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Original Article

Antimicrobial, antioxidant activities and chemical composition of selected Thai spices

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Abstract

Nine volatile oils and six methanol extracts from *Ocimum americanum, O. basilicum, O. sanctum*, *Citrus hystrix*, *Alpinia galanga, Curcuma zedoaria, Kaempferia parviflora* and *Zingiber cassumunar* were assessed for antimicrobial and antioxidant activities. The volatile oils and extracts were investigated against eight bacteria and three fungi. The results illustrated that *O. americanum* volatile oil exhibited broad spectrum activity against tested bacteria with the MICs ranging 1.4-3.6 mg/ml and *Candida spp*. with the MICs ranging from 0.5-0.6 mg/ml. The *O. sanctum* volatile oil showed a considerable activity against only *Candida spp*. with the MICs ranging from 0.8-1.4 mg/ml. Interestingly, growth of *Mycobacterium phlei* was inhibited by the volatiles of *O. americanum*, *C. hystrix* peel, and *C. zedoaria* with MIC of 1.7, 3.5 and 1.2 mg/ml, respectively. For antioxidant activity evaluation, the methanol extracts of *C. hystrix* (leaf and peel) and *K. parviflora* had potent antioxidant activity by the radical-scavenging DPPH method with IC_{50} of 24.6, 66.3 and 61.5 mg/ml, respectively. GC-MS analysis revealed the typical chemical profiles of the volatile oils. The major component showed the characteristics of the volatile oils and was probably responsible for the antimicrobial effect.

Keywords: antimicrobial, antioxidant, chemical composition, GC-MS

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1. Introduction

Essential oils and various extracts of spices and herbs have recently attracted scientific interest as sources of natural medicine. They have been screened for their potential use as traditional medicine for treatment of many infectious diseases and food preservatives (Candan *et al.*, 2003). Thai spices are commonly used daily as food ingredients and flavoring agents. Many reports demonstrated an array of biological activities of compounds and volatile oils isolated from the spices (Bakkali *et al.*, 2008). They also showed variation of the chemical profiling dependent upon geographical and seasonal changes (Ndounga *et al.*, 1997; Cimanga *et al.*, 2002; Ficker *et al.*, 2003; Jirovetz *et al.*, 2003; Silva *et al.*, 2003). For instance, the Turkish basils appeared in different morphology and had at least seven chemotypes based on the major constituents (Telci *et al.*, 2006).

In this study, we determined the chemical composition, antimicrobial and antioxidant activities of the methanol extracts and the volatile oils, isolated from Thai spices including *Ocimum americanum*, *O. basilicum*, *O. sanctum* (Lamiaceae), *Citrus hystrix* peel and leaf (Rutaceae), *Alpinia galanga*, *Curcuma zedoaria*, *Kaempferia parviflora* and *Zingiber cassumunar* (Zingiberaceae). Some of them are recommended by the Ministry of Health as herbs for primary healthcare such as mucopolysaccharide from *O. americanum* seed used as bulk laxative, *O. basilicum*, *O. sanctum* leaves and *A. galanga* rhizome used for antiflatulence, *Z. cassumunar* rhizome used as an anti-inflammatory. The last, *Z. cassumunar* rhizome, is also being included in the National Herbal List of Thailand for emmenogouge in the recipe of Prasa-Plai. *C. hystrix* has been widely used in the food, flavor and fragrance industries. Its peel oil is traditionally used in hair treatment preparations. Its leaf is a major component of the aroma in the well-known Thai soup. *K. parviflora* is traditionally used as a tonic drink for health promotion (Yenjai *et al.*, 2004). As they used as spices, the safety warranty is recognized. Therefore, we evaluated their potential for antimicrobial and antioxidant activities. Comparison of the activities and chemical profiling is discussed.

2. Materials and Methods

2.1 Plant materials

Plant materials were obtained from the local market as shown in Table 1. The plants were identified and the voucher specimens were deposited at Faculty of Pharmaceutical Sciences, Khon Kaen University, Prince of Songkla University and Srinakharinwirot University, Thailand.

2.2 Preparations of volatile oils and methanol extracts

2.2.1 Volatile oils

Volatile oil was extracted from 1 kg fresh plants (Table 1) by hydrodistillation using Clevenger apparatus for 3 h. The volatile oil from *Citrus hystrix* peel was extracted by cold press method. The volatile oils obtained were dried with anhydrous Na_2SO_4, kept under N_2 gas, in light-resistant containers, and stored at -20ºC until use.

2.2.2 Methanol extracts

Fresh plants (0.5 kg) were macerated with 2.5 l of methanol for 3-4 days at room temperature (30±2°C) and filtered. The filtrate was evaporated under reduced pressure and the residue kept at -20°C until use.

Table 1. List of selected Thai spices.

Plant name	Plant part	Source
LAMIACEAE*		
Ocimum americanum L.	aerial part	Patthalung
O. basilicum L.	aerial part	Patthalung
O. sanctum L.	aerial part	Patthalung
RUTACEAE**		
Citrus hystrix DC.	leaf	Khon Kaen
C. hystrix DC.	fruit peel	Khon Kaen
ZINGIERACEAE**		
Alpinia galanga Willd.	rhizome	Songkhla
Curcuma zedoaria Rosc.	rhizome	Yala
Kaempferia parviflora Wall. Ex Baker	rhizome	Loei
Zingiber cassumunar Roxb.	rhizome	Yala

* volatile oils
**volatile oils and methanol extracts

2.3 Antimicrobial activity

2.3.1 Microbial strains

The following strains of bacteria and fungi were used as test organisms: gram positive bacteria, *Bacillus subtilis* (ATCC6051), *Staphylococcus epidermidis* (ATCC12228), *S. aureus* (ATCC25923); gram negative bacteria: *Escherichia coli* (ATCC25922), *Enterococcus faecalis* (ATCC1406), *Proteus mirabilis* (ATCC14153), *Pseudomonas aeruginosa* (ATCC27853); Mycobacterium, *Mycobacterium phlei* (ATCC 11758) and fungi: *Candida albicans* (ATCC10231), *C. parasilosis* (ATCC90018) and *C. tropicalis* (ATCC13803). Bacteria and fungi were prepared as described in Brantner and Grein (1994).

2.3.2 Agar disc diffusion method

Filter paper discs (9 mm in diameter) were impregnated with 5 ml (approx. 4-5 mg) of the volatile oils and 100 ml (approx. 1.65 mg) of the diluted methanol extracts. Discs were placed on agars as appropriate, which had been spread with suspension of test microorganism (1×10^8 CFU/ml). Plates were incubated at 37°C, for 24 h for bacteria and 26°C, for 48 h for fungi. The diameter of the inhibition zones was measured in mm. The results were evaluated by comparison to standard antibiotics.

2.3.3 Minimal inhibitory concentration (MIC)

The MICs were determined using microdilution broth method (Brantner and Grein, 1994). The oil solution was prepared in methanol to obtain concentrations of 1.0-3.5 mg/ml for antibacterial testing and concentrations of 0.1-1.6 mg/ml for antifungal testing. Bacterial and fungal suspensions (5×10^4 CFU/ml) were added onto a 96-well microtiter plate in the presence of Mueller Hinton broth. The plates were incubated. The MIC was defined as the lowest antimicrobial concentration that completely inhibited growth.

2.4 Antioxidant activity

2.4.1 2,2-diphenyl-1-picryl-hydrazine (DPPH) assay

DPPH assay was carried out with slight modification (Hatano *et al.*, 1988). The free radical scavenging capacity of the oils and extracts was compared to the antioxidant activity of rutin as a positive control. Test solutions were prepared in methanol to obtain concentrations of 2-250 mg/ml. After mixing with DPPH solution and standing for 10 min, the absorbance was measured at 517 nm (Jasco 7800 spectrophotometer). Samples were measured in triplicate. The IC_{50} was determined.

2.4.2 Lipid peroxidation assay

The method of non-enzymatic lipid peroxidation in liposome assay was followed according to the previous report (Houghton *et al.*, 1995). Test oils and extracts were diluted in DMSO to obtain the concentration of 25-200 mg/ml. The absorbance was measured at 532 nm. The inhibition of lipid peroxidation was calculated in percent by comparing to control tubes without extract added. Fisetin was used as a standard. Test samples were carried out in triplicate.

2.5 GC and GC-MS analysis

GC was performed using a Varian CP-3800 gas chromatograph (Varian Chromatography Systems, Walnut Creek, California, USA) equipped with a 1079 universal capillary injector operating at 250°C in the split mode (1:20), and a flame ionisation detector (FID) running at 310°C. The samples were analyzed on a fused silica Permabond SE 54 capillary column (50 m x 0.25 mm i.d.; film thickness 0.27 μm; Macherey-Nagel, Düren, Germany). The helium carrier gas had a delivery rate of 1.8 ml/min. Oven temperature programming was 50°C during injection, and gradient from 50°C to 300°C at the rate of 5°C/min.

GC/MS was performed on a Hewlett Packard (Hewlett Packard, Palo Alto, California, USA) G1800A GCD system (Electron impact voltage: 70 eV, injector temperature: 280°C, detector temperature: 320°C, foreline pressure: 4 Pa, mass range 30-425 amu). The sample was analyzed on a HP-5 (30 m x 0.25 mm i.d.; film thickness 0.25 μm) capillary column (Hewlett Packard). The helium carrier gas had a delivery rate of 1 ml/min and the column temperature programming was as follows: 40°C initial temperature maintained for 5 min, and the temperature was increased from 40°C to 300°C at the rate of 4°C/min.

Compounds were identified using both chromatographic and mass spectroscopic criteria. The WILEY275 database was used for automatic identification of GC-MS peaks, and linear retention indices were compared with published data. Whenever possible, mass spectra and retention indices were also compared with data obtained from authentic compounds (Dragoco, Holzminden, Germany; Fluka Chemie GmbH, Buchs Switzerland). Quantitative results were achieved from GC-FID profiles using the area percent method without consideration of calibration factors (i.e. F = 1.0) for all compounds.

3. Results

3.1 Antimicrobial activity

Eight bacteria and three fungi were investigated for susceptibility tests with the volatile oils and the methanol extracts. As shown in Table 2, *O. americanum* volatile oil inhibited growth of *B. subtilis*, *S. aureus*, *S. epidermidis*, *E. coli*, *P. mirabilis*, *C. albicans*, *C. parapilosis* and *C. tropicalis*. The MICs of *O. americanum* against *Candida spp.* were rather low (0.5-0.6 mg/ml), whereas the MICs against bacteria ranged from 1.4-3.6 mg/ml. This result suggested that the

Table 2. Antimicrobial activity screening of the volatile oils and the methanol extracts

Plants by family	Extracts	Inhibition zone diameter (mm)[b]										
		B. s.[a]	S. a.	S. e.	E. c.	E. f.	P. a.	P. m.	M. p.	C. a.	C. p.	C. t.
LAMIACEAE												
Ocimum americanum	Volatile oil	15	17	15	13	<11	<11	15	15	24	19	20
O. basilicum	Volatile oil	<11	<11	<11	<11	<11	<11	<11	<11	<11	<11	<11
O. sanctum	Volatile oil	<11	12	<11	<11	<11	<11	<11	<11	17	13	14
RUTACEAE												
Citrus hystrix peel	Volatile oil	<11	<11	<11	<11	<11	<11	<11	14	<11	<11	<11
C. hystrix peel	MeOH	<11	<11	<11	<11	<11	<11	<11	<11	<11	<11	<11
C. hystrix leaf	Volatile oil	<11	<11	<11	<11	<11	<11	<11	<11	<11	<11	<11
C. hystrix leaf	MeOH	<11	<11	<11	<11	<11	<11	<11	<11	<11	<11	<11
ZINGIBERACEAE												
Alpinia galanga	Volatile oil	<11	<11	<11	<11	<11	<11	<11	<11	12	<11	<11
A. galanga	MeOH	<11	<11	<11	<11	<11	<11	<11	<11	<11	<11	<11
Curcuma zedoaria	Volatile oil	<11	12	<11	<11	<11	<11	<11	18	<11	<11	<11
C. zedoaria	MeOH	<11	<11	<11	<11	<11	<11	<11	<11	<11	<11	<11
Kaempferia parviflora	Volatile oil	<11	<11	<11	<11	<11	<11	<11	12	<11	<11	<11
K. parviflora	MeOH	<11	<11	<11	<11	<11	<11	<11	<11	<11	<11	<11
Zingiber cassumunar	Volatile oil	<11	<11	<11	<11	<11	<11	<11	13	<11	<11	<11
Z. cassumunar	MeOH	<11	<11	<11	<11	<11	<11	<11	<11	<11	<11	<11
Antibiotics		27[k]	22[k]	25[k]	18[k]	14[am]	22[g]	20[k]	20[ox]	24[n]	26[n]	25[n]

[a] bacterial species: B. s. = *Bacillus subtilis*; S. a. = *Staphylococcus aureus*; S. e. = *S. epidermidis*; E. c. = *Escherichia coli*; E. f. = *Enterococcus faecalis*; P. a. = *Pseudomonas aeruginosa*; P. m. = *Proteus mirabilis*; M. p. = *Mycobacterium phlei*; C. a. = *Candida albicans*; C. p. = *C. parapilosis*; C. t. = *C. tropicalis*.

[b] As the diameter of the disc was 9 mm, inhibition zone <11 mm was not evaluated.

[c] Antibiotics: k = kanamycin; am = ampicillin; g = gentamycin; ox = oxacillin; n = nystatin

volatile oil of *O. americanum* remarkably exhibited broad spectrum of activity, especially toward the fungi. The volatile oil of *O. sanctum* showed moderate activity against *C. albicans*, *C. parapilosis* and *C. tropicalis* with the MICs of 0.8, 1.3 and 1.4 mg/ml, respectively. Against *M. phlei*, the volatile oils of *O. americanum*, *C. hystrix peel* and *C. zedoaria* exhibited the inhibitory activity with the MICs of 1.7, 3.5 and 1.2 mg/ml, respectively. The volatile oil of *O. basilicum*, *C. hystrix* leaf, *A. galanga*, *K. parviflora* and *Z. cassumunar* did not show any inhibitory activity against tested organisms.

3.2 Antioxidant activity

Plant extracts and volatile oils have been evaluated for their antioxidant activity according to radical scavenging property. In the DPPH method, as shown in Table 4, the methanol extract of *C. hystrix* leaf exhibited the highest radical scavenging activity with an IC_{50} of 24.6 μg/ml. The extracts of *C. hystrix* peel and *K. parviflora* rhizome scavenged the hydroxyl radical similarly with IC_{50} of 66.3 and 61.5 μg/ml, respectively. The oils of *C. hystrix* (peel and leaf), *O. americanum* and *O. sanctum* showed weak activity with an IC_{50} > 250 μg/ml. The other test samples did not show any activity against the DPPH radical. The reference standard rutin had an IC_{50} of 0.9 μg/ml. In the lipid peroxidation method, the results showed that at 50 μg of a reference standard fisetin could inhibit lipid peroxide at the level of 10%, whereas methanol extracts of *C. hystrix* leaf, *C. hystrix* peel and *K. parviflora* showed less activity. The level of inhibition was higher than 90% of control (data not shown). Other tested samples did not show any scavenging activity on the lipid peroxide in the range of amount tested (25-200 μg).

3.3 Chemical composition

GC and GC-MS analysis provided the chemical profiling of the volatile oils and suggested the chemotype of the spices. The *O. americanum* oil, which exhibited the broad range of antibacterial and antifungal activities, contained (*E*)-citral (29.76%) and (*E*)-citral (23.74%) as major components, followed by methyl chavicol (9.43%), L-linalool (8.76%), cis-α-bisabolene (4.44%) and β-caryophyllene (4.05%). The largest component found in the *O. basilicum* oil was methyl

Table 3. Minimum inhibitory concentrations (MICs) of the effective volatile oils

Tested organisms	MIC (mg/ml)				
	C. hystrix peel	*C. zedoaria*	*O. americanum*	*O. sanctum*	Antibiotics[a]
Bacillus subtilis			3.6		0.0002[k]
Escherichia coli			1.6		0.0005[k]
Mycobacterium phlei	3.5	1.2	1.7		0.0001[ox]
Proteus mirabilis			2.3		0.0005[k]
Staphylococcus aureus			1.4		0.0002[k]
S. epidermidis			1.4		0.0002[k]
Candida albicans			0.5	0.8	0.0001[n]
C. parapilosis			0.5	1.3	0.0004[n]
C. tropicalis			0.6	1.4	0.0005[n]

[a]Antibiotics: k = kanamycin; ox = oxacillin; n = nystatin

chavicol (75.61%) and trace components were α-zingiberene (5.21%), trans-β-ocimene (2.62%) and 1,8-cineole (1.81%). The *O. sanctum* oil contained methyl eugenol (65.36%) as major component, following β-elemene (9.38%), d-germacrene (4.90%) and α-humulene (2.48%) (Table 5).

C. hystrix peel and leaf showed similar patterns of chemical compositions as shown in Table 6. The major constituent was citronellal (about 23%). Trace components in the oil of *C. hystrix* peel were L-linalool (4.22%), β-pinene (1.82%) and limonene (1.13%). The oil of *C. hystrix* leaf had a similar pattern to that found in the oil of *C. hystrix* peel. The chemical profilings of *A. galanga*, *C. zedoaria*, *K. parviflora* and *Z. cassumunar* oils are listed in Table 7. The principal components were 1,8-cineole (26.33%) in *A. galanga* oil, unknown component (23.95%) in *C. zedoaria* oil, D-germacrene (9.55%) and camphene (9.22%) for *K. parviflora* oil, and terpinene-4-ol (40.65%) in *Z. cassumunar* oil.

Table 4. Antioxidant activity of the volatile oils and the extracts by DPPH method

Plant extracts	IC_{50}(μg/ml)
C. hystrix peel extract	66.3
C. hystrix peel oil	>250
C. hystrix leaf extract	24.6
C. hystrix leaf oil	>250
K. parviflora extract	61.5
O. americanum oil	>250
O. sanctum oil	>250
Rutin	0.9

4. Discussion

The use of Thai spices is not only for food additives but also for acting as medicinal plants as recommended of the Ministry of Health of Thailand. We performed the antimicrobial, antioxidant activities and chemical composition of Thai spices. The most active volatile oil for antimicrobial activity was *O. americanum* oil, since it contained (*E*)-citral and (*Z*)-citral, which possesses a wide range of antimicrobial properties as demonstrated in the tea tree oil (Hayes and Markovic, 2002). It can be proposed to use *O. americanum* oil for anti-infective preparation. However, in our study, we could not detect the inhibitory activity of *O. basilicum* oil, which has been reported to possess activity against various bacteria. A previous report suggested that there is variability in composition of *O. basilicum* oil, that can be divided into 7 chemotypes based on principal component: linalool, methyl cinnamate, methyl cinnamate/linalool, methyl eugenol, citral, methyl chavicol and methyl chavicol/citral types. The most active components against bacteria are linalool, citral and methyl cinnamate types (Telci *et al.*, 2006). Hussain and his co-worker demonstrated that *O. basilicum* produced the essential oils that varied dependent upon the season. The linalool-rich *O. basilicum* oil exhibited antibacterial and antifungal activities, including activities against *S. aureus*, *E. coli* and *B. subtilis* and pathogenic fungi *Aspergillus niger* (Hussain *et al.*, 2008). In this study, *O. basilicum* oil is categorized in the methyl chavicol chemotype and, therefore, was inactive against tested bacteria. On the other hand, *O. sanctum* oil is classified to the methyl eugenol chemotype, which showed to have broad range in antimicrobial activity and had good antifungal activity.

In this study, the volatile oils and methanol extracts of *C. hystrix* (peel and leaf), *A. galanga*, *C. zedoaria*, *K. parviflora* and *Z. cassumunar* did not show any antimicrobial activity, except the volatile oils of *C. hystrix* peel and *C. zedoaria* against *M. phlei*. It is hypothesized that the active ingredient is probably absent from the volatile oils and methanol extracts. On the other hand, the extracts of *A. galanga* rhizome with acetone and ethyl acetate containing 1-acetoxychavicol acetate possessed potent antibacterial activity

Table 5. Chemical compositions of the *Ocimum* oils.

Substance	retention indice[a]	Composition (%)		
		O. americanum	*O. basilicum*	*O. sanctum*
Dodecane	975	-	0.21	-
1-Octen-3-ol	979	0.10	-	-
No matches found	986	-	0.60	-
6-Methyl-5-hepten-2-one	988	6.00	-	-
1,8-cineole	1023	0.79	1.81	-
Limonene	1024		-	-
Cis-ocimene	1032	-	0.05	-
Trans-beta-ocimene	1043	1.41	2.62	-
Alpha-terpinolene	1079	-	0.12	-
Fenchone	1081	0.39	-	-
No matches found	1091	-	-	-
L-linalool	1095	8.76	0.80	0.33
L-camphor	1133	-	1.83	-
Citronellal	1149	0.56	-	-
(-)-Borneol	1155	-	-	1.39
Alpha-terpineol	1188	-	-	-
Methyl Chavicol	1205	9.43	75.61	0.72
Beta-citronellol	1220	-	-	-
Z-citral	1252	29.76	-	-
Geraniol	1265	0.58	-	-
Piperitone	1261	0.47	-	-
Trans-anethole	1277	-	0.36	-
E-citral	1286	23.74	-	-
Eugenol	1356	0.25	-	2.32
Neryl acetate	1363	0.49	-	-
Beta-elemene	1382	0.32	1.08	9.38
Geranyl acetate	1382	0.99	-	-
Methyl eugenol	1396	0.11	1.35	65.36
Beta-caryophyllene	1412	4.05	0.60	-
Alpha-zingiberene	1426	1.83	5.21	-
Alpha-humulene	1443	1.05	0.39	2.48
Trans-beta-farnesene	1447	0.32	0.21	-
Epi-bicyclosesqui-phellandrene	1452	-	0.14	-
d-Germacrene	1471	1.90	0.28	4.90
Alpha-amorphene	1504	-	-	-
Beta-sesquiphellandrene	1517	-	0.28	-
Elemol	1543	-	-	0.37
Cis-alpha-bisabolene	1544	4.44	-	
Caryophyllene oxide	1580	0.56	-	0.70
Beta-eudesmol	1645	0.12	-	-
Oxygenated sesquiterpene	1645	-	-	-
Alpha-bisabolol	1679	0.21	-	-

[a] Linear retention indices were compared with published data and data obtained from authentic compounds.
[b] - = absence

against such as *E. coli, Salmonella typhi*, vancomycin resistant *Enterococcus faecalis*, *Propionibacterium acnes*, *S. aureus*, and *S. epidermidis* (Latha *et al.*, 2009; Niyomkam *et al.*, 2010). However, Natta *et al.* (2009) reported that *A. galanga* oil, which was composed of methyl chavicol as the major component, did not inhibit growth of *E. coli, S. aureus*,

Table 6. Chemical compositions (%) of the *Citrus hystrix* oils.

Substance	retention indice[a]	Composition (%)	
		C. hystrix leaf	*C. hystrix* peel
Octane	792	9.57	-[b]
1-ethyl-2-methyl-benzene	957	3.94	-
Sabinene	968	2.21	1.55
Beta-pinene	970	1.67	1.82
1, 2, 3-trimethyl-benzene	975	1.26	-
1, 3, 5-trimethyl-benzene	988	5.47	1.01
1, 2, 4-trimethyl-benzene	989	-	4.90
Alpha-terpinene	1012	0.20	-
p-cymene	1019	0.55	-
Limonene	1023	1.18	1.13
Trans-sabinene hydrate	1061	2.48	2.35
L-linalool	1095	4.36	4.22
Citronellal	1150	23.41	23.85
Alpha-terpineol	1185	5.40	5.15
Citronellol	1223	1.40	1.48
Citronellyl acetate	1348	3.75	3.82
Alpha-copaene	1369	2.35	2.16
Geranyl acetate	1379	4.45	5.12
Beta-cubebene	1383	2.46	2.34
Beta-caryophyllene	1412	3.51	3.73
Alpha-humulene	1446	0.94	1.09
d-Germacrene	1475	1.82	2.01
Delta-cadinene	1518	4.74	5.69
Elemol	1543	4.17	6.59
Neronidol	1557	0.51	0.91
1,6-Germacradien-5-ol	1568	0.65	1.23

[a] Linear retention indices were compared with published data and data obtained from authentic compounds.
[b] - = absence

Table 7. Chemical compositions (%) of the Zingiberaceous oils.

Substance	retention indice[a]	Composition (%)			
		A. galanga	*C. zedoaria*	*K. parviflora*	*Z. cassumunar*
Alpha-thujene	924	-[b]	-	-	1.16
Alpha-pinene	931	2.90	0.15	8.31	1.61
Camphene	943	0.10	-	9.22	-
Beta-pinene	971	1.69	-	11.10	1.22
Sabinene	974	-	-	-	18.03
Myrcene	989	1.49	-	1.26	1.65
Alpha-terpinene	1013	0.54	-	-	3.42
Limonene	1022	-	-	2.58	-
p-cymene	1022	-	-	-	5.72
Beta-phellandrene	1025	-	-	-	1.52
Beta-ocimene	1032	-	-	-	-
1,8-cineole	1036	26.33	3.57	0.88	-
Gamma-terpinene	1055	0.95	-	0.09	7.02

Table 7. (Continued)

Substance	retention indice[a]	Composition (%)			
		A. galanga	*C. zedoaria*	*K. parviflora*	*Z. cassumunar*
Alpha-terpinolene	1084	-	3.52	0.34	1.52
L-linalool	1094	-	-	3.67	-
Camphor	1139	-	2.98	-	-
1-Terpineol	1141	-	-	-	0.66
Borneol	1159	-	0.45	6.49	-
Terpinene-4-ol	1173	2.34	0.22	0.19	40.65
Alpha-terpineol	1184	-	0.26	0.22	1.15
Chavicol	1259	0.69	-	-	-
Endobornyl acetate	1276	-	-	-	-
Piperitenone	1336	-	1.70	-	-
No matches found	1346	13.93	-	-	-
Alpha-copaene	1371	0.29	-	6.83	-
Geranyl acetate	1381	0.74	-	-	-
Beta-elemene	1389	2.39	0.57	6.03	-
Methyl eugenol	1403	0.57	-	-	-
Beta-caryophyllene	1418	5.48	0.69	6.76	-
Epi-bicyclosesquiphellandrene	1423	-	-	-	-
Gamma-elemene	1431	2.33	1.70	-	-
Alpha-humulene	1451	2.81	0.04	2.00	-
Beta-farnesene	1457	4.27	-	-	-
AR-curcumene	1474	-	0.86	-	-
D-germacrene	1477	-	-	9.55	-
Alpha-guaiene	1489	-	-	0.38	-
Bicyclogermacrene	1489	-	-	3.00	-
Beta-selinene	1486	5.14	-	-	-
Alpha-selinene	1494	3.31	-	-	-
No matches found	1494	-	4.91	-	-
Beta-bisabolene	1508	4.78	-	-	-
Delta-cadinene	1516	-	-	2.17	-
Beta-sesquiphellandrene	1520	0.76	1.56	-	1.02
Eugenol acetate	1524	0.45	-	-	-
No matches found	1550	-	0.77	-	-
No matches found	1555	-	1.01	-	-
No matches found	1575	-	2.99	-	-
No matches found	1579	-	1.94	-	-
No matches found	1605	-	8.31	-	-
No matches found	1631	-	-	6.21	6.21
No matches found	1637	-	1.10	-	-
Oxygenated sesquiterpene	1664	-	-	-	-
No matches found	1681	-	23.95	-	-
No matches found	1683	-	3.09	-	-
No matches found	1697	-	3.17	-	-
No matches found	1711	-	9.19	-	-

[a]Linear retention indices were compared with published data and data obtained from authentic compounds.
[b]- = absence

B. cereus or *Listeria monocytogenes*. Interestingly, the characteristics of their *A. galanga* oil were different from ours, which has cineole as a principal component. This evidence is also occurred in the case of *C. hystrix* peel. Waikedre *et al.* (2010) reported that *C. hystrix* peel oil contained terpinen-4-ol (13.0%), β-pinene (10.9%), α-terpineol (7.6%), 1,8-cineole

(6.4%), citronellol (6.0%) and limonene (4.7%). Ethyl acetate extract of *C. hystrix* peel had stronger antibacterial activity than the volatile oil obtained from hydrodistillation (Chanthaphon *et al.*, 2008).

For radical scavenging activity, the extracts from *C. hystrix* (peel and leaf) and *K. parviflora* have promising an antioxidant activity. Several studies reported that flavonoids have the potential for antioxidant properties (Pietta, 2000). Hesperidin is a component, which responsible for radical scavenging activity in *C. hystrix* (Berhow *et al.*, 1996). Yenjai *et al.* (2004) demonstrated that *K. parviflora* contained a variety of flavonoids, which play an important role as radical scavengers.

From this study, we realized that the biological activity is variable according to the chemical compositions, the presence of an active ingredient in particular. Standardization of the component, which is responsible for the biological activity, is important. The results obtained from this study provide the information of distinct chemical profiling of Thai spices. Specifically, the *O. americanum* oils can be applied for a wide range of antibacterial purposes and can be used as a preservative instead of synthetic preservatives.

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References

Bakkali, F., Averbeck, S., Averbeck, D and Idaomar, M. 2008. Biological effected of essential oils-A review. Food and Chemical Toxicology. 46(2), 446-475.

Berhow, M., Fong, C.H. and Hasegawa, S. 1996. Limonoid and flavonoid composition in varieties of Papeda and Papedocitrus. Biochemical Systematics and Ecology. 24, 237-242.

Brantner, A. and Grein, E. 1994. Antibacterial activity of plant extracts used externally in traditional medicine. Journal of Ethnopharmacology. 44, 35-40.

Candan, F., Unlu, M., Tape, B., Daferera, D., Polissiou, M., Sokmen, A. and Akpulat, H.A. 2003. Antioxidant and antimicrobial activity of the essential oil and methanol extract of *Achillea millefolium* subsp. *Millefolium Afam* (Asteraceae). Journal of Ethnopharmacology 87, 215-220.

Chanthaphon, S., Chanthachum, S. and Hongpattarakere, T. 2008. Antimicrobial activities of essential oils and crude extracts from tropical *Citrus spp*. Against food-related microorganisms. Songklanakarin Journal of Science and Technology, 30 (SUPPL. 1), 125-131.

Cimanga, K., Kambu, K., Tona, L., Apers, S., Bruyne, T.D., Hermans, N., Totte, J., Pieters, L. and Vlietinck, A. J. 2002. Correlation between chemical composition and antibacterial of essential oils of some aromatic medicinal plants growing in the Democratic Republic of Congo. Journal of Ethnopharmacology. 79, 213-220.

Ficker, C.E., Smith, M., Susiarti, S., Leaman, D.J., Irawati, C. and Arnason, J.T. 2003. Inhibition of human pathogenic fungi by members of Zingiberaceae used by the Kenyah (Indonesian Borneo). Journal of Ethnopharmacology. 85, 289-293.

Hatano, T., Kagawa, H., Yasuhara, T. and Okada, T. 1988. Two new flavonoids and other constituents in licorice root: their relative astringency and radical scavenging effects. Chemical & Pharmaceutical Bulletin. 36, 2090-2097.

Hayes, A.J. and Markovic, B. 2002. Toxicity of Austrian essential oil *Backhousia citriodora* (Lemon myrtle). Part 1. Antimicrobial activity and *in vitro* cytotoxicity. Food and Chemical Toxicology. 40, 535-543.

Houghton, P.J. Zarka, R., De La Heras, B. and Hoult, J.R. 1995. Fixed oil of *Nigella sativa* and derived thymoquinone inhibit eicosanoid generation in leukocytes and membrane lipid peroxidation. Planta Medica. 61, 33-36.

Hussain, A.I., Anwar, F., Hussain Sherazi, S.T., and Przybylski, R. 2008. Chemical composition, antioxidant and antimicrobial activities of basil (*Ocimum basilicum*) essential oils depends on seasonal variations. Food Chemistry. 108(3), 986-995.

Jirovetz, L., Buchbauer, G., Shafi, M.P. and Kaniapady, M.M. 2003. Chemotaxonomical analysis of the essential oil aroma compounds of four different *Ocimum* species from southern India. European Food Research Tachnology. 217, 120-124.

Latha, C., Shriram, V.D., Jahagirdar, S.S., Dhakephalkar, P.K. and Rojatkar, S.R. 2009. Antiplasmid activity of 1'-acetoxychavicol acetate from *Alpinia galanga* against multi-drug resistant bacteria. Journal of Ethnopharmacology. 123(3), 522-525.

Natta, L., Orapin, K., Krittika, N., and Pantip, B. 2009. Essential oil from five Zingiberaceae for anti food-borne bacteria. International Food Research Journal. 16(3), 337-346.

Ndounga, M. and Onamba, J.M. 1997. Antibacterial and antifungal activities of essential oil of *Ocimum grastissimum* and *O. basilicum* from Congo. Fitoterapia. 68(2), 190-191.

Niyomkam, P., Kaewbumrung, S., Kaewnopparat, S. and Panichayupakaranant, P. 2010. Antibacterial activity of Thai herbal extracts on acne involved microorganism. Pharmaceutical Biology. 48(4), 375-380.

Pietta, P.G. 2000. Flavanoids as antioxidants. Journal of Natural Product. 63, 1035-1042.

Silva, M.G.D., Dos Santos, R.N., Matos, F.J.A. and Machado, M.I.L. 2003. Volatile oil constituents from leaf, inflorescene and root oils of *Ocimum americanum* L. grown in north-eastern Brazil. Flavour and Fragrance Journal. 18, 303-304.

Suppakul, P., Miltz, J., Sonneveld, K. and Bigger, S.W. 2003.

Antimicrobial properties of basil and its possible application in food packaging. Journal of Agricultural & Food Chemistry. 51, 3197-3207.

Telci, I., Emine, B., Güngör, Y. and Betül A. 2006. Variability in essential oil composition of Turkish basils (*Ocimum basilicum* L.). Biochemical Systematics and Ecology. 34(6), 489-497.

Waikedre, J., Dugay, A., Barrachina, I., Herrenknecht, C, Cabalion, P., and Fournet, A. 2010. Chemical composition and antimicrobial activity of the essential oils from New Caledonian *Citrus macroptera* and *Citrus hystrix*. Chemistry and Biodiversity. 7 (4), 871-877.

Yenjai, C., Prasanphen, K., Daodee, S., Wongpanich, V. and Kittikoop, P. 2004. Bioactive flavanoids from *Kaempferia parviflora*. Fitoterapia 75, 89-92.