International Food Research Journal 15(3): 337-346 (2008)

Essential oil from five Zingiberaceae for anti food-borne bacteria

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Abstract: Essential oil from five Zingiberaceae species: ginger (*Zingiber officinale* Roscoe.), galanga (*Alpinia galanga* Sw.), turmeric (*Curcuma longa* L.), kaempferia (*Boesenbergia pandurata* Holtt.) and bastard cardamom (*Amomum xanthioides* Wall.) obtained by hydrodistillation and two solvent extractions (petroleum ether and ethanol) was characterized. Their antibacterial effects towards *Escherichia coli*, *Staphylococcus aureus, Bacillus cereus* and *Listeria monocytogenes* were tested by a disc diffusion assay. Essential oil of kaempferia and bastard cardamom obtained by hydrodistillation extraction could inhibit growth of all tested bacteria. Essential oil of ginger extracted by hydrodistillation had the highest efficiency against three positive strains of bacteria (*S. aureus, B. cereus* and *L. monocytogenes*), with a minimum concentration to inhibit *B. cereus* and *L. monocytogenes* of 6.25 µg/ml. Volatile compounds of all extracts were analyzed by gas chromatography-mass spectrometry (GC-MS). The major components of ginger, galanga, turmeric, kaempferia, and bastard cardamom obtained by hydrodistillation, were zingiberene, methyl chavicol, turmerone, γ-terpinene, and methyl chavicol, respectively.

Keywords: Ginger, galanga, turmeric, kaempferia, bastard cardamom, antibacteria

INTRODUCTION

Food-borne diseases are still a major problem of the world, even in well-developed countries (Mead *et al.*, 1999). A variety of microorganisms also lead food spoilage that is encountered as one of the most important matter concerning the food industry. So far, many pathogenic microorganisms, such as *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Listeria monocytogenes* and *Campylobacter jejuni* have been reported as the causal agents of food-borne diseases and/ or food spoilage (Deak and Beuchat, 1996; Betts *et al.*, 1999). Thus, at present, it is a necessity to use the chemical preservatives to prevent the growth of food spoiling microbes in the food industry (Sagdıc and Ozcan, 2003). Due to the consumers concerned about the safety of food containing preservative as synthetic chemicals, therefore, there is a growing interest to use natural antibacterial compounds. Extracts of herbs and spices can be the preservation of foods, as these possess a characteristic flavor and sometimes show antioxidant activity as well as antimicrobial activity (Smid and Gorris, 1999). For centuries, indigenous plants have been used in herbal medicine for curing various diseases (Cowan, 1999). Recently, the acceptance of traditional medicine as an alternative form for health care and the development of microbial resistance to the available antibiotics (Srinivasan *et al.*, 2001; Kumarasamy *et al.*, 2002) have led authors to investigate the antimicrobial activity of medicinal plants.

Zingiberaceae is among the plant families that are widely distributed throughout the tropics, particularly in Southeast Asia. It is an important natural resource that provides man with many useful products for food, spices, medicines, dyes, perfume and aesthetics (Burkill, 1966). Thailand is a country of high plant biodiversity as a result of its geographical position in the tropics and the climatic variation between north and south. There are 200 species of Zingiberaceae belonging to 20 genera found in Thailand. In recent years, several reports have been published concerning the composition and/or the biological properties (antimicrobial, antioxidant, anticancer and a stimulated effect on the immune system) of Zingiberaceae extracts (Negi *et al.*, 1999; Scartezzini and Speroni, 2000; Youko *et al.*, 2000; Patricia *et al.*, 2003; Jirovetz *et al.*, 2003; Bendjeddou *et al.*, 2003; Nguefack *et al.*, 2004). These studies have emphasized the existence of marked chemical differences among oils extracted from different species or varieties. These variations are likely to influence the antimicrobial activity of the oil and are generally a function of three factors: genetically determined properties, the age of the plant and the environment.

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The objectives of this study were to compare the antimicrobial activity of the essential oils and extracts from Zingiberaceae against common food-borne pathogen and/or spoilage bacteria, including *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus* and *Listeria monocytogenes*. Evaluating minimal inhibitory concentrations and the main components of the extracts by GC/MS, in an attempt to contribute to the use of these as alternative products for microbial control and food preservation were determined.

MATERIALS AND METHODS

Plant material

Fresh rhizomes of five Zingiberaceae (ginger, galanga, turmeric, kaempferia, bastard cardamom) were purchased from a local vegetable and fruit market of the Tungkru District, Bangkok, Thailand.

Extraction procedure

Hydrodistillation

Essential oils of five Zingiberaceae species were extracted by hydrodistillation, and all operations were carried out at room temperature. The fresh rhizomes of Zingiberaceae were washed to remove soil, peeled and sliced. Sliced rhizomes of fresh Zingiberaceae (2 Kg) were mixed with distilled water (5 L). The essential oils were extracted by hydrodistillation using a vertical hydrodistillation unit. A flask containing the homogenate was heated during 24 h and the vapor condensed and separated throughout an auto-oil/water separator. Each essential oil extraction was running in triplicate. Yield percentages were recorded as dry basis material.

Solvent extraction

Plant material was oven dried at 50°C for 24 h to reduce water content. Extracts were prepared by blending preserved plant material (approximately 200 g dry weight) in 99% ethanol and petroleum ether (1:3 w/v ratio). After 24 h, the mixture was filtered through Whatman filter paper (No.1) using a Buchner funnel. The solvent was removed with a rotary vacuum evaporator at 40°C (30 mmHg). The oil was stored in dark vials at 4°C before analyzing. The waste or residue after extracted by petroleum ether of plant materials was repeated once with ethanol, called secondary extraction, similar to the above condition.

Gas chromatography/mass spectrometry analysis (GC-MS)

The volatile composition of plant extracts were analyzed using GC-MS system (GC-8000, FISONS Co., Italy), equipped with a 30 m x 0.25 mm i.d. x 0.25 µm film thickness, ZB-5 capillary column. The electron impact technique (70 eV) was used. The carrier gas was helium at flow rate of 1.3 ml/min, and 1 µl of sample was injected. The injector and detector temperatures were 250°C and 230°C, respectively. The other analytical conditions were as follows:

Galanga, turmeric, kaempferia: Temperature programming: 60°C, as initial temperature, for 5 min, 8°C/min to 180°C, 10°C/min to 240°C, holding for 5 min.

Bastard cardamom: Temperature programming: 60°C, as initial temperature, for 5 min, 8°C/min to 180°C, 10°C/min to 240°C, holding for 15 min.

Ginger: Temperature programming: 50°C, as initial temperature, for 1 min, 3°C/min to 240°C, holding for 2 min.

A mixture of C_8-C_{25} *n*-alkanes used as standard was previously separated under the conditions mentioned in the Experimental section. Retention indices (RIs) for all components were determined from the results of the chromatography of these mixtures and extracts according to the Van den Dool method (Dool and Kratz, 1963). The identification of compounds was based on a comparison of their retention times with those of authentic standards, by comparison of their mass spectra with data in the Wiley 175 and National Institute of Standards and Technology (NIST) libraries and comparison with literature data (Luger *et al.*, 1996; Jirovetz *et al.*, 2003; Isidorov and Vinogorova, 2003; Acree and Arn, 2004; Hochmuth, 2006; Alma *et al.*, 2007).

Preparation of bacteria strain

Four different food-borne bacteria were used. Three species of Gram positive bacteria, *S. aureus, B. cereus* and *L. monocytogenes*, and one Gram negative bacteria, *E. coli*, were obtained from stock cultures of the Department of Applied Microbiology, King Mongkut's University of Technology Thonburi, Thailand. Bacteria were sub-cultured on nutrient agar at 37°C prior to being grown in nutrient broth overnight. All overnight (ON) cultures were standardized by matching to the McFarland 0.5 turbidity standard using sterile saline to produce approximately $1.5x10^8$ colony forming units (cfu) per ml.

Antibacterial screening

The antibacterial activity of the plant extracts was carried out by disc diffusion assay that was used to screen (Kumar *et al.*, 2001; Gulluce *et al.*, 2003). Muller Hinton agar (MHA) plates were swabbed with the respective broth culture of the organisms (diluted to 0.5 McFarland Standard with saline) and kept for absorption to take place. Sterile 6 mm diameter filter paper discs were impregnated with 100 mg/ml of plants extract that dissolved in sterile dimethylsulfoxide (DMSO). Negative controls were prepared using the same solvents employed to dissolve the plant extracts. Streptomycin (5 µg/ml) was used as positive reference standards to determine the sensitivity of one strain in each bacterial species tested. The plates were incubated overnight at 37°C. The antimicrobial activity was evaluated by measuring the zone expressed as mm of inhibition against test organism. Five discs per plate and three plates were used, and each test was run in triplicate.

Determination of minimum inhibitory concentration (MIC)

The minimum inhibition concentration (MIC) values were also studied for the bacteria which were determined as sensitive to the extracts in disc diffusion assay. The inoculated bacteria as prepared from 24 h nutrient broth cultures and suspensions were adjusted to 0.5 McFarland turbidity standard. Plant extracts dissolved in DMSO were first diluted to the highest concentration (50 µg/ml) to be tested, and then serial two fold dilutions were made in a concentration range from 6.25 µg/ml to 50 µg/ml. The least concentration of each extract showing a clear of inhibition was taken as the MIC levels.

Statistical analysis

Analysis of variance of antibacterial activities of plant extracts from ginger, galanga, turmeric, kaempferia, bastard cardamom were analyzed using SAS Program. Mean separation was performed by Protected LSD method at $p \leq 0.05$ (SAS, 1991).

RESULTS AND DISCUSSION

Volatile compounds of the plant extracts

Essential oils and extracts of Zingiberaceae obtained from four extraction method (A; hydrodistillation, B; extracted by petroleum ether, C; secondary extraction or waste of plant extracted by ethanol and D; extracted by ethanol), were analyzed using GC-MS system. The component of ginger are given in Table 1, zingiberene (A; 30.7%, B; 51.4%, C; 46.0%, D; 41.5%) was found as main constituent in all essential oils, that according with reported by Kelly *et al.* (2002). The second major component was identified as β-farnesene (A; 15.2%, B; 16.0%, C; 17.6%, D; 22.8%).

The main constituent of galanga extracted by hydrodistillation was methyl chavicol (37.9%), whereas in galanga extracted by solvent as it was

Table 1: % Relative peak areas and RIs of volatile compounds in ginger extracts

Compound	RIs	% Relative peak area			
		A	B	C	D
calminol	1233	1.4	3.3	-	-
neral	1247	10.2	4.8	1.3	1.1
chavicol	1249	1.2	-	-	-
geranial	1261	15.1	-	1.9	0.6
linalool acetate	1277	0.5	-	-	-
curcumene	1471	6.3	3.3	4.2	2.9
zingiberene	1489	30.7	51.4	46.0	41.5
β-farnesene	1491	15.2	16.0	17.6	22.8
β-bisabolene	1518	6.9	7.3	9.0	6.9
β-sesquiphellandrene	1560	11.3	12.2	15.1	17.6
guaiol	1589	0.7	0.6	1.3	0.9
gingerone	1718	0.5	1.1	3.6	5.7

A, B. C and D refer to the different extraction methods used (A: hydrodistillation; B: extraction with petroleum ether; C: secondary extraction with ethanol of plant residue after extraction by method B and D: extraction with ethanol).

ethyl-p-methoxycinnamate (B; 49.8%, C; 68.2%, D; 74.6%) (Table 2). For the main constituents of this plant differences were observed with that obtained in India (Jirovetz *et al.*, 2003). These discrepancies may be explained by factors such as soil and climatic conditions (Baydar *et al.*, 2004).

The result obtained by GC-MS analysis of turmeric is presented in Table 3. Fifteen compounds were identified. The oil profile shows turmerone as the main compound (A; 50.0%, B; 58.5%, C; 66.7%, D; 64.7%); other major compounds were curlone, α-farnesene and α-zingiberene, respectively.

Table 2: % Relative peak areas and RIs of volatile compounds in galanga extracts

Compound	RIs	% Relative peak area			
		A	B	C	D
1,8-cineole	1029	33.6	4.1	2.4	3.3
methyl chavicol	1033	37.9	-	-	-
Camphor	1139	4.5	16.0	9.8	5.0
β-thujene	1158	0.7	2.4	-	-
β-caryophyllene	1432	4.2	5.9	7.4	1.5
β-farnesene	1491	4.2	4.6	4.8	1.8
γ-elemene	1494	1.2	3.5	-	0.3
β-selinene	1497	3.0	2.4	2.2	1.6
α-farnesene	1500	5.9	6.7	2.4	5.3
γ-selinene	1503	4.2	4.3	2.0	2.4
γ-cadinene	1507	0.8	0.5	-	-
ethyl p-methoxy-cinnamate	1711	-	49.8	68.2	74.6
eugenol acetate	1935	-	-	0.8	4.2

A, B. C and D refer to the different extraction methods used (A: hydrodistillation; B: extraction with petroleum ether; C: secondary extraction with ethanol of plant residue after extraction by method B and D: extraction with ethanol).

Table 3: % Relative peak areas and RIs of volatile compounds in tumeric extracts

Compound	RIs	% Relative peak area			
		A	B	C	D
1,8-cineole	1025	1.1	1.4	-	-
γ-terpinene	1055	5.5	1.7	-	-
isocaryophyllene	1409	1.8	0.7	-	0.5
β-caryophyllene	1449	1.1	0.4	-	0.4
ar-curcumene	1471	2.9	1.2	1.3	1.3
α-zingiberene	1489	7.8	3.6	1.8	3.5
β-bisabolene	1494	1.7	0.5	-	0.5
α-farnesene	1500	10.8	4.1	2.5	4.1
cubenol	1602	3.1	0.9	1.4	1.1
ar-turmerol	1610	0.9	1.9	2.5	2.1
turmerone	1643	50.0	58.5	66.7	64.7
curlone	1645	12.9	20.9	21.4	20.1
(6S,7R)-bisabolene	1666	-	0.9	-	-
α-atlantone	1669	0.5	1.3	0.9	0.7
(E)-α-atlantone	1689	-	1.8	1.1	1.0

A, B. C and D refer to the different extraction methods used (A: hydrodistillation; B: extraction with petroleum ether; C: secondary extraction with ethanol of plant residue after extraction by method B and D: extraction with ethanol).

The profile of kaempferia in Table 4 shows γ-terpinene (44.0%), which was only found most in this essential oil by hydrodistillation. The other major compounds of kaempferia extracts were geraniol (A; 20.6%, B; 55.3%, C; 37.2%, D; 40.7%) and 6-camphenone (A; 18.7%, B; 29.6%, C; 25.2%, D; 31.3%).

The most abundant compound in rhizome of bastard cardamom obtained by hydrodistillation and petroleum ether was methyl chavicol (93.1% and 48.7%, respectively), whereas in the ethanol extract and secondary extraction with ethanol was anethole (Table 5).

Table 4: % Relative peak areas and RIs of volatile compounds in kaemferia extracts extracts

Compound	RIs	% Relative peak area			
		A	B	C	D
limonene	1025	0.7	-	-	-
1,8-cineole	1029	12.8	1.8	3.6	5.2
γ-terpinene	1055	44.0	3.5	1.9	4.7
linalool	1090	0.8	1.2	0.8	1.2
terpineol	1135	0.3	-	-	-
6-camphenone	1141	18.7	29.6	25.2	31.3
borneol	1154	-	-	0.3	0.4
nerol	1234	-	-	0.6	0.6
citral	1254	-	0.3	-	-
geraniol	1276	20.6	55.3	37.2	40.7
methyl cinnamate	1320	2.1	2.6	3.7	4.2
β-farnesene	1448	-	0.8	0.2	0.3
nerolidol	1543	-	-	0.3	0.4
11-dodecen-1-ol	1692	-	2.2	1.1	1.1
pinostrobin chalcone	2502	-	2.7	25.1	10.1

A, B. C and D refer to the different extraction methods used (A: hydrodistillation; B: extraction with petroleum ether; C: secondary extraction with ethanol of plant residue after extraction by method B and D: extraction with ethanol).

Table 5: % Relative peak areas and RIs of volatile compounds in bastard cardamom extracts

Compound	RIs	% Relative peak area			
		A	B	C	D
methyl chavicol	1175	93.1	48.7	5.2	19.2
anethole	1282	0.3	44.2	79.4	63.4
α-copaene	1378	1.0	1.1	-	0.5
aromadendrene	1443	0.2	0.3	0.8	0.2
α-himachalene	1450	0.2	-	-	-
caryophellene	1432	0.4	-	-	-
α-farnesene	1448	0.3	0.3	-	0.6
α-selinene	1494	0.6	0.5	1.0	0.8
γ-cadinene	1508	3.3	4.2	4.8	13.5
β-chamigrene	1547	0.3	0.3	1.0	0.5
β-farnesol	1697	0.2	0.5	-	-
bornyl benzoate	1766	-	-	1.5	0.3
eugenolacetate	1935	-	-	6.4	1.1

A, B. C and D refer to the different extraction methods used (A: hydrodistillation; B: extraction with petroleum ether; C: secondary extraction with ethanol of plant residue after extraction by method B and D: extraction with ethanol).

Antibacterial activity

The antibacterial activity of essential oils and extracts from five Zingiberaceae species against the microorganisms considered in the present study were qualitatively and quantitatively assessed evaluating the presence of inhibition zone and zone diameter (Table 6). Among Gram-positive bacteria, *B. cereus* was the most sensitive organism to plant extracts that this finding is in agreement with a previous report (Alzoreky *et al.*, 2003). Gram-negative bacteria showed resistance (no inhibition zone) to the 18 extracts of plant. Water-distilled essential oils of kaempferia (%yield = 0.26 dry basis (d.b.)) and bastard cardamom (%yield = 0.27 d.b.) were only inhibitory for *E. coli*. Kaempferia produced an average zone of inhibition (ZOI) of 9.0 mm while bastard cardamom produced an average ZOI of 10.0 mm, from triplicate assays. On the growth of *B. cereus*, ginger extracted by hydrodistillation was most effective to inhibitory, that average ZOI of 20.0 mm. Furthermore, *S. aureus* was inhibited by ginger as well as kaempferia. *L. monocytogenes* was the bacterium most sensitive to essential oil of ginger, that the largest inhibition zone diameter was 22.0 mm. Based on these results, it is possible to conclude that the essential oil has a stronger activity and broader spectrum than those of solvent extracts. As emphasized elsewhere, Gram-positive bacteria are more sensitive to plant oil and extracts than Gram-negative bacteria (Cosentino

Table 6: Results of the antibacterial tests of the investigated plants in agar diffusion assay

Plants species	Extract	Inhibition zones (mm)* against			
		S. aureus	*B. cereus*	*E. coli*	*L. monocytogenes*
Zingiber officinale (ginger)	Hydrodistillation	16^{a}	20^{a}	0^{c}	22^{a}
	Ethanol	9^{hi}	10^{h}	0^{c}	10^{i}
	Petroleum ether	10^{g}	12^{f}	0^{c}	11^{h}
	Secondary extraction with ethanol after extraction by petroleum ether	8^{j}	11^{g}	0^{c}	8^{l}
Alpinia galangal (galanga)	Hydrodistillation	8^{k}	9^{hi}	0^{c}	11^{h}
	Ethanol	12^{e}	13^{d}	0^{c}	9^{k}
	Petroleum ether	12^{e}	12^{f}	0^{c}	9^{k}
	Secondary extraction with ethanol after extraction by petroleum ether	13^{d}	14^{cd}	0^{c}	10^{i}
Curcuma longa (turmeric)	Hydrodistillation	9^{i}	10^{h}	0^{c}	16^{c}
	Ethanol	11^{fg}	12^{f}	0^{c}	13^{f}
	Petroleum ether	10^{h}	10^{g}	0^{c}	10^{i}
	Secondary extraction with ethanol after extraction by petroleum ether	11^{fg}	11^{e}	0^{c}	11^{g}
Boesenbergia pandurata (kaempferia)	Hydrodistillation	15^{ab}	16^{b}	9^{b}	19^{b}
	Ethanol	11^{f}	14^{c}	0^{c}	15^{d}
	Petroleum ether	14^{b}	12^{cf}	0^{c}	13^{f}
	Secondary extraction with ethanol after extraction by petroleum ether	14^{b}	16^{b}	0^{c}	14^{e}
Amomum xanthioides (bastard cardamom)	Hydrodistillation	12^{c}	13^{d}	10^{a}	13^{f}
	Ethanol	0^{l}	8^{i}	0^{c}	0^{m}
	Petroleum ether	8^{j}	9^{hi}	0^{c}	0^{m}
	Secondary extraction with ethanol after extraction by petroleum ether	0^{l}	0^{j}	0^{c}	0^{m}
Streptomycin, 5 mg/ml		24	26	22	24

* Inhibition zone including the diameter of the paper disc (6 mm).
a,b,c,…the letters in the same column are significant difference at $p \leq 0.05$.

et al., 1999; Karaman *et al.*, 2003). The basis of varying degree of sensitivity of test organisms of bacteria may be due to the intrinsic tolerance of microorganisms and the nature and combinations of phytocompounds present in the essential oil. The bioassay guided fractionation procedure showed that the plant essential oil was rich in terpenes (monoterpene, oxygenated monoterpene and sesquiterpene). At present, however, the mode of action of terpenic constituents on microorganism is not fully understood. Nevertheless, in view of their hydrophobicity, it is generally considered that they are involved in such mechanism as cytoplasmic membrane, coagulation of cell contents and disruption of the proton motive force (Burt, 2004).

In this study, the major compounds in five Zingiberaceae essential oils were terpenes which effect on membrane of bacteria (Gram positive and negative), as shown in Figure 1. Terpenes in ginger, galanga, turmeric, kaempferia, bastard cardamom were zingiberene and farnescene; methyl chavicol and ethyl-p-methoxycinnamate; tumerone, farnescene, curlone and zingiberene; terpinene, geraniol, and 6-camphenone; methyl chavicol, respectively.

Minimum inhibitory concentration (MIC)

The MICs of each plant extracts are presented in Table 7. Of the 7 plant extracts tested, ginger, turmeric, and bastard cardamom extracted by hydrostillation, kaempferia extracted by

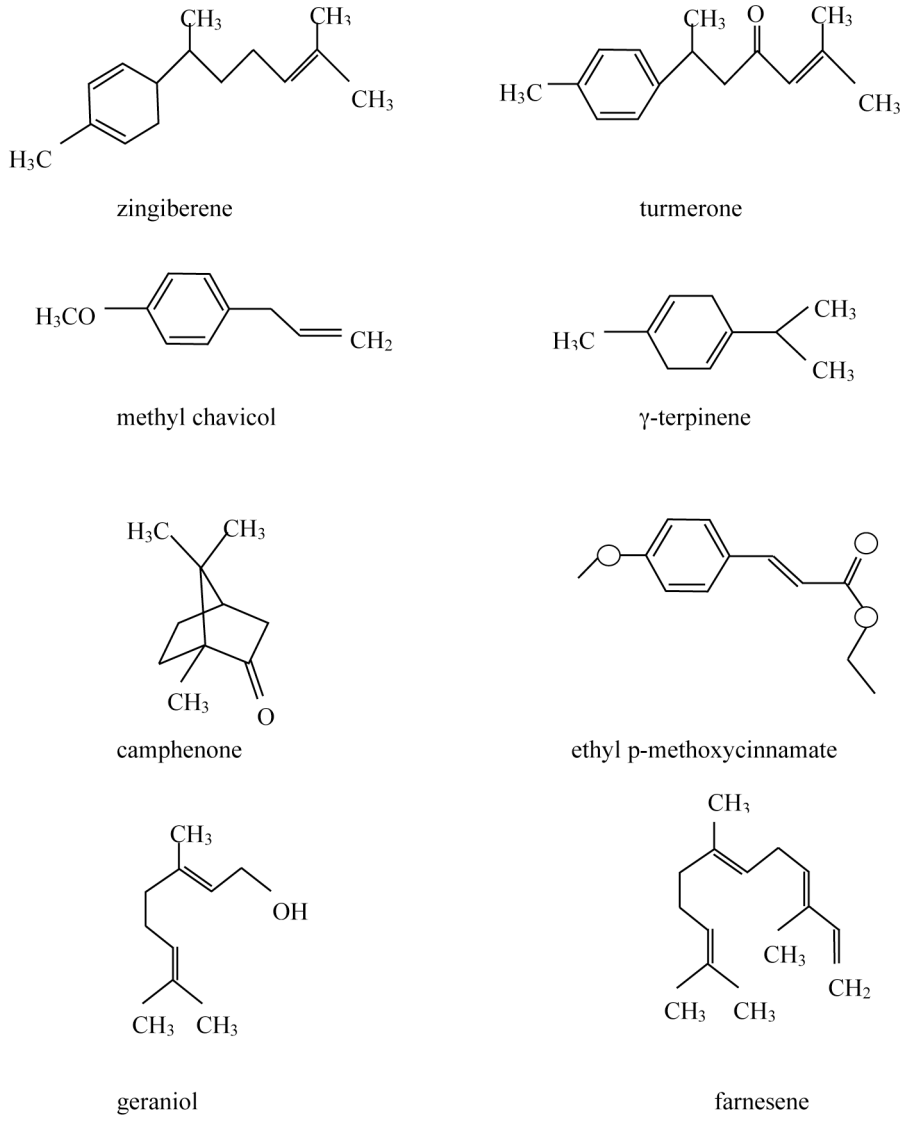

Figure 1: Structures of major components in essential oils from five *Zingiberaceace* sp.

Table 7: Minimum inhibitory concentration (MIC) of plant extracts against bacteria

Bacterial species	Minimum inhibitory concentration (mg ml - 1)						
	Hydrodistillation				Secondary extraction with ethanol after extraction by petroleum ether		Ethanol
	ginger	turmeric	kaempferia	bastard cardamom	galanga	kaempferia	kaempferia
S. aureus	12.5	none	12.5	none	100	50	12.5
B. cereus	6.25	none	12.5	none	25	12.5	12.5
E. coli	none	none	50.0	25	none	none	none
L. monocytogenes	6.25	25	6.25	none	none	6.25	6.25

hydrodistillation and ethanol, waste of kaempferia and galanga extracted by ethanol seem to be the most efficient plant extracts against the four pathogenic bacteria tested. The results demonstrate a wide range of activities of the different herbs and extracts against the bacteria tested. Relatively high levels of activity (MIC of 12.5 – 6.25 µg/ml) against this pathogen were present in plants extracts from all herbs except galanga. The MIC values indicate that the oil of ginger was more efficient than that of others. By minimum concentration to inhibit *B. cereus* and *L. monocytogenes* was 6.25 µg/ml. As was the case with the water extracts, *L. monocytogenes* proved to be most sensitive of the tested bacteria. The ethanolic extracts of kaempferia showed better growth inhibition against *L. monocytogenes* than *B. cereus* and *S. aureus*. Numerous herbs, spices and plants have been reported to be potential sources of antimicrobial agents but not many have been studied with respect to levels and range of activity (Hsieh *et al.*, 2001). In particular, plants of limited distribution, such as those restricted to particular regions or countries, are poorly studied.

CONCLUSION

Volatile compounds of all extracts were analyzed by GC-MS, were terpenes. Most abundant terpenes in ginger, galanga, turmeric, kaempferia, and bastard cardamom were zingiberene and farnescene; methyl chavicol and ethyl-p-methoxycinnamate; tumerone, farnescene, curlone, and zingiberene; terpinene, geraniol, and 6-camphenone; methyl chavicol, respectively. Essential oil of kaempferia and bastard cardamom obtained by hydrodistillation extraction could inhibit growth of all tested bacteria. Essential oil of ginger extracted by hydrodistillation had the highest efficiency against three positive strains of bacteria (*S. aureus, B. cereus* and *L. monocytogenes*). The results demonstrate a wide range of activities of the different herbs and extracts against the bacteria tested. Relatively high levels of activity (MIC of 6.25-12.5 µg/ml) against these bacteria were observed for plant extracts from all herbs except *Alpinia galangal*. The MIC values indicated that ginger oil from hydrodistillation was more efficient than the others at which MIC values for inhibition of *B. cereus* and *L. monocytogenes* were 6.25 µg/ml. The ethanolic extracts of kaempferia showed better growth inhibition against *L. monocytogenes* than *B. cereus* and *S. aureus*. Among the tested bacteria, *L. monocytogenes* was the most sensitive bacteria when it was subjected by oils obtained from various extraction methods.

ACKNOWLEDGEMENTS

The authors would like to thank Department of Applied Microbiology, King Mongkut's University of Technology Thonburi in Thailand for supplying the bacterial strains.

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