75 (7.5-11.0)	14.25 (11.0-18.0)

* There were 12 replicates of each test.

4. Field repellent study

Two repellent products, 25% LHEv and 25% DEETv, with the longest-lasting protection times established from the laboratory repellent study were the candidates tested in the field assessment.

4.1 Preliminary survey

The preliminary human-baited-trap surveys (PS) were performed for 2 times, PS I and PS II, in the hot season from March to April 2013, at Sunpesua subdistrict located approximately 6 km north of Muang district, Chiang Mai province. In addition to many breeding habitats of mosquitoes, this field location consisted of human residences with domestic animals such as cats and dogs, which did not receive any protections from mosquito bites, thus supplying plentiful blood sources for mosquitoes. However, this area had not been previously reported because of risk from mosquito-borne diseases.

From the results obtained it was demonstrated that a total of 925 adult female mosquitoes consisting of 5 genera were caught during the preliminary surveys (Table 3.6). In the PS I, a total of 615 adult females comprising 5 genera were collected and the most abundant mosquitoes were *Armigeres* and *Culex*, which counted as 302 (49.10%) and 300 (48.78%), respectively. This finding was relatively corresponded to that of PS II showing a total of 310 adult females comprising 4 genera, and *Armigeres* was the most predominant (232, 74.83%), followed by *Culex* (70, 22.60%). While, *Anopheles* were collected from the PS I, none of this mosquito was obtained from the PS II.

According to the results, it was indicated that there were large and mixed mosquito populations, of which some species comprising *Armigeres*, *Aedes*, *Anopheles*, *Culex*, and *Mansonia* were abundant and available for repellency evaluation. During the study period, sunset at the testing area occurred at ≈ 19.30 hr local time and the mosquitoes were crowded ≈ 90 min before and after sunset (18.00-21.30 hr). Therefore, the suitable period for collecting mosquitoes in the field repellent assessments was arranged between 18.00 and 21.30 hr.

Table 3.6 Mosquitoes collected on human volunteers during the preliminary surveys at Sunpesua subdistrict, Chiang Mai province, northern Thailand, March-April, 2013

Mosquitoes	**No. of mosquitoes collected (%)**					
	PS I		**PS II**		**Total**	
Aedes spp.	8	(1.30)	6	(1.93)	14	(1.51)
Armigeres spp.	302	(49.10)	232	(74.83)	534	(57.73)
Anopheles spp.	3	(0.49)	0	(0.00)	3	(0.33)
Culex spp.	300	(48.78)	70	(22.60)	370	(40.00)
Mansonia spp.	2	(0.33)	2	(0.64)	4	(0.43)
Total	**615**	**(100)**	**310**	**(100)**	**925**	**(100)**

4.2 Field repellent assessment

Field study also was carried out in the hot season from April to May 2013, at the same place of preliminary trials; showing large and varied populations of field mosquitoes, which were abundant enough for repellency assessment. For each collection, the volunteers were exposed to natural populations of mosquitoes for 180 min, between 18.00 hr and 21.30 hr, which was indicated in the preliminary study as the suitable period for mosquito collection.

The results of 2 selected repellent products, 25% LHEv and 25% DEETv, when applied on human skin under field conditions are illustrated in Table 3.7 and Table 3.8. It was found that 25% LHEv and 25% DEETv exerted a similar strong repellency, with a complete protection (100%) against all the mosquito species. There was a highly significant difference between the mean number of mosquito collected on the controls and testers treated with 25% LHEv or 25% DEETv in every collecting sites (CS); nine 20-min exposure sites (Table 3.7). The mean collecting rates of mosquitoes on the control volunteers at CS1, CS2, CS3, and CS4 were increased dramatically from 2.2±3.6 to 21.4±18.6, 29.4±22.6, and 37.4±21.9, respectively; then decreased, but were still relatively high, to 24.3±8.9, 24.5±9.6, 22.8.4±10.5, 22.2±13.3, and 20.1±10.0 at CS5, CS6, CS7, CS8, and CS9, respectively. These results indicated that the maximum mean collecting rate was that of CS4 (19.06-19.26 hr) and the crowded mosquitoes were observed between 18.22-21.16 hr. This finding likely corresponded to that of the preliminary trials.

Table 3.7 Number of mosquitoes collected on human volunteers during field repellent bioassays at Sunpesua subdistrict, Chiang Mai province, northern Thailand, April-May, 2013

Collecting site (CS): Time	**Treatment**	**No. of mosquitoes collected**	**Mosquito collecting rate (Mean ± S.E.) ***
CS 1: 18.00-18.20 hr	Control	56	2.2±3.6 a
	25% LHEv	0	0.0±0.0 b
	25% DEETv	0	0.0±0.0 b
CS 2: 18.22-18.42 hr	Control	559	21.4±18.6 a
	25% LHEv	0	0.0±0.0 b
	25% DEETv	0	0.0±0.0 b
CS 3: 18.44-19.04 hr	Control	752	29.4±22.6 a
	25% LHEv	0	0.0±0.0 b
	25% DEETv	0	0.0±0.0 b
CS 4: 19.06-19.26 hr	Control	980	37.4±21.9 a
	25% LHEv	0	0.0±0.0 b
	25% DEETv	0	0.0±0.0 b
CS 5: 19.28-19.48 hr	Control	629	24.3±8.9 a
	25% LHEv	0	0.0±0.0 b
	25% DEETv	0	0.0±0.0 b
CS 6: 19.50-20.10 hr	Control	638	24.5±9.6 a
	25% LHEv	0	0.0±0.0 b
	25% DEETv	0	0.0±0.0 b
CS 7: 20.12-20.32 hr	Control	593	22.8±10.5 a
	25% LHEv	0	0.0±0.0 b
	25% DEETv	0	0.0±0.0 b
CS 8: 20.34-20.54 hr	Control	577	22.2±13.3 a
	25% LHEv	0	0.0±0.0 b
	25% DEETv	0	0.0±0.0 b
CS 9: 20.56-21.16 hr	Control	522	20.1±10.0 a
	25% LHEv	0	0.0±0.0 b
	25% DEETv	0	0.0±0.0 b
Total	Control	5,306	204.1±76.0 a
	25% LHEv	0	0.0±0.0 b
	25% DEETv	0	0.0±0.0 b

***** Mean in each column for each sample followed by the same letter is not significantly different ($P > 0.05$).

Table 3.8 Results obtained from field repellent assessment of 25% LHEv and 25% DEETv, conducted at Sunpesua subdistrict, Chiang Mai province, northern Thailand, April-May, 2013

Mosquito species	Control	25% LHEv		25% DEETv	
	No. of mosquitoes collected (%)	No. of mosquitoes collected (%)	Protection (%)	No. of mosquitoes collected (%)	Protection (%)
Ae. aegypti	4 (0.08)	0(0)	100	0(0)	100
Ae. albopictus	41 (0.77)	0(0)	100	0(0)	100
Ae. lineatopenis	8 (0.15)	0(0)	100	0(0)	100
Ae. vexans	110 (2.07)	0(0)	100	0(0)	100
An. barbirostris	12 (0.23)	0(0)	100	0(0)	100
Ar. subalbatus	2,269 (42.76)	0(0)	100	0(0)	100
Cx. gelidus	17 (0.32)	0(0)	100	0(0)	100
Cx. quinquefasciatus	2,190 (41.27)	0(0)	100	0(0)	100
Cx. tritaeniorhynchus	65 (1.23)	0(0)	100	0(0)	100
Cx. vishnui	514 (9.69)	0(0)	100	0(0)	100
Ma. annulifera	23 (0.43)	0(0)	100	0(0)	100
Ma. indiana	41 (0.77)	0(0)	100	0(0)	100
Ma. uniformis	12 (0.23)	0(0)	100	0(0)	100
Total	5,306 (100)	0(0)	100	0(0)	100

Regarding the results demonstrated in Table 3.8, it appeared that both repellent products, 25% LHEv and 25% DEETv, afforded excellent personal protection against a wide range of mosquito species belonging to five genera, i.e., *Aedes*, *Anopheles*, *Armigeres*, *Culex*, and *Mansonia*. A total of 5,306 adult female mosquitoes comprising 13 species were collected during the field trials. The most predominant species were *Ar. subalbatus*, *Cx. quinquefasciatus*, and *Cx. vishnui*, which made up 42.76%, 41.27%, and 9.69%, respectively. No mosquito bite was observed on the volunteers treated with 25% LHEv and 25% DEETv throughout the field study. Based on these findings, it was clearly demonstrated that the protective effect of 25% LHEv and 25% DEETv appeared to be complete against natural populations of mosquitoes.

5. Physical and biological stability of *L. sinense* hexane extract

The samples of *L. sinense* hexane extract were determined for physical characteristics and repellent activity against *Ae. aegypti* after it had been kept at various temperatures [4 ˚C, ambient temperature (AT: 21-35 ˚C), and 45 ˚C] for different durations (1, 2, and 3 months). According to results demonstrated in Table 3.9, it appeared that the physical characteristics such as appearance, color, and odor of all samples kept at 4 ˚C for 1, 2, and 3 months were similar to those of the fresh sample. Although, the samples stored at ambient temperature and 45 ˚C for 1, 2, and 3 months were still viscous, with a pleasant aromatic odor, their color had changed from light- to dark-brown. Repellent activities against *Ae. aegypti* of the stored samples of *L. sinense* were presented for a period of at least 3 months and slightly different (Table 3.9). Surprisingly, the median complete-protection times of samples kept at 4 ˚C, ambient temperature, and 45 ˚C for 1 month were insignificantly increased to 7.5 (5.0-9.0), 7.25 (5.0-10.5), and 8.0 (4.5-8.5) hr, respectively. However, most samples stored at each temperature for 2 and 3 months offered slightly lower repellency than the fresh sample and those kept for 1 month.

Table 3.9 Physical characteristics and repellency against *Ae. aegypti* of the fresh and stored samples kept at 4 ˚C, ambient temperature (AT), and 45 ˚C for 1, 2, and 3 months of *L. sinense* rhizome hexane extract

Plant samples (Temperature/Duration)		**Physical characteristics**			**Median complete–protection time (Range, hr)**
		Appearance	**Color**	**Odor**	
Fresh sample		Viscous	Light-brown	Aromatic	6.5 (5.0-8.0)
Stored sample					
4 ˚C	1 month	Viscous	Light-brown	Aromatic	7.5 (5.0-9.0)
	2 months	Viscous	Light-brown	Aromatic	5.25 (3.5-6.5)
	3 months	Viscous	Light-brown	Aromatic	4.25 (3.0-6.5)
AT (21-35 ˚C)	1 month	Viscous	Dark-brown	Aromatic	7.25 (5.0-10.5)
	2 months	Viscous	Dark-brown	Aromatic	6.5 (3.5-8.0)
	3 months	Viscous	Dark-brown	Aromatic	5.5 (3.0-6.5)
45 ˚C	1 month	Viscous	Dark-brown	Aromatic	8.0 (4.5-8.5)
	2 months	Viscous	Dark-brown	Aromatic	4.25 (3.0-6.5)
	3 months	Viscous	Dark-brown	Aromatic	3.5 (2.5-5.5)

6. Chemical composition of *L. sinense* hexane extract

L. sinense rhizome hexane extract, the most effective sample, was analyzed by gas chromatography coupled to mass spectrometry (GC/MS), of which gas chromatogram and its chemical compositions are demonstrated in Figure 3.1 and Table 3.10, respectively. A total of 18 compounds accounting 99.99% in the *L. sinense* hexane extract were identified. The major components were 3-n-butylphthalide (31.46%), 2, 5-lutidin (21.94%), and linoleic acid (16.41%), followed by minor constituents of 4-hydroxyindole (7.05%), butylidene phthalide (6.25%), bis (2-ethylhexyl) phthalate (4.84%), and β-selinene (2.41%).

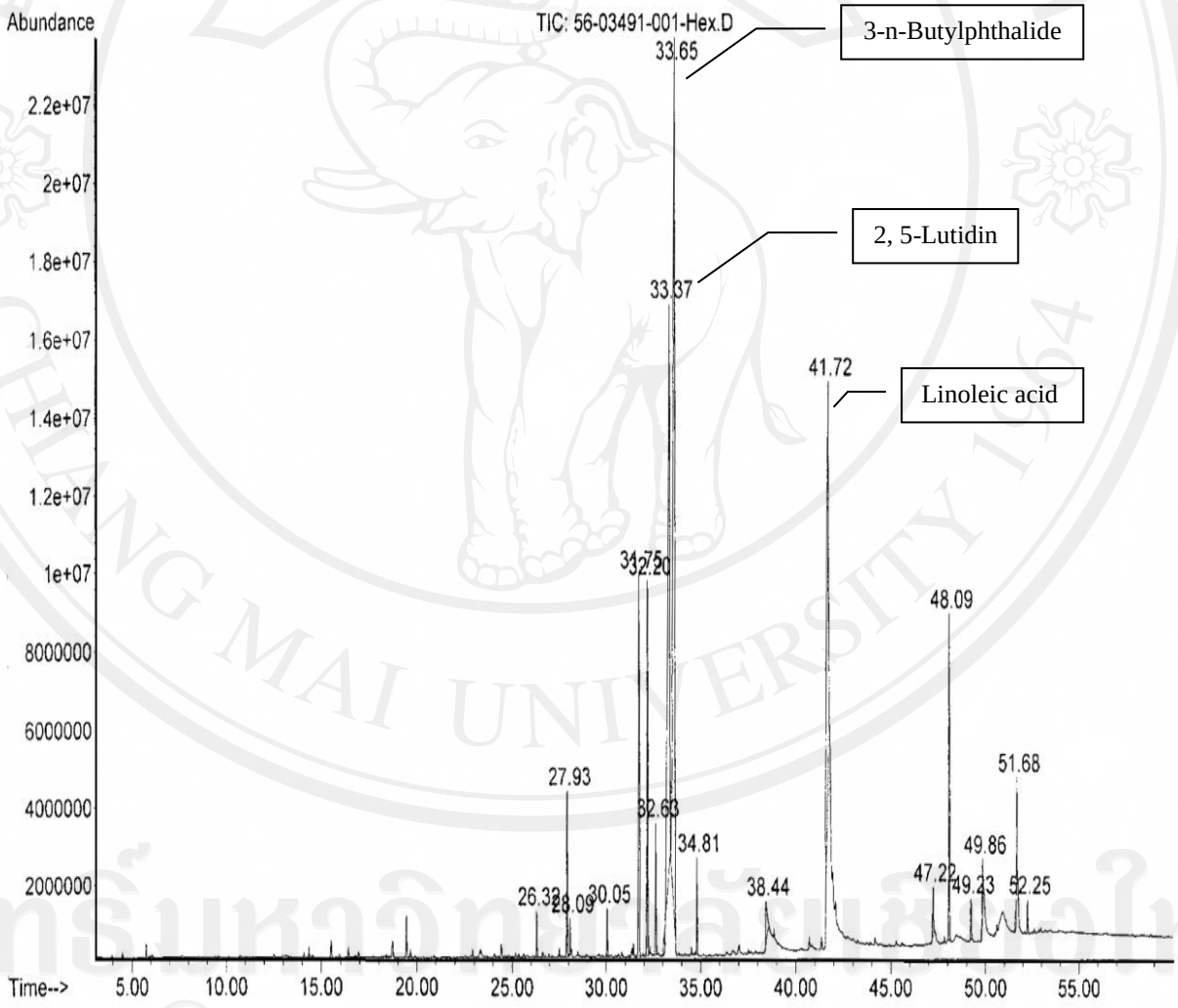

Figure 3.1 GC/MS total ion chromatogram of *L. sinense* rhizome hexane extract

Table 3.10 Chemical compositions of *L. sinense* rhizome hexane extract as determined by GC/MS analysis

Peak	Retention time (min)	Compounds (CAS No.)	Quality (Peak purity)	Content (%)
1	26.319	*α*-Cedrene (000469-61-4)	86	0.63
2	27.930	*β*-Selinene (917066-67-0)	99	2.41
3	28.094	α-Selinene (000473-13-2)	97	0.46
4	30.049	(-)-Spathulenol (077171-55-2)	95	0.66
5	31.751	4-Hydroxyindole (002380-94-1)	64	7.05
6	32.196	Butylidene phthalide (000551-08-6)	94	6.25
7	32.633	1, 3, 5-Undecatriene, (E, E)- (019883-29-5)	52	2.03
8	33.370	2, 5-Lutidin (000589-93-5)	59	21.94
9	33.651	3-n-Butylphthalide (006066-49-5)	95	31.46
10	34.810	3-n-Butylphthalide (006066-49-5)	95	1.43
11	38.443	Palmitinic acid (000057-10-3)	99	1.24
12	41.721	Linoleic acid (000060-33-3)	97	16.41
13	47.223	7-Methylindole (000933-67-5)	43	0.70
14	48.093	Bis (2-ethylhexyl) phthalate (000117-81-7)	91	4.84
15	49.233	Aldehyd (000103-95-7)	22	0.55
16	49.857	3- (2, 2-Dideuterobutyl)-thiophene-1, 1-dioxide (000000-00-0)	50	0.51
17	51.676	2-(3-Methoxy-2-(trimetthylsilyl)phenyl)-4, 4-dimethyl-2-oxazoline (088932-58-5)	83	0.93
18	52.349	Docosa-2, 6, 10, 14, 18-pentaen-22-al, 2, 6, 10, 15, 18-pentamethyl-, alltrans (000000-00-00)	64	0.49