Volatile Constituents of the Essential Oils from Aerial Parts of Three *Ocimum* spp.

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Abstract

Three species of *Ocimum* spp. including *O. tenuiflorum* L., *O. basilicum* L. and *O. americanum* L. were investigated for their volatile constituents. Aerial parts of each specie were collected from Phitsanulok, Thailand and their essential oils were obtained by hydrodistillation. The chemical composition of the essential oil was analyzed by means of gas chromatography-mass spectrometry using standard *n*-alkane mixtures as internal standards. The major components of *O. tenuiflorum* were methyl eugenol (76.3%), beta-caryophyllene (9.9%), germacrene D (3.9%), germacrene A (3.0%) and borneol (1.0%). The major components of *O. basilicum* were methyl chavicol (89.8%), (*E*)-beta-ocimene (2.1%), 1,8-cineol (1.1%), *epi*-alpha-cadinol (1.1%) and linalool (1.0%). Finally, the major components of *O. americanum* were geranial (40.0%), neral (33.6%), linalool (3.8%), beta-caryophyllene (2.5%), nerol (1.7%), geraniol (1.4%), germacrene D (1.4%) and alpha-humulene (1.1%).
Keywords: *Ocimum*; Essential oil; Constituents

Introduction

The *Ocimum* genus belonging to the Lamiaceae comprises annual and 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. In 1995, Pushpangadan and Bradu recognized more than 150 species; however in 1999, Paton et al. proposed that *Ocimum* had only 65 species and other attributions should be considered as synonyms (Telci et al., 2006). In Thailand, only five species of this genus are recorded including *O. americanum* L. (Hoary basil), *O. basilicum* L. (Sweet basil), *O. gratissimum* L., *O. kilimandscharicum* Baker ex Gurke and *O. tenuiflorum* L. (Holy basil) (Phuphathanaphong et al., 2001). All of them were traditionally used as culinary herbs and medicinal plants for various purposes such as for cough, expectorant, headache, anti-flatulence, antifungal and anti-emetic (Bunyapraphatsara, 1999). Some species were considered to be a source of aroma compounds and essential oils containing biologically active constituents that showed insect repellent (Seyoum et al., 2002; Tawatsin et al., 2001), insecticidal (Kéita et al., 2001), antibacterial (Arnal-Schnebelen et al., 2004; Lertsatitthanakorn et al., 2006; Opalchenova & Obreshkova, 2003), antifungal (Vieira et al., 2003), antioxidant (Lee et al., 2004; Politeo et al., 2007) and anti-ulcerogenic properties (Dharmani et al., 2004).

According to the literature, the composition of the volatile oils of these plants when collected from different areas showed some differences in types of compounds present (Labra et al., 2004; Sifola & Barbieri, 2006; Telci et al., 2006; Tognolini et al., 2006). In this study, we have used GC/MSD analysis to determine the composition of the oils obtained by hydrodistillation of the aerial parts of *O. tenuiflorum*, *O. basilicum* and *O. americanum* collected from the northern part of Thailand.

Materials and Methods

Plant materials

The fresh aerial parts of *O. americanum*, *O. basilicum* and *O. tenuiflorum* were collected from local market in Muang District, Phitsanulok Province, Thailand in June 2005. Voucher specimens of these plants were deposited at Natural Product Research Laboratory, Faculty of Pharmaceutical Sciences, Naresuan University, Phitsanulok.

Essential oil isolation

Fresh leaf and stems of *O. americanum*, *O. basilicum* and *O. tenuiflorum*, 2.0 kg each, were separately cut and subjected to hydrodistillation for 4-6 h to yield pale yellow oil of 0.5, 1.4 and 0.7% v/w, respectively. Briefly, the plant was immersed in water and heated to boiling, after which the essential oil was evaporated together with water vapour and finally collected through the condenser. The distillate was separated and dried over anhydrous sodium sulfate. The oils were kept in air-tight light protection vials and stored at temperature 4 ^{o}C until analysis by GC/MSD.

Analysis of the essential oils

Samples of essential oil were diluted in dichloromethane (1:10) and analyzed by GC/MSD. 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. Samples were injected in the split mode at a ratio of 1:20. The injector was kept at 220 ^{o}C and the transfer line at 240 ^{o}C. The oven temperature was maintained at 60 ^{o}C for 1 min and then programmed to 260 ^{o}C at a rate of 3 ^{o}C/min and held for 10 min at 260 ^{o}C. Programmed-temperature Kováts retention indices (RI) were obtained by GC/MSD 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 Kováts RIs with those in the literature (Adams, 2001; Davies, 1990).

Results

The essential oils obtained from the aerial parts of *O. americanum*, *O. basilicum* and *O. tenuiflorum* were analyzed by mean of GC-MSD. According to the GC-MS analysis of *O. tenuiflorum*, 21 components were identified, representing 99.6% of all compositions. The major components were methyl eugenol (76.3%) which was phenylpropanoid derivative; beta-caryophyllene (9.9%), germacrene D (3.9%) and germacrene A (3.0%) which were sesquiterpene derivatives. The GC-MS analysis of *O. basilicum* oil showed 22 components, representing 99.7% of the total oil in which methyl chavicol (phenylpropanoid derivative) was the major component (89.8%). Finally, the GC-MS analysis of *O. americanum* revealed 27 compounds, representing 90.8% of total peak areas. The major compositions were geranial (40.0%), neral (33.6%) and linalool (3.8%) in which were classified into the monoterpene group. The volatile components of all essential oils 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 these species are also compared (Table 4-6).

Table 1. Volatile components in aerial parts of *O. tenuiflorum*

Compounds	RA[a] (%)	RI (exp.)[b]	RI (lit.)[c]	MW[d]	Identification method[e]
alpha-pinene	0.5	932	939	136	1, 2
camphene	0.5	947	954	136	1, 2
sabinene	0.1	972	975	136	1, 2
beta-pinene	0.3	975	979	136	1, 2
limonene	0.2	1027	1029	136	1, 2
linalool	0.3	1100	1097	136	1, 2
borneol	1.0	1165	1169	152	1, 2
methyl chavicol	0.1	1198	1196	148	1, 2
eugenol	0.4	1359	1359	164	1, 2
alpha-copaene	0.7	1375	1377	204	1, 2
beta-bourbonene	0.1	1384	1388	204	2
beta-cubebene	0.5	1390	1388	204	1, 2
beta-elemene	0.5	1391	1391	204	1, 2
methyl eugenol	76.3	1416	1404	178	2
beta-caryophyllene	9.9	1422	1419	204	1, 2
alpha-humulene	0.7	1454	1455	204	1, 2
germacrene D	3.9	1482	1485	204	1, 2
beta-selinene	0.1	1486	1490	204	1, 2
alpha-selinene	0.2	1498	1498	204	1, 2
germacrene A	3.0	1506	1509	204	1, 2
delta-cadinene	0.3	1524	1523	204	1, 2

[a]RA, relative area (peak area relative to total peak area); [b]RI (exp.), programmed temperature retention index from this experiment; [c]RI (lit.), programmed temperature retention index 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 in aerial parts of *O. basilicum*

Compounds	RA[a] (%)	RI (exp.)[b]	RI (lit.)[c]	MW[d]	Identification method[e]
alpha-pinene	0.1	932	939	136	1, 2
beta-pinene	0.1	975	979	136	1, 2
myrcene	0.2	990	991	136	1, 2
limonene	0.1	1027	1029	136	1, 2
1,8-cineol	1.1	1030	1031	154	1, 2
(*Z*)-beta-ocimene	0.1	1036	1037	136	1, 2
(*E*)-beta-ocimene	2.1	1047	1050	136	1, 2
alpha-terpinolene	0.1	1088	1089	136	1, 2
linalool	1.0	1101	1097	154	1, 2
camphor	0.7	1144	1146	152	1, 2
borneol	0.2	1165	1169	154	1, 2
methyl chavicol	89.8	1207	1196	148	2
beta-elemene	0.1	1391	1391	204	1, 2
methyl eugenol	0.6	1406	1404	178	1, 2
beta-caryophyllene	0.2	1418	1419	204	1, 2
trans-alpha-bergamotene	0.5	1435	1435	204	1, 2
alpha-humulene	0.2	1452	1455	204	1, 2
germacrene D	0.4	1480	1485	204	1, 2
bicyclogermacrene	0.2	1495	1500	204	1, 2
alpha-bulnesene	0.5	1504	1510	204	2
gamma-cadinene	0.3	1513	1514	204	1, 2
epi-alpha-cadinol	1.1	1640	1640	222	1, 2

[a]RA, relative area (peak area relative to total peak area); [b]RI (exp.), programmed temperature retention index from this experiment; [c]RI (lit.), programmed temperature retention index 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 in aerial parts of *O. americanum*

Compounds	RA[a] (%)	RI (exp.)[b]	RI (lit.)[c]	MW[d]	Identification method[e]
alpha-pinene	0.1	932	939	136	1, 2
6-metyhl-5-hepten-2-one	0.6	986	986	126	1, 2
myrcene	0.1	990	991	136	1, 2
para-cymene	0.1	1023	1025	134	1, 2
limonene	0.2	1027	1029	136	1, 2
(*E*)-beta-ocimene	0.9	1046	1050	136	1, 2
linalool	3.8	1101	1097	154	1, 2
endo-fenchol	0.2	1113	1117	154	1, 2
cis-*para*-mentha-2,8-dien-1-ol	0.1	1138	1138	152	1, 2
(*E*)-myroxide	0.1	1143	1145	152	1, 2
citronellal	0.1	1153	1153	154	1, 2
unknown	1.3	1165	-	152	
unknown	1.9	1184	-	152	
alpha-terpineol	0.2	1191	1189	154	1, 2
methyl chavicol	0.2	1199	1196	148	1, 2
nerol	1.7	1231	1230	154	1, 2
neral	33.6	1247	1238	152	2
geraniol	1.4	1258	1253	154	1, 2
geranial	40.0	1279	1267	152	2
alpha-copaene	0.2	1375	1377	204	1, 2
beta-caryophyllene	2.5	1418	1419	204	1, 2
trans-alpha-bergamotene	0.9	1435	1435	204	1, 2
cis-beta-farnesene	0.1	1443	1443	204	1, 2
alpha-humulene	1.1	1452	1455	204	1, 2
trans-beta-farnesene	0.5	1458	1457	204	1, 2
germacrene D	1.4	1480	1485	204	1, 2
beta-bisabolene	0.1	1508	1506	204	1, 2
delta-cadinene	0.1	1523	1523	204	1, 2
unknown	3.1	1543	-	204	-
caryophyllene oxide	0.5	1581	1583	220	1, 2

[a]RA, relative area (peak area relative to total peak area); [b]RI (exp.), programmed temperature retention index from this experiment; [c]RI (lit.), programmed temperature retention index 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 major volatile components of *O. tenuiflorum* with previous citation*

Compounds	Percentage relative area						
	EXP.	I	II	III	IV	V	VI
eugenol	0.4	59.4	7.3	8.5	4.4	53.1	
beta-elemene	0.5		2.5	2.8	1.7		
methyl eugenol	76.3		75.0	72.9	78.4		56.2
beta-caryophyllene	9.9	29.4	4.8	2.7	8.0	24.4	16.6
germacrene D	3.9						5.1
germacrene A	3.0	8.1					
(*E*)-cinnamyl acetate			3.8	4.8	2.0		

*component which was more than 2% relative area of the total; **EXP**. = this experiment; **I** from Trevisan et al., 2006; **II-IV** from Kothari et al., 2004; **V** from Asha et al., 2001; **VI** from Jirovetz et al., 2003.

Table 5. Comparison of major volatile components of *O. basilicum* with previous citation*

Compounds	Percentage relative area												
	EXP.	**I**	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**	**X**	**XI**	**XII**
1,8-cineol	1.1	4.2		6.1	10.3		2.7			5.1	11.0		
(*E*)-beta-ocimene	2.1												
linalool	1.0	48.3	21.5	23.2	12.3	4.3	5.1	2.7		49.9	42.5	69.3	28.4
camphor	0.7												13.1
methyl chavicol	89.8						68.3	41.8			33.1	2.4	
beta-elemene	0.1									3.31			
methyl eugenol	0.6				34.2	2.0	2.4						
beta-caryophyllene	0.2					3.4		2.5					
trans-alpha-bergamotene	0.5									4.1			
germacrene D	0.4	3.3		2.9	2.2		2.6						
gamma-cadinene	0.3	3.2		3.6	2.6					2.5		2.5	
epi-alpha-cadinol	1.1									4.0			
eugenol		10.1		3.1	4.2				44.0	12.3			
delta-cadinene		7.1		5.5	3.9		2.7						
methyl cinnamate			61.0	30.3	4.6								
alpha-cadinol			2.7									2.6	
zingiberene				3.4	2.2	2.8							
citral						61.0		33.9					
alpha-bisabolene						2.8		2.1					
geraniol						2.5							3.8
benzyl alcohol									5.7				
2-phenyl ethanol									2.7				
chavicol									29.5				
vanillin									2.9				
terpinen-4-ol											3.3		
alpha-muurolol											2.5		
trans-beta-guaiene												2.1	
(*Z*)-methyl cinnamate													34.5
(*E*)-methyl cinnamate													6.9

*component which was more than 2% relative area of the total; **EXP**. = this experiment; **I-VII** from Telci et al., 2006; **VIII** from Politeo et al., 2007; **IX** from Tognolini et al., 2006; **X** from Trevisan et al., 2006; **XI** from Soković & Griensven, 2006; **XII** from Jirovetz et al., 2003.

Discussion

The essential oil of *O. tenuiflorum* contained high percentage of methyl eugenol in consistent with previous reports from India (Jirovetz et al., 2003; Kothari et al., 2004). The high in methyl eugenol, phenylpropanoid derivative, content of this essential oil may be useful as pharmacological tools or applications for certain pharmacological activities such as antiplatelet activity (Tognolini et al., 2006), antinociceptive effect (Yano et al., 2006) and cardiovascular effect (Lahlou et al., 2004). This compound also might be used for other purposes including for attracting fruit flies for pollination (Tan et al., 2006) and in food and perfume industry (Kothari et al., 2004).

The chemical analysis of essential oils derived from *O. basilicum* has been the subject of many studies. The results showed great variation in essential oil components (see Table 5). One of them (Telci et al., 2006) investigated 18 Turkish basil essential oils composition and revealed that seven different chemotypes could be identified. In this study, the essential oil-enriched methyl chavicol (89.8%) was revealed and was considered as the highest percentage yield comparing to the other collections ranging from 0-68.3% (see Table 5). Because of high methyl chavicol content, this essential oil may be used to control *Tyrophagus putrescentiae*, a stored-food mite (Lee, 2005), to treat Candida infections

Table 6. Comparison of major volatile components of *O. americanum* with previous citation*

Compounds	Percentage relative area							
	EXP.	**I**	**II**	**III**	**IV**	**V**	**VI**	**VII**
alpha-pinene	0.1	8.3	2.7	2.6				4.7
myrcene	0.1		2.1			2.4		2.0
para-cymene	0.1			3.2		2.0		
limonene	0.2	7.8				2.0		41.5
(*E*)-beta-ocimeme	0.9							3.5
linalool	3.8						44.9	
alpha-terpineol	0.2		6.5					6.9
neral	33.6							
geraniol	1.4						38.2	
geranial	40.0						2.6	
beta-caryophyllene	2.5					22.4		
trans-alpha-bergamotene	0.9		2.8					
alpha-humulene	1.1							2.2
(*E*)-beta-farnesene	0.5					6.8	2.0	
delta-cadinene	0.1		2.5					4.0
unknown	3.1							
sabinene		8.0						
camphor		26.7			6.0			
gamma-selinene		10.9						
1,8-cineol			60.2	3.2				10.1
beta-pinene			5.7					7.7
eugenol				8.1				
thymol				43.5		2.33		
beta-selinene					3.4			
(*Z*)-methyl cinnamate					72.0			
(*E*)-methyl cinnamate					9.1			
gamma-terpinene						5.9		
sabinene hydrate						5.5		2.0
terpinene-4-ol						26.9		2.0
beta-sesquiphellandrene						2.9		
alpha-farnesene						4.0		

*component which was more than 2% relative area of the total; **EXP**. = this experiment; **I** from Upadhyay et al., 1991; **II** from Djibo et al., 2004; **III** from Cimanga et al., 2002; **IV** from Jirovetz et al., 2003; **V** from Oussou et al., 2004; **VI-VII** from Ngassoum et al., 2004.

in the combination with antifungal drugs (Shin & Pyun, 2004) and to use as local anesthetic agent (Leal-Cardoso et al., 2004).

The major composition of *O. americanum* essential oil was monoterpenes including geranial (40.0%), neral (33.6%) and linalool (3.8%). According to the literature, great differences in chemical compositions of *O. americanum* were revealed and their chemical patterns did not show the relationship with each other. For example, there are two chemotypes of *O. americanum* in Cameroon in which one had linalool (44.9%) and geraniol (38.2%) as major compositions, another contained limonene (41.5%) and 1,8-cineol (10.1%) as major compositions (Ngassoum et al., 2004). Two collections from India were also showed variation, one had a strong camphoraceous odor (Upadhyay et al., 1991), another possessed herbal-fruity and sweet-balsamic odor (Jirovetz et al., 2003). Some reports revealed that high amount of geranial together with neral in the essential oil were related to the antibacterial activity (Cimanga et al., 2002). Furthermore, some monoterpenes and sesquiterpenes such as alpha-pinene, beta-pinene, germacrene D, beta-caryophyllene and myrcene have shown antibacterial and antifungal activities which might be useful for medicinal purposes (Filipowicz et al., 2003; Magiatis et al., 1999;

Medina et al., 2005; Porter & Wilkins, 1999; Shafi et al., 2002).

The chemotaxomical study of the plant in genus *Ocimum* revealed great variation in their volatile components. That could be due to the difference 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). Therefore, research and development of essential oil from this genus such as the dosage formulation and other applications should be done carefully and their chemical composition profile should be investigated in each batch of collection for the preliminary quality control.

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