CHEMICAL COMPOSITIONS AND ANTIOXIDATIVE ACTIVITIES OF ESSENTIAL OILS FROM FOUR *OCIMUM* SPECIES ENDEMIC TO THAILAND

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ABSTRACT: *Ocimum basilicum*, *O. canum*, *O. gratissimum*, and *O. sanctum* were studied for their essential oil constituents by hydrodistillation from their aerial parts and then analyzed by Gas Chromatography-Mass Spectrometry (GC-MS). It was found that the individual essential oil contained both terpenoids and phenylpropanoids of which methyl chavicol (92.48 %), citral (71.99 %), and (*Z*)-β-ocimene (48.28 %) were major constituents of *O. basilicum*, *O. canum*, and *O. gratissimum*, respectively, while both red kaprao and white kaprao (*O. sanctum*) contained the same major constituent, methyl eugenol, 47.18 % and 53.67 % respectively. All essential oils were further studied on antioxidative activities, compared with standard vitamin E and butylated hydroxyltoluene (BHT), by DPPH (1,1-diphenyl-2-picrilhydrazyl) radical scavenging method and then the absorbance of DPPH was detected at 520 nm by UV spectrophotometer. The results had been shown in the effective concentration that decreases 50% of free radical (EC_{50}). It was found that EC_{50} of α-tocopherol, BHT, essential oil of *O. basilicum*, *O. canum*, *O. gratissimum*, red kaprao, and white kaprao (*O. sanctum*) were 62.77, 19.77, 47057.45, 8343.19, 30.20, 343.56, and 767.82 µg/ml, respectively. Among these essential oils, *O. gratissimum* had shown the highest antioxidative activity which is higher than that of standard α-tocopherol.

Key words: *Ocimum basilicum*, *O. canum*, *O. gratissimum*, *O. sanctum*, chemical constituent, antioxidative activities

INTRODUCTION: Due to synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and *tert*-butyl hydroquinone (TBHQ), which are widely used in food industry and cosmetic, have been growing concern over the possible carcinogenic effects[1]. Thus interest in natural antioxidant has increased considerably. Nowadays, it is well known that natural antioxidants extracted from herbs and spices have high antioxidant properties and are used in many food applications[2]. Various plants are proposed to be antioxidant according to their essential oils contain phenylpropanoids in high contents. Essential oils of plant genus *Ocimum* belonged to family Lamiaceae such as *Ocimum basilicum* L., *O canum* Sims., *O gratissimum* L. and *Ocimum sanctum* L. composed of interesting phenyl-propenes e.g. eugenol, methyl eugenol and methyl chavicol. Essential oils of these plants have been broadly used as culinary herbs and also used in folk medicine to treat various diseases. For example, the aerial part of *O. basilicum* is traditionally used as antispasmodic, aromatic, carminative, digestive, stomachic, and tonic agents[3-4]. They are also used externally for treatment acne, insect stings, snake bites, and skin infections[5]. In addition *O. gratissimum* are commonly used for treat upper respiratory tract infections, diarrhea, headache, ophthalmic, skin diseases, and pneumonia[6-7]. Moreover various parts of *O. sanctum* are traditionally used in Ayurveda for treating infections, skin diseases, hepatic disorder, cold, cough, malarial fever and as antidote for snake bite[8]. Although, these essential oils exhibited various

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biological activities such as antibacterial[9-11], anti helmintic[12], antifugal[13], however they have not been evaluated antioxidative activity. The purpose of this study was to determine the essential oil components of these plant species and their antioxidative activity.

MATERIALS AND METHODS:

Plant Materials

Arial part of four species in genus *Ocimum* including with *O. basilicum* L., *O. canum* Sims., *O gratissimum* L., and two varieties of *O. sanctum* L., red and white kaprao, were collected from plant garden of the Faculty of Pharmacy, Rangsit University, Pathum-thani, Thailand. All these explants were authenticated by one of us (N.R.).

Hydrodistillation

All aerial parts of these plants were hydrodistilled by a Clevenger-type apparatus. The essential oils were collected and stored at 4 °C until being analysed for its chemical constituents by Gas Chromatography/ Mass Spectrometry (GC/MS).

GC-MS Analysis

Finnigan Trace GC ultra (Thermo Electron Corpo-ration, USA) with quadrupole mass spectrometer was used for gas chromatography/mass spectrometry analysis. The column was ZB-5 fused silica linked methyl silicon capillary column (30 m. x 0.22 mm. i.d.; 0.25 µM); oven temperature programming was 50-250 °C at 7 °C/min; injector and detector temperature were 250 and 280 °C, respectively; sample volume injected was 1 µl; split ratio was 100:1; and the carrier gas was He (2 ml/min).

Compound Identification

Compounds were identified by comparing the Kovats gas chromatographic retention indices of the peaks on the HP-5MS column with literature values, computer matching using the Masslynx database, and comparison of the fragmentation patterns of the mass spectra with those reported in the literature[14,15].

Antioxidative Activities

All these essential oils were studied for the ability to scavenge the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical by comparison with a well known synthetic antioxidants, butylated hydroxytoluene (BHT) and α-tocopherol (vitamin E). Briefly, a portion of sample solution was mixed with the same volume of 6×10^{-5} M DPPH in methanol and allowed to stand in dark condition at room temperature for 30 minutes. The absorbances were then measure at 517 nm[16].

RESULTS AND DISCUSSION: The essential oil contents obtained from hydrodistillation on each fresh leave were shown in Table 1. They were exhibited clear, pale yellow with characteristic odor.

Table 1 Essential oil contents obtained from each fresh leaves hydrodistillation.

Plant species	Yield (% v/w)
Ocimum basilicum	0.15
Ocimum canum	0.05
Ocimum gratissimum	0.15
Ocimum sanctum	
White kaprao	0.06
Red kaprao	0.06

Individual essential oil was identified their components by GC-MS and the results were revealed in Table 2-5. As shown in these Tables, it was found that individual essential oil contained both terpenoids and phenylpropanoids of which methyl chavicol (92.48 %), citral (71.99 %), and (*Z*)-β-ocimene (48.28 %) were major constituents of *O. basilicum*, *O. canum*, and *O. gratissimum*, respectively, while both red and white kaprao (*O. sanctum*) contained the same major constituent, methyl eugenol, 47.18 % and 53.67 % respectively. Silva and coworker (2004) reviewed that the essential oil of *O. gratissimum* obtained by steam distillation possessed eugenol (54.0%), 1,8-cineol (21.6%) as the major constituents whereas (*Z*)-β-ocimene contained only 4.1%[17]. Meanwhile, the

Table 2 Chemical constituents of *O. basilicum* essential oil obtained from hydrodistillation

Compound	Kovat's Index	% Area
Monoterpenes		
β-myrcene	0991	0.24
limonene	1031	0.09
(*E*)- β-ocimene	1040	2.27
Oxygenated monoterpenes		
1,8-cineole	1033	0.93
fenchone	1087	0.07
linalool	1098	0.10
camphor	1143	0.33
Sesquiterpenes		
trans-α-bergamotene	1436	2.14
α-bulnesene	1505	0.22
γ-cadinene	1513	0.24
Oxygenated sesquiterpenes		
cubenol	1642	0.47
Phenylpropenes		
methyl chavicol	1195	92.48

essential oil of *O. gratissimum* obtained by hydro-distillation in the present study possessed (*Z*)-β-ocimene (48.28%) and eugenol (25.02%) as the main compounds, however 1,8-cineol had not been identified. Asha and coworkers (2001) reported that the oil of *O. sanctum* possessed eugenol (53.10%) as the main compound[12] whereas those of the present work along with Kothari and coworkers (2004) were methyl eugenol[18]. These results are somewhat different from our result which might imply that the climate and geographic conditions in different areas may affect the essential oil constituent production. Essential oil contents of two varieties of *O. sanctum* which are white and red kaprao are same (0.06 % v/w). Most of chemical constituents in both essential oils are same, but different in ratio. Moreover, there is one different minor compound, linalool, which had been only identified in red kaprao. Therefore both of them are smelled little different. Chemical structures of main compounds in these essential oils are shown in Fig. 1.

The essential oils were then determined antioxidative activity by DPPH radical scavenging method. The absorbances were then measure at 517 nm and calculated in the effective concentration that

Table 3 Chemical constituents of *O. canum* essential oil obtained from hydrodistillation

Compound	Kovat's Index	% Area
Monoterpenes		
limonene	1031	0.52
(*E*)- β-ocimene	1050	2.48
Oxygenated monoterpenes		
fenchone	1087	1.11
linalool	1098	5.65
nerol	1228	1.69
neral	1240	34.01
geraniol	1255	1.01
geranial	1270	37.89
Sesquiterpenes		
β-caryophyllene	1418	6.79
trans-α-bergamotene	1436	0.71
α-humulene	1454	2.33
γ-muurolene	1477	1.48
(*Z*)- α-bisabolene	1504	1.91
Phenylpropenes		
methyl eugenol	1401	1.14
Other		
unidentified	-	2.88

Table 4 Chemical constituents of *O. gratissimum* essential oil obtained from hydrodistillation

Compound	Kovat's Index	% Area
Monoterpenes		
β-myrcene	0991	0.54
(*Z*)- β-ocimene	1040	48.28
(*E*)- β-ocimene	1050	1.74
Oxygenated monoterpenes		
linalool	1098	0.72
Sesquiterpenes		
α-copaene	1376	0.82
β-cubebene	1390	0.33
β-caryophyllene	1418	3.06
trans-α-bergamotene	1436	4.36
α-humulene	1454	0.24
γ-muurolene	1477	9.32
(*E,E*)- α-farnesene	1508	4.33
δ-cadinene	1524	0.44
Phenylpropenes		
eugenol	1356	25.02
methyl eugenol	1401	0.61
Other		
unidentified	-	0.18

Table 5 Chemical constituents of *O. sanctum* essential oil obtained from hydrodistillation

Compound	Kovat's Index	% Area	
		white kaprao	red kaprao
Monoterpenes			
α-thujene	0931	0.53	0.73
camphene	0953	0.42	0.69
sabinene	0976	0.13	0.15
β-pinene	0980	0.34	0.51
limonene	1031	0.44	0.24
Oxygenated monoterpenes			
linalool	1098	-	0.34
borneol	1165	0.35	0.37
Sesquiterpenes			
α-copaene	1376	0.58	0.42
β-elemene	1391	1.15	0.82
β-caryophyllene	1418	35.2	37.83
α-humulene	1454	1.78	1.87
γ-muurolene	1477	2.14	3.49
α-bulnesene	1505	1.56	3.38
Phenylpropenes			
eugenol	1356	1.71	1.99
methyl eugenol	1401	53.67	47.18

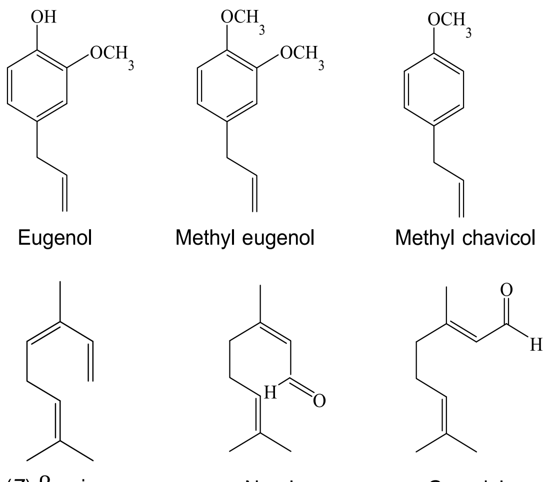

Fig.1 The chemical structure of main compounds in *Ocimum* spp. essential oil

decreases 50% of free radical (EC_{50}). The results were shown in Table 6. Compare to the synthetic antioxidant α-tocopherol (vitamin E) and butylated hyroxytoluene (BHT), essential oil of *O. gratissimum* had shown the best antioxidant which is double active more than standard α-tocopherol. Meanwhile essential oils of red and white kaprao (*O. sanctum*), *O. canum*, and *O. basilicum* were next in rank. It has established that the antioxidant effects are mainly because of phenolic compounds[19] meanwhile some monoterpene aldehydes and ketones such as citral, citronellal, isomenthone, and menthone are also powerful scavenging compounds[20]. Due to the main compounds in *O. gratissimum* essential oil are eugenol (25.02%) and (*Z*)-β-ocimene (48.28%) which eugenol has the hydroxyl radical possess antioxidative activity[21], and (*Z*)- β-ocimene had been reported to be antioxidant[22], so *O. gratissimum* essential oil had shown the highest antioxidative activity.

Even though essential oil of *O. canum* had contained fewer amounts of phenylpropenes, however it had been composed of highest content of citral which can be possessed antioxidant activity. Jilini and Simon (2002) had reported antioxidant activity of five green basil (*O. basilicum*) cultivars and breeding line[21]. Either essential oil contained high yield of eugenol or low yield of methyl chavicol would be high antioxidant activity. Our results had shown that *O. basilicum* essential oil contained high yield of methyl chavicol (92.48%) with no eugenol had not shown antioxidative activity. β-Caryophyllene is shown the high antioxidant activity[20] and Jilini and Simon[21] had also reported which essential oils consisted of higher yield of linalool, it would be higher antioxidant activity. Our results had shown that *O. sanctum* which contained high content of β-Caryophylline possessed antioxidant activity and red kaprao had been higher antioxidant activity than white kaprao according to its essential oil contained linalool and higher yield of eugenol.

Table 6 The EC_{50} of each essential oil and synthetic antioxidant

Name compound		EC_{50} (mcg/ml)
Synthetic antioxidant	Standard vitamin E	62.77
	Standard BHT	19.67
Essential oil	*Ocimum basilicum* L.	47057.45
	Ocimum canum Sims.	8485.29
	Ocimum gratissimum L.	30.20
	Ocimum sanctum L.	
	White kaprao	767.82
	Red kaprao	343.56

CONCLUSION: Each essential oil of four species in genus *Ocimum* contained both terpenoids and phenylpropanoids. Among these compounds methyl chavicol (92.48 %) and citral (71.99 %) were major constituents in essential oil of *O. basilicum* and *O. canum*, respectively. Meanwhile (*Z*)-β-ocimene (48.28 %) and eugenol (25.02%) were major constituents in essential oil of *O. gratissimum*. Both red and white kaprao (*O. sanctum*) contained same major constituent methyl eugenol, 47.18 % and 53.67 %, respectively. To determine antioxidative activity by DPPH radical scavenging, essential oil of *O. gratissimum* had shown the highest antioxidative activity which is which is higher than that of standard α-tocopherol. Therefore this essential oil may have some benefit in food industry and cosmetic.

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องค์ประกอบเคมีและฤทธิ์ต้านอนุมูลอิสระของน้ำมันระเหยจากพืชสกุล *Ocimum* 4 ชนิดในประเทศไทย

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บทคัดย่อ: ศึกษาองค์ประกอบเคมีของน้ำมันระเหยจาก โหระพา แมงลัก ยี่หร่า และกะเพรา โดยกลั่นส่วนเหนือดินของพืชแต่ละชนิดด้วยน้ำและนำน้ำมันระเหยที่ได้มาวิเคราะห์โดยใช้วิธี Gas Chromatography - Mass Spectrometry (GC-MS) พบสารประกอบเทอร์ปีนอยด์และฟีนิลโพรพานอยด์ และมี methyl chavicol (92.48 %), citral (71.99 %) และ (*Z*)-β-ocimene เป็นองค์ประกอบเคมีหลักในน้ำมันระเหยโหระพา แมงลัก และยี่หร่า ตามลำดับ ในขณะที่ทั้งกะเพราแดงและกะเพราขาวมี methyl eugenol เป็นองค์ประกอบเคมีหลักเหมือนกัน ซึ่งคิดเป็น 47.18 % และ 53.67 % ตามลำดับ ศึกษาฤทธิ์ต้านอนุมูลอิสระของน้ำมันระเหยโดยเปรียบเทียบกับสารมาตรฐานวิตามินอีและ butylated hydroxyltoluene (BHT) ด้วยวิธี DPPH (1,1-diphenyl-2-picrilhydrazyl) radical scavenging วัดค่าการดูดกลืนแสงของ DPPH ที่ 520 นาโนเมตร โดย UV spectrophotometer แสดงผลเป็นค่าความเข้มข้นของน้ำมันระเหยที่สามารถต้านอนุมูลอิสระได้ร้อยละ 50 (EC_{50}) พบว่าค่า EC_{50} ของสารมาตรฐานวิตามินอี BHT น้ำมันระเหยของโหระพา แมงลัก ยี่หร่า กะเพราแดง และกะเพราขาว เท่ากับ 62.77, 19.77, 47057.45, 8343.19, 30.20, 343.56 และ 767.82 ไมโครกรัมต่อมิลลิลิตร ตามลำดับ และในบรรดาน้ำมันระเหยของพืชที่ศึกษาทั้งหมดนั้นน้ำมันระเหยยี่หร่าแสดงฤทธิ์ต้านอนุมูลอิสระได้ดีที่สุด ซึ่งมีฤทธิ์ดีกว่าสารมาตรฐานวิตามินอี 2 เท่า

คำสำคัญ: โหระพา แมงลัก ยี่หร่า กะเพรา องค์ประกอบเคมี ฤทธิ์ต้านอนุมูลอิสระ

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