

Full Paper

Antibacterial Effect of Five Zingiberaceae Essential Oils

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Abstract: Essential oil obtained by hydrodistillation and two different solvent extractions (petroleum ether and ethanol) 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.) was characterized. Volatile components of all extracts were analyzed by gas chromatography-mass spectrometry (GC-MS). The major components of ginger, turmeric, galangal, bastard cardamom and kaempferia were zingiberene, turmerone, methyl chavicol, and γ -terpinene, respectively. 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 mg/mL.

Keywords: Antibacterial activity; ginger; galangal; turmeric; kaempferia; bastard cardamom

Introduction

Food-borne diseases are still a major problem in the World, even in well-developed countries [1]. A variety of microorganisms also lead food spoilage which is one of the most important concerns of 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 foodborne diseases and/or food spoilage [2, 3]. Thus, at present, chemical preservatives must be used to prevent the growth of food spoiling microbes in the food industry [4]. Due to consumer concerns about the safety of food containing synthetic chemicals as preservatives, there is a growing interest in the use of natural antibacterial compounds, like extracts of herbs and spices, for the preservation of foods, as these possess characteristic flavors and sometimes show antioxidant activity as well as antimicrobial activity [5]. For centuries, indigenous plants have been used in herbal medicine for curing various diseases [6]. Recently, the acceptance of traditional medicine as an alternative form for health care and the development of microbial resistance to the available antibiotics has led authors to investigate the antimicrobial activity of medicinal plants [7, 8].

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 [9]. 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 [10-16]. 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.

The objectives of this study were to compare the antimicrobial activity of the essential oils and extracts from Zingiberaceae against common foodborne 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.

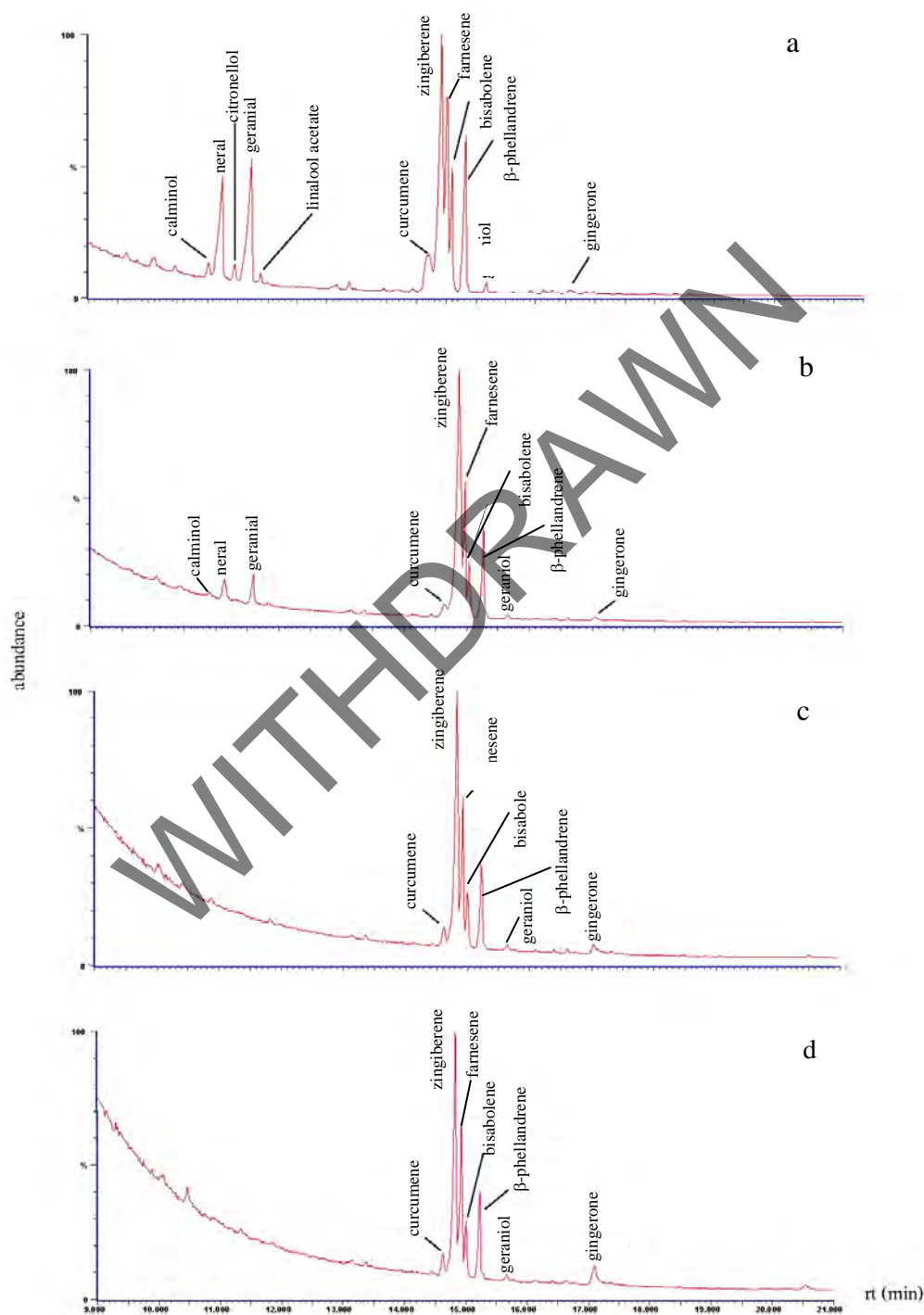
Results and Discussion

Chemical composition of the plant extracts.

Essential oils and extracts of Zingiberaceae obtained by four extraction methods (A: hydrodistillation, B: extracted with petroleum ether, C: secondary extraction with ethanol of plant residue after extraction by method B and D: extracted with ethanol), were analyzed using GC-MS system. The components of ginger are given in Figure 1. Zingiberene (A: 30.7%, B: 51.1%, C: 46.0%,

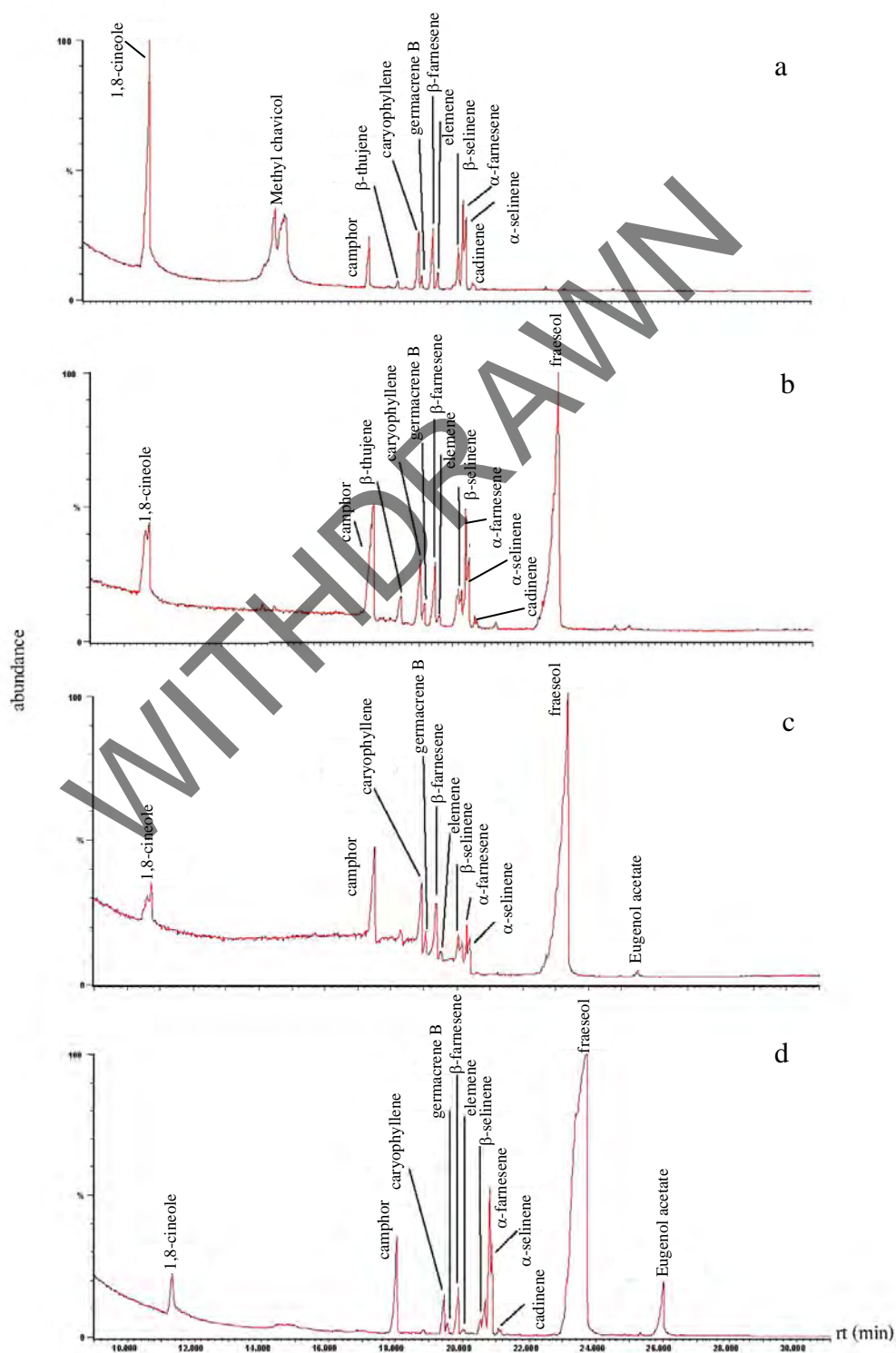
D: 41.5%) was found as main constituent in all essential oils, that according with reported by [17]. The second major component was identified as farnesene (A: 15.2%, B: 16.0%, C: 17.6%, D: 22.8%).

Figure 1. Volatile compounds in ginger extracts obtained by (a) hydrodistillation (b) petroleum ether (c) secondary extraction with ethanol and (d) ethanol.



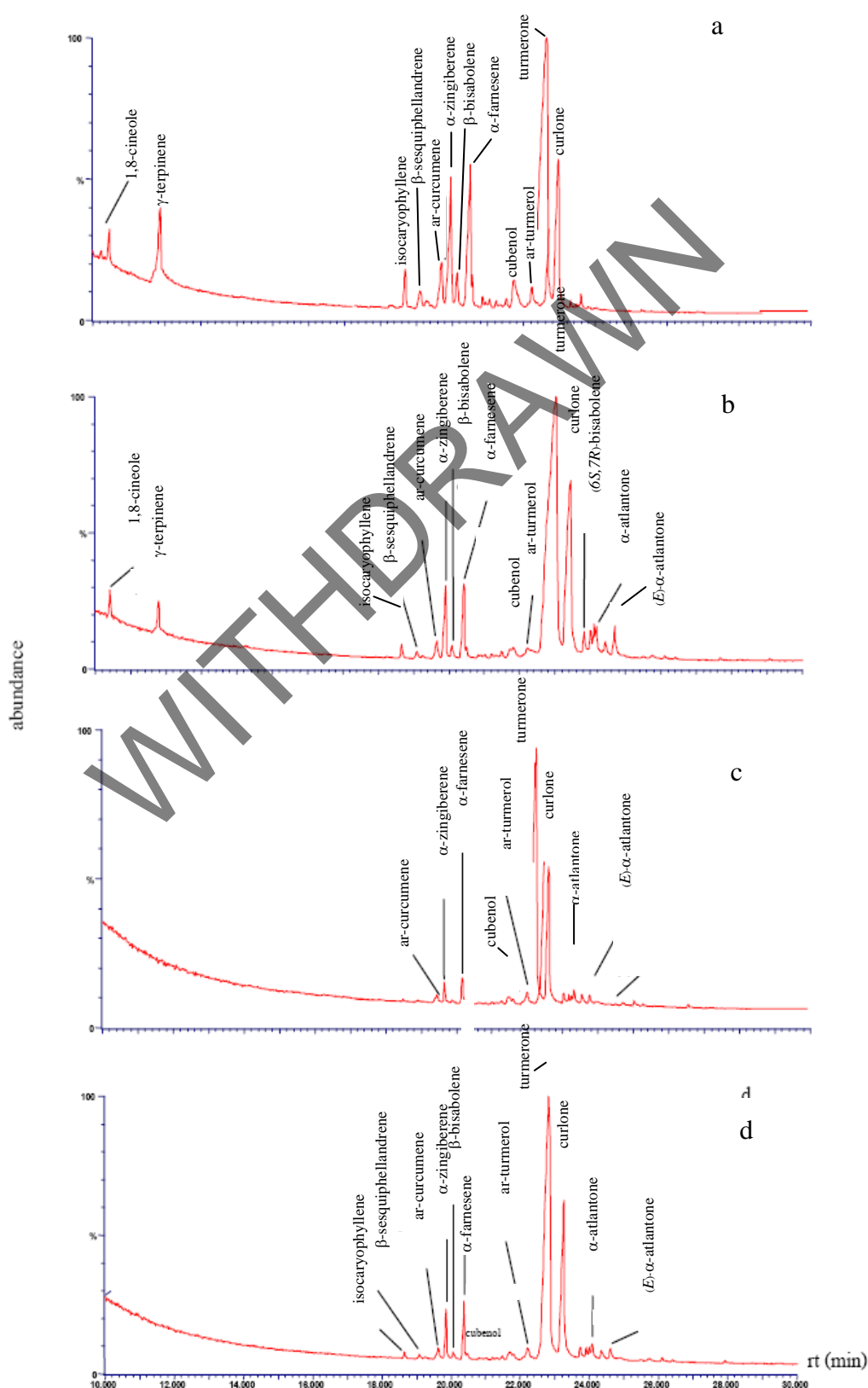
The main constituent of galanga extracted by hydrodistillation was methyl chavicol (37.9%), whereas in solvent extracts it was fraesol (B: 49.8%, C: 68.2%, D: 74.6%, Figure 2). Differences in the main constituents of this plant were observed, compared to with those obtained with plants from India [13]. These discrepancies may be explained by such factors as soil and climatic conditions [18].

Figure 2. Volatile compounds in galangal extracts obtained by: (a) hydrodistillation (b) petroleum ether (c) secondary extraction with ethanol and (d) ethanol.



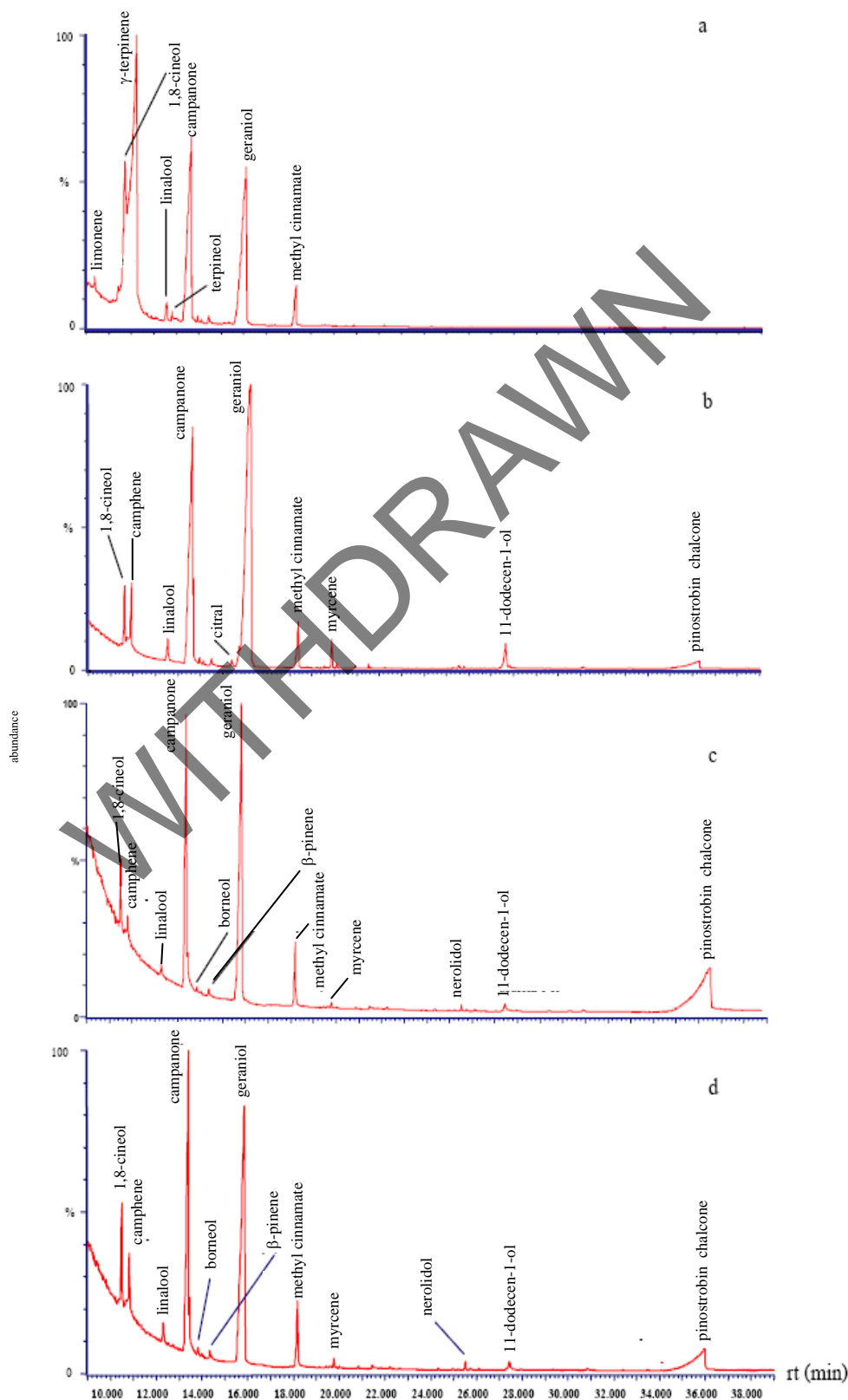
The GC-MS analysis results of turmeric are presented in Figure 3. Fifteen compounds were identified. The oil profile shows turmerone as the main compound (A: 50.0%, B: 58.5%, C: 66.7%, D: 64.7%); other major compounds were curlone, α -farnesene and α -zingiberene, respectively.

Figure 3. Volatile compounds of turmeric extracts obtained by (a) hydrodistillation (b) petroleum ether (c) secondary extraction with ethanol and (d) ethanol.



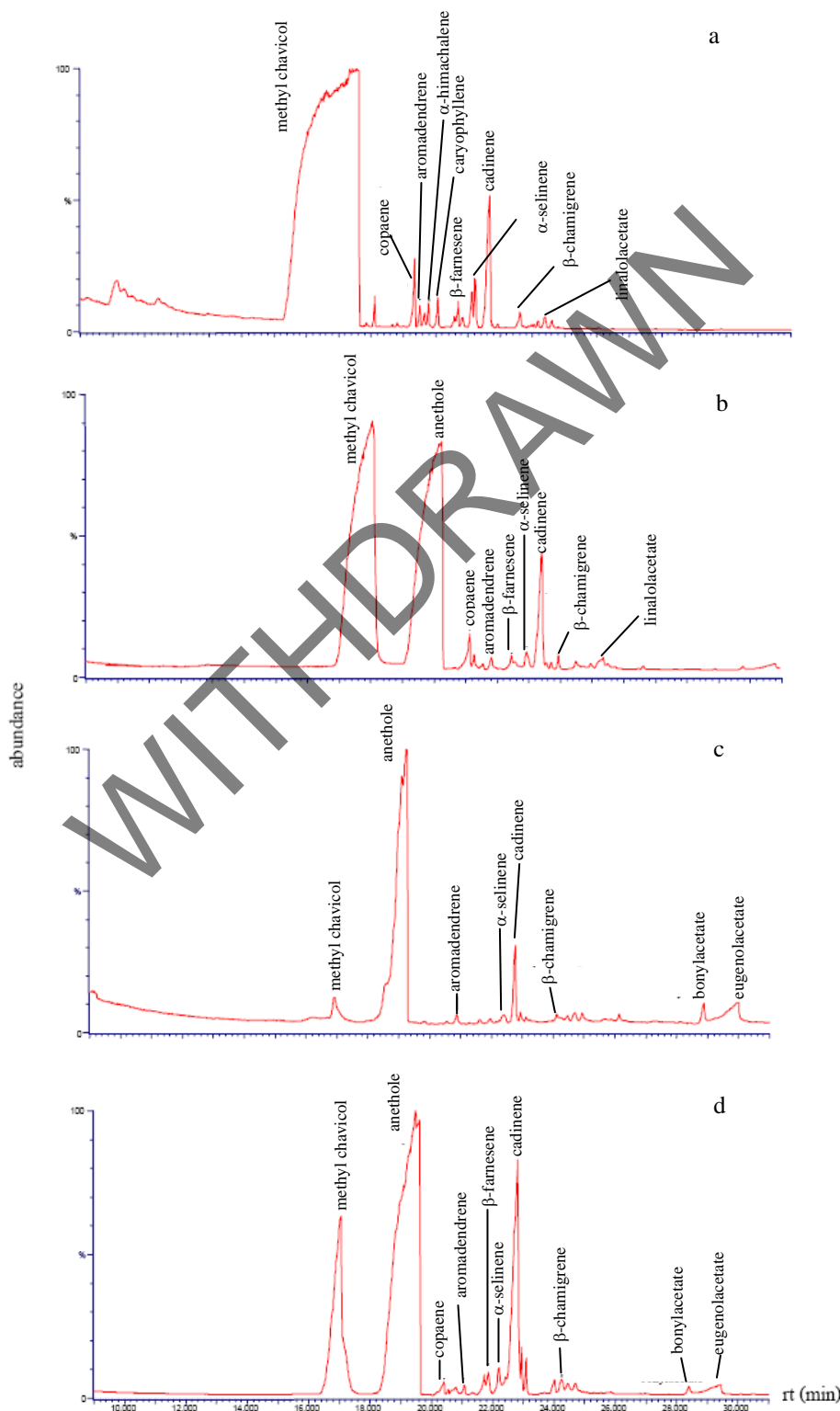
The profile of kaempferia (Figure 4) shows γ -terpinene (44.0%), which was only found in this essential oil, as the major peak. The other significant compounds of kaempferia extracts were geraniol (B: 55.3%, C: 37.2%, D: 40.7%) and 2-camphanone (B: 29.6%, C: 25.2%, D: 31.3%).

Figure 4. Volatile compounds of kaempferol extracts obtained by (a) hydrodistillation (b) petroleum ether (c) secondary extraction with ethanol and (d) ethanol.



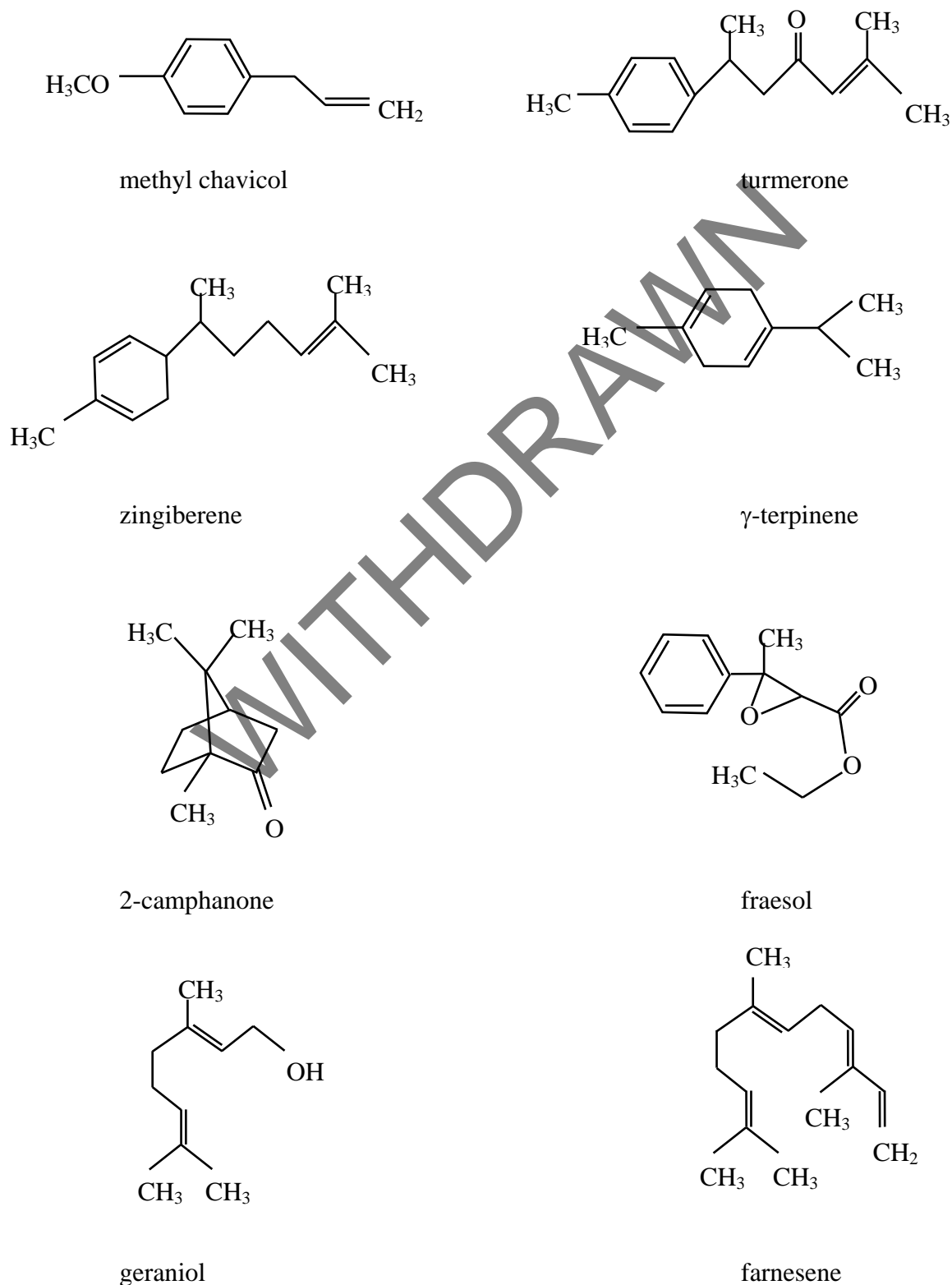
The most abundant compound obtained from bastard cardamom rhizome by hydrodistillation and petroleum ether extraction was methyl chavicol (93.1% and 48.7%, respectively), whereas in the ethanol extract it was anethole (Figure 5).

Figure 5. Volatile compounds of bastard cardamom extracts obtained by (a) hydrodistillation (b) petroleum ether (c) secondary extraction with ethanol and (d) ethanol.



In summary, the major compounds in five Zingiberaceae essential oils (ginger, galangal, turmeric, kaempferia, bastard cardamom) were terpenes (zingiberene and farnescene; methyl chavicol and fraesol; tumerone, farnescene, and zingiberene; terpinene, geraniol, 2-camphanone and methyl chavicol, respectively, Figure 6), which display effects on membrane of bacteria (gram positive and negative).

Figure 6. Structures of major components in essential oils from five Zingiberaceae sp.



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 by evaluating the presence of inhibition zones and zone diameter measurements (Table 1).

Table 1. Results of the antibacterial tests of the investigated plants in agar diffusion assay.

Plant species	Extract	Yield ^{a*} : %	Inhibition zones (mm) ^{b*} against			
			<i>S. aureus</i>	<i>B. cereus</i>	<i>E. coli</i>	<i>L. mono cytogenes</i>
<i>Zingiber officinale</i> (ginger)	Water	0.27 ^l	16 ^a	20 ^a	0 ^c	22 ^a
	Ethanol	3.55 ^b	9 ^{hi}	10 ^h	0 ^c	10 ⁱ
	Secondary extraction	2.65 ^c	8 ^g	11 ^g	0 ^c	8 ^l
	Petroleum ether	1.08 ^{fg}	10 ^g	12 ^f	0 ^c	11 ^h
<i>Alpinia galangal</i> (galanga)	Water	0.18 ⁿ	8 ^k	9 ^{hi}	0 ^c	11 ^h
	Ethanol	1.15 ^f	12 ^e	13 ^d	0 ^c	9 ^k
	Secondary extraction	1.03 ^g	13 ^d	14 ^{cd}	0 ^c	10 ⁱ
	Petroleum ether	0.48 ^j	12 ^e	12 ^f	0 ^c	9 ^k
<i>Curcuma longa</i> (turmeric)	Water	0.64 ⁱ	9 ⁱ	10 ^h	0 ^c	16 ^c
	Ethanol	3.65 ^a	11 ^{fg}	12 ^f	0 ^c	13 ^f
	Secondary extraction	3.60 ^{ab}	11 ^{fg}	11 ^e	0 ^c	11 ^g
	Petroleum ether	1.65 ^d	10 ^h	10 ^g	0 ^c	10 ⁱ
<i>Boesenbergia pandurata</i> (kaempferia)	Water	0.26 ^m	15 ^{ba}	16 ^b	9 ^b	19 ^b
	Ethanol	1.25 ^e	11 ^f	14 ^c	0 ^c	15 ^d
	Secondary extraction	0.83 ^h	14 ^b	16 ^b	0 ^c	14 ^e
	Petroleum ether	0.40 ^{kj}	14 ^b	12 ^{cf}	0 ^c	13 ^f
<i>Amomum xanthioides</i> (bastard cardamom)	Water	0.27 ^{lm}	12 ^c	13 ^d	10 ^a	13 ^f
	Ethanol	0.48 ^j	0 ^l	8 ⁱ	0 ^c	0 ^m
	Secondary extraction	0.35 ^k	0 ^l	0 ^j	0 ^c	0 ^m
	Petroleum ether	0.65 ⁱ	8 ^j	9 ^{hi}	0 ^c	0 ^m
Streptomycin, 5 mg/mL	—		24	26	22	24

^{a*} Percentage extract yield (w/w) was estimated as extract weight/starting material weight x 100

^{b*} Inhibition zone including the diameter of the paper disc (6 mm).

Negative controls did not show any activity. -: no activity

a, b, c,.....means with the same letters in columns are not significant difference at $p \leq 0.05$.

Among Gram-positive bacteria, *B. cereus* was the most sensitive organism to the plant extracts, in agreement with a previous report [19]. Gram-negative bacteria showed resistance (i.e. no inhibition

zone) towards the 18 plant extracts. Water-distilled essential oils of kaempferia (yield 0.26%) and bastard cardamom (yield 0.27%) were only inhibitory towards *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). Ginger extracted by hydrodistillation was the most inhibitory towards growth of *B. cereus*, with an average ZOI of 20.0 mm. Furthermore, *S. aureus* was inhibited by ginger as well as kaempferol. *L. monocytogenes* was the bacterium most sensitive to essential oil of ginger, and the largest inhibition zone diameter was 22.0 mm. Based on these results, we may conclude that the essential oils presented stronger activity and broader spectrum of activity than the solvent extracts. As emphasised elsewhere, Gram-positive bacteria are more sensitive to plant oil and extract than Gram-negative bacteria [20, 21]. The varying degrees of sensitivity of the bacterial test organisms may be due to both 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 (monoterpenes, oxygenated monoterpenes and sesquiterpenes). At present, however, the mode of action of terpenic constituents on microorganisms 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 [22].

Minimum inhibitory concentration (MIC)

The MICs of each plant extracts are presented in Table 2. Of the seven plant extracts tested, *Zingiber officinale*, *Curcuma longa* and *Amomum xanthioides* extracted by hydrostillation, *Boesenbergia pandurata* extracted by hydrostillation and with ethanol, secondary extraction with ethanol of *Boesenbergia pandurata* and *Alpinia galangal* extracted with ethanol seem to be the most efficient plant extracts against the four pathogenic bacteria tested.

Table 2. Minimum inhibition concentration (MIC) of plant extracts against bacteria.

Bacterial species	Minimum inhibition concentration (mg mL ⁻¹)						
	Hydrodistillation				Secondary extraction with ethanol		Ethanol
	<i>Z. o.</i>	<i>C. l.</i>	<i>B. p.</i>	<i>A. x.</i>	<i>A. g.</i>	<i>B. p.</i>	<i>B. p.</i>
<i>S. aureus</i>	12.5	N.T.	12.5	N.T.	100	50	12.5
<i>B. cereus</i>	6.25	N.T.	12.5	N.T.	25	12.5	12.5
<i>E. coli</i>	N.T.	N.T.	50	25	N.T.	N.T.	N.T.
<i>L. monocytogenes</i>	6.25	25	6.25	N.T.	N.T.	6.25	6.25

Z. o., *Zingiber officinale*; *A. g.*, *Alpinia galangal*; *C. l.*, *Curcuma longa*; *B. p.*, *Boesenbergia pandurata*; *A. x.*, *Amomum xanthioides*. N.T., not 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 mg/L) were observed for plant extracts from all herbs except *Alpinia galangal*. The MIC values indicate that ginger oil was more

efficient than the others. The minimum concentration for inhibition of *B. cereus* and *L. monocytogenes* was 6.25 mg/L. As was the case with the water extracts, *L. monocytogenes* proved to be most sensitive of the bacteria tested. The ethanolic extracts of *Boesenbergia pandurata* 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 [23]. In particular, plants of limited distribution, such as those restricted to particular regions or countries, are poorly studied.

Experimental

1. Plant material

Fresh Zingiberaceae rhizomes (ginger, galanga, turmeric, kaempferia, bastard cardamom) were purchased at a local vegetable and fruit market in Tungku 63, Bangkok, Thailand.

2. Extraction procedure

Hydrodistillation: Essential oils 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 mixture 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 duplicate.

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 extraction of plant materials with petroleum ether was extracted again with ethanol (so-called secondary extraction, method C), under conditions similar to those described above.

2.3 Gas chromatography/mass spectrometry analysis (GC-MS)

The chemical composition of plants extract were analyzed using a GC-8000 GC-MS system (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 a 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. The identification of compounds was based on a comparison of their retention times with those of authentic standards and by comparison of their mass spectra with those of data in the Wiley Registry of Mass Spectral Data and National Institute of Standards and Technology (NIST) libraries.

2.4 Preparation of bacterial strains

Four different foodborne bacteria were used. Three species of Gram positive bacteria: *Staphylococcus aureus*, *Bacillus cereus* and *Listeria monocytogenes* and one Gram negative bacterium, *Escherichia 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 standardised by matching to the McFarland 0.5 turbidity standard using sterile saline to produce approximately 1.5×10^8 colony forming units (cfu) per mL.

2.5 Antibacterial screening

The antibacterial activity of the plant extracts was carried out by disc diffusion assay as previously reported [24-25]. Muller Hinton agar (MHA) plates were swabbed with the respective broth cultures of the organisms (diluted to 0.5 McFarland Standard with saline) and stored for absorption to take place. Sterile 6 mm diameter filter paper discs were impregnated with plant extract (100 mg/mL) dissolved in sterile dimethylsulfoxide (DMSO). Negative controls were prepared using the same solvents employed to dissolve the plant extracts. Streptomycin (5 mg/mL) was used as positive reference standard 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.

2.6 Determination of minimum inhibitory concentrations (MICs)

The minimum inhibition concentration (MIC) values were also studied for the bacteria which were determined in the disc diffusion assay to be sensitive to the extracts. 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 mg/mL) to be tested, and then serial two-fold dilutions were made in a concentration range from 6.25 mg/mL to 50 mg/mL. The least concentration of each extract showing a clear of inhibition was taken as the MIC level.

2.7 Statistical analysis

Analysis of variance of antibacterial activities of plant extracts from ginger, galanga, turmeric, kaempferia, bastard cardamom were analyzed using the SAS Program. Mean separation was performed by the Protected LSD method at $p \leq 0.05$ [26].

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Sample Availability: Contact the authors.