CHAPTER V

DISSCUSSION

Hydrodistillation of leaves from selected Thai Lamiaceous plants yielded various amounts of essential oils ranging from 0.01 % to 0.9 % v/w of fresh weight. It was found that the leaves of *M. cordifolia* and *M. arvensis* var *piperascens* yielded the lowest and the highest amounts, respectively. In terms of the chemical composition analyzed by GC/MS, most of the constituents were quite different. No clear relationship between the distribution of particular components of these plants was observed.

It was found that carvacrol (77.95 %) was the major component of the essential oil from *C. amboinicus*. These was in agreement with the result previously reported (Prudent *et al.*, 1995). The major components of the essential oil from *H. suaveolens* were also in agreement with those reported recently (Ahmed *et al.*, 1995). They were 1,8-cineol (21.70 %), *(E)*-caryophyllene (17.87 %) and sabinene (16.92 %). However, the percentage of 1,8-cineole in our oil were quite different from those of Ahmed *et al.*, 1994 (38.70 % 1,8-cineole).

It was found that major components in the essential oil of *Mentha arvensis* var. *piparascens* and *M. cordifolia* were oxygenated monoterpenes. They were menthol (79.44 %) and piperitenone oxide (73.20 %), respectively. Most of the constituents of *Mentha arvensis* var. *piparascens* found in this study were similar to those recently reported (Pino *et al.*, 1995). However the percentage of menthol in our oil, were quite different from that report (menthol 51.68 %). For *Mentha cordifolia* leaf oil, this study is the first report on its chemical composition.

Comparision of the essential oil composition among *Ocimum* species was shown in Table 24. It was found that α-humulene and δ-cadinene were present in all of four *Ocimun* species including *O. basilicum, O. canum, O. gratissimun* and *O. sanctum*. Some major components were present specifically in individual species. For example geranial (22.36 %) and geranyl acetate (13.10 %) were present only in

Table 24 Chemical composition of essential oil hydrodistilled from *Ocimum* leaves

Compound	% Area			
	O. basilicum	*O. canum*	*O. gratissimum*	*O. sanctum*
Monoterpene				
α-thujene	-	-	-	0.20
camphene	-	-	-	0.21
sabinene	-	0.26	-	t
β-pinene	-	-	-	0.14
myrcene	0.11	-	0.29	-
δ-3-carene	-	0.14	-	-
O-cymene	-	0.13	-	-
limonene	0.15	1.11	-	0.07
Z-β-ocimene	-	-	20.35	-
(*E*)-β-ocimene	1.29	0.78	1.13	-
neo-allo-ocimene	-	-	0.07	-
Oxygenated monoterpene				
1,8-cineole	0.31	0.63	-	0.07
fenchone	-	0.21	-	-
linalool	-	5.42	0.64	0.32
(exo)-fenchol	-	0.56	-	-
camphor	0.71	-	-	-
β-pinene oxide	-	0.57	-	-
trans-verbenol	-	0.36	-	-
cis-chrysanthenol	-	0.60	-	-
borneol	0.16	-	-	0.55
α-terpineol	-	0.66	-	-
linalool acetate	-	2.98	-	-
cis-carveol	-	1.70	-	-
geranial	-	22.36	-	-
geranyl acetate	-	13.10	-	-
Sesquiterpene				
α-copaene	-	0.30	0.83	-
β-bourbonene	-	-	0.15	-

Table 24 Chemical composition of essential oil hydrodistilled from *Ocimum* leaves (continued)

Compound	% Area			
	O. basilicum	*O. canum*	*O. gratissimum*	*O. sanctum*
β-cubebene	-	-	0.11	-
β-elemene	0.15	-	-	2.65
*9-epi-(E)*caryophyllene	-	-	-	**23.69**
(*E*)-caryophyllene	0.18	9.55	2.19	-
α-transbergamotene	1.98	2.26	3.28	-
α-humuene	0.25	4.81	0.24	1.53
γ-muurolene	-	1.88	-	0.39
germacrene D	0.38	-	7.65	-
β-selinene	-	0.32	0.35	0.12
α-selinene	-	0.10	-	0.14
(*Z*)-α-bisabolene	0.13	-	-	-
E,E,α-farnesene	-	-	2.95	-
β-bisabolene	0.35	0.20	-	-
germacrene A	0.27	-	0.26	-
α-bulnesene	0.41	-	-	4.51
γ-cadinene	0.54	-	-	-
δ-cadinene	0.17	0.14	0.41	0.11
β-sesquiphellandrene	-	-	0.18	-
germacrene B	-	6.57	-	-
Oxygenated sesquiterpene				
caryophyllene oxide	-	0.70	-	-
humulene epoxide II	-	0.22	-	-
1-epi-cubenol	0.24	-	-	-
epi-α-cadinol	2.38	-	-	-
α-cadinol	0.41	-	-	-
Phenyl propane				
methyl chavicol	**88.40**	0.38	-	-
eugenol	-	-	-	**19.18**
(*E*)-isoeugenol	-	-	**58.92**	-
methyl eugenol	0.75	-	-	**46.08**

O. canum, *(Z)-β*-ocimene (20.35 %) was only in *O.gratissimum* and *9-epi-(E)* caryophyllene (23.69 %) was only in *O. sanctum*. These components may potentially be used as markers for identification of these individuals.

In terms of the proportion of terpenoid compounds, only *O. canum* leaf oil was found to contain the major component in the oxygenated monoterpene group (50 %). The major components in leaf oil of the other three species were in the phenylpropanoid group, and the non-terpenoid compound. It was found that essential oils in these species were composed of uncommon major constituents. That of *O. basilicum* was composed of methyl chavicol (88.40 %), epi-α-cadinol (2.38 %) and α-trans–bergamotene (1.98 %). That of *O. canum* contained geranial (22.36 %), geranyl acetate (13.10 %) and *(E)* caryophyllene (9.55 %). That of *O. gratissimum* contained (*E*)-isoeugenol (58.92 %), (*Z*)-*β*-ocimene (20.35 %) and germacrene D (7.66 %). Most of the constituents found in this study were different from other reports. *O. basilicum* leaf oil has been previously reported to contain linalool, (*E*)-methyl cinnamate and 1,8-cineole (Ozek *et al.*, 1995).

It was found that perilla aldehyde (57.31 %) was the major component of *Perilla frutescens*. This was in agreement with the findings by Kang *et al.*, 1992. In contrast, it was different from the findings by Nguyen *et al.*, 1995 (28.4 % limonene, 25.9 % piperitone). For *Pogostemon cablin* oil, its major component was patchouli alcohol. This agreed with the previous report by Nguyen *et al.*, 1990.

Leaves of selected Lamiaceous plants of western origin contained the essential oil yields comparable to those of indigenous species. They were 0.1-0.9 % v/w of fresh leaves. The plants with the lowest essential oil content included *Melissa officinalis* and *Origanum majorana*. Those containing the highest content were *Rosemarinus officinalis*, *Thymus* sp1. (summer Thyme) and *Thymus* sp.2 (winter Thyme).

In consideration to the chemical composition, the major constituents of oils from two plants of western origin, *Mentha piperita* L. and *M. spicata* L., and two indigenous spp., *M. arvensis* L. var *piperascens* Malinvaud and *M. cordifolia* Opiz, were different except menthol and menthone in oils of *M. piperita* L. and *M. arvensis* L. var *piparascens* Malinvaud. However, their amounts were different. The latter contained very high amount of menthol (79.44 %) but low amount of menthone (9.44 %) whereas the former contained nearly equal amounts of menthol (30.57 %) and menthone (24.63 %). It was found that most of the constituents of the essential oils in *M. piperita* and *M. spicata* in this study were similar to previous reports of Tisserand and Balacs (1995).

Origanum majorana oil was obtained in 0.1 % v/w. This was different from previous report (3% v/w) (Wren, 1988). The GC/MS analysis indicated terpin 4-ol (28.37 %), linalool (20.70 %) and β-phellandrene (10.75 %). Most of the constituents found in this study were different from previously reported results. *Origanum majorana* leaf oil had been previously reported to contain carvacrol and thymol (Sarer, E., Scheffer, J.J.C., and Baerheim, S.A., 1982).

Origanum vulgare oil was obtained in 0.2 % v/w. The GC/MS analysis indicated carvacrol (75.63 %) as the major component, together with *p*-mentha-2,4(8)-diene (10.11 %) and *o*-cymene (2.34 %). The presence of carvacrol as the major component was in agreement with the results from a recently reported study (Roengsumran *et al.*, 1997).

Rosemary oil was obtained in 0.9 % v/w. This was quite different from the previous reported 2.35 % v/w by Perez-Alonso *et al.*, 1995. It was found to contain α-pinene (22.48 %), camphor (20.07 %) and bornyl acetate (8.67 %). The presence of α-pinene as the major component was in agreement with the results from earlier reports (Tisserand and Balacs, 1995 ; Perez-Alonso *et al.*, 1995). However, its percentage was quite different.

Salvia officinalis leaf oil was obtain in 0.3 % v/w. This was quite different from the previously reported value (1.4 % v/w) (Tsankova, E.T., Konaktchiev. and Genova, E.M., 1995). The GC/MS analysis indicated that *cis*-thujone (37.49 %) was the major component, together with camphor 13.79 % and α-humulene 9.46 %. These were in agreement with those reported recently (Tisserand and Balacs, 1995).

Thymus vulgaris leaves oil was obtained in 0.2 % v/w. The GC/MS analysis indicated that thymol (47.87%) was the major component, This was in agreement with that previously reported (Tisserand and Balacs, 1995). The essential oils obtained from the leaves of *Thymus* sp.1 and *Thymus* sp. 2 were similar in terms of both yields (0.9 % v/w) and major chemical components. However, their percentages were different : thymol (26.43 % and 59.27 %, respectively), γ-terpinene (21.63 % and 15.20 %, respectively) and *o*-cymene (15.87% and 9.37%, respectively). This is the first report on their chemical composition.

Essential oils of selected indigenous Lamiaceous plants used in this study showed activity against *S. aureus* and *B. subtilis* except the oil from *Mentha arvensis* L. var *piperascens* and *Ocimum basilicum* L. They showed no activity against *P. aeruginosa*, *C. albicans* and *M.gypseum*. *Coleus amboinicus* Lour. leaf oil also inhibited *E. coli* and *E. faecalis*. This was in agreement with the previous report (Prudent *et al.*, 1995). The antibacterial activities of *C. amboiaincus* Lour. oil might come from its major constituent, carvacrol, a phenolic compound. However, it was previously reported that carvacrol showed activities against *B. cereus* and *E. coli* but it had no activities against *S. aureus* and *P. aeruginosa* (Ross, S.A., El-Keltawi, N.E., and Megalla, S.E., 1980). This suggested that the activity of *C. amboinicus* Lour. oil against *S. aureus* might come from other constituents. On the other hand, the difference of result on *S. aureus* might come from the difference in tested strains.

Hyptis suaveolens leaf oil showed antibacterial effect on gram-positive bacteria (*S. aureus* and *B. subtilis*) but it had no activity against gram-negative bacteria. The effect on gram-positive bacteria agreed with a previous report but its effect on gram-negative bacteria was different (Iwu *et al.*, 1990). Its antibacterial effect might be due to 1,8-cineole, its major component. Previously, 1,8-cineole was found to have antibacterial activity (Prudent *et al.*, 1995).

Essential oil from *Mentha arvensis* L.var. *piperascens* Malinvaud showed no activity against both bacteria and fungi despite the presence of menthol as its major component. Previously, menthol was found to show weak activities against *S. aureus, E. coli* and *C. albicans* (Morris, *et al.* 1979; Ross,*et al.*, 1980). The reasons underlying the negative results of this oil might be the followings. First, some constituents in the oil might interfere with menthol activity. Or second, the test organisms used in this study might be different from those used in the previous reports. In contrast to essential oil of *M. arvensis* Linn var *piperascens* Malinvaud, that of *Mentha cordifolia* Opiz showed activities against some bacteria, *S. aureus* and *B. subtilis*.

Essential oil of *Ocimum basilicum* L. leaves showed no activity against test bacteria and fungi. This was different from the finding by Dikshit and Husain (1984). They found that *Ocimum bacillicum* L. oil inhibited mycelial growth of *M. gypseum*. The different results of this study and the previous study might come from the difference in chemical composition of the oils and /or in sensitivity of techniques used in antimicrobial activity screening. In the previous study, they determined the percentage of mycelial inhibition but measurement of clear inhibition zone was used in this study.

In addition to the activities against *S.aureus* and *B. subtilis*, *Ocimum canum* L. oil also showed activity against *E. faecalis*. Its effects might come from its major constituent, geranial, an aldehyde compound. Several aldehydes were shown to possess antimicrobial activity (Russell A.D., Hugo, W,B. and Ayliffe, A. J. 1992). Instead of inhibiting *E. faecalis, O. gratissimum* L. oil could inhibit the gram-negative bacterium, *E. coli*. It showed strong activity against *S. aureus* (MIC 0.078 %). Strong antibacterial activities of *O. gratissimum* L. oil might be due to its major component , *(E)*-isoeugenol. Isoeugenol was found to have antibacterial activities against *S. aureus* and *E. coli* (Morris *et al.*, 1979). Essential oil of *Ocimum sanctum* L. leaves showed activities against *S. aureus* and *B. subtilis*. Its activity might be due to its major constituents, methyl eugenol and eugenol. Both major constituents were found to showed activities against some bacteria (Morris, *et.al*, 1979; Ross, *et al.*, 1980).

Besides *S. aureus* and *B. subtilis*, essential oil of *Perilla frutescens* also showed activity against the gram-negative bacterium, *E. coli*. This was in agreement with a previous report (Kang *et al.*, 1992). Perilla aldehyde, its major constituent, seemed to be the active component. It was found to showed activities against several bacteria (Kang *et al.*, 1992).

Essential oil of *Pogostemon cabin* only showed activies against *S. aureus* and *B. subtilis*. Its major constituent was patchouli alcohol. This agreed with the previous report on activies of Patchuli oil (Morris *et al.*, 1979).