CHAPTER 4

Results and discussion

4.1 Extraction yield

Essential oils from aerial parts of *Elsholtzia* and leaves of *Clausena* species were extracted by steam distillation technique. The highest yield of essential oil was obtained from *E.* sp., followed by *E. griffithii*, *E. communis*, *E. stachyodes*, *C. harmandiana*, and *C. lansium* at 1.21, 0.58, 0.44, 0.26, 0.10, and 0.01% (w/w) on fresh weight basis, respectively (Table 4.1). The majority of oils have been shown yellow colour while the oil of *C. harmandiana* have been revealed colourless.

Table 4.1 Physical properties and percentage yields (% yield) of essential oils from *Elsholtzia* and *Clausena* species

Parts of used	Plants	Appearance	Colour	% yield (w/w)
aerial parts	*E. stachyodes*	liquid	pale yellow	0.26
	E. communis	liquid	light yellow	0.44
	E. griffithii	liquid	pale yellow	0.58
	E. sp.	liquid	light yellow	1.21
leaves	*C. lansium*	liquid	light yellow	0.01
	C. harmandiana	liquid	colourless	0.10

After the completion of steam distillation, solid residues were collected and air-dried. The dried solid residues were re-extracted using solvent extraction on the basis of polarity order of different organic solvents. The residual crude extracts from each plant were obtained (Table 4.2). Although all of plant materials were extracted by the same conditions, the percentage yields of residual crude extracts were varied appearance. This may be due to the difference in plant compositions such as cellulose, chlorophyll, tannin, and protein in the materials.[6]

Moreover, the principle of solvent extraction is the basis of like-dissolve-like.[58] Therefore, chemical components in the residual crude extracts were different. These results depend on polarity of organic solvents used and plant species. Non polar substances such as wax, lipids, and fatty acids were dissolved by hexane whereas moderate and polar substances were extracted by acetone and ethanol, respectively.

Table 4.2 Percentage yield (% yield) of residual crude extracts from *Elsholtzia* and *Clausena* species

Plants	**Residual crude extracts**	**% yield (w/w)**
E. stachyodes	hexane extract	0.29
	acetone extract	0.41
	ethanol extract	0.91
E. communis	hexane extract	0.56
	acetone extract	1.01
	ethanol extract	1.59
E. griffithii	hexane extract	0.55
	acetone extract	0.73
	ethanol extract	2.81
E. sp.	hexane extract	0.90
	acetone extract	2.04
	ethanol extract	1.19
C. lansium	hexane extract	4.14
	acetone extract	4.53
	ethanol extract	0.94
*C. harmandiana**	hexane extract	0.92
	ethyl acetate extract	0.66
	methanol extract	0.56

* Only the solid residue of *C. harmandiana* was re-extracted with hexane, ethyl acetate and methanol, respectively.

4.2 Determination of chemical compounds of essential oils

The chemical components of essential oils from six plants, including *E. stachyodes*, *E. communis*, *E. griffithii*, *E.* sp., *C. lansium*, and *C. harmandiana*, were analyzed using gas chromatography-mass spectrometry (GC-MS) technique on the HP-5MS column. The identification of each compound was assigned by the comparison of their retention index with relative to a standard mixture of n-alkanes (C_8-C_{22}) under the same experimental conditions by comparing with the MS literature data of Adams, 2007.[21] As well as, the mass spectral database such as NIST 98 (National Institute of Standards and Technology, Gaithersburg, MD, USA) and WILEY7n (Wiley, New York, USA) were also used for spectral matching. The relative percentage amounts of individual components were calculated based on the peak area from total ion chromatography by a computerized integrator.

4.2.1 Chemical constituents of essential oil from *E. stachyodes*

The aerial part of *E. stachyodes* produced clear and pale yellow essential oil. The Total Ion Chromatogram (TIC) of the essential oil is shown in Figure 4.1. *E. stachyodes* oil revealed thirty-five compounds, amounting for 98.79% of the total essential oil content that are identified in Table 4.3. The results indicated that the essential oil was dominated by monoterpene hydrocarbons (47.91%) and oxygenated monoterpenes (46.80%). The monoterpenes consisted mainly of γ-terpinene (24.39%), α-terpinene (6.57%), *p*-cymene (6.09%) and β-(*Z*)-ocimene (3.40%). In addition, the oxygenated monoterpenes were characterized by the presence of carvacrol (43.75%) as main constituents. There was reported that the essential oil of oregano, which is contained carvacrol as main components, can cause cytotoxicity to pulmonary epithelial cells and anti-influenza virus activity.[59] According to the literature,[60,61] bioactivities of carvacrol, which was the major compound of this oil, have been shown strongly antibacterial and antifungal activities. It was also exhibited radical scavenging effect, anti-inflammatory activity, acetylcholine esterase inhibition, white blood cell macrophage stimulant and antispasmodic effects.

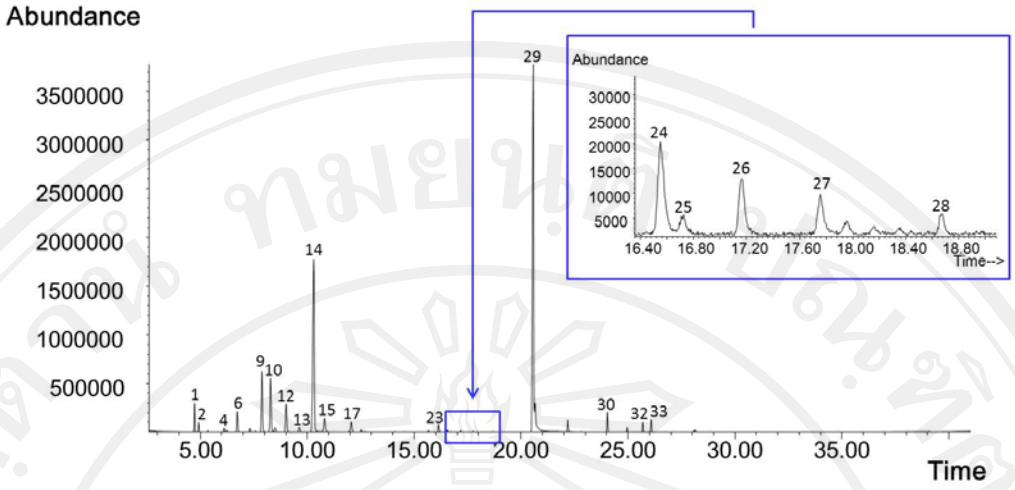


Figure 4.1 Total Ion Chromatogram (TIC) of essential oil from *E. stachyodes*

Table 4.3 Chemical constituents of the essential oil from *E. stachyodes*

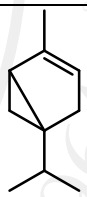
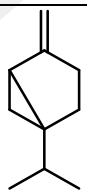
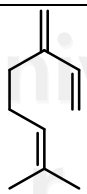
Peak No.	RT (min)	Compounds	RI Exp.	RI Ref.	% ± SD Components	Structures
1	4.711	α-Thujene	922	924	1.78 ± 0.08	
2	4.911	α-Pinene	928	932	0.60 ± 0.04	
3	5.339	Camphene	942	946	0.07 ± 0.01	
4	6.101	Sabinene	967	969	0.31 ± 0.01	
5	6.234	β-Pinene	971	974	0.14 ± 0.00	
6	6.715	Myrcene	987	988	1.84 ± 0.07	

Table 4.3 Chemical constituents of the essential oil from *E. stachyodes* (continued)

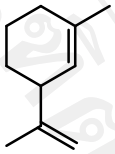
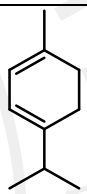
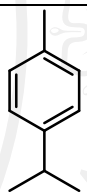
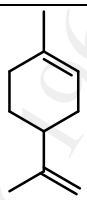
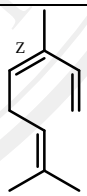
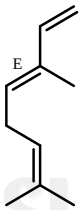
Peak No.	RT (min)	Compounds	RI Exp.	RI Ref.	% ± SD Components	Structures
7	7.311	α-Phellandrene	1004	1002	0.34 ± 0.01	
8	7.558	*iso*-Sylvestrene	1008	1007	0.07 ± 0.01	
9	7.863	α-Terpinene	1013	1014	6.57 ± 0.17	
10	8.273	*p*-Cymene	1021	1020	6.09 ± 0.14	
11	8.482	Limonene	1024	1024	0.49 ± 0.01	
12	9.006	β-*(Z)*-Ocimene	1034	1032	3.40 ± 0.13	
13	9.620	β-*(E)*-Ocimene	1045	1044	0.60 ± 0.02	
14	10.292	γ-Terpinene	1057	1054	24.39 ± 0.55	

Table 4.3 Chemical constituents of the essential oil from *E. stachyodes* (continued)

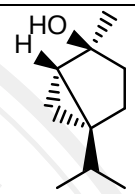
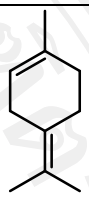
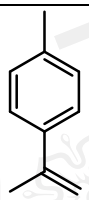
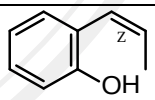
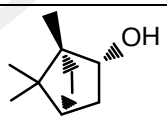
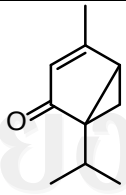
Peak No.	RT (min)	Compounds	RI Exp.	RI Ref.	% ± SD Components	Structures
15	10.796	*cis*-Sabinene hydrate	1066	1065	1.70 ± 0.03	
16	11.954	Terpinolene	1086	1086	0.12 ± 0.00	
17	12.049	*p*-Cymenene	1088	1089	1.11 ± 0.03	
18	12.511	*trans*-Sabinene hydrate	1096	1098	0.24 ± 0.01	
19	12.696	Linalool	1100	1095	0.05 ± 0.01	
20	14.515	*2*-(*1Z*)-Propenyl-phenol	1141	1146	0.03 ± 0.00	
21	15.658	Borneol	1166	1165	0.13 ± 0.00	
22	15.925	Umbellulone	1172	1167	0.02 ± 0.01	

Table 4.3 Chemical constituents of the essential oil from *E. stachyodes* (continued)

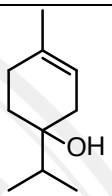
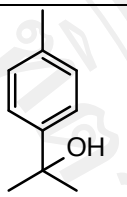
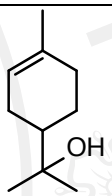
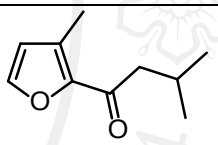
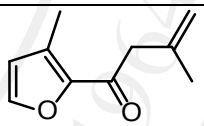
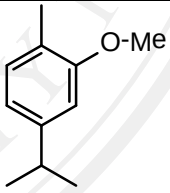
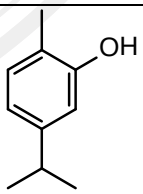
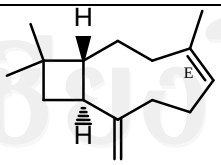
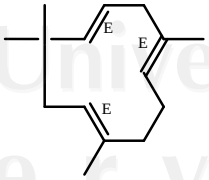
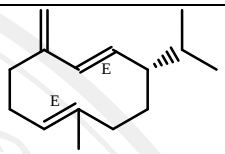
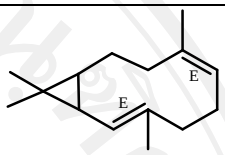
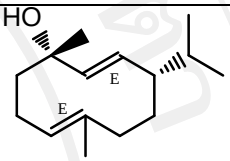
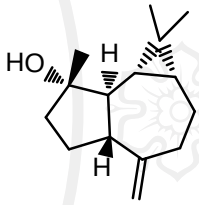
Peak No.	RT (min)	Compounds	RI Exp.	RI Ref.	% ± SD Components	Structures
23	16.130	Terpinen-4-ol	1177	1174	0.70 ± 0.02	
24	16.549	*p*-Cymene-8-ol	1186	1179	0.18 ± 0.00	
25	16.725	*α*-Terpineol	1190	1186	0.03 ± 0.00	
26	17.163	Elsholtzia ketone	1200	1202	0.10 ± 0.00	
27	17.754	*α*-Dehydro elsholtzia ketone	1217	1217	0.06 ± 0.01	
28	18.673	Methyl ether	1244	1241	0.03 ± 0.00	
29	20.568	Carvacrol	1299	1298	43.75 ± 1.30	
30	24.030	(*E*)-Caryophyllene	1418	1417	1.53 ± 0.07	
31	24.954	*α*-Humulene	1453	1452	0.34 ± 0.01	

Table 4.3 Chemical constituents of the essential oil from *E. stachyodes* (continued)

Peak No.	RT (min)	Compounds	RI Exp.	RI Ref.	% ± SD Components	Structures
32	25.687	Germacrene D	1481	1484	0.73 ± 0.01	
33	26.078	Bicyclogermacrene	1496	1500	1.05 ± 0.04	
34	28.063	Germacrene D-4-ol	1577	1574	0.06 ± 0.01	
35	28.125	Spathulenol	1580	1577	0.16 ± 0.02	
		Total identified			98.79 %	
		Monoterpene hydrocarbons			47.91 %	
		Oxygenated monoterpenes			46.80 %	
		Sesquiterpene hydrocarbons			3.64 %	
		Oxygenated sesquiterpenes			0.22 %	
		Acyl furan			0.16 %	
		Others			0.06 %	

RT: Retention Time (min)

RI exp.: Retention Indices from the experiment

RI ref.: Retention Indices from the literature data (Adam, 2007) [21]

% ± SD Components: Relative percentage of components are calculated on GC peak areas on HP-5MS column; values expressed are mean values ± standard deviation of triplicate.

4.2.2 Chemical constituents of essential oil from *E. communis*

The aerial part of *E. communis* gave clear and light yellow essential oil. The Total Ion Chromatogram (TIC) of the essential oil is shown in Figure 4.2. Twenty compounds were identified from *E. communis* oil, accounting for 97.93% of the total oil content that are shown in Table 4.4. The results revealed that major components were trans-4-caranone (2.99%), nerol (2.07%), neral (35.53%), geraniol (1.72%), geranial (45.43%) and (*E*)-caryophyllene (2.76%). This essential oil consisted mainly of oxygenated monoterpenes (91.53%) such as neral (35.53%) and geranial (45.43%). These compounds have a strong lemon odor and they are used in perfumes and flavoring.[62] They have been demonstrated in cytotoxic and antitumoural effects such as HL-60 human promyelocytic leukaemia cells, K562 human erythroleukemic cells, HepG2 human hepatocellular liver carcinoma cells, and HeLa human cervix epithelioid carcinoma cells.[63] Moreover, antioxidant, anticancer, and antimicrobial activity of this compound were also reported.[64]

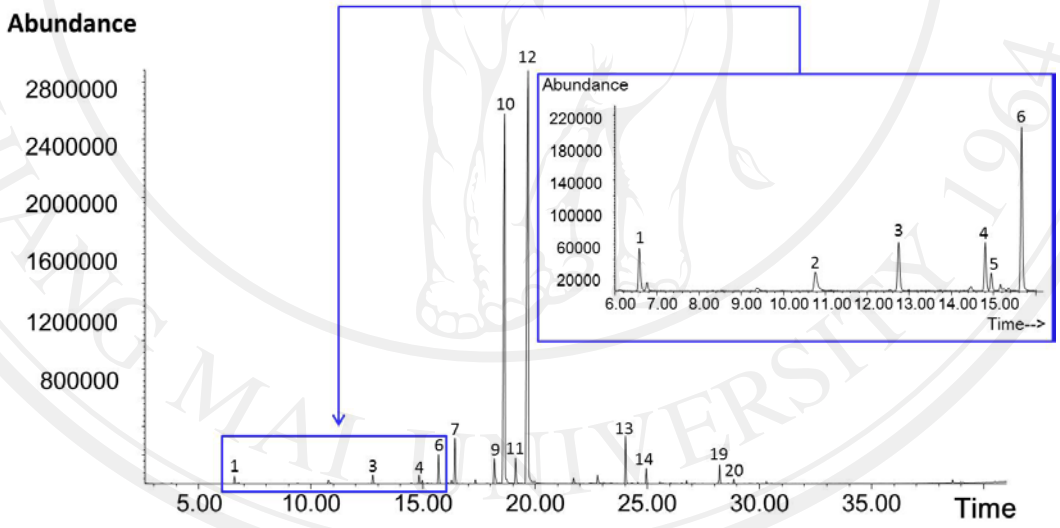


Figure 4.2 Total Ion Chromatogram (TIC) of essential oil from *E. communis*

Table 4.4 Chemical constituents of the essential oil from *E. communis*

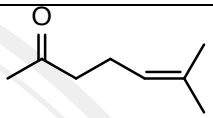
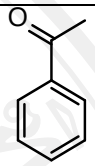
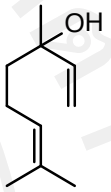
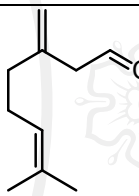
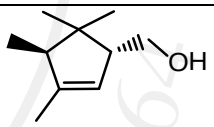
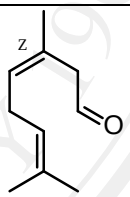
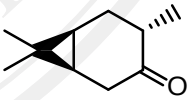
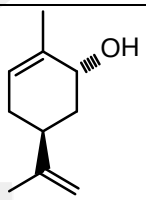
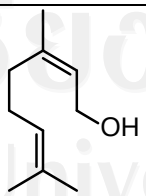
Peak No.	RT (min)	Compounds	RI Exp.	RI Ref.	% ± SD Components	Structures
1	6.568	6-Methyl-5-hepten-2-one	982	981	0.53 ± 0.01	
2	10.763	Acetophenone	1065	1059	0.34 ± 0.03	
3	12.744	Linalool	1101	1095	0.72 ± 0.01	
4	14.806	*exo*-Isocitral	1147	1140	0.57 ± 0.02	
5	14.949	*α-trans*-Necrodol	1150	1144	0.22 ± 0.00	
6	15.668	(*Z*)-Isocitral	1167	1160	1.94 ± 0.04	
7	16.401	*trans-4*-Caranone	1183	1196	2.99 ± 0.04	
8	17.316	*trans*-Carveol	1205	1215	0.32 ± 0.03	
9	18.163	Nerol	1229	1227	2.07 ± 0.06	

Table 4.4 Chemical constituents of the essential oil from *E. communis* (continued)

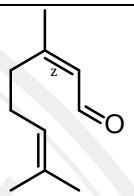
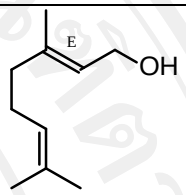
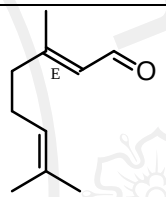
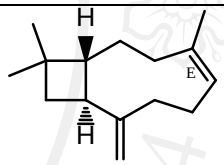
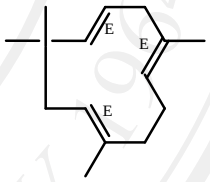
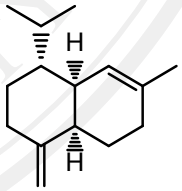
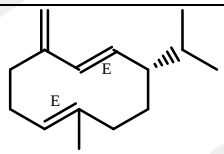
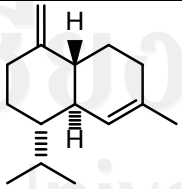
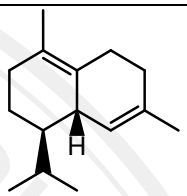
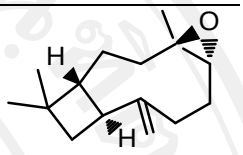
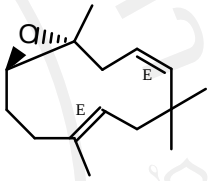
Peak No.	RT (min)	Compounds	RI Exp.	RI Ref.	% ± SD Components	Structures
10	18.620	Neral	1242	1235	35.53 ± 0.66	
11	19.111	Geraniol	1257	1249	1.72 ± 0.05	
12	19.668	Geranial	1273	1264	45.43 ± 0.45	
13	24.035	*(E)*-Caryophyllene	1418	1417	2.76 ± 0.07	
14	24.959	α-Humulene	1453	1452	0.90 ± 0.04	
15	25.563	γ-Muurolene	1476	1478	0.10 ± 0.01	
16	25.678	Germacrene D	1480	1484	0.05 ± 0.00	
17	26.525	γ-Cadinene	1514	1513	0.05 ± 0.00	

Table 4.4 Chemical constituents of the essential oil from *E. communis* (continued)

Peak No.	RT (min)	Compounds	RI Exp.	RI Ref.	% ± SD Components	Structures
18	26.754	δ-cadinene	1523	1522	0.16 ± 0.00	
19	28.225	Caryophyllene oxide	1584	1582	1.19 ± 0.06	
20	28.854	Humulene epoxide II	1610	1608	0.30± 0.02	
		Total identified			97.93 %	
		Monoterpene hydrocarbons			0.00 %	
		Oxygenated monoterpenes			91.53 %	
		Sesquiterpene hydrocarbons			4.03 %	
		Oxygenated sesquiterpenes			1.49 %	
		Acyl furan			0.00 %	
		Others			0.88 %	

RT: Retention Time (min)

RI exp.: Retention Indices from the experiment

RI ref.: Retention Indices from the literature data (Adam, 2007) [21]

% ± SD Components: Relative percentage of components are calculated on GC peak areas on HP-5MS column; values expressed are mean values ± standard deviation of triplicate.

4.2.3 Chemical constituents of essential oil from *E. griffithii*

The pale yellow essential oil was obtained from *E. griffithii*. The chemical compounds were analyzed by GC-MS. The Total Ion Chromatogram (TIC) of the essential oil is displayed in Figure 4.3. Eighteen different compounds were identified, representing 99.01% of the total oil content that are presented in Table 4.5. This essential oil was dominated by acyl furan such as elsholtzia ketone (83.87%) and *β*-dehydroelsholtzia ketone (6.12%). In addition, (*E*)-caryophyllene (5.41%) and *α*-humulene (1.22%) were the minor components in this oil. Previous research[52] reported that the *E. splendens* essential oil consisted mainly of elsholtzia ketone (5.96%) and dehydroelsholtzia ketone (82.46%). This essential oil showed anti-acne-inducing bacterial activity against *P. acnes* and anti-inflammatory effect.

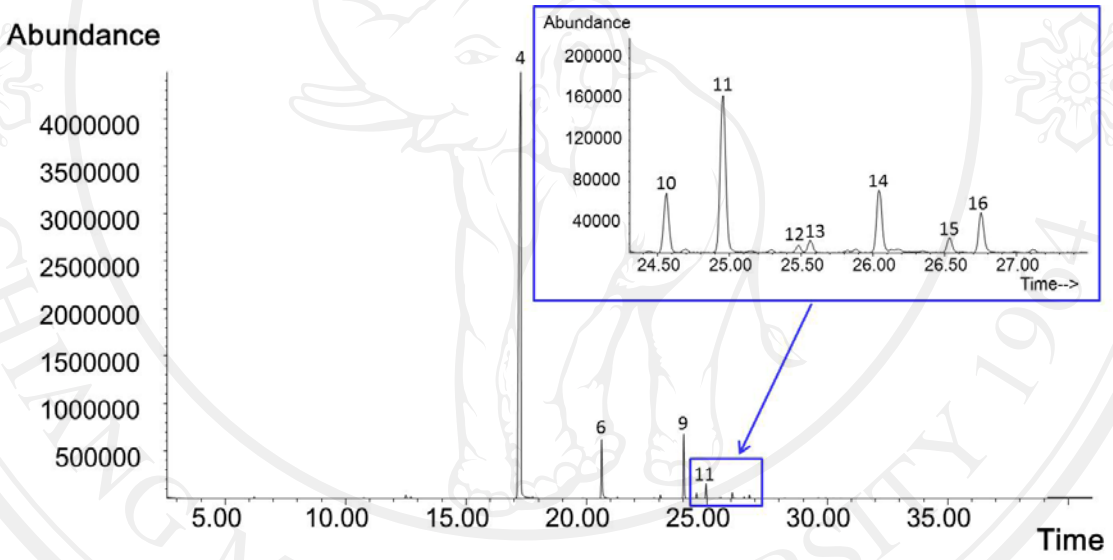


Figure 4.3 Total Ion Chromatogram (TIC) of essential oil from *E. griffithii*

Table 4.5 Chemical constituents of the essential oil from *E. griffithii*

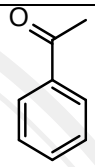
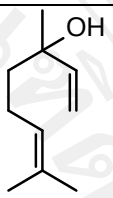
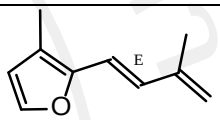
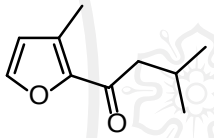
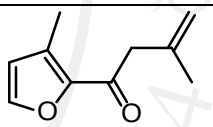
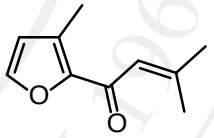
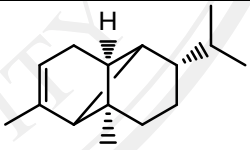
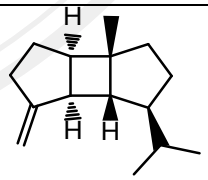
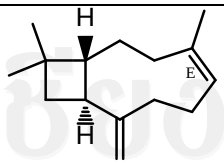
Peak No.	RT (min)	Compounds	RI Exp.	RI Ref.	% ± SD Components	Structures
1	10.739	Acetophenone	1065	1059	0.11 ± 0.01	
2	12.701	Linalool	1100	1095	0.18 ± 0.01	
3	17.096	Shisofuran	1199	1198	0.07 ± 0.02	
4	17.268	Elsholtzia ketone	1203	1202	83.87 ± 0.54	
5	17.758	α-Dehydro-elsholtzia ketone	1218	1217	0.14 ± 0.02	
6	20.625	β-Dehydro-elsholtzia ketone	1300	1302	6.12 ± 0.42	
7	22.801	α-Copaene	1374	1374	0.06 ± 0.01	
8	23.058	β-Bourbonene	1383	1387	0.31 ± 0.02	
9	24.030	*(E)*-Caryophyllene	1418	1417	5.41 ± 0.26	

Table 4.5 Chemical constituents of the essential oil from *E. griffithii* (continued)

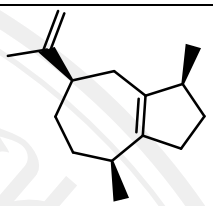
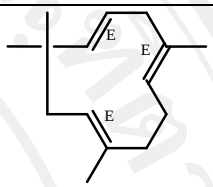
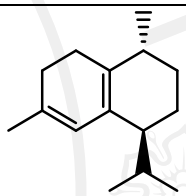
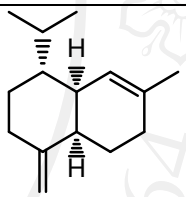
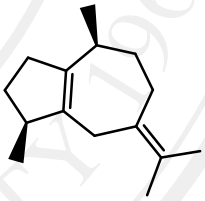
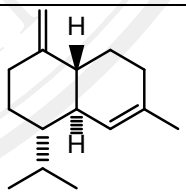
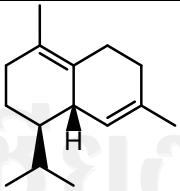
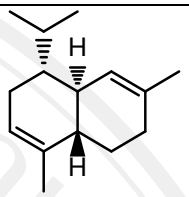
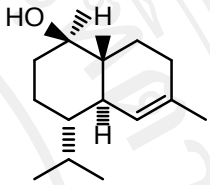
Peak No.	RT (min)	Compounds	RI Exp.	RI Ref.	% ± SD Components	Structures
10	24.563	α-Guaiene	1438	1437	0.42 ± 0.04	
11	24.954	α-Humulene	1453	1452	1.22 ± 0.06	
12	25.477	*trans*-Cadina-1(6),4-diene	1473	1475	0.05 ± 0.00	
13	25.563	γ-Muurolene	1476	1478	0.09 ± 0.00	
14	26.044	β-*cis*-Guaiene	1494	1492	0.48 ± 0.02	
15	26.535	γ-Cadinene	1514	1513	0.10 ± 0.01	
16	26.754	δ-Cadinene	1523	1522	0.29 ± 0.02	

Table 4.5 Chemical constituents of the essential oil from *E. griffithii* (continued)

Peak No.	RT (min)	Compounds	RI Exp.	RI Ref.	% ± SD Components	Structures
17	27.111	α-Cadinine	1538	1537	0.02 ± 0.00	
18	29.920	α-Cadinol	1657	1652	0.05 ± 0.01	
		Total identified			99.01 %	
		Monoterpene hydrocarbons			0.00 %	
		Oxygenated monoterpenes			0.18 %	
		Sesquiterpene hydrocarbons			8.46 %	
		Oxygenated sesquiterpenes			0.05 %	
		Acyl furan			90.20 %	
		Others			0.12 %	

RT: Retention Time (min)

RI exp.: Retention Indices from the experiment

RI ref.: Retention Indices from the literature data (Adam, 2007) [21]

% ± SD Components: Relative percentage of components are calculated on GC peak areas on HP-5MS column; values expressed are mean values ± standard deviation of triplicate.

4.2.4 Chemical constituents of essential oil from *E.* sp.

E. sp. gave clear and light yellow Essential oil. The Total Ion Chromatogram (TIC) of the essential oil is presented in Figure 4.4. The chemical constituents of *E.* sp. oil could be identified as thirty-five compounds, accounting for 85.68% of the total oil compositions that are shown in Table 4.6. This complex mixture consisted mainly of oxygenated monoterpenes (26.89%), sesquiterpene hydrocarbons (17.30%), and acyl furan (38.53%). The results indicated that the oxygenated monoterpenes dominated by linalool (1.49%), neral (9.98%), and geranial (12.03%). Furthermore, the major compounds of sesquiterpene were (*E*)-caryophyllene (9.92%), α-humulene (1.66%), and α-zingiberene (3.80%) whereas the acyl furan were characterized by the presence of elsholtzia ketone (38.21%) as main constituents.

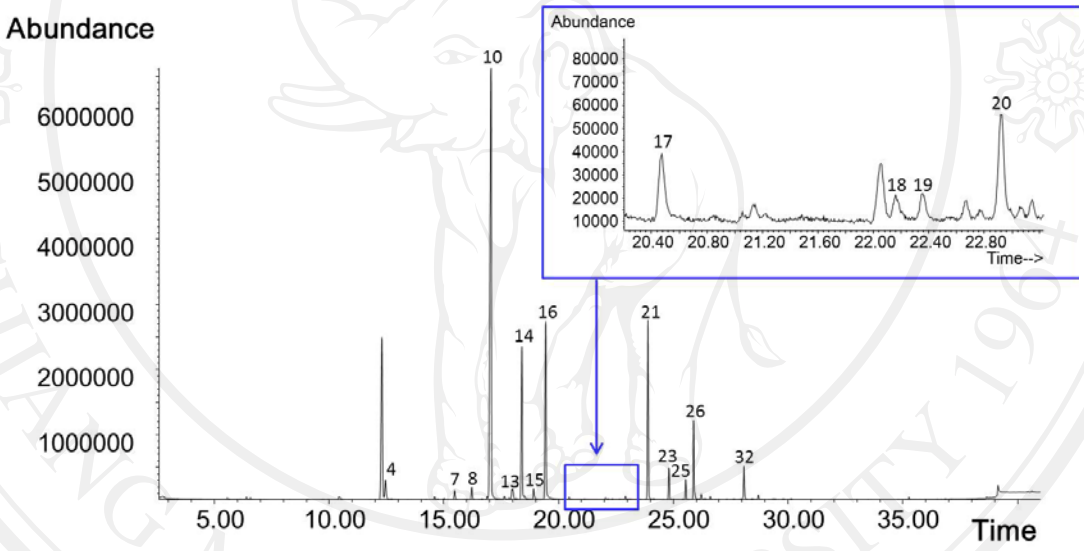


Figure 4.4 Total Ion Chromatogram (TIC) of essential oil from *E.* sp.

Table 4.6 Chemical constituents of the essential oil from *E.* sp.

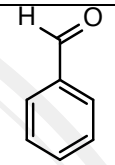
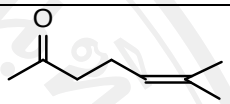
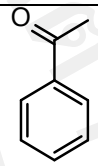
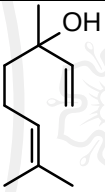
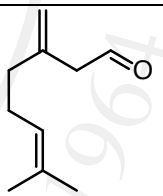
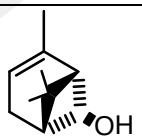
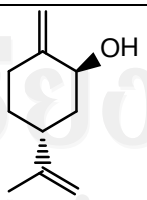
Peak No.	RT (min)	Compounds	RI Exp.	RI Ref.	% ± SD Components	Structures
1	5.563	Benzaldehyde	953	952	0.11 ± 0.01	
2	6.396	6-Methyl-5-hepten-2-one	980	981	0.15 ± 0.01	
3	10.434	Acetophenone*	1062	1059	0.33 ± 0.05	
4	12.458	Linalool*	1098	1095	1.49 ± 0.07	
5	14.606	*exo*-Isocitral	1146	1140	0.18 ± 0.01	
6	15.025	Nerol oxide	1155	1154	0.08 ± 0.00	
7	15.477	*cis*-Chrysanthenol	1166	1160	0.60 ± 0.02	
8	16.225	*p-trans*-Mentha-1(7),8-dien-2-ol	1182	1187	0.75 ± 0.01	

Table 4.6 Chemical constituents of the essential oil from *E.* sp. (continued)

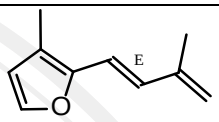
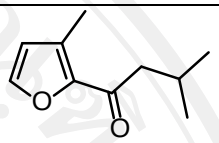
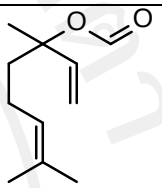
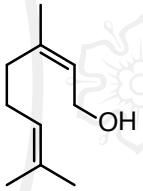
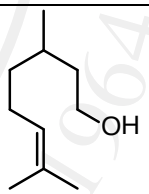
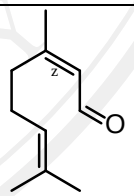
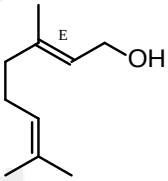
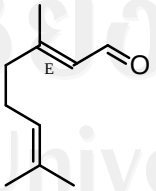
Peak No.	RT (min)	Compounds	RI Exp.	RI Ref.	% ± SD Components	Structures
9	16.892	Shisofuran	1197	1198	0.20 ± 0.01	
10	17.063	Elsholtzia ketone*	1201	1202	38.21 ± 0.16	
11	17.649	Linalool formate	1218	1214	0.19 ± 0.01	
12	17.973	Nerol*	1228	1227	0.42 ± 0.01	
13	18.006	Citronellol	1229	1223	0.58 ± 0.03	
14	18.411	Neral*	1240	1235	9.98 ± 0.05	
15	18.920	Geraniol*	1255	1249	0.72 ± 0.04	
16	19.449	Geranial*	1270	1264	12.03 ± 0.04	

Table 4.6 Chemical constituents of the essential oil from *E.* sp. (continued)

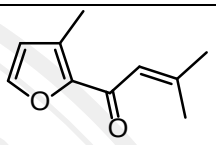
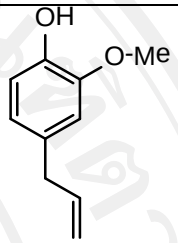
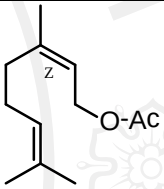
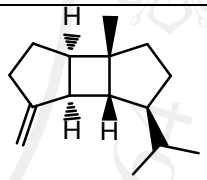
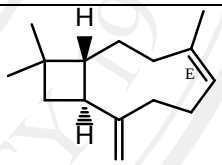
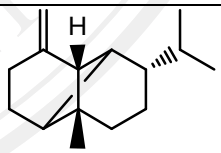
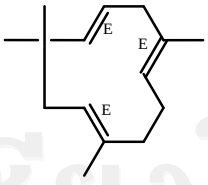
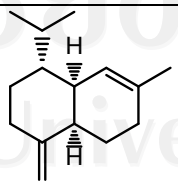
Peak No.	RT (min)	Compounds	RI Exp.	RI Ref.	% ± SD Components	Structures
17	20.473	β-Dehydro elsholtzia ketone	1300	1302	0.12 ± 0.01	
18	22.158	Eugenol	1357	1356	0.06 ± 0.02	
19	22.354	Neryl acetate	1364	1359	0.06 ± 0.00	
20	22.925	β-Bourbonene	1383	1387	0.19 ± 0.01	
21	23.901	(*E*)-Caryophyllene*	1418	1417	9.92 ± 0.03	
22	24.158	β-Copaene	1428	1430	0.06 ± 0.00	
23	24.811	α-Humulene*	1453	1452	1.66 ± 0.03	
24	25.425	γ-Muurolene	1476	1478	0.04 ± 0.01	

Table 4.6 Chemical constituents of the essential oil from *E.* sp. (continued)

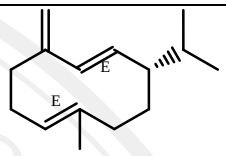
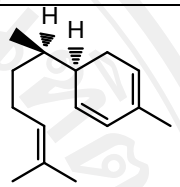
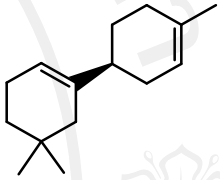
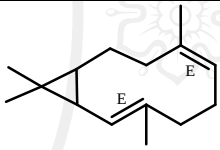
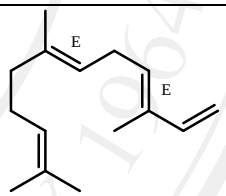
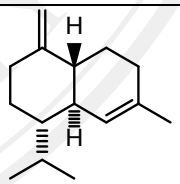
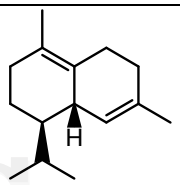
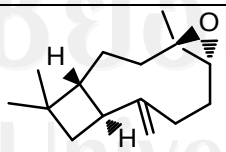
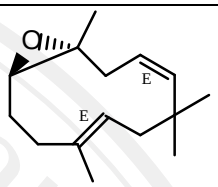
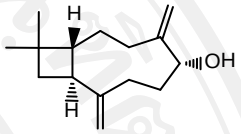
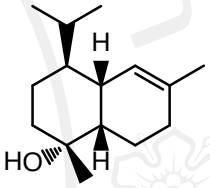
Peak No.	RT (min)	Compounds	RI Exp.	RI Ref.	% ± SD Components	Structures
25	25.539	Germacrene D*	1480	1484	1.02 ± 0.02	
26	25.887	α-Zingiberene	1493	1493	3.80 ± 0.08	
27	25.939	β-Macrocarpene	1495	1499	0.08 ± 0.06	
28	25.968	Bicyclogermacrene	1497	1500	0.06 ± 0.00	
29	26.22	α-(E,E)-Farnesene	1507	1505	0.26 ± 0.03	
30	26.392	γ-Cadinene	1514	1513	0.06 ± 0.01	
31	26.616	δ-Cadinene	1523	1522	0.15 ± 0.01	
32	28.078	Caryophyllene oxide	1584	1582	1.78 ± 0.07	

Table 4.6 Chemical constituents of the essential oil from *E.* sp. (continued)

Peak No.	RT (min)	Compounds	RI Exp.	RI Ref.	% ± SD Components	Structures
33	28.701	Humulene epoxide II	1610	1608	0.24 ± 0.00	
34	29.344	Caryophylla-4(12), 8(13)-dien-5a-ol	1639	1639	0.05 ± 0.02	
35	29.449	*α*-Muurolol	1643	1644	0.05 ± 0.02	
		Total identified			85.68 %	
		Monoterpene hydrocarbons			0.00 %	
		Oxygenated monoterpenes			26.89 %	
		Sesquiterpene hydrocarbons			17.30 %	
		Oxygenated sesquiterpenes			2.13 %	
		Acyl furan			38.53 %	
		Others			0.83 %	

RT: Retention Time (min)

RI exp.: Retention Indices from the experiment

RI ref.: Retention Indices from the literature data (Adam, 2007) [21]

% ± SD Components: Relative percentage of components are calculated on GC peak areas on HP-5MS column; values expressed are mean values ± standard deviation of triplicate.

* The retention time of these components shifted to the lower retention time comparison with *E. stachyodes*, *E. communis*, and *E. griffithii*. However, the results could be explained that the components showed the similar Relative Retention Time (RRT) value with the compounds contained in three species. (The calculation of RRT values used *E*-caryophylene as reference peak presented in Appendix A table A1.3)

4.2.5 Chemical constituents of essential oil from *Elsholtzia* species

The chemical constituents of essential oils from four *Elsholtzia* species; *E. stachyodes*, *E. communis*, *E. griffithii*, and *E.* sp. were clearly different. The results are shown in Table 4.7. The major chemical components of essential oils from these species were presented by monoterpene, oxygenated monoterpene, sesquiterpene, and acyl furan. The dominant compounds of *E. stachyodes* oil were *γ*-terpinene (24.39%) and carvacrol (43.75%). The essential oil of *E. communis* consisted mainly of neral (35.53%) and geranial (45.43%) whereas elsholtzia ketone (83.87%) and *β*-dehydroelsholtzia ketone (6.12%) were the major components of *E. griffithii* oil. Moreover, the essential oil of *E.* sp. was dominated by elsholtzia ketone (38.21%), neral (9.98%), geranial (12.03%), and (*E*)-caryophyllene (9.92%).

Comparison to the previous study,[65] the results exhibited that the main chemical compositions of *E. stachyodes*, *E. communis*, and *E. griffithii* oil were similar in this research whereas the quantity of major compounds were different. However, some chemical compositions in *E. communis* oil in previous study such as *p*-cymene, elsholtzia ketone, piperitenone, geranyl acetate, and caryophyllene oxide were not detected in this study. Likewise, *α*-thujene, *α*-terpinene, *p*-cymene, *γ*-terpinene, and carvacrol were also not detected in *E. griffithii* oil in this study. The reason could be explained that the sample materials were collected from different area at Mae Hong Son province; the earlier study,[65] these plants was collected from the market, while in this study the plants was obtained from cultivated area. Moreover, these might depend on the several environment, agronomic conditions, and the harvest which are mostly correlated to differences in the chemical components of the oils.[60,66]

In this research, the results were according to the many reports[67,68] that the chemical constituents of various *Elsholtzia* species from different origins showed the presence of acyl furans; elsholtzia ketone and dehydro elsholtzia ketone, and oxygenated monoterpenes; linalool, geranyl acetate, carvacrol, neral, and geranial, as representative marker constituents. There are many reports indicated that *E. ciliata*, *E. cristata*, *E. flava*, *E.densa*, *E. patrini*, and *E. splendens* contained rosefuran, elsholtzia ketone, and dehydro elsholtzia ketone as the main components. In addition, neral, geranial, and isocitral were reported as a major compound in *E. blanda*.

Table 4.7 Chemical constituents of the essential oil from *Elsholtzia* species

Compounds	RI Ref.	% components			
		E. stachyodes	*E. communis*	*E. griffithii*	*E.* sp.
Monoterpene hydrocarbons					
α-Thujene	924	1.78	-	-	-
α-Pinene	932	0.60	-	-	-
Camphene	946	0.07	-	-	-
Sabinene	969	0.31	-	-	-
β-Pinene	974	0.14	-	-	-
Myrcene	988	1.84	-	-	-
α-Phellandrene	1002	0.34	-	-	-
iso-Sylvestrene	1007	0.07	-	-	-
α-Terpinene	1014	6.57	-	-	-
p-Cymene	1020	6.09	-	-	-
Limonene	1024	0.49	-	-	-
β-(*Z*)- Ocimene	1032	3.40	-	-	-
β-(*E*)-Ocimene	1044	0.60	-	-	-
γ-Terpinene	1054	24.39	-	-	-
Terpinolene	1086	0.12	-	-	-
p-Cymenene	1089	1.11	-	-	-
Total	**-**	**47.91**	**0.00**	**0.00**	**0.00**
Oxygenated monoterpenes					
cis-Sabinene hydrate	1065	1.70	-	-	-
Linalool	1095	0.24	0.72	0.18	1.49
trans-Sabinene hydrate	1098	0.05	-	-	-
exo-Isocitral	1140	-	0.57	-	0.18
α-trans-Necrodol	1144	-	0.22	-	-
Nerol oxide	1154	-	-	-	0.08

Table 4.7 Chemical constituents of the essential oil from *Elsholtzia* species (continued)

Compounds	RI Ref.	% components			
		E. stachyodes	*E. communis*	*E. griffithii*	*E.* sp.
Oxygenated monoterpenes					
cis-Chrysanthenol	1160	-	1.94	-	0.60
Borneol	1165	0.13	-	-	-
Umbellulone	1167	0.02	-	-	-
Terpinen-4-ol	1174	0.70	-	-	-
p-Cymene-8-ol	1179	0.18	-	-	-
α-Terpineol	1186	0.03	-	-	-
trans-*p*-Mentha-1(7),8-dien-2-ol	1187	-	-	-	0.75
trans-4-Caranone	1196	-	2.99	-	-
trans-Carveol	1215	-	0.32	-	-
Citronellol	1223	-	-	-	0.58
Nerol	1227	-	2.07	-	0.42
Neral	1235	-	35.53	-	9.98
Geraniol	1249	-	1.72	-	0.72
Geranial	1264	-	45.43	-	12.03
Carvacrol	1298	43.75	-	-	-
Eugenol	1356	-	-	-	0.06
Total	**-**	**46.80**	**91.53**	**0.18**	**26.89**
Sesquiterpene hydrocarbons					
α-Copaene	1374	-	-	0.06	-
β-Bourbonene	1387	-	-	0.31	0.19
(*E*)-Caryophyllene	1417	1.53	2.76	5.41	9.92
β-Copaene	1430	-	-	-	0.06
α-Guaiene	1437	-	-	0.42	-
α-Humulene	1452	0.34	0.90	1.22	1.66

Table 4.7 Chemical constituents of the essential oil from *Elsholtzia* species (continued)

Compounds	RI Ref.	% components			
		E. stachyodes	*E. communis*	*E. griffithii*	*E.* sp.
Sesquiterpene hydrocarbons					
trans-Cadina-1(6),4-diene	1475	-	-	0.05	-
γ-Muurolene	1478	-	0.10	0.09	0.04
Germacrene D	1484	0.73	0.05	-	1.02
β-*cis*-Guaiene	1492	-	-	0.48	-
α-Zingiberene	1493	-	-	-	3.80
β-Macrocarpene	1499	-	-	-	0.08
Bicyclogermacrene	1500	1.05	-	-	0.06
α-(*E*,*E*)- Farnesene	1505	-	-	-	0.26
γ-Cadinene	1513	-	0.05	0.10	0.06
δ-Cadinene	1522	-	0.16	0.29	0.15
α-Cadinine	1537	-	-	0.02	-
Total	**-**	**3.64**	**4.03**	**8.46**	**17.30**
Oxygenated sesquiterpenes					
Germacrene D-4-ol	1574	0.06	-	-	-
Spathulenol	1577	0.16	-	-	-
Caryophyllene oxide	1582	-	1.19	-	1.78
Humulene epoxide II	1608	-	0.30	-	0.24
Caryophylla-4(12),8(13)-dien-5a-ol	1639	-	-	-	0.05
α-Muurolol	1644	-	-	-	0.05
α-Cadinol	1652	-	-	0.05	-
Total	**-**	**0.22**	**1.49**	**0.05**	**2.13**

Table 4.7 Chemical constituents of the essential oil from *Elsholtzia* species (continued)

Compounds	RI Ref.	% components			
		E. stachyodes	*E. communis*	*E. griffithii*	*E.* sp.
Acyl furans					
Shisofuran	1198	-	-	0.07	0.20
Elsholtzia ketone	1202	0.10	-	83.87	38.21
α-Dehydroelsholtzia ketone	1217	0.06	-	0.14	-
β-Dehydroelsholtzia ketone	1302	-	-	6.12	0.12
Total	-	**0.16**	**0.00**	**90.21**	**38.53**
Others					
Benzaldehyde	952	-	-	-	0.11
6-Methyl-5-hepten-2-one	981	-	0.53	-	0.15
Acetophenone	1059	-	0.34	0.11	0.33
2-(*1Z*)-Propenyl-phenol	1146	0.03	-	-	-
Linalool formate	1214	-	-	-	0.19
Methyl ether	1241	0.03	-	-	-
Neryl acetate	1359	-	-	-	0.06
Total	-	**0.06**	**0.88**	**0.11**	**0.83**

RI ref.: Retention Indices from the literature data (Adam, 2007) [21]

From these results could classify the chemical compositions of the oils from *Elsholtzia* plants as monoterpene hydrocarbon, oxygenated monoterpene, sesquiterpene hydrocarbon, oxygenated sesquiterpene and acyl furan (Table 4.8). The results exhibited that the essential oil of *E. stachyodes* was dominated by almost as monoterpene hydrocarbon as oxygenated monoterpene, representing 47.91 and 46.80%, respectively. The *E. communis* oil was dominated by significantly oxygenated monoterpene (91.53%) higher than other compounds whereas the oil of *E. griffithii* was possessed of significantly acyl furan (90.20%) higher than other components. In addition, the essential oil of *E.* sp. consisted mainly of oxygenated monoterpene, sesquiterpene hydrocarbon, and acyl furan, accounting for 26.89, 17.30, and 38.53%, respectively.

Table 4.8 The comparison of chemical constituents of essential oil from *Elsholtzia* species

Compounds	% Components			
	E. stachyodes	*E. communis*	*E. griffithii*	*E.* sp.
Total identified	98.79	97.93	99.01	85.68
Monoterpene hydrocarbons	47.91	0.00	0.00	0.00
Oxygenated monoterpenes	46.80	91.53	0.18	26.89
Sesquiterpene hydrocarbons	3.64	4.03	8.46	17.30
Oxygenated sesquiterpenes	0.22	1.49	0.05	2.13
Acyl furans	0.16	0.00	90.21	38.53
Others	0.06	0.88	0.11	0.83

4.2.6 Chemical constituents of essential oil from *C. lansium*

The leaves of *C. lansium* produced clear and light yellow essential oil. The Total Ion Chromatogram (TIC) of the essential oil is presented in Figure 4.5. In *C. lansium* oil, thirty components were identified, accounting for 92.14% of the total oil content that are shown in Table 4.9. The essential oil contained mainly oxygenated sesquiterpenes (47.54%) such as α-(*Z*)-santalol (24.81%), *trans*-sesquisabinene hydrate (10.63%), (*E*)-nerolidol (5.36%), α-(*Z*)-*trans*-bergamotol (2.54%) and bisabolol (1.84%), followed by sesquiterpene hydrocarbons (41.10%) such as (*E*)-caryophyllene (18.66%), α-(*E*,*E*)-farnesene (8.73%), β-(*E*)-farnesene (5.56%), α-humulene (2.18%) and γ-(*Z*)-bisabolene (0.86%). A few of monoterpene hydrocarbons and oxygenated monoterpenes were also presented. In this study indicated that α-(*Z*)-santalol is the major compound in this plant. This compound has been reported the effect to the central nervous system in mice and some tumors.[49] In addition, β-bisabolol and sabinene have been shown larvicidal activity against *Aedes aegypti*, *Culex quinquefasciatus* and *Anopheles Stephensi* larvae.[43]

In this research, the results was significantly different in comparison to the previous study.[49] The essential oil of *C. lansium* collected from Hainan island, China was dominated by oxygenated sesquiterpenes that β-santalol, bisabolol, methyl santalol, ledol and sinensal were reported as the main compounds. Moreover, amounts of sesquiterpene hydrocarbons were found in this study higher than the oil of Hainan island whereas ledol and sinensal were not detected in this study. In addition, volatile compounds of *C. lansium* leaves, which was obtained through headspace sampler, were reported.[69] The volatile components were dominated by sesquiterpene hydrocarbons such as sabinene, β-bisabolene, β-caryophyllene, and α-zingiberene. Santalol and bisabolol were not found in the volatile compound in comparison with the oil in this study.

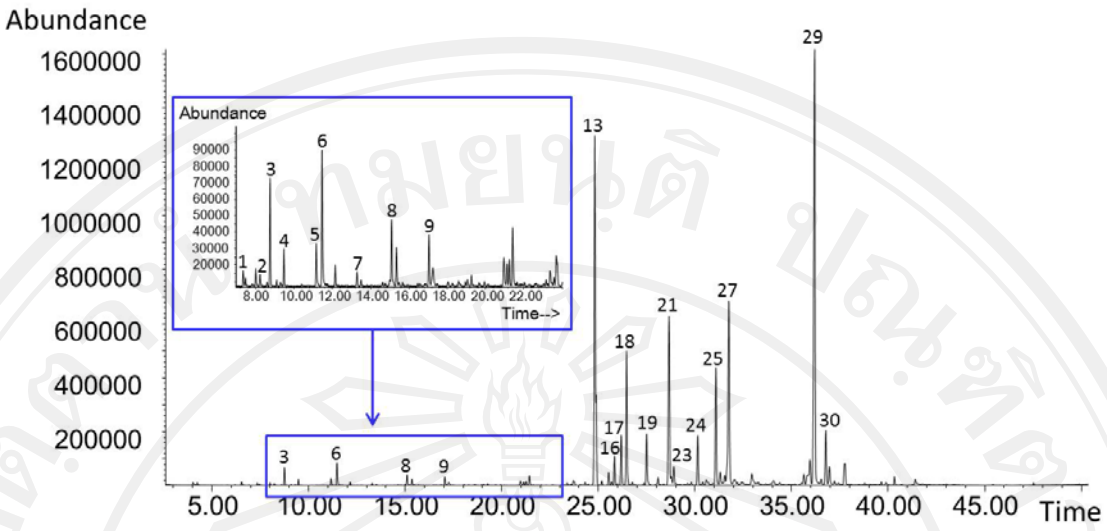


Figure 4.5 Total Ion Chromatogram (TIC) of essential oils from *C. lansium*

Table 4.9 Chemical constituents of the essential oil from *C. lansium*

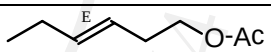
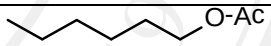
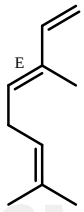
Peak No.	RT (min)	Compounds	RI Exp.	RI Ref.	% ± SD Components	Structures
1	7.968	Hexenyl acetate	1007	1001	0.08 ± 0.01	
2	8.192	Hexyl acetate	1013	1007	0.06 ± 0.00	
3	8.730	Phellandrene	1027	1025	0.58 ± 0.03	
4	9.458	β-(*E*)-Ocimene	1047	1044	0.19 ± 0.01	
5	11.130	Nonanone	1091	1087	0.24 ± 0.02	

Table 4.9 Chemical constituents of the essential oil from *C. lansium* (continued)

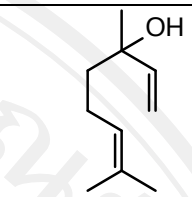
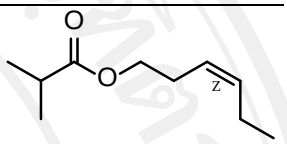
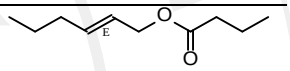
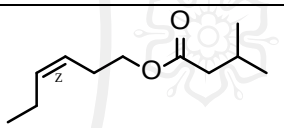
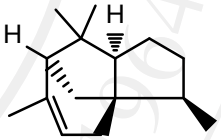
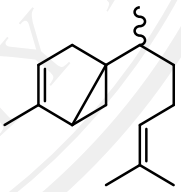
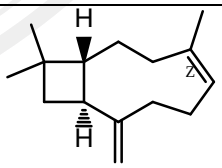
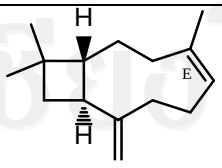
Peak No.	RT (min)	Compounds	RI Exp.	RI Ref.	% ± SD Components	Structures
6	11.430	Linalool	1099	1095	0.85 ± 0.16	
7	13.282	(3Z)-Hexenyl isobutanoate	1143	1142	0.08 ± 0.03	
8	15.111	(2E)-Hexenyl isobutanoate	1187	1193	0.39 ± 0.04	
9	17.039	Hexenyl 3-methyl butanoate	1232	1232	0.31 ± 0.02	
10	23.697	Funebrene	1389	1380	0.17 ± 0.02	
11	23.721	Sesquithujene	1390	1390	0.15 ± 0.02	
12	24.325	(Z)-Caryophyl-lene	1404	1408	0.14 ± 0.00	
13	24.882	(E)-Caryophyl-lene	1417	1417	18.66 ± 0.07	

Table 4.9 Chemical constituents of the essential oil from *C. lansium* (continued)

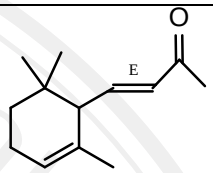
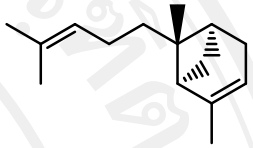
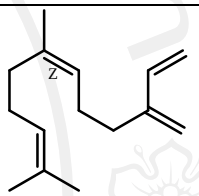
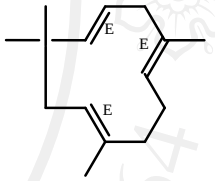
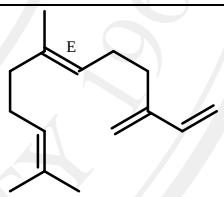
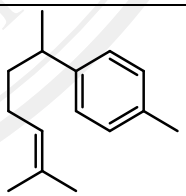
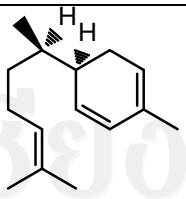
Peak No.	RT (min)	Compounds	RI Exp.	RI Ref.	% ± SD Components	Structures
14	25.187	α-(*E*)-Ionone	1425	1428	0.18 ± 0.01	
15	25.497	Bergamotene	1432	1432	0.54 ± 0.02	
16	25.825	β-(*Z*)-Farnesene	1440	1440	1.17 ± 0.02	
17	26.206	α-Humulene	1449	1452	2.18 ± 0.02	
18	26.421	β-(*E*)-Farnesene	1454	1454	5.56 ± 0.06	
19	27.454	Curcumene	1479	1479	2.29 ± 0.06	
20	28.054	Zingiberene	1493	1493	0.28 ± 0.02	

Table 4.9 Chemical constituents of the essential oil from *C. lansium* (continued)

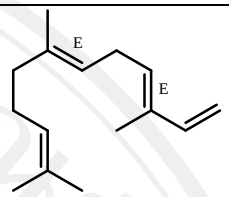
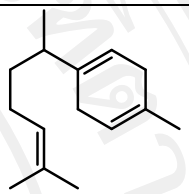
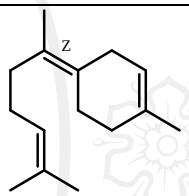
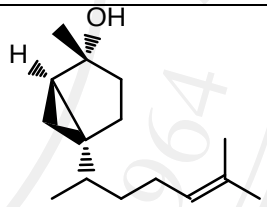
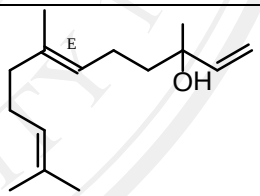
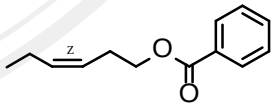
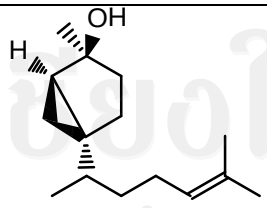
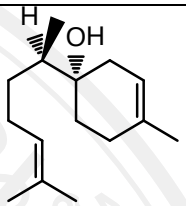
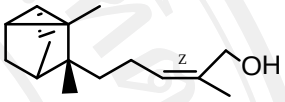
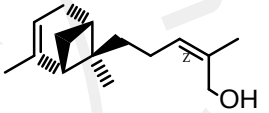
Peak No.	RT (min)	Compounds	RI Exp.	RI Ref.	% ± SD Components	Structures
21	28.597	α-(*E*,*E*)-Farnesene	1506	1505	8.73 ± 0.19	
22	28.787	β-Curcumene	1510	1514	0.36 ± 0.04	
23	28.325	γ-(*Z*)-Bisabolene	1513	1514	0.86 ± 0.08	
24	30.173	*cis*-Sesqui-sabinene hydrate	1541	1542	2.36 ± 0.03	
25	31.073	(*E*)-Nerolidol	1561	1561	5.36 ± 0.04	
26	31.282	(*3Z*)-Hexenyl benzoate	1566	1565	0.55 ± 0.03	
27	31.740	*trans*-Sesquisabinene hydrate	1576	1577	10.63 ± 0.12	

Table 4.9 Chemical constituents of the essential oil from *C. lansium* (continued)

Peak No.	RT (min)	Compounds	RI Exp.	RI Ref.	% ± SD Components	Structures
28	35.940	Bisabolol	1670	1670	1.84 ± 0.02	
29	36.102	*α*-(*Z*)-Santalol	1674	1674	24.81 ± 0.06	
30	36.768	*α*-(*Z*)-*trans*-Bergamotol	1689	1690	2.54 ± 0.02	
		Total identified			92.14 %	
		Monoterpene hydrocarbons			0.77 %	
		Oxygenated monoterpenes			0.85 %	
		Sesquiterpene hydrocarbons			41.10 %	
		Oxygenated sesquiterpenes			47.54 %	
		Fatty acids			0.00 %	
		Others			1.88 %	

RT: Retention Time (min)

RI exp.: Retention Indices from the experiment

RI ref.: Retention Indices from the literature data (Adam, 2007) [21]

% ± SD Components: Relative percentage of components are calculated on GC peak areas on HP-5MS column; values expressed are mean values ± standard deviation of triplicate.

4.2.7 Chemical constituents of essential oil from *C. harmandiana*

The leave of *C. harmandiana* gave clear and colourless essential oil. The Total Ion Chromatogram (TIC) of the essential oil is shown in Figure 4.6. Twenty-nine compounds were identified, accounting for 96.50% of the total oil content that are presented in Table 4.10. The oil consisted of monoterpene hydrocarbons (28.67%) and fatty acids (59.35%) such as *β*-(*Z*)-ocimene (12.41%), *β*-(*E*)-ocimene (13.56%), decanoic acid (15.74%), undecanoic acid (6.86%), and dodecanoic acid (35.65%). Previous researches[70,71] reported that decanoic acid (capric acid) and dodecanoic acid (lauric acid) have been shown powerful bactericidal agents *in vitro*. Moreover, both capric acid and lauric acid exhibited significantly antimicrobial and anti-inflammatory activities against *P. acnes*.

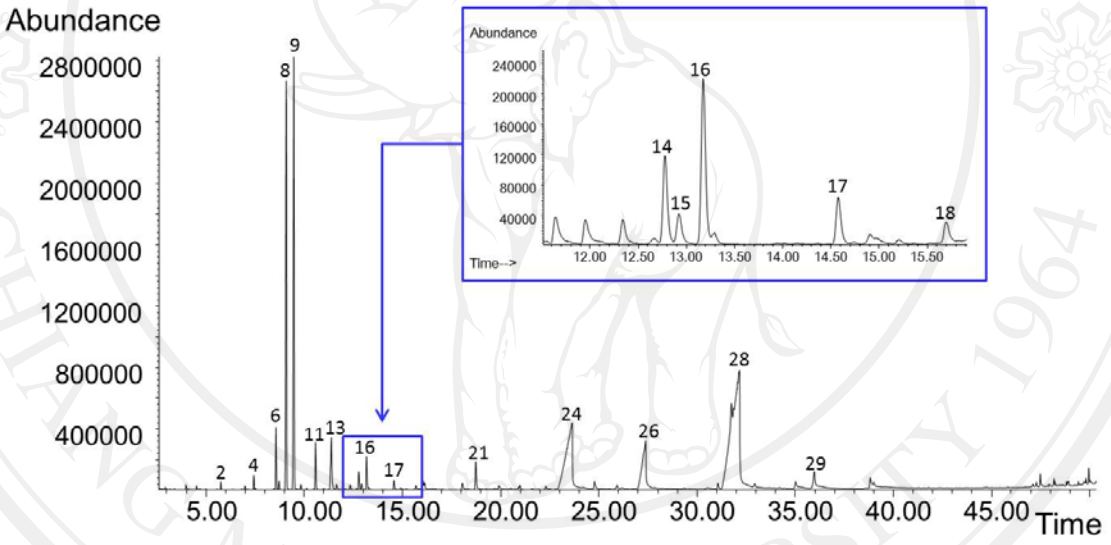


Figure 4.6 Total Ion Chromatogram (TIC) of essential oils from *C. harmandiana*

Table 4.10 Chemical constituents of the essential oil from *C. harmandiana*

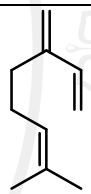
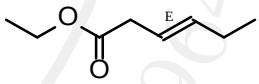
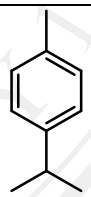
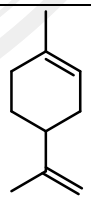
Peak No.	RT (min)	Compounds	RI Exp.	RI Ref.	% ± SD Components	Structures
1	5.553	*α*-Thujene	925	924	0.02 ± 0.00	
2	5.739	*α*-Pinene	932	932	0.17 ± 0.00	
3	6.977	*β*-Pinene	975	974	0.09 ± 0.00	
4	7.420	Myrcene	990	988	0.34 ± 0.01	
5	7.501	Ethyl-*(3E)*-hexenoate	993	1003	0.04 ± 0.00	
6	8.553	*o*-Cymene	1023	1022	1.64 ± 0.03	
7	8.706	Limonene	1027	1024	0.22 ± 0.01	
8	9.077	*β*-(Z)-Ocimene	1037	1032	12.41 ± 0.20	

Table 4.10 Chemical constituents of the essential oil from *C. harmandiana* (continued)

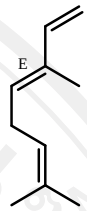
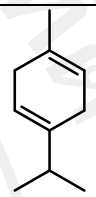
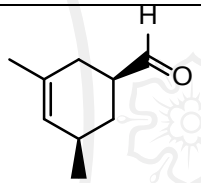
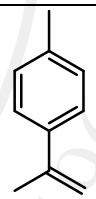
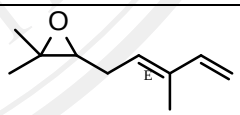
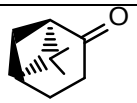
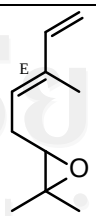
Peak No.	RT (min)	Compounds	RI Exp.	RI Ref.	% ± SD Components	Structures
9	9.463	*β*-(E)-Ocimene	1047	1044	13.56 ± 0.21	
10	9.825	*γ*-Terpinene	1057	1054	0.15 ± 0.00	
11	10.573	*cis*-Vertocitral C	1076	1076	1.35 ± 0.04	
12	11.001	*p*-Cymenene	1088	1089	0.06 ± 0.01	
13	11.377	*α*-Pinene oxide	1098	1099	1.58 ± 0.14	
14	12.773	*(E)*-Epoxy-ocimene	1131	1137	0.56 ± 0.02	
15	12.920	Nopinone	1135	1135	0.20 ± 0.01	
16	13.168	*(E)*-Myroxide	1141	1140	1.06 ± 0.03	

Table 4.10 Chemical constituents of the essential oil from *C. harmandiana* (continued)

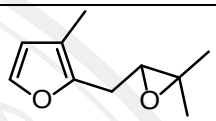
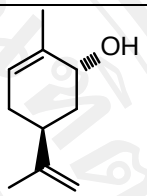
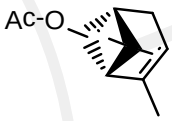
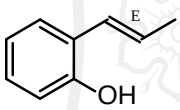
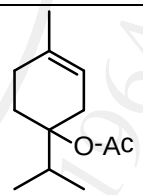
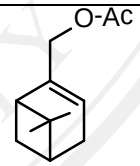
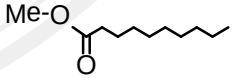
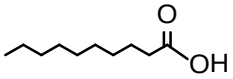
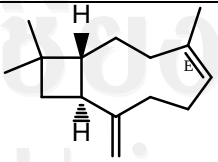
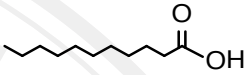
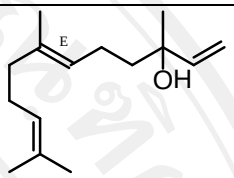
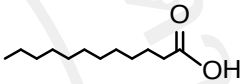
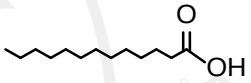
Peak No.	RT (min)	Compounds	RI Exp.	RI Ref.	% ± SD Components	Structures
17	14.573	Rosefuran epoxide	1174	1173	0.34 ± 0.01	
18	15.692	*trans*-Carveol	1203	1215	0.16 ± 0.01	
19	16.001	*trans*-Chrysanthenyl acetate	1210	1235	0.95 ± 0.06	
20	18.744	2-(*1E*)-Propenyl - phenol	1272	1264	1.15 ± 0.05	
21	19.920	Terpinene-4-ol acetate	1299	1299	0.22 ± 0.01	
22	20.906	Myrtenyl acetate	1323	1324	0.06 ± 0.01	
23	20.992	Methyl decanoate	1325	1323	0.20 ± 0.03	
24	23.654	n-Decanoic acid*	1388	-	15.74 ± 0.64	
25	24.787	*(E)*-Caryophyllene	1415	1417	0.38 ± 0.01	

Table 4.10 Chemical constituents of the essential oil from *C. harmandiana* (continued)

Peak No.	RT (min)	Compounds	RI Exp.	RI Ref.	% ± SD Components	Structures
26	27.392	Undecanoic acid*	1478	-	6.86 ± 0.12	
27	31.073	*(E)*-Nerolidol	1561	1561	0.26 ± 0.03	
28	32.173	Dodecanoic acid*	1586	-	35.65 ± 0.81	
29	35.973	Tridecanoic acid*	1671	-	1.09 ± 0.10	
		Total identified			96.50 %	
		Monoterpene hydrocarbons			28.67 %	
		Oxygenated monoterpenes			3.35 %	
		Sesquiterpene hydrocarbons			0.38 %	
		Oxygenated sesquiterpenes			0.26 %	
		Acyl furan			0.34 %	
		Acid compound			59.35 %	
		Others			4.16 %	

RT: Retention Time (min)

RI exp.: Retention Indices from the experiment

RI ref.: Retention Indices from the literature data (Adam, 2007) [21]

% ± SD Components: Relative percentage of components are calculated on GC peak areas on HP-5MS column; values expressed are mean values ± standard deviation of triplicate.

* The Retention Index value of the components have not been reported in database of GCMS therefore, the components were identified by comparing with the MS literature data of NIST 98 with match quality as 95%.

4.2.8 Chemical constituents of essential oil from *Clausena* species

The chemical constituents of essential oils from *Clausena* species; *C. lansium* and *C. harmandiana* were clearly different as shown in Table 4.11. The result revealed that the essential oil of *C. lansium* was dominated by sesquiterpene hydrocarbons (41.10%) and oxygenated sesquiterpenes (47.54%) such as (*E*)-caryophyllene (18.66%), α-(*E*,*E*)-farnesene (8.73%), β-(*E*)-farnesene (5.56%), α-(*Z*)-santalol (24.81%), *trans*-sesquisabinene hydrate (10.63%), and (*E*)-nerolidol (5.36%). Furthermore, A few of monoterpene hydrocarbon were presented whereas fatty acids were not detected in *C. lansium* oil. In contrast, A few of sesquiterpene hydrocarbons and oxygenated sesquiterpenes were presented in *C. harmandiana* oil in comparison to the *C. lansium* oil. Moreover, the essential oil of *C. harmandiana* consisted mainly of fatty acids (59.35%) such as decanoic acid (15.74%), undecanoic acid (6.86%), and dodecanoic acid (35.65%), followed by β-(*Z*)-ocimene (12.41%) and β-(*E*)-ocimene (13.56%) as monoterpene hydrocarbon (28.67%).

Many researches on the other species of *Clausena*, were reported. The various *Clausena* species showed the difference in the chemical compositions. For instance, Cheng *et al*.[44] concluded that the main constituents of *C. excavata* essential oil from the leaves were terpinolene (17.86%), and safrole (75.85%). Likewise, Rajkumar *et al*.[43] reported that the oil from the leaves of *C. dentata* were dominated by sabinene (21.27%), borneol (18.57%), β-bisabolol (17.68%), and biofloratriene (19.61%). Furthermore, the essential oil of *C. suffruticosa* leaves consisted mainly of estragole (58.23%) and anethole (33.20%) reported by Rahman *et al*.[45]

Table 4.11 Chemical constituents of the essential oil from *Clausena* species

Compounds	RI Ref.	% Components	
		C. lansium	*C. harmandiana*
Monoterpene hydrocarbons			
α-Thujene	924	-	0.02
α-Pinene	932	-	0.17
β-Pinene	974	-	0.09
Myrcene	988	-	0.34
o-Cymene	1022	-	1.64
Limonene	1024	-	0.22
Phellandrene	1025	0.58	-
β-(*Z*)-Ocimene	1032	-	12.41
β-(*E*)-Ocimene	1044	0.19	13.56
γ-Terpinene	1054	-	0.15
p-Cymenene	1089	-	0.06
Total	-	**0.77**	**28.67**
Oxygenated monoterpenes			
Linalool	1095	0.85	-
α-Pinene oxide	1099	-	1.58
(*E*)-Epoxy-ocimene	1137	-	0.56
(*E*)-Myroxide	1140	-	1.06
trans-Carveol	1215	-	0.16
Total	-	**0.85**	**3.35**
Sesquiterpene hydrocarbons			
Funebrene	1380	0.17	-
Sesquithujene	1390	0.15	-
(*Z*)-Caryophyllene	1408	0.14	-
(*E*)-Caryophyllene	1417	18.66	0.38
Bergamotene	1432	0.54	-
β-(*Z*)-Farnesene	1440	1.17	-

Table 4.11 Chemical constituents of the essential oil from *Clausena* species (continued)

Compounds	RI Ref.	% Components	
		C. lansium	*C. harmandiana*
Sesquiterpene hydrocarbons			
α-Humulene	1452	2.18	-
β-(*E*)-Farnesene	1454	5.56	-
Curcumene	1479	2.29	-
Zingiberene	1493	0.28	-
α-(*E*,*E*)-Farnesene	1505	8.73	-
β-Curcumene	1514	0.36	-
γ-(*Z*)-Bisabolene	1514	0.86	-
Total	-	**41.10**	**0.38**
Oxygenated sesquiterpenes			
cis-Sesquisabinene hydrate	1542	2.36	-
(*E*)-Nerolidol	1561	5.36	0.26
trans-Sesquisabinene hydrate	1577	10.63	-
Bisabolol	1670	1.84	-
α-(*Z*)-Santalol	1674	24.81	-
α-(*Z*)-*trans*-Bergamotol	1690	2.54	-
Total	-	**47.54**	**0.26**
Fatty acids			
n-Decanoic acid*	-	-	15.74
Undecanoic acid*	-	-	6.86
Dodecanoic acid*	-	-	35.65
Tridecanoic acid*	-	-	1.09
Total	-	**0.00**	**59.35**
Others			
Hexenyl acetate	1001	0.08	-
ethyl-(*3E*)-hexenoate	1003	-	0.04
Hexyl acetate	1007	0.06	-

Table 4.11 Chemical constituents of the essential oil from *Clausena* species (continued)

Compounds	RI Ref.	% Components	
		C. lansium	*C. harmandiana*
Others			
cis-vertocitral C	1076	-	1.35
Nonanone	1087	0.24	-
Nopinone	1135	-	0.20
(*3Z*)-Hexenyl isobutanoate	1142	0.08	-
rosefuran epoxide	1173	-	0.34
(*2E*)-Hexenyl isobutanoate	1193	0.39	-
Hexenyl 3-methyl butanoate	1232	0.31	-
trans-Chrysanthenyl acetate	1235	-	0.95
2-(*1E*)-Propenyl- phenol	1264	-	1.15
Terpinene-4-ol acetate	1299	-	0.22
Myrtenyl acetate	1324	-	0.06
Methyl decanoate	1323	-	0.20
α-(*E*)-Ionone	1428	0.18	-
(*3Z*)-Hexenyl benzoate	1565	0.55	-
Total	**-**	**1.88**	**4.50**

RI ref.: Retention Indices from the literature data (Adam, 2007) [21]

* The Retention Index value of the components have not reported in database of GCMS therefore, the components were identified by comparing with the MS literature data of NIST 98 with match quality as 91%.

Moreover, the chemical composition of *C. lansium* and *C. harmandiana* oils could be classified as monoterpene, oxygenated monoterpene, sesquiterpene, oxygenated sesquiterpene and fatty acids (Table 4.12). The results indicated that the essential oil of *C. lansium* was dominated by almost as sesquiterpene hydrocarbon as oxygenated sesquiterpene, representing 41.10 and 47.54%, respectively while, the *C. harmandiana* oil was characterized by significantly fatty acids (59.35%) followed by monoterpene hydrocarbon (28.67%).

Table 4.12 The comparison of chemical constituents of essential oil from *Clausena* species

Compounds	% Components	
	C.lansium	*C.harmandiana*
Total identified	92.14	96.50
Monoterpene hydrocarbons	0.77	28.67
Oxygenated monoterpenes	0.85	3.35
Sesquiterpene hydrocarbons	41.10	0.38
Oxygenated sesquiterpenes	47.54	0.26
Fatty acids	-	59.35
Others	1.88	4.50

4.3 Antibacterial activity

The antibacterial activity of the essential oils and residual crude extracts from *E. stachyodes*, *E. communis*, *E. griffithii*, *E.* sp., *C. lansium*, and *C. harmandiana* against two strains acne inducing bacteria; *Staphylococcus aureus* ATCC25923 and *Staphylococcus epidermidis* TISTR518 were investigated by the disc diffusion and broth dilution method.

4.3.1 Antibacterial activity of the essential oils

The *in vitro* antibacterial activity against *S. aureus* and *S. epidermidis* of the essential oils from *Elsholtzia* species; *E. stachyodes*, *E. communis*, *E. griffithii*, and *E.* sp., were presented by disc diffusion method. The results are shown in Table 4.13.

The essential oils of *E. stachyodes* showed the highest activity to inhibit the growth of *S. aureus* and *S. epidermidis* (39±1.00 and 41±1.00 mm, respectively) followed by *E. communis*, *E.* sp., and *E. griffithii* oils, respectively. The *E. stachyodes* oil was very effective to inhibit the growth of *S. aureus* and *S. epidermidis* with MIC 0.78 and 1.56 µl/ml, respectively whereas the MBC values were 1.56 and 3.12 µl/ml, respectively. Moreover, the essential oil of *E. communis* also displayed strong antibacterial activity against *S. aureus* and *S. epidermidis* with the same value of MIC and MBC at 3.91 µl/ml. The antibacterial activity of the essential oils was related to the amount of monoterpene hydrocarbons and oxygenated monoterpenes which contained in the oils.[72]

The results in Table 4.7 showed that the essential oil of *E. stachyodes* consisted mainly of carvacrol (43.75%) which is a phenolic compound. The previous study have been reported that the phenolic compounds can across the bacterial cytoplasmic membrane, allowing ions to leave the cytoplasm.[75] This information could explain the strong activity against several microorganisms of the carvacrol.[60,73] Moreover, this oil also dominated by monoterpene hydrocarbons such as *γ*-terpinene, *α*-terpinene, *p*-cymene, limonene, and ocimene which could accumulate in the lipid bilayer and distort the lipid–protein interaction of bacteria.[75] In addition, the type of hydrocarbons group has been reported to influence the activity. For example, *p*-cymene which is a precursor of carvacrol showed a very weak antibacterial effect. The compound swells bacterial cell membranes to a greater extent than carvacrol does. The greater efficiency of *p*-cymene probably facilitates transport of carvacrol across the cytoplasmic membrane into the cell. Hence, a synergistic effect is achieved when these compounds are worked together.[75,76] These results could be the reason for the highest antibacterial activity of the *E. stachyodes* essential oil. Likewise, *E. communis* oil contained a high proportion of oxygenated monoterpene consisting mainly of neral (35.53%) and geranial (45.43%). Indeed, these compounds have been reported that they showed strong antimicrobial activity.[8] This led to the high activity of *E. communis* essential oil.

The essential oil of *E.* sp. showed moderate efficacy to inhibit the growth of *S. aureus* and *S. epidermidis* with the same value of MIC at 15.62 µl/ml whereas *E. griffithii* oil showed weakly activity. The activity of *E.* sp. oil correlated to the amount of oxygenated monoterpenes and sesquiterpene hydrocarbons which contained in the oil. This oil had oxygenated monoterpenes less than *E. communis* and *E. stachyodes* essential oil. Furthermore, it was also presented a little sesquiterpene hydrocarbons such as (*E*)-caryophyllene (9.92%), *α*-humulene (1.66%), *α*-zingiberene (3.80%), and germacrene D (1.02%) which showed moderate antimicrobial activity.[13] In contrast, the essential oil of *E. griffithii* which dominated by acyl furan showed the lowest of antibacterial activity in this study. However, the previous research[52] reported that *E. splendens* essential oil, which mainly contained acyl furan (88.24%), showed anti-acne inducing bacterial activity against *P. acnes* at MIC 0.31 µl/ml. This report suggests that *E. griffithii* oil may inhibit this bacterium.

Table 4.13 Diameter of inhibition zones (mm) mean ± SD, MIC, and MBC of essential oils from *Elsholtzia* species

plants	Bacterial strains					
	S. aureus ATCC25923			*S. epidermidis* TISTR518		
	Clear zone (mm)	MIC (µl/ml)	MBC (µl/ml)	Clear zone (mm)	MIC (µl/ml)	MBC (µl/ml)
E. stachyodes	39 ± 1.00	0.78	1.56	41 ± 1.00	1.56	3.12
E. communis	34 ± 0.50	3.91	3.91	34 ± 1.50	3.91	3.91
E. griffithii	8 ± 1.00	1000	1000	8 ± 1.00	1000	1000
E. sp.	16 ± 0.58	15.62	31.25	17 ± 1.00	15.62	15.62
Erythromycin*	22 ± 0.00	3.91	15.62	20 ± 0.00	7.81	15.62

Erythromycin* (250 µl/ml) were used as positive control.

C. lansium produced a less of essential oil yield. The antibacterial activity of *C. lansium* oil is shown in Table 4.14. The essential oil inhibited the growth of *S. aureus* and *S. epidermidis* with the low concentration at 125 and 62.50 µl/ml, respectively by disc diffusion method. The bacteriostasis of this oil based on the amount of oxygenated sesquiterpenes and sesquiterpene. In part of the essential oil from *C. harmandiana* did not investigate for antibacterial activity in this case because the quantitative limitation of this oil.

Table 4.14 Diameter of inhibition zones (mm) mean ± SD of *C. lansium* oil at different concentrations.

Concentrations	Zones of inhibition (mm)	
(µl/ml)	*S. aureus* ATCC25923	*S. epidermidis* TISTR518
1000	15 ± 1.00	24 ± 1.53
500	12 ± 0.58	21 ± 1.15
250	11 ± 1.00	18 ± 2.00
125	9 ± 0.58	12 ± 1.00
62.50	ND	10 ± 0.58
31.25	ND	ND
15.62	ND	ND
DMSO*	ND	ND
Erythromycin**	22 ± 0.00	20 ± 0.00

DMSO* were used as negative control.

Erythromycin** (250 µl/ml) were used as positive control.

ND: Not Detection

The antibacterial activity of essential oils of *E. stachyodes*, *E. communis*, *E. griffithii*, *E.* sp., and *C. lansium* were different based on the chemical constituents included in each essential oil. These finding were supported by the several researches that the essential oils, which were highly amount of phenols; thymol and carvacrol content, were presented strongly antimicrobial activity.[8,73] The majority of oxygenic compounds in essential oil usually showed higher antibacterial activity than hydrocarbon compounds.[8] The activity rank of essential oil compositions is as follows; phenols > aldehydes > ketones > alcohols > ethers > hydrocarbons.[77]

However, some studies have been reported that whole essential oils have a better antibacterial activity than the mixing of major compounds.[74] These results could infer that the antibacterial activity of essential oil attributed to the synergistic of components in the oil rather than single compound. This can be suggested that the minor components are helpful to the activity and may have a synergistic effect or potentiating influence.[76] Nevertheless, the mechanism of essential oil action towards microorganisms is complex and has not been fully explained. It is generally recognized that the essential components may destroy the bacterial cell wall and cytoplasmic membrane, which results in a leakage of the cytoplasm. Moreover, terpenoids with functional groups such as phenolic alcohols or aldehydes, affect the activities of membrane catalysed enzymes which action on respiratory pathways.[8] In this study, the antibacterial activity against *S. aureus* and *S. epidermidis* of *E. stachyodes* and *E. communis* essential oils demonstrated a strong activity. However, the essential oils of lemongrass and eucalyptus have been shown the higher activity against *S. aureus* with 0.30 and 0.41 µl/ml, respectively. In contrast, tea tree oil which is usually used as ingredient for acne treatment showed the lower antibacterial activity against these bacteria at MIC 2500 and 6300 µl/ml, respectively.[8] This could be indicated that the oils of *E. stachyodes* and *E. communis* have more effective than tea tree oil.

4.3.2 Antibacterial activity of the residual crude extracts

Antibacterial activity of residual crude extracts from *Elsholtzia* and *Clausena* species were determined. All of residual crude extracts presented weakly antibacterial activity in the comparison with essential oils as shown in Table 4.15. The residual crude extracts from *C. lansium* and *C. harmadiana* could inhibit the growth of *S. aureus* and *S. epidermidis*. The activity against two bacterial strains of hexane residual crude extracts of *C. lansium* displays the highest activity with the same value of MIC at 1.56 mg/ml, followed by acetone and ethanol. These finding were supported by the previous research[34-42] that *Clausena* species contained various non-volatile chemical constituents including carbazole alkaloids, flavonoids, coumarins, and amides. These compounds have been reported biological activities such as antimalarial activity, anti-inflammatory activity, antimicrobial activity, and antitumor promoting activity.

Likewise, the residual crude hexane extract of *E. communis* showed anti-acne inducing bacterial activity. *Elsholtzia* species were consisted of flavonoids, coumarins, triterpenoids, steroids, and fatty acids. These compounds have been reported antibacterial activity, antiviral activity, and anti-inflammatory activity.[11] Therefore, the antibacterial effect of the residual crude extracts may relate to these compounds. However, the active compounds of these plant species may decompose from steam distillation process affected weakly antibacterial activity of the residual crude extracts in this study.

Table 4.15 Diameter of inhibition zones (mm) mean ± SD, MIC, and MBC of residual crude extracts (100 mg/ml) from *Elsholtzia* and *Clausena* species

Residual crude extracts	*S. aureus* ATCC25923			*S. epidermidis* TISTR518		
	Clear zone (mm)	MIC (mg/ml)	MBC (mg/ml)	Clear zone (mm)	MIC (mg/ml)	MBC (mg/ml)
E. stachyodes						
hexane	ND	>100	>100	ND	>100	>100
acetone	ND	>100	>100	ND	>100	>100
ethanol	ND	>100	>100	ND	>100	>100
E. communis						
hexane	14 ± 0.00	6.25	12.5	14 ± 0.00	6.25	12.5
acetone	ND	>100	>100	ND	>100	>100
ethanol	ND	>100	>100	ND	>100	>100
E. griffithii						
hexane	ND	>100	>100	ND	>100	>100
acetone	ND	>100	>100	ND	>100	>100
ethanol	ND	>100	>100	ND	>100	>100
***E.* sp.**						
hexane	ND	>100	>100	ND	>100	>100
acetone	ND	>100	>100	ND	>100	>100
ethanol	ND	>100	>100	ND	>100	>100

Table 4.15 Diameter of inhibition zones (mm) mean ± SD, MIC, and MBC of residual crude extracts (100 mg/ml) from *Elsholtzia* and *Clausena* species(continued)

Residual crude extracts	*S. aureus* ATCC25923			*S. epidermidis* TISTR518		
	Clear zone (mm)	MIC (mg/ml)	MBC (mg/ml)	Clear zone (mm)	MIC (mg/ml)	MBC (mg/ml)
C. lansium						
hexane	14 ± 0.00	1.56	1.56	14 ± 0.00	1.56	1.56
acetone	11 ± 0.00	3.12	3.12	12 ± 0.00	3.12	3.12
ethanol	9 ± 0.00	3.12	6.25	12 ± 0.00	3.12	6.25
C. harmandiana						
hexane	11 ± 0.00	100	100	10 ± 0.00	100	100
ethyl acetate	10 ± 0.00	100	100	9 ± 0.00	100	100
methanol	8 ± 0.00	100	100	8 ± 0.00	100	100
Erythromycin*	22 ± 0.00	0.98	3.90	20 ± 0.00	1.95	15.62

Erythromycin* (250 µl/ml) were used as positive control.
ND: Not Detection