



CHAPTER V

DISCUSSION

1. Essential Oil Content in Each Selected Rutaceous Plants.

The 18 Rutaceous plants selected for this study appear to contain their leaf essential oils in highly variable percentage on the fresh weight basis (Table 20). The two highest essential oil containing plants include *Zanthoxylum limonella* and *Clausena anisata* which have the leaf oil content of 4.3% and 3.5%, respectively. The plants with the lowest essential oil content include *Clausena excavata*, *Ferronia limonia* and *Glycosmis pentaphylla*, each contain only 0.1% of fresh weight.

The genus *Citrus* also shows high variation in their essential oil content while *Citrus reticulata* and *Citrus medica* have the oil content of approximately 1.5 %, those of *Citrus hystrix*, *Citrus maxima* and *Citrus aurantifolia* are all less than 0.8%.

Table 20 Essential oil content in the selected Rutaceous plants (in alphabetical order).

Species	Essential oil content (% v/w of fresh weight)
<i>Aegle marmelos</i>	1.8
<i>Atalantia monophylla</i>	0.2
<i>Citrus aurantifolia</i>	0.5
<i>Citrus hystrix</i>	0.8
<i>Citrus maxima</i>	0.4
<i>Citrus medica</i>	1.5
<i>Citrus reticulata</i>	1.5
<i>Clausena anisata</i>	3.5
<i>Clausana excavata</i>	0.1

Table 20 (Continued)

<i>Species</i>	Essential oil content (%v/w of fresh weight)
<i>Ferronia limonia</i>	0.1
<i>Glycosmis pentaphylla</i>	0.1
<i>Hesperethusa crenulata</i>	0.4
<i>Micromelum minutum</i>	0.2
<i>Murraya paniculata</i>	0.9
<i>Paramignya scandens</i>	0.6
<i>Toddalia asiatica</i>	0.5
<i>Triphasia trifolia</i>	0.2
<i>Zanthoxylum limonella</i>	4.3

2. Distribution of Essential Oil Components in the Selected Rutaceous Plants.

Data analysis of various essential oil components found in the 18 Rutaceous species selected for this study showed that overall, there are overall, more than 140 components identified in these 18 essential oils. For the purpose of discussion, The distribution of each component in the selected plants was classified into 5 categories as follows:

1. Those components present in high content of more than 10%
2. Those components present in moderate content of 2-10%
3. Those components present in low content of less than 2%
4. Those components present in only one single plant
5. Those components present in most selected Rutaceous plants

2.1 High- content components

The components present in high content (more than 10%) are shown in Fig. 38. There are 18 components including 12 monoterpenoids, 5 sesquiterpenoids and 1 phenylpropanoids. The most prominent ones are: (*E*)-anethol which is 98.4% of the

Monoterpene

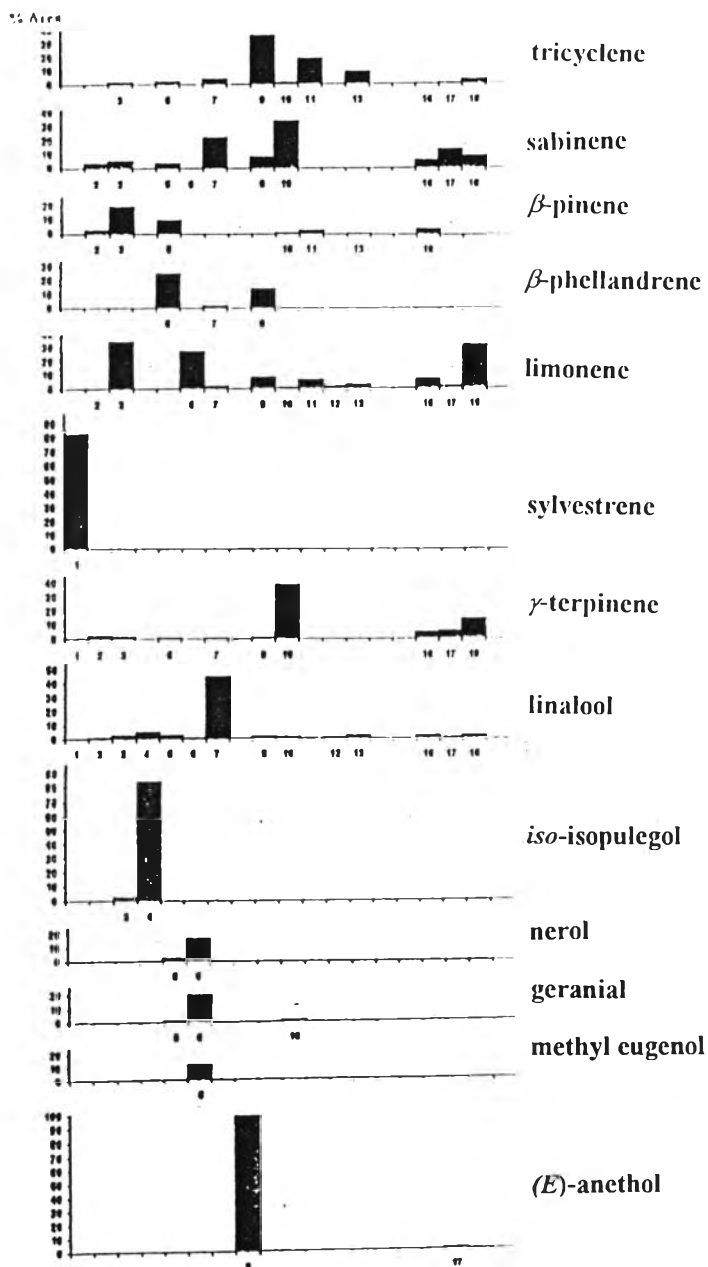
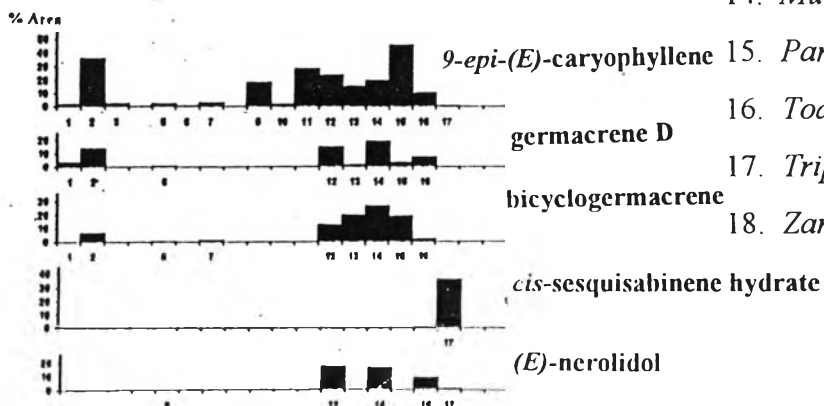


Figure 38 The distribution of the major essential oil components (more than 10 %) in the selected Rutaceous plants.

1. *Aegle marmelos*
2. *Atalantia monophylla*
3. *Citrus aurantifolia*
4. *Citrus hystrix*
5. *Citrus maxima*
6. *Citrus medica*
7. *Citrus reticulata*
8. *Clausena anisata*
9. *Clausena excavata*
10. *Ferronia limonia*
11. *Glycosmis pentaphylla*
12. *Hesperethusa crenulata*
13. *Micromelum minutum*
14. *Murraya paniculata*
15. *Paramignya scanden*
16. *Toddalia asiatica*
17. *Triphasia tripholia*
18. *Zanthoxylum limonella*

Sesquiterpene



essential oil of *Clausena anisata*, iso-isopulegol (83.8%) in the oil of *Citrus hystrix*, sylvestrene (82.5%) in the oil of *Aegle marmelos* and *cis*-sesquisabinene hydrate (35.9%) in that of *Triphasia trifolia*. The other components which are considered to be present in high content are shown in Table 21.

Table 21 Some major essential oil components found in the selected Rutaceous plants.

Component	Percentage in oil	Plant species
(<i>E</i>)-anethol	98.40	<i>Clausena anisata</i>
iso-isopulegol	83.82	<i>Citrus hystrix</i>
sylvestrene	82.49	<i>Aegle marmelos</i>
<i>cis</i> -sesquisabinene hydrate	35.86	<i>Triphasia trifolia</i>
tricyclene	35.35	<i>Clausena excavata</i>
sabinene	33.99	<i>Ferronia limonia</i>
bicyclogermacrene	26.25	<i>Murraya paniculata</i>
β -phellandrene	24.84	<i>Citrus maxima</i>
β -pinene	19.64	<i>Citrus aurantifolia</i>
methyl eugenol	11.47	<i>Citrus medica</i>

2.2 Moderate Content Components

The essential oil components of this group are present in the concentration range of 2-10%. It includes 24 monoterpenoids and 21 sesquiterpenoids (Fig. 39). Some of these components are distributed specifically in a few plants (Table 22) and some are widely distributed in the Rutaceous plants investigated (Table 23).

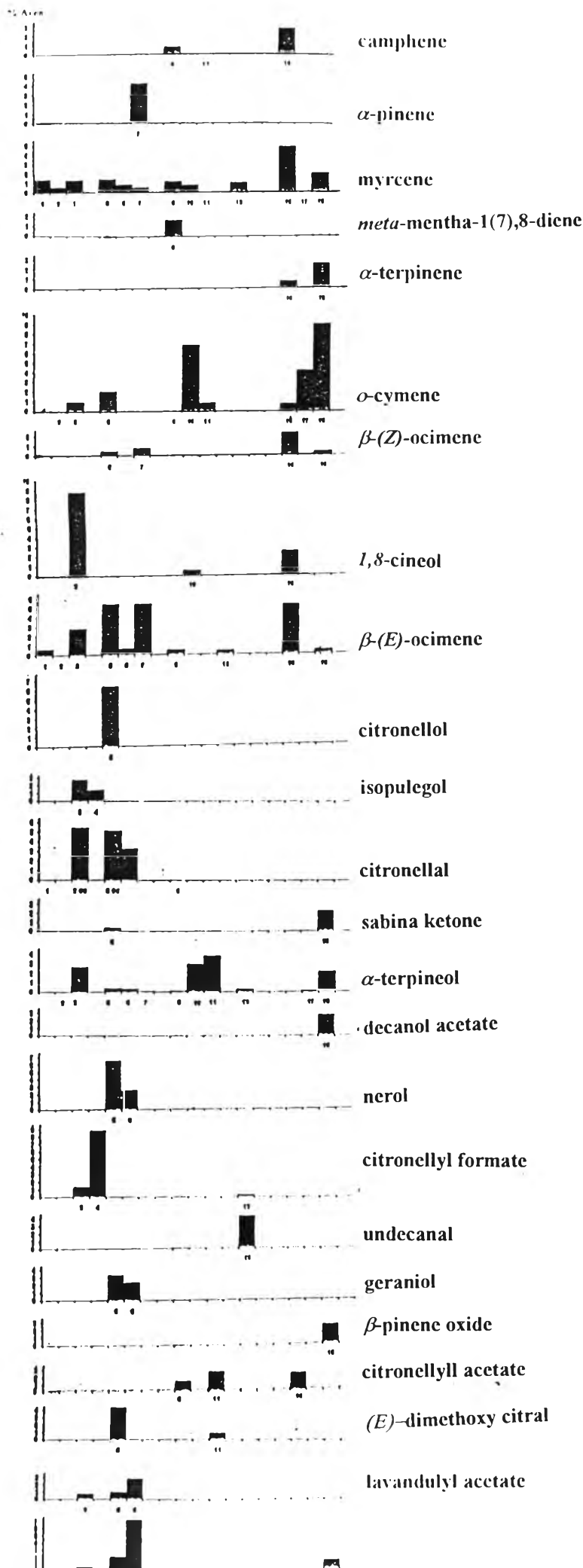


Figure 39 The distribution of the moderate essential oil components (2-10 % in the selected Rutaceous plants.

1. *Aegle marmelos*
2. *Atalantia monophylla*
3. *Citrus aurantifolia*
4. *Citrus hystrix*
5. *Citrus maxima*
6. *Citrus medica*
7. *Citrus reticulata*
8. *Clausena anisata*
9. *Clausena excavata*
10. *Ferronia limonia*
11. *Glycosmis pentaphylla*
12. *Hesperethusa crenulata*
13. *Micromelum minutum*
14. *Murraya paniculata*
15. *Paramignya scanden*
16. *Toddalia asiatica*
17. *Triphasia tripholia*
18. *Zanthoxylum limonella*

Sesquiterpene

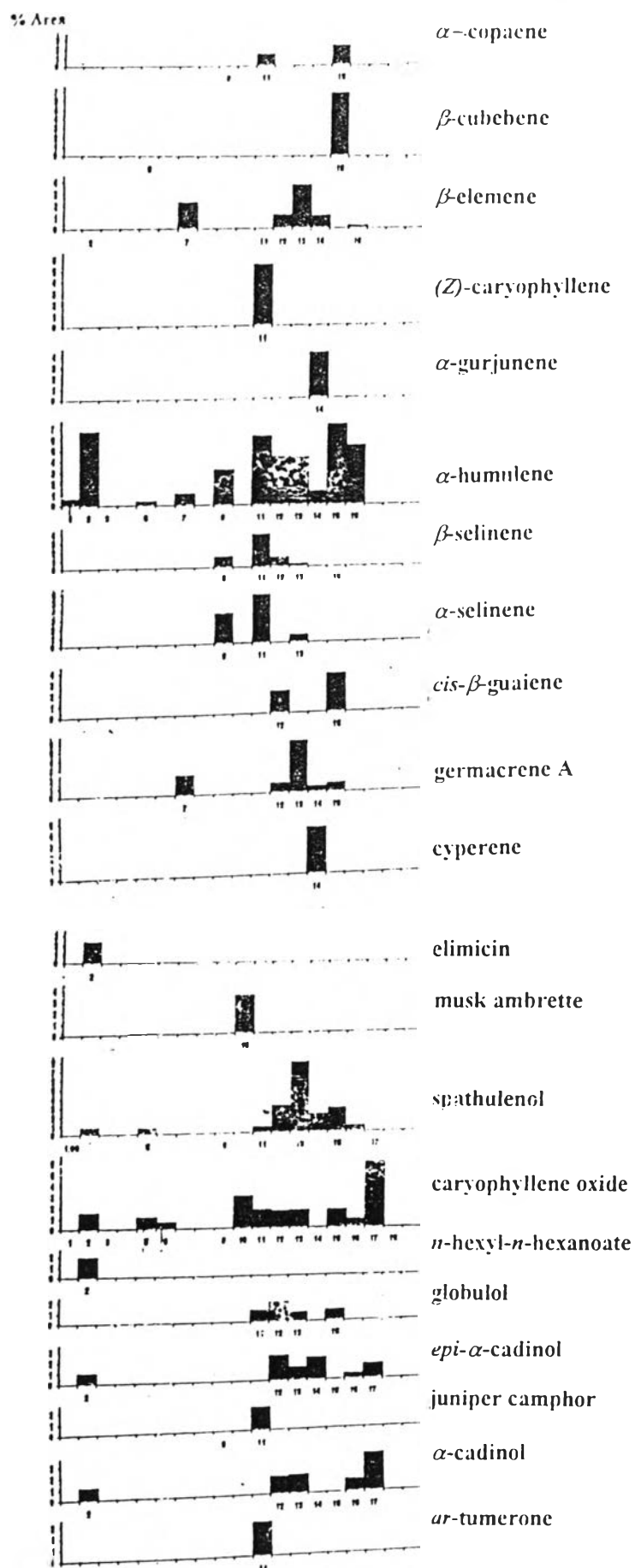


Figure 39 (Continued)

1. *Aegle marmelos*
2. *Atalantia monophylla*
3. *Citrus aurantifolia*
4. *Citrus hystrix*
5. *Citrus maxima*
6. *Citrus medica*
7. *Citrus reticulata*
8. *Clausena anisata*
9. *Clausena excavata*
10. *Ferocnia limonia*
11. *Glycosmis pentaphylla*
12. *Hesperethusa crenulata*
13. *Micromelum minutum*
14. *Murraya paniculata*
15. *Paramignya scandens*
16. *Toddalia asiatica*
17. *Triphasia tripholia*
18. *Zanthoxylum limonioides*

Table 22 The essential oil components present only in one single plant out of the 18 selected Rutaceous plants.

Component	Relative conc. (%)	Species
sylvestrene	82.50	<i>Aegle marmelos</i>
elimicin	2.30	<i>Atalantia monophylla</i>
<i>trans</i> - β -guaiene	0.75	
<i>cis</i> -sesquisabinenehydrate	0.47	<i>Citrus aurantifolia</i>
<i>iso</i> -isopulegol	83.82	<i>Citrus hystrix</i>
citronellal	5.10	<i>Citrus maxima</i>
<i>cis</i> -verbenol	0.94	<i>Citrus medica</i>
<i>trans</i> -verbenol	0.94	
<i>(E)</i> -anethol	98.40	<i>Clausena anisata</i>
methyl chavicol	1.60	
<i>meta</i> -mentha-1(7),8-diene	1.70	<i>Clausena excavata</i>
musk ambrette	3.40	<i>Ferronia limonia</i>
<i>cis</i> -sabinene hydrate	0.47	
piperitol	0.60	
<i>(Z)</i> -caryophyllene	5.40	<i>Glycosmis pentaphylla</i>
<i>ar</i> -turmerone	2.90	
β -eudesmol	0.53	
hinesol	0.74	<i>Hesperethusa crenulata</i>
linalool oxide	0.50	
β -eudesmol acetate	0.45	
guaiol acetate	0.26	
α -gurjunene	5.10	<i>Murraya paniculata</i>
cyperene	4.08	

Table 22 (Continued)

Component	Relative conc. (%)	Species
β -oplopenone	1.74	<i>Micromelum minutum</i>
<i>epi</i> - α -muurolol	1.12	
β -caryophyllene	0.92	
<i>n</i> -undecanol	0.89	
viridiflorene	0.71	
<i>n</i> -decanal	0.66	
(<i>Z</i>)-nerolidol	0.64	
14-hydroxy-9- <i>epi</i> -(<i>E</i>)- caryophyllene	0.64	
β -bisabolenal	0.30	
β -cubebene	6.80	
α -eudesmol acetate	1.34	
aromadendrene	0.86	
<i>epi</i> -cubebol	0.53	
<i>cis</i> -sesquisabinene hydrate	35.90	<i>Triphasia trifolia</i>
γ -elemene	1.67	
<i>ar</i> -curcumene	1.61	
<i>cis</i> -bornyl acetate	1.20	
α -acrorenol	1.16	
α -terpinyl acetate	1.15	
<i>trans</i> -pinocarvyl acetate	0.84	
<i>cis</i> -pinocarvyl acetate	0.82	
2,3,6 trimethyl benzaldehyde	0.71	
humulene epoxide II	0.66	
β -pinene oxide	2.40	<i>Zanthoxylum limonella</i>
2-undecanone	1.08	
<i>trans</i> -verbenol	0.97	
carvone	0.60	
<i>trans</i> -thujone	0.58	
<i>para</i> -mentha-2,4(8)-diene	0.53	
carvacrol	0.52	
<i>cis</i> -thujone	0.31	

2.3 Low-content Components

This group includes 39 monoterpenoids and 46 sesquiterpenoids. Again, these components are distributed either specifically in particular plant species (Table 22) or widely in a number of Rutaceous plants (Table 23).

2.4 Unique Components

This group of essential oil components is classified based on the occurrence of the components detected in only a single plant of the selected Rutaceous plants. Table 22 shows a list of these components. It can be seen that some selected Rutaceous plants have only one unique essential oil component, some have only a few and some have almost ten unique components. The latter includes *Zanthoxylum limonella*, *Triphasia trifolia* and *Micromelum minutum*. The presence of a high number of the unusual essential oil components suggests that the biosynthetic pathways of the C₅ and C₁₀ terpenoids operating in these 3 plants are quite different from the other Rutaceous plants.

The presence of the unique component(s) in the selected Rutaceous plants (Table 22) is also useful as chemical marker(s) for plant identification in the future. It should be noted from Table 22 that all of the selected plants have their own characteristic component(s). Particularly, each of the five *Citrus* species appears to have its own unique component (Table 22).

2.5 Widely distributed components.

Among the over 140 components identified in the essential oils of the 18 selected Rutaceous plants, there are only 9 components found to be distributed in most of the study (Table 23). *9-epi-(E)-Caryophyllene* appears to be present in most plants except *Citrus hystrix*, *Clausena anisata*, *Toddalia asiatica* and *Zanthoxylum limonella*. The absence of this component in *Citrus hystrix* and *Clausena anisata* is not surprising since these plants seem to devote themselves to the formation of isopulegol (84%) and (*E*)-anethol (98.40%), respectively.

Monoterpene

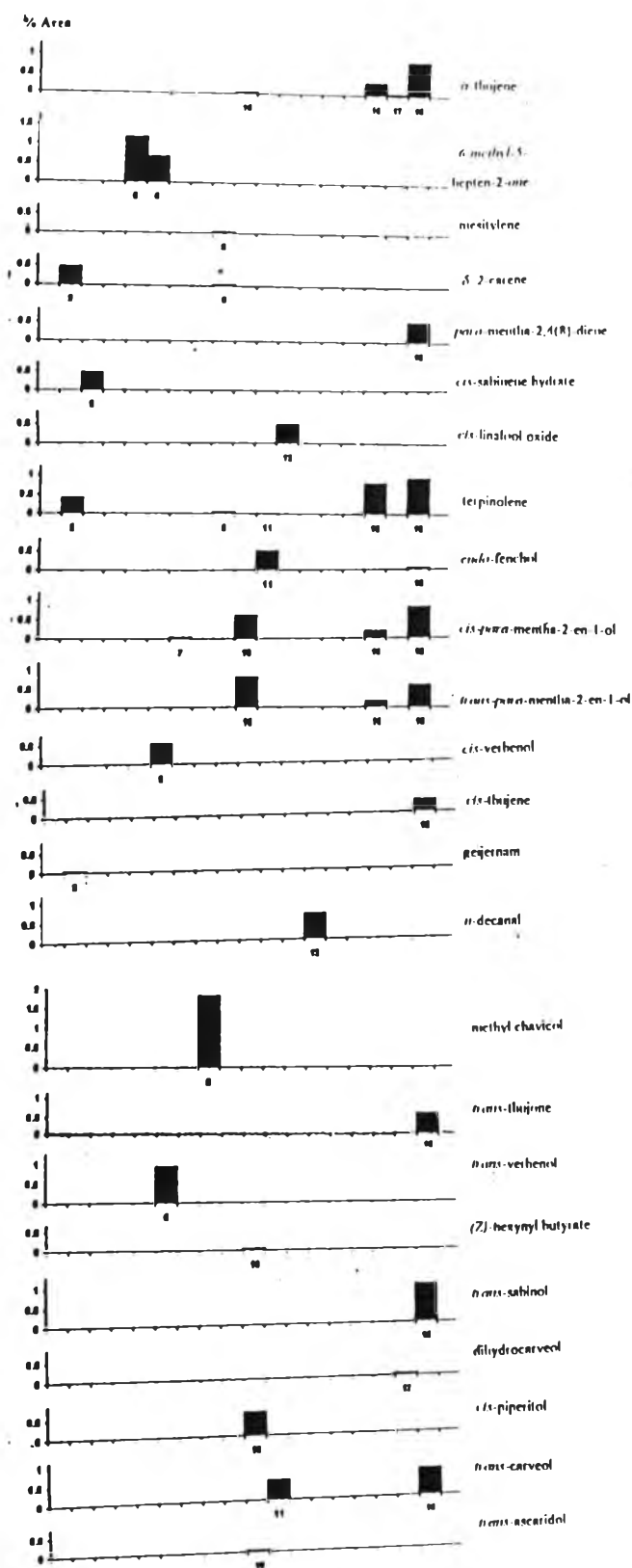


Figure 40 The distribution of the low essential oil components (less than 2 %) in the selected Rutaceous plants.

1. *Aegle marmelos*
2. *Atalantia monophylla*
3. *Citrus aurantifolia*
4. *Citrus hystrix*
5. *Citrus maxima*
6. *Citrus medica*
7. *Citrus reticulata*
8. *Clausena anisata*
9. *Clausena excavata*
10. *Ferronia limonia*
11. *Glycosmis pentaphylla*
12. *Hesperethusa crenulata*
13. *Micromelum minutum*
14. *Murraya paniculata*
15. *Paramignya scanden*
16. *Toddalia asiatica*
17. *Triphasia tripholia*
18. *Zanthoxylum limonella*

Monoterpene

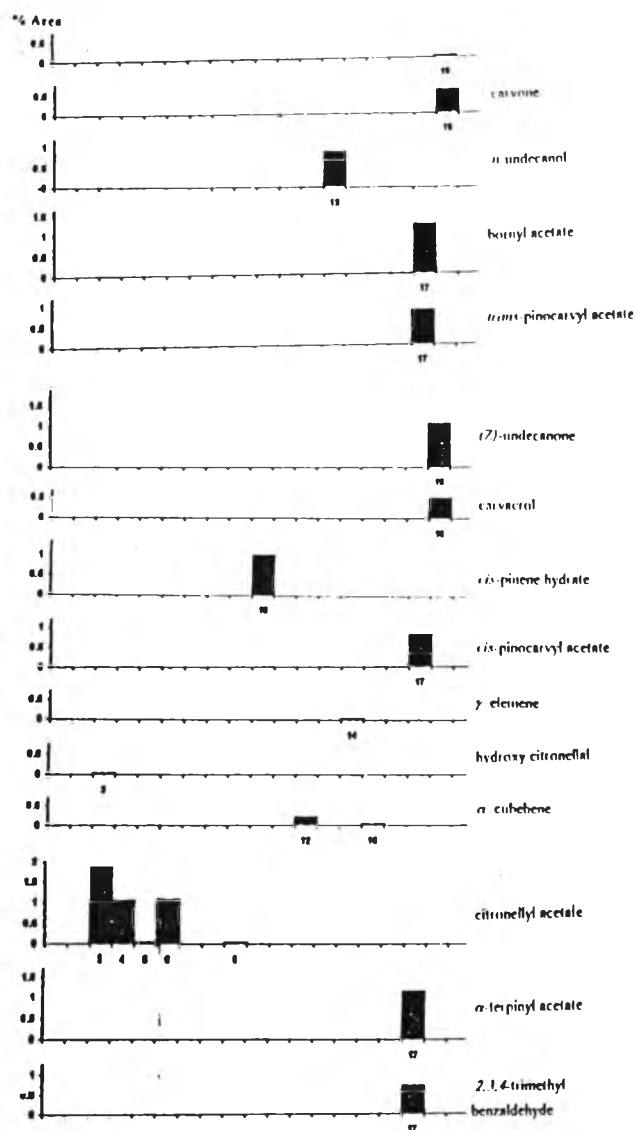


Figure 40 (Continued)

1. *Aegle marmelos*
2. *Atalantia monophylla*
3. *Citrus aurantifolia*
4. *Citrus hystrix*
5. *Citrus maxima*
6. *Citrus medica*
7. *Citrus reticulata*
8. *Clausena anisata*
9. *Clausena excavata*
10. *Ferronia limonia*
11. *Glycosmis pentaphylla*
12. *Hesperethusa crenulata*
13. *Micromelum minutum*
14. *Murraya paniculata*
15. *Paramignya scanden*
16. *Toddalia asiatica*
17. *Triphasia tripholia*
18. *Zanthoxylum limonella*

Sesquiterpene

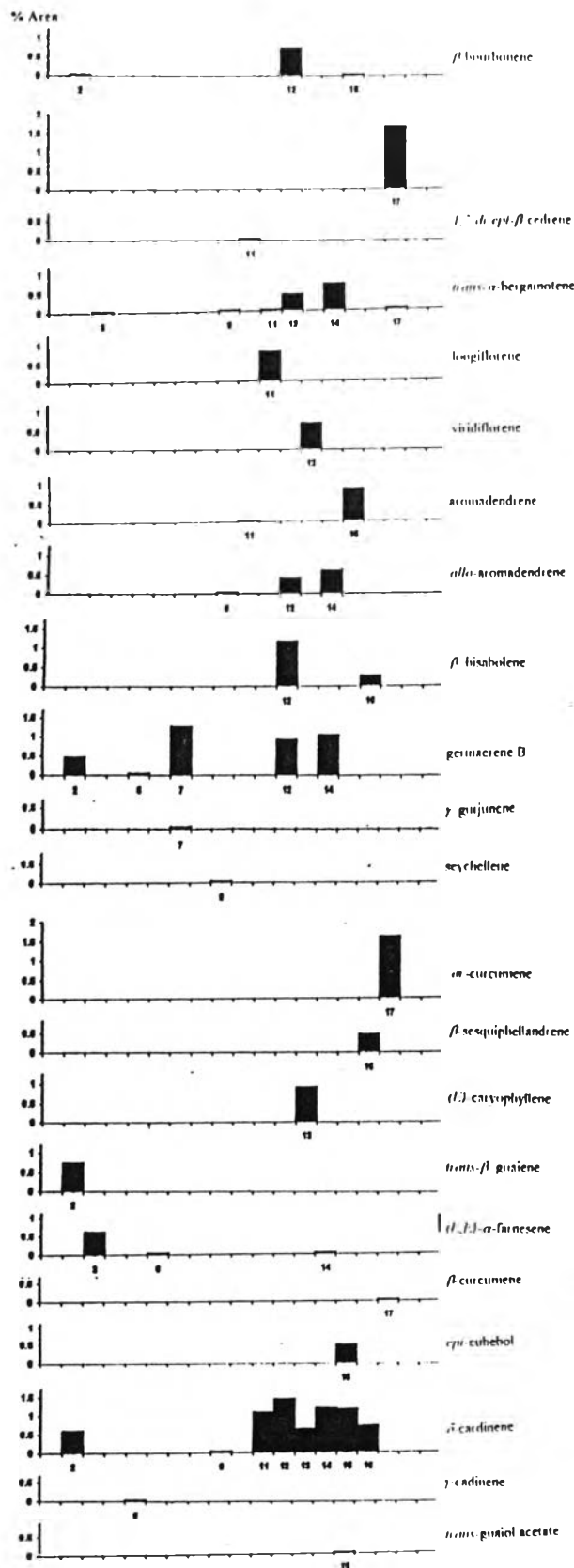


Figure 40 (Continued)

1. *Aegle marmelos*
2. *Atalantia monophylla*
3. *Citrus aurantifolia*
4. *Citrus hystrix*
5. *Citrus maxima*
6. *Citrus medica*
7. *Citrus reticulata*
8. *Clausena anisata*
9. *Clausena excavata*
10. *Ferronia limonia*
11. *Glycosmis pentaphylla*
12. *Hesperethusa crenulata*
13. *Micromelum minutum*
14. *Murraya paniculata*
15. *Paramignya scanden*
16. *Toddalia asiatica*
17. *Triphasia tripholia*
18. *Zanthoxylum limonella*

Sesquiterpene

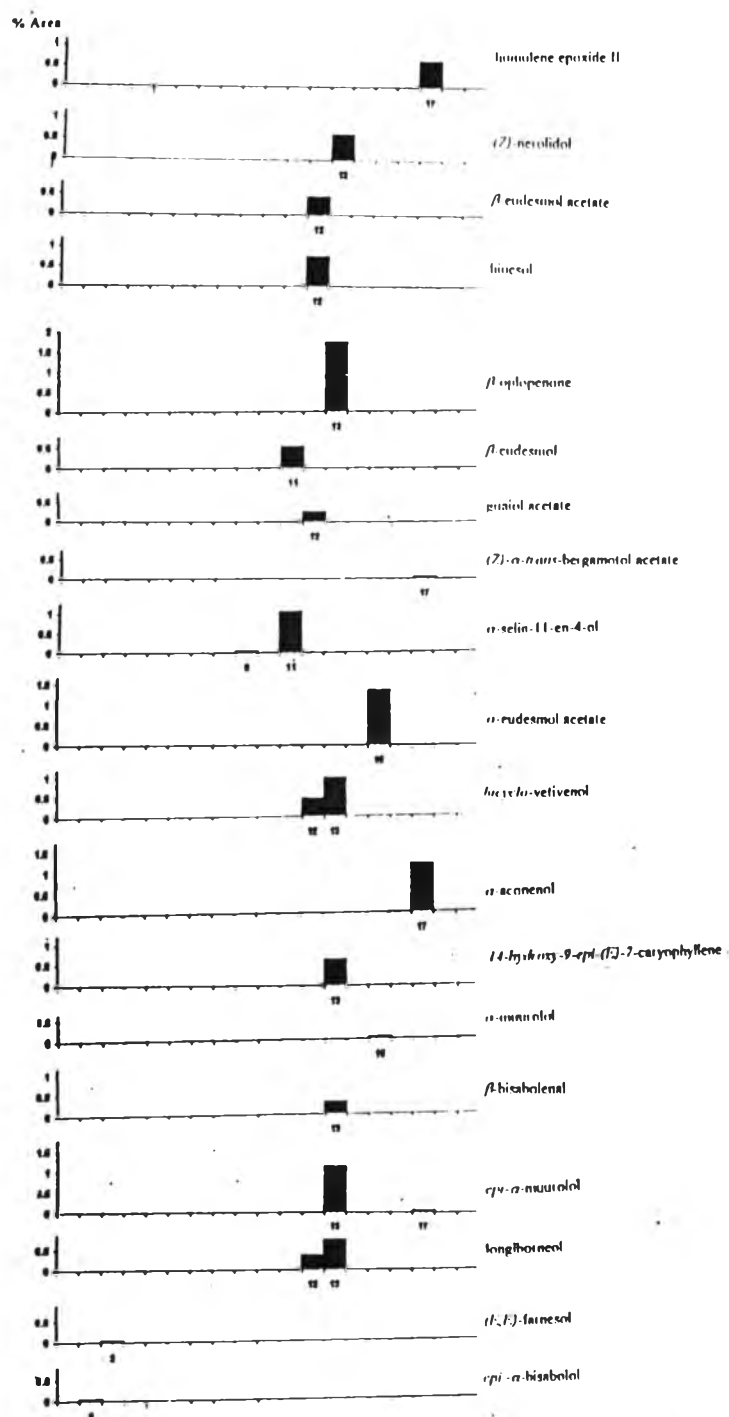


Figure 40 (Continued)

1. *Aegle marmelos*
2. *Atalantia monophylla*
3. *Citrus aurantifolia*
4. *Citrus hystrix*
5. *Citrus maxima*
6. *Citrus medica*
7. *Citrus reticulata*
8. *Clausena anisata*
9. *Clausena excavata*
10. *Ferronia limonia*
11. *Glycosmis pentaphylla*
12. *Hesperethusa crenulata*
13. *Micromelum minutum*
14. *Murraya paniculata*
15. *Paramignya scanden*
16. *Toddalia asiatica*
17. *Triphasia tripholia*
18. *Zanthoxylum limonella*

Table 23 The essential oil components that are widely distributed in the selected Rutaceous plants.

Plant species	Relative conc.(%)								
	sabinene	limonene	linalool	9-epi-(E)-caryophyllene	myrcene	(E)- β -ocimene	α -terpineol	α -humulene	caryophyllene oxide
<i>Aegle marmelos</i>	8.93	-	0.39	1.65	1.33	0.59	-	0.60	t
<i>Atalantia monophylla</i>	3.39	t	1.24	35.67	0.54	t	t	6.66	1.46
<i>Citrus aurantifolia</i>	5.07	33.88	2.68	2.22	1.29	-	2.62	t	t
<i>Citrus hystrix</i>	-	-	4.95	-	-	-	-	-	-
<i>Citrus maxima</i>	3.46	-	2.71	2.08	1.36	5.35	0.36	0.35	1.03
<i>Citrus medica</i>	0.28	27.05	0.95	0.48	0.81	0.63	0.33	-	0.57
<i>Citrus reticulata</i>	22.25	2.04	47.77	2.82	0.52	5.36	t	1.03	-
<i>Clausena anisata</i>	-	-	-	-	-	-	-	-	-
<i>Clausena excavata</i>	8.08	8.10	1.56	18.17	1.14	0.48	t	3.18	t
<i>Ferronia limonia</i>	33.99	0.73	1.10	1.67	0.75	-	2.91	-	2.88
<i>Glycosmis pentaphylla</i>	-	6.21	-	28.55	t	-	3.80	6.22	1.61
<i>Hesperethusa crenulata</i>	-	1.50	0.79	23.92	-	-	-	4.30	-
<i>Micromelum minutum</i>	-	2.58	1.90	15.65	0.95	-	0.26	4.23	1.56
<i>Murraya paniculata</i>	-	-	-	20.05	-	-	-	1.16	t
<i>Paramignya scandens</i>	-	-	-	45.98	-	-	-	7.22	1.58
<i>Toddalia asiatica</i>	5.57	6.82	2.24	-	4.70	5.28	-	5.32	0.68
<i>Triphasia trifolia</i>	13.52	1.57	0.75	0.44	t	-	-	-	5.82
<i>Zanthoxylum limonella</i>	9.13	31.09	2.48	-	1.92	0.38	2.10	-	t

t = Trace

3. Comparison of Essential Oil Composition among *Citrus* species.

Fig. 41 shows the distribution of various essential oil components in 5 *Citrus* species including *C. aurantifolia*, *C. hystrix*, *C. maxima*, *C. medica* and *C. reticulata*. From this figure, it can be seen that linalool is the only component present in all the 5 species. Particularly in *C. reticulata*, linalool appears to be the major component, with the content of almost 50 %.

On the other hand, some major components seem to be present specifically in individual species. For example, 1,8-cineol (8.75%) is present only in *C. aurantifolia*, citronellol (6.32%) and (E)-dimethoxy citral (4.30%) only in *C. maxima*, methyl eugenol (11.47%) in *C. medica* and α -pinene (4.20%) and β -elemene (2.22 %) are present only in *C. reticulata*. These components may potentially be used as markers for the identification of these individuals.

It should also be noted that the terpenoid group of acyclic monoterpenoids such as nerol, geraniol, neral, geranial, citronellal, myrcene are mostly present in the species of *C. maxima*, *C. medica* and , in lesser components, *C. aurantifolia*. On the other hand, only a few of cyclic monoterpenoids (eg. linalool and (E)- β -ocimene) are present in *C. reticulata*.

Among the 5 *Citrus* species, *C. hystrix* seems to be the most unique one. It contains very high content of *iso*-isopulegol (83.82%). *iso*-Isopulegol belongs to the terpenoid group of menthane. Unlike the other *Citrus* species, *C. hystrix* contains only two acyclic monoterpenoids: linalool and citronellyl acetate.

C. reticulata also shows relatively unique essential oil composition. It is the only *Citrus* species containing sabinene (2%). The plant also has more sesquiterpenoids than the other *Citrus* species.

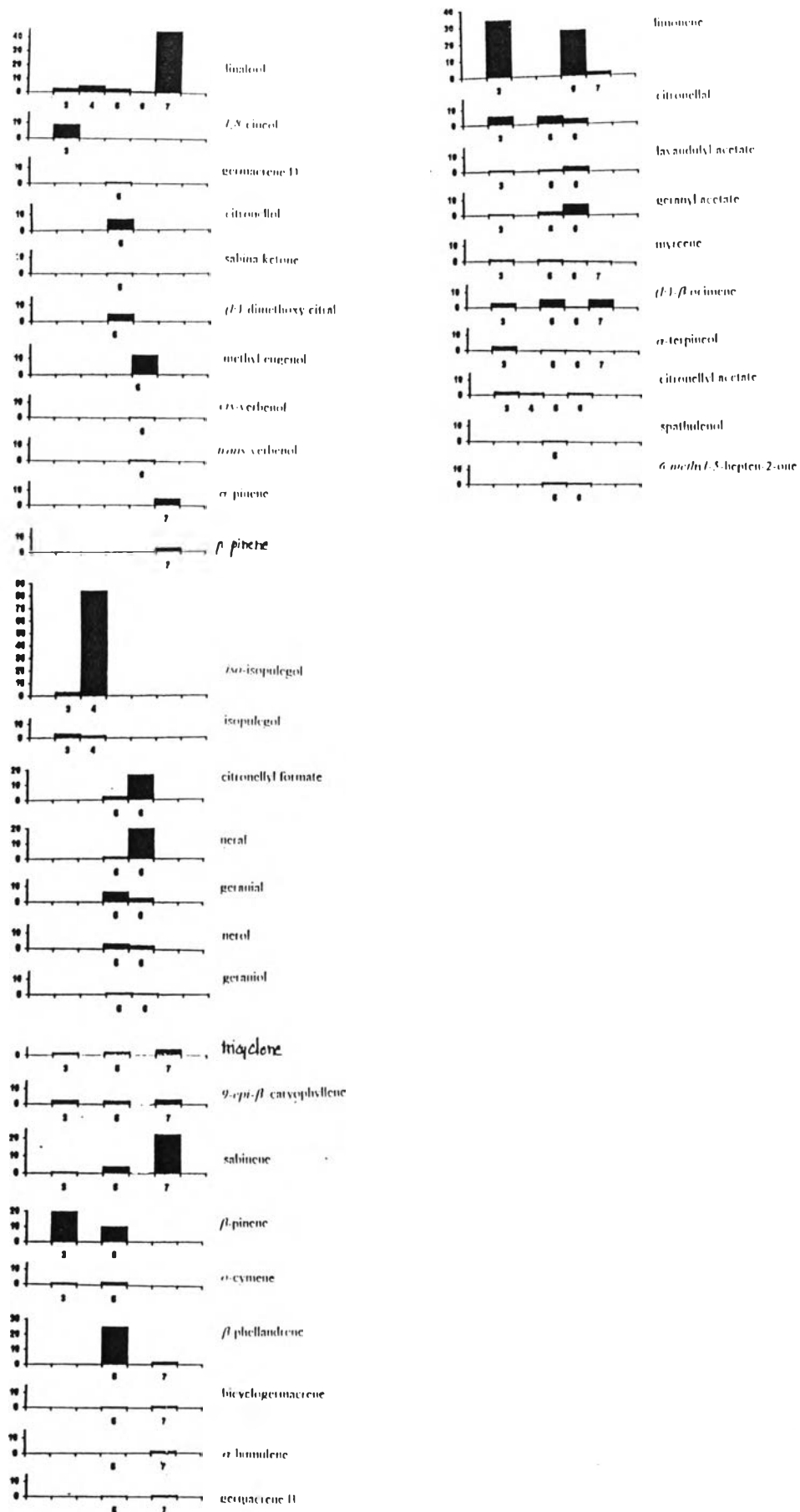


Figure 41 The distribution of the essential oil components in the *Citrus* species.

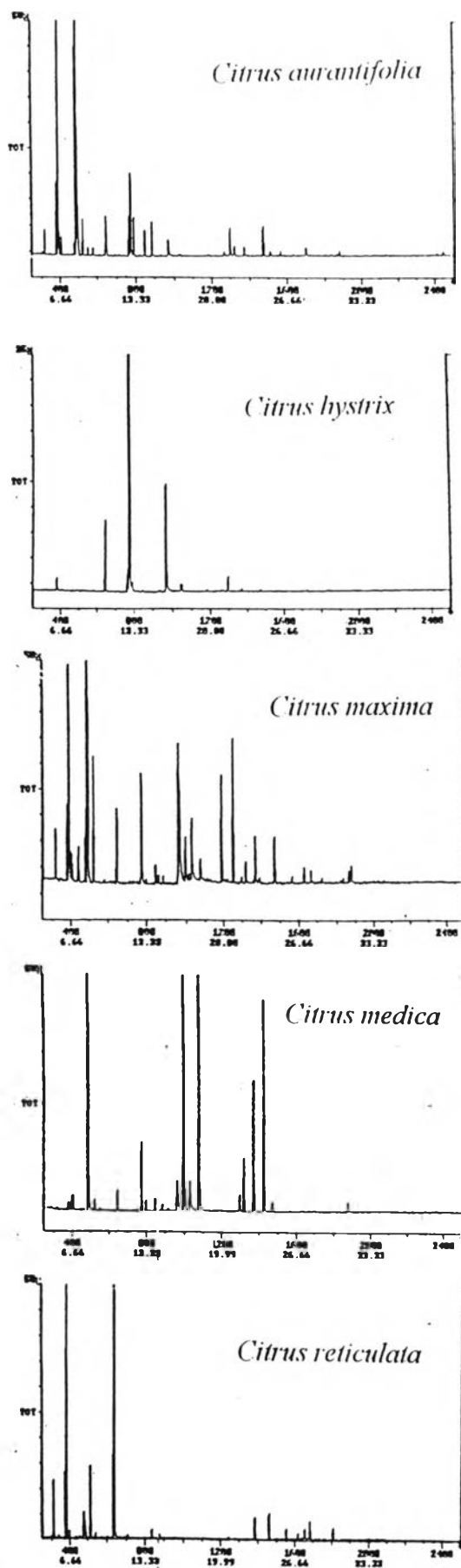


Figure 42 GC chromatogram of essential oil from Citrus species.

4. Comparison of the Essential Oil Compositions of some Rutaceous Plants of the sub family Aurantioideae

Study on the distribution of the essential oil components in some related Rutaceous plants was also carried out. The plants selected for this comparative study belong to 2 tribes: Clauseneae and Citreae. The Clauseneous plants include *Glycosmis pentaphylla*, *Micromelum minutum* and *Murraya paniculata* whereas the Citreous plants include *Atalantia monophylla*, *Hesperethusa crenulata* and *Paramignya scandens*. These species were selected based on their apparent similarity of their essential oil compositions.

As showed in Fig. 43, 9-epi-(E)-caryophyllene appears to be the major and common component of all these plants. Its content is mostly over 20 % of each of the isolated oil. In addition, bicyclogermacrene is also another major component of these plants, except *P. scandens*. For other minor common components, it appears to be α -humulene, spathulenol, δ -cadinene and epi- α -cadinol. These components are usually present in less than 10 %.

For the other components, no clear relationship between the distribution of particular components of these plants is observed.

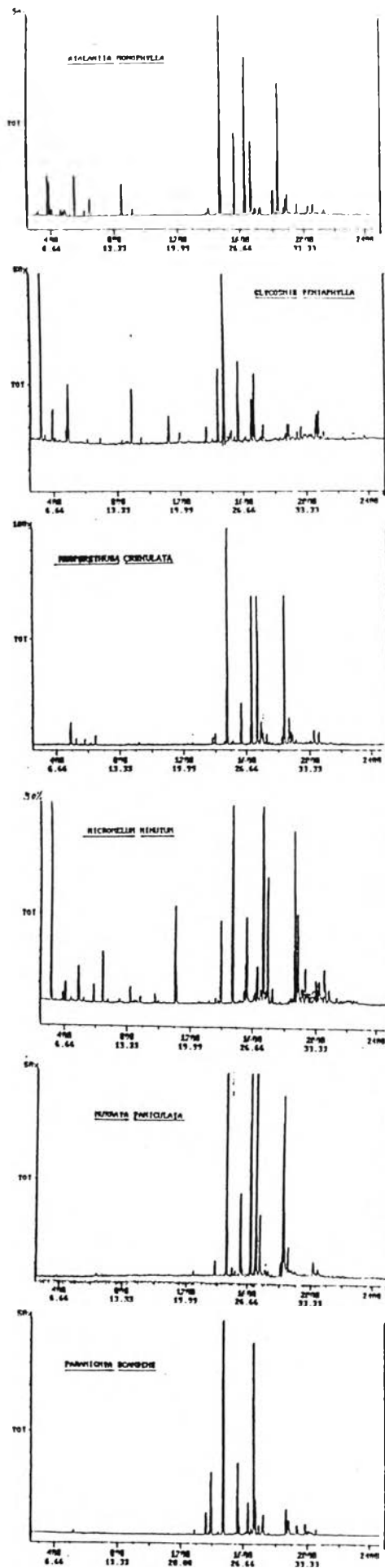


Figure 43 GC chromatogram of essential oil from subfamily Aurantioideae

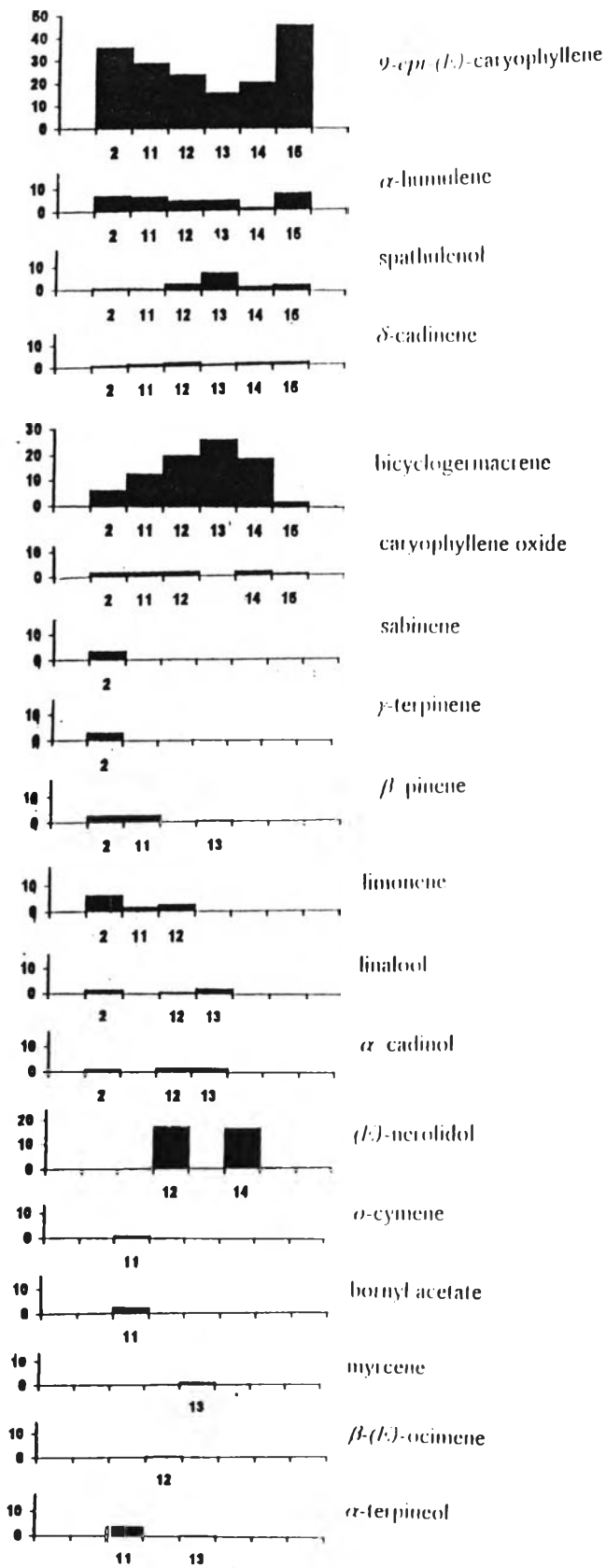


Figure 44 The distribution of the essential oil components in subfamily Aurantioideae

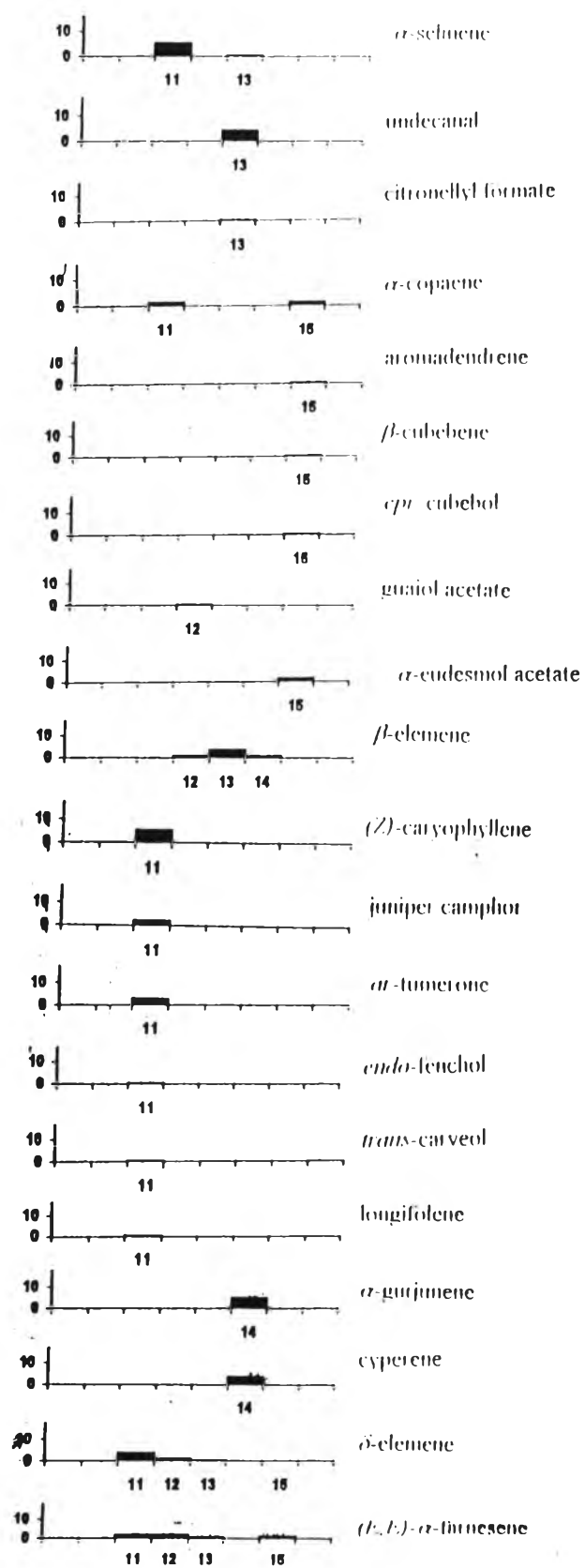


Figure 44 (Continued)

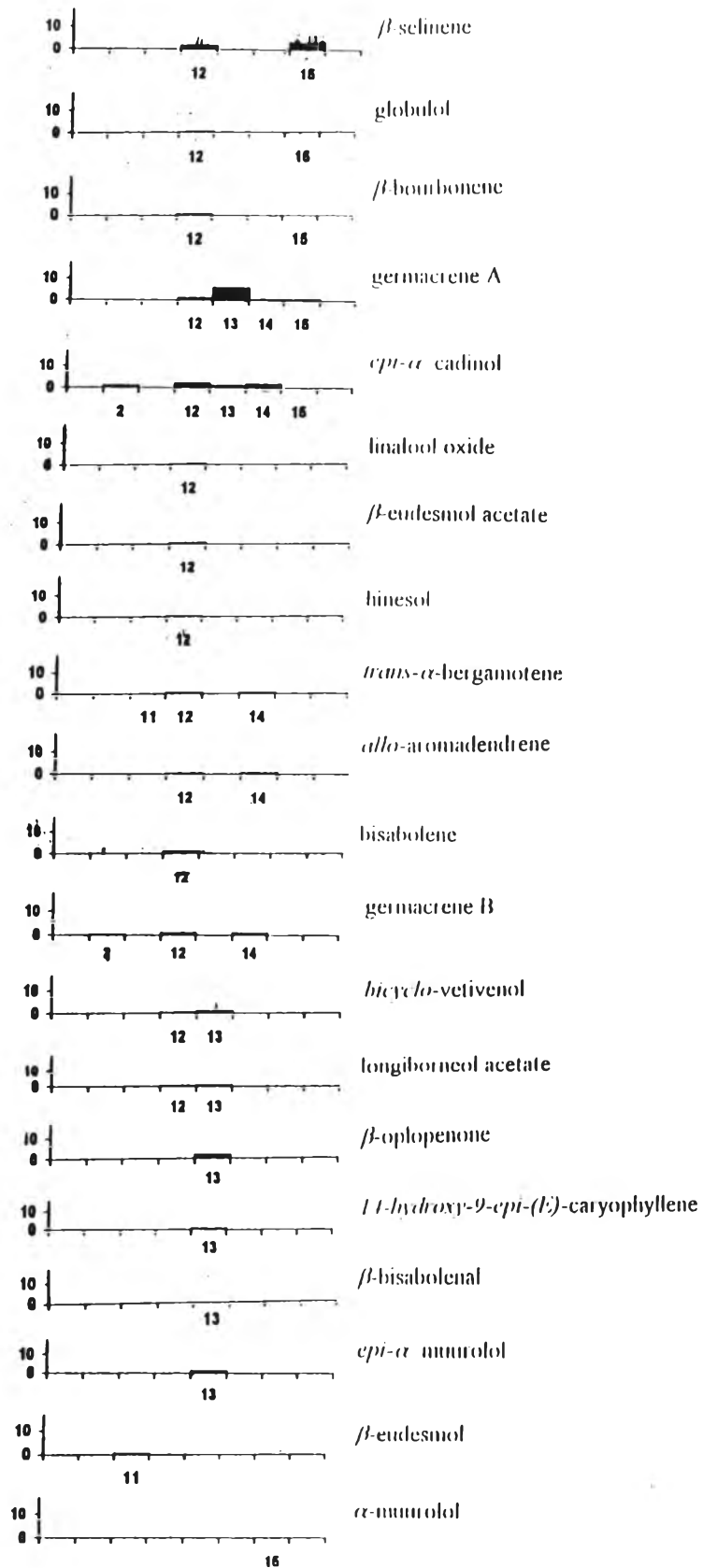


Figure 44 (Continued)