Variability of essential oil constituents of *Ocimum africanum*

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Abstract

Aerial parts of *Ocimum africanum* Lour. (Lamiaceae) collecting from three different locations of Thailand, including the northern, north-eastern and southern parts, were evaluated for their volatile constituents. Their essential oils were obtained by hydrodistillation method. The chemical composition of the essential oil was analyzed by means of gas chromatography-mass spectrometry using standard *n*-alkane mixtures as internal standards. All samples of Hoary basil essential oil were characterized by a high percentage of monotepenes, represented especially by neral (range from 21.1% to 36.8%) and geranial (range from 15.6% to 33.4%). The major components of essential oil from the northern part were neral (36.8%), geranial (33.4%), (*E*)-caryophyllene (3.5%), and linalool (3.1%), whereas the oil from the north-eastern part comprised neral (21.1%), geranial (15.6%), linalool (8.9%), (*E*)-caryophyllene (7.7%), germacrene D (5.5%), and alpha-humulene (3.5%). Finally, the essential oil from the southern part contained neral (24.4%), geranial (22.8%), methyl chavicol (9.4%), linalool (5.6%), and (*E*)-caryophyllene (5.5%). Therefore, all three essential oils of *O. africanum* collected from different locations of Thailand could be considered as the same neral/gernial chemotype. According to the literatures, eight different chemotypes were recorded for the essential oil of *O. africanum* and the result of this study revealed that high neral/geranial contents in the essential oil of this species could be identified as a novel chemotype.

Keywords: *Ocimum africanum*; Essential oil; Constituents; Gas chromatography/mass spectrometry; retention indices

Introduction

The genus *Ocimum* belonging to the Lamiaceae comprises annual, perennial herbs and shrubs native to the tropical and subtropical Regions of Asia, Africa and South America. The taxonomy of *Ocimum* is complex due to interspecific hybridization and polyploidy of the species in the genus. According to the literature, some of the species of this genus showed great variation in both morphology and essential oil components. For example, seven different chemotypes were identified from 18 Turkish basil essential oils (*O. basilicum*) whereas only two basil oil types were commercially available (Schulz et al., 2003; Telci et al., 2006). In Thailand, five species of this genus were recorded including *O. americanum* L. (syn. *O. canum* Sims, Hoary basil), *O. basilicum* L. (Sweet basil), *O. tenuiflorum* L. (syn. *O. sanctum* L., Holy basil), *O. gratissimum* L. and *O. kilimandscharicum* Baker ex Gurke (Phuphathanaphong et al., 2001). Among the species of this genus, *O. americanum* L., an aromatic annual herb commonly called "Maeng Lak" or "Mang Lak" in Thai, is one of the most popular culinary herb which is widely distributed in every parts of Thailand and is sold as a fresh-cut in almost local markets. In Thailand and Cambodian, the nutlets, which produce mucilage when wet, are used for making soup or a sweet desert. The fresh leaves are the main side dish for the traditional Thai rice noodle dish called "Khanom Chean". Recently, Suddee (2005) revealed the taxonomic revision of the tribe *Ocimeae*, including the genus *Ocimum*, in continental South East Asia in which "Maeng Lak" was currently revised to be *O. africanum* Lour.. Several scientific names had been used for this species and were indicated as the synonyms including *Ocimum pilosum* Willd., *O. americanum* L., *O. americanum* L. var. *pilosum* (Willd.) A. J. Paton, *O. basilicum* L. var. *pilosum* (Willd.) Benth., *O. citratum* Rumph., *O. minimum sensu* Burm. f., *O. basilicum* L. var. *anisatum* Benth., *O. graveolens* A. Br., O. x *citriodorum* Vis., *O. petitianum* A. Rich., *O. menthaefolium sensu* Hochst ex Benth., *O. basilicum sensu* Hook. f., *O. basilicum sensu* Doan, *O. americanum sensu* Keng, *O. americanum sensu* Pushpangadan and *O. basilicum sensu* Paton (Suddee et al., 2005).

In Thailand, besides of the culinary herb usage, this plant was also used as a Thai traditional medicine for various purposes such as for cough, expectorant, headache, anti-flatulence, antifungal and anti-emetic (Bunyapraphatsara, 1999). In Africa, its leaves have been used as an insecticide for protection against post-harvest insect damage. The active ingredients are likely to be associated with the essential oils. The essential oil of this plant were reported to exhibit strong fungicidal activity against certain plant pathogens (Vieira et al., 2003) and antibacterial activity against *Propionibacterium acnes* (Viyoch et al., 2006). It also exhibited larvicidal activity against *Aedes aegypti* and mosquitoes repellant property (Cavalcanti et al., 2004; Seyoum et al., 2002; Tawatsin et al., 2001). Methanolic extract of this plant was also showed tyrosinase inhibitory activity (Khanom et al., 2000). According to the literature, the non-volatile compositions of *O. africanum* (syn. *O. americanum*, *O. americanum* var. *pilosum*) especially the flavonoids when collected from different areas showed some difference types of compounds. However, some results showed that it was possible to authenticate the leaf samples of this plant on the basis of external flavonoid profiles (Grayer et al., 2001; Grayer et al., 2002; Vieira et al., 2003). The volatile components were also investigated in several studies. The occurrence of volatile chemical in aromatic plants is not only an indication of chemical diversity but may also help solve taxonomical problems (Başer, 2002). Various vibrational spectroscopy methods have also been used for identification of basil oil chemotypes (Schulz et al., 2003). However, there is little data available about the chemical characterization of the essential oils of the *O. africanum* (syn. *O. americanum*) growing in Thailand, except those reported by Viyoch (2006). The purpose of this study was to determine chemical composition of the essential oils obtained by hydrodistillation of the aerial parts of *O. africanum* which were collected from three different locations including the northern, north-eastern and southern parts of Thailand. The comparison on volatile constituents with other collections of this plant was also evaluated in this study.

Materials and Methods

Plant Materials

The fresh aerial parts of *O. africanum* (Thai names: Mang Lak or Maeng Lak) were purchased from three local markets in Muang district, Phitsanulok province (representing the northern part of Thailand); Muang district, Khon Kaen province (representing the north-eastern part of Thailand); and Hat-Yai district, Songkla province (representing the southern part of Thailand) in April 2005. Voucher specimens of this plant were deposited at the Faculty of Pharmaceutical Sciences, Naresuan University, Phitsanulok.

Distillation of Essential Oil

Fresh leaf and stems of *O. africanum* collected from Phitsanulok, Khon Kaen and Songkla provinces, approx. 2.0 kg each, were cut and hydrodistillated for 4-6 h. The appearance of essential oils showed variation in color from deep yellow to pale yellow. All of them were dried over anhydrous sodium sulfate, kept in air-tight light protection vials and stored at temperature 4°C until analysis.

Analysis of the essential oils

Samples of essential oils were diluted in dichloromethane (1:10) and analyzed by GC/MS. The analyses were carried out using an Agilent technologies 6890N gas chromatograph fitted with a fused silica capillary column (HP-5ms; 30 m x 0.25 mm i.d., 0.25 mm film thickness; Agilent J&W) coupled to an Agilent 5973 mass selective detector (EI, 70eV). The carrier gas was helium introduced at a rate of 1.02 ml/min. The samples were injected in the split mode at a ratio of 1:20. The injector was kept at 220 °C and the transfer line at 240 °C. The oven temperature was maintained at 60°C for 1 min and then programmed to 260 °C at a rate of 3 °C/min and held for 10 min at 260 °C. Programmed-temperature Kovats RIs were obtained by GC/MS analysis of an aliquot of the essential oil spiked with an *n*-alkane mixture containing each homologous from n-C_8 to n-C_{40}. Identification of oil constituents was accomplished by a comparison of mass spectra with literature data (wiley and NIST) and by a comparison of their programmed-temperature Kov ts retention indices (RIs) with those in the literature (Adams, 2001; Davies, 1990).

Results and Discussion

The essential oils of *O. africanum* obtained from three different sources were analyzed by means of GC-MS. The volatile components of them are summarized and listed in the order of their elution times (table 1-3) and previous reports of identification of main components in the essential oil from other collections of this species are also compared (table 4). The aromatic character of each basil chemotype depends mainly on the individual composition of the essential oil fraction; generally chemotypes are classified on the basis of prevalent compounds or components with an amount higher than 20% of the total (Schulz et al., 2003). As shown in table 1-3, all three samples of the Hoary basil oils contained the same major chemical compositions including neral (range from 21.1% to 36.8%) and geranial (range from 15.6% to 33.4%). Thus the essential oils of *O. africanum* collected from three different locations of Thailand could be considered as the same neral/geranial chemotype. Some investigation on chemical composition and antibacterial activity relationship of several essential oils has revealed that the antibacterial activity of the essential oil was related to the high amount of neral and geranial (Cimanga et al., 2002). This result can support partly the use of *O. africanum* as traditional remedies for the treatment of some infections.

Table 1. Volatile components of *O. africanum* collected from the northern part of Thailand.

Compounds	RA[a] (%)	RI[b] (Exp.)	RI[c] (Lit.)	MW[d]	Identification method[e]
alpha-pinene	0.1	932	939	136	1, 2
beta-pinene	0.1	976	979	136	1, 2
6-methyl-5-hepten-2-one	1.0	991	986	126	1, 2
limonene	0.2	1028	1029	136	1, 2
1,8-cineole	0.1	1031	1031	154	1, 2
(*Z*)-beta-ocimene	0.1	1037	1037	136	1, 2
(*E*)-beta-ocimene	1.2	1049	1050	136	1, 2
linalool	3.1	1101	1097	154	1, 2
endo-fenchol	0.3	1119	1117	154	1, 2
citronellal	0.2	1156	1153	154	1, 2
unknown	1.8	1171	-	152	-
unknown	2.5	1191	-	152	-
alpha-terpineol	0.2	1196	1189	154	1, 2
methyl chavicol	0.4	1202	1196	148	1, 2
endo-fenchyl acetate	0.1	1222	1220	196	1, 2
neral	36.8	1231	1238	152	1, 2
geranial	33.4	1306	1267	152	2
methyl geranate	0.1	1339	1325	182	2
eugenol	0.2	1369	1359	164	1, 2
alpha-copaene	0.2	1383	1377	204	1, 2
beta-cucubene	1.3	1399	1388	204	1, 2
(*E*)-caryophyllene	3.5	1430	1419	204	1, 2
alpha-*trans*-bergamotene	1.3	1444	1435	204	1, 2
alpha-humulene	1.6	1462	1455	204	1, 2
(*E*)-beta-farnesane	0.6	1465	1457	204	1, 2
gamma-muurolene	2.0	1489	1480	204	1, 2
beta-selinene	0.1	1492	1490	204	1, 2
alpha-selinene	0.1	1499	1498	204	1, 2
germacrene A	0.1	1509	1509	204	1, 2
delta-cadinene	0.2	1527	1523	204	1, 2
unknown	4.0	1556	-	204	-
caryophyllene oxide	1.0	1589	1583	220	1, 2
beta-eudesmol	0.2	1653	1651	222	1, 2

[a]RA, relative area (peak area relative to total peak area); [b]RI, programmed temperature retention index; [c]RI, programmed temperature RI from literature data; [d]MW, molecular weight from GC/MS (EI) data. [e]1, based on retention index; 2, based on comparison of mass spectra.

Table 2. Volatile components of *O. africanum* collected from the north-eastern part of Thailand.

Compounds	RA[a] (%)	RI[b] (Exp.)	RI[c] (Lit.)	MW[d]	Identification method[e]
3-hexen-1-ol	0.3	-	-	100	2
alpha-pinene	0.3	932	939	136	1, 2
beta-pinene	0.2	976	979	136	1, 2
6-methyl-5-hepten-2-one	2.4	990	986	126	1, 2
limonene	0.4	1028	1029	136	1, 2
1,8-cineole	0.3	1032	1031	154	1, 2
(*Z*)-beta-ocimene	0.1	1037	1037	136	1, 2
benzene acetaldehyde	0.1	1045	1042	120	1, 2
(*E*)-beta-ocimene	1.9	1050	1050	136	1, 2
fenchone	0.7	1090	1087	152	1, 2
linalool	8.9	1101	1097	154	1, 2
endo-fenchol	0.8	1120	1117	154	1, 2
(*E*)-myroxide	0.5	1146	1145	152	1, 2
citronellal	0.3	1157	1153	154	1, 2
unknown	1.8	1171	-	152	-
rosefuran epoxide	0.3	1179	1177	166	1, 2
unknown	2.2	1191	-	152	-
alpha-terpineol	1.2	1198	1189	154	1, 2
methyl chavicol	0.6	1202	1196	148	1, 2
endo-fenchyl acetate	0.3	1223	1220	196	1, 2
neral	21.1	1231	1238	152	1, 2
geranial	15.6	1303	1267	152	2
methyl geranate	0.3	1339	1325	182	2
eugenol	0.4	1369	1359	164	1, 2
alpha-copaene	0.6	1382	1377	204	1, 2
beta-cucubene	0.3	1398	1388	204	1, 2
(*E*)-caryophyllene	7.7	1430	1419	204	1, 2
alpha-*trans*-bergamotene	2.8	1445	1435	204	1, 2
(*Z*)-beta-farnesene	0.3	1452	1443	204	1, 2
alpha-humulene	3.5	1463	1455	204	1, 2
germacrene D	5.5	1491	1485	204	1, 2
beta-selinene	0.3	1494	1490	204	1, 2
alpha-selinene	0.3	1500	1498	204	1, 2
germacrene A	0.2	1510	1509	204	1, 2
beta-bisabolene	0.4	1514	1506	204	1, 2
delta-cadinene	0.5	1528	1523	204	1, 2
unknown	8.5	1556	-	204	-
caryophyllene oxide	1.3	1588	1583	220	1, 2
beta-eudesmol	0.4	1653	1651	222	1, 2
alpha-cadinol	0.1	1657	1654	222	1, 2
unknown	1.0	1754	-	220	-
4,6-bis(4'-methylpent-3'-en-1'-yl) -6-methylcyclohexa-1,3-diene-1-carbaldehyde	0.7	2112	-	286	2

[a]RA, relative area (peak area relative to total peak area); [b]RI, programmed temperature retention index; [c]RI, programmed temperature RI from literature data; [d]MW, molecular weight from GC/MS (EI) data. [e]1, based on retention index; 2, based on comparison of mass spectra.

Table 3. Volatile components of *O. africanum* collected from the southern part of Thailand.

Compounds	RA[a] (%)	RI[b] (Exp.)	RI[c] (Lit.)	MW[d]	Identification method[e]
3-hexen-1-ol	0.1	-	-	100	2
6-methyl-5-hepten-2-one	2.2	991	986	126	1, 2
(*E*)-beta-ocimene	0.4	1048	1050	136	1, 2
fenchone	0.4	1090	1087	152	1, 2
linalool	5.6	1101	1097	154	1, 2
endo-fenchol	0.3	1120	1117	154	1, 2
pinene oxide	0.5	1154	1159	152	1, 2
citronellal	0.3	1157	1153	154	1, 2
methyl chavicol	9.4	1202	1196	148	1, 2
neral	24.4	1231	1238	152	1, 2
geranial	22.8	1303	1267	152	2
methyl geranate	0.2	1339	1325	182	2
eugenol	0.2	1369	1359	164	1, 2
neryl acetate	0.6	1380	1362	196	2
alpha-copaene	0.5	1383	1377	204	1, 2
beta-bourbonene	0.2	1392	1388	204	1, 2
unknown	0.2	1398	-	204	-
methyl eugenol	0.5	1420	1404	178	2
(*E*)-caryophyllene	5.5	1430	1419	204	1, 2
alpha-*trans*-bergamotene	2.6	1444	1435	204	1, 2
(*Z*)-beta-farnesene	0.2	1452	1443	204	1, 2
alpha-humulene	2.8	1462	1455	204	1, 2
allo-aromadendrene	0.1	1468	1460	204	1, 2
germacrene D	2.0	1490	1485	204	1, 2
alpha-zingiberene	0.4	1501	1494	204	1, 2
beta-bisabolene	0.4	1514	1506	204	1, 2
gamma-cadinene	0.2	1519	1514	204	1, 2
delta-cadinene	0.5	1529	1523	204	1, 2
unknown	5.2	1556	-	204	-
spathulenol	0.5	1588	1578	220	1, 2
humulene epoxide II	0.5	1616	1608	220	1, 2
epi-alpha-cadinol	0.5	1647	1640	222	1, 2
beta-eudesmol	0.3	1656	1651	222	1, 2
alpha-cadinol	0.7	1657	1654	222	1, 2
4,6-bis(4'-methylpent-3'-en-1'-yl) -6-methylcyclohexa-1,3-diene-1-carbaldehyde	1.4	2112	-	286	2

[a]RA, relative area (peak area relative to total peak area); [b]RI, programmed temperature retention index; [c]RI, programmed temperature RI from literature data; [d]MW, molecular weight from GC/MS (EI) data. [e]1, based on retention index; 2, based on comparison of mass spectra.

Table 4. Comparison of the major volatile components of *O. africanum** with previous citation.**

Compounds	Percentage Relative Area										
	N	NE	S	I	II	III	IV	V	VI	VII	VIII
alpha-pinene	0.1	0.3	-	8.3	2.6	0.8	1.3	0.2	4.7	2.7	-
beta-pinene	0.1	0.2	-	-	0.5	0.8	0.3	-	7.7	5.7	-
6-methyl-5-hepten-2-one	1.0	2.4	2.2	-	-	-	-	1.2	-	-	-
limonene	0.2	0.4	-	7.8	1.5	7.5	2.0	0.1	41.5	1.9	-
1,8-cineole	0.1	0.3	-	-	3.2	7.3	-	0.2	10.1	60.2	-
(*E*)-beta-ocimene	1.2	1.9	0.4	-	-	-	tr	-	3.5	-	-
linalool	3.1	8.9	5.6	-	-	1.8	-	44.9	0.4	0.9	-
alpha-terpineol	0.2	1.2	-	-	-	15.0	-	tr	6.9	6.5	-
methyl chavicol	0.4	0.6	9.4	-	-	17.3	-	-	-	-	-
neral	36.8	21.1	24.4	-	-	-	-	1.7	-	-	-
geranial	33.4	15.6	22.8	-	-	-	-	2.7	-	-	-
eugenol	0.2	0.4	0.2	-	8.1	28.5	-	-	-	-	-
(*E*)-caryophyllene	3.5	7.7	5.5	-	-	-	22.4	1.0	0.8	1.9	4.6
alpha-*trans*-bergamotene	1.3	2.8	2.6	-	-	-	-	0.1	1.9	2.8	6.8
alpha-humulene	1.6	3.5	2.8	-	0.6	-	-	0.3	2.2	-	-
(*E*)-beta-farnesene	0.6	-	-	-	-	-	6.8	2.1	0.2	-	-
germacrene D	-	5.5	2.0	-	-	-	-	0.8	1.1	-	-
beta-bisabolene	-	0.4	0.4	-	-	4.5	-	tr	-	-	-
gamma-muurolene	2.0	-	-	-	-	-	0.4	0.1	-	-	-
beta-selinene	0.1	0.3	-	-	-	-	-	-	-	-	-
delta-cadinene	0.2	0.5	0.5	-	-	-	0.3	tr	4.0	2.5	-
camphor	-	-	-	26.7	-	1.7	0.6	-	-	-	-
sabinene	-	-	-	8.0	-	-	-	tr	-	-	-
gamma-selinene	-	-	-	10.9	-	-	-	-	-	-	-
para-cymene	-	-	-	-	3.2	-	2.0	tr	tr	-	-
thymol	-	-	-	-	43.5	-	2.3	-	-	-	-
beta-myrcene	-	-	-	-	0.2	1.0	2.4	0.7	2.0	2.1	-
gamma-terpinene	-	-	-	-	1.2	-	5.9	-	0.7	0.4	-
terpinene-4-ol	-	-	-	-	1.4	-	26.9	-	2.0	1.3	-
sabinene hydrate	-	-	-	-	-	-	5.5	-	2.0	-	-
geraniol	-	-	-	-	-	-	0.3	38.2	tr	-	-
beta-sesquiphellandrene	-	-	-	-	-	-	2.9	-	-	-	-
alpha-farnesene	-	-	-	-	-	9.1	4.1	-	-	-	-
(*Z*)-methyl cinnamate	-	-	-	-	-	-	-	-	-	-	8.8
(*E*)-methyl cinnamate	-	-	-	-	-	-	-	-	-	-	70.9

*the synonyms of this plant using for the chemical comparison are including *O. americanum, O. canum*, and *O. americanum* var. *pilosum*.

**component which was more than 2% relative area of the total; N = oil from the northern part of Thailand; NE = oil from the north-eastern part of Thailand; S = oil from the southern part of Thailand; I = Upadhyay et al., 1991; II = Cimanga et al., 2002; III = Abd El-Aziz et al., 2007; Jirovetz et al., 2003; IV = Oussou et al., 2004; V-VI = ; VII = Djibo, et al., 2004; VIII = Cavalcanti et al., 2004.

The major components of essential oil from the northern part were neral (36.8%), geranial (33.4%), (*E*)-caryophyllene (3.5%), and linalool (3.1%), whereas the oil from the north-eastern part comprised neral (21.1%), geranial (15.6%), linalool (8.9%), (*E*)-caryophyllene (7.7%), germacrene D (5.5%), and alpha-humulene (3.5%). Finally, the essential oil from the southern part contained neral (24.4%), geranial (22.8%), methyl chavicol (9.4%), linalool (5.6%), and (*E*)-caryophyllene (5.5%). One other significant component (RI 1556, table 1-3) which was found in all three *O. africanum* essential oil remains unknown. The tentative molecular weight from the mass spectral data (EI) was 204. The mass-spectral fragmentation pattern and RI could not be matched to any reported data for essential oil components. Further investigations by using the combination of isolation and spectroscopic techniques, especially nuclear magnetic resonance, are required to identify this component.

In conclusion, the essential oil profiles of *O. africanum* collected from three different sources including the northern, north-eastern and southern part of Thailand, showed no chemical variation and this evidence might be used to support the taxonomic study of this species in Thailand. According to the literature, there are different levels of classification in the genus *Ocimum*, i.e. to distinguish species, and to characterize sections or subgenera (Grayer et al., 2002). Recently, Suddee (2005) has extensively taxonomic studied of this genus over the full range of its geographic distribution in South-East Asia. The results revealed that the former scientific name of Mang Lak, *Ocimum americanum* var. *pilosum* has been changed to be *O. africanum* and the remainder of the names has been considered as synonyms (Suddee et al., 2005).

In order to compare the volatile chemical profiles of this plant with the other collections, the reports which its synonyms have been used should be concerned such as *O. americanum*, *O. canum*, and *O. americanum* var. *pilosum*. Using principal component analysis on the aromatic volatile oils, the *O. africanum* accessions could be separated into 8 different chemotypes: (i) camphor, (ii) thymol, (iii) eugenol, (iv) terpinene-4-ol/(*E*)-caryophyllene, (v) linalool/geraniol, (vi) limonene, (vii) 1,8-cineol, and (viii) (*E*)-methyl cinnamate. Whereas, the high neral and geranial contents in the *O. africanum* essential oils from Thailand could be considered as a new neral/geranial chemotype. The chemotaxomical study of *O. africanum* and other species in this genus revealed great variation in their volatile components. That could be due to the different in interspecific hybridization (polymorphism), infraspecific variation (genetic), geographic origin (climate and nutrition) and method of harvest (Jirovetz et al., 2003; Kothari et al., 2004; Labra et al., 2004; Masi et al., 2006; Misra et al., 2006; Sifola & Barbieri, 2006; Telci et al., 2006; Zheljazkov et al., 2008). Therefore, the chemical composition profile of the essential oil from this plant should be investigated for the preliminary quality control especially in the early step of research and development of the essential oil.

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