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Research Article

Antimicrobial activity of essential oils extracted from Thai herbs and spices

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Abstract

A preliminary analysis of antimicrobial activities of 28 essential oils against food poisoning and food spoilage bacteria was conducted. The results revealed that the essential oils could be classified into five groups according to their inhibition effects against the tested bacteria. In addition, the essential oils obtained from phlai (*Zingiber cassumunar* Roxb.), lemon grass (*Cymbopogon citratus* (De Can dolle) Stapf), mint (*Mentha arvensis* L.), hairy basil (*Ocimum americanum* Linn.), clove (*Syzygium aromaticum*) and cinnamon (*Cinnamomum zeylanicum* Nees) were capable of inhibiting 5-7 types of tested bacteria since they presented similar or larger sizes of clear zones than the ones obtained from the antibiotics. However, the essential oils obtained from phlai and hairy basil were found to be unsuitable to use according to their colour, odor and cost. Therefore, five essential oils including cinnamon, clove, mint, ginger and lemon grass were selected for further study.

Extraction of the essential oils by distillation technique was carried out prior to the analysis of chemical constituents and physical properties i.e. refractive index, optical rotation and relative density. The results showed that clove, cinnamon, mint, lemon grass and ginger produced 4.0, 1.53, 1.0, 0.25 and 0.2% yield, respectively. In addition, different major constituents were found in each essential oil. For example, cinnamon contains a high amount of methyl cinnamate; clove has 87.66% eugenol; mint has 87.55% menthol, while the major constituents of lemon grass and ginger are Neral (citral b) and Geranial (citral a). An analysis of minimum inhibitory concentration (MIC) indicated that all tested bacteria except *P. aeruginosa* ATCC 25922 were easily inhibited by the essential oils obtained from lemon grass, mint, cinnamon and clove, where the MIC values were approximately 0.4-2 mg/ml.

Keywords: food spoilage bacteria, *Cymbopogon citrates*, *Mentha arvensis, Syzygium aromaticum, Cinnamomum zeylanicum*, Thailand**,** refractive index, optical rotation, relative density.

Introduction

Essential oils are organic substances produced by plants which can be obtained from roots, stem, bark, leaves, flowers, fruits and seeds [1]. They are volatile and have a special odor which helps protect the plant from insect, bacteria and other microbial attacks, or helps to induce insects for pollination purposes.

The essential oils have complex constituents. There are more than 50-500 compounds in one type of essential oil [2]. The main compositions are terpenes, including monoterpenes, sesquiterpenes, phenylpropene and terpenes which are composed of oxygen in the form of alcohols, aldehydes, esters and ketones, e.g. geranial menthol. Limonene, citral, geraniol, menthol and camphor are examples of monoterpenes, while β-bisabolene and β-caryophyllene are sesquiterpenes [2].

Herbs or spices have been widely used as cooking ingredients to help preserve food quality for a long time because the essential oils extracted from some herbs and spices have some antimicrobial activities. Considerable research has been undertaken on the antimicrobial potential of Thai medicinal plants, including some herbs and spices grown domestically in Thailand. Smith-Palmer, Stewart and Fyfe [3], found that most Gram-positive bacteria were more sensitive to inhibition by plant essential oils than the Gram-negative bacteria. In earlier work, Wannissorn *et al* [4] focused on evaluating the effects of plant essential oils against zoonotic enteropathogens which are an important factor for certifying broiler export. Matan *et al* [5] looked at incorporating a mixture of essential oils from Thai clove and cinnamon into active packaging for food. Khunkitti *et al* [6] examined the potential use of essential oils for acne control, while Gasaluck *et al* [7] focused on the antimicrobial properties of galangal extract against a specific pathogen. A more general contribution was made by Thongson *et al* [8] on the effect of Thai spices against *Listeria* and *Salmonella*. In another study, Thongson *et al* [9] also conducted work using ultrasound-assisted extraction techniques, while Nanasombat and Lohasupthawee [10] focused their study on crude extracts and essential oils. Huang *et al* [11] examined the herbs and spices commonly used in Thai *Tom Yum*, including lemon grass, for their antimicrobial properties. Kerdchoechuen *et al* [12] focused their study on the antibacterial effects of the

Zingiberaceae species, while another cosmetic study undertaken by Viyoch *et al* [13] looked at the potential of basil, including hairy basil in micro-emulsion formulas.

The object of this study was thus to investigate the antimicrobial activity of 28 plants found commonly in Thailand in order to select five suitable essential oils for further study against a range of food-borne pathogens. The physical and chemical properties of the five selective essential oils were then investigated.

Materials and Methods

Determination of antimicrobial activity of essential oils against food spoilage and food poisoning bacteria using disc diffusion assay [14]

Preparation of test disc

A required number of paper discs were placed into sterile Petri dishes. Each disc was then loaded with 15 µl of cinnamon oil or other tested essential oils and dried under laminar flow for 10 min.

Preparation of inoculum

Each bacterium was cultured on suitable solid media according to the strain used and incubated under appropriate conditions for 18-24 h (Table 1). With a sterile cotton swab, each overnight culture was thoroughly suspended in normal saline (0.85% sodium chloride solution). The inoculum was prepared by diluting the culture suspension with normal saline to obtain the turbidity in accordance with McFarland Standard No.1.

Preparation of test media

Twenty millilitres of the suitable medium were poured into each Petri dish. After solidification, the media were dried under biohazard standards for 15 – 20 min and used as the basal medium plates. To prepare the test media, each inoculum was uniformly spread over the surface of basal media by using a sterile cotton swab.

Antimicrobial assay

The test media was used immediately after preparation. Test discs impregnated with essential oils were placed onto the surface of test media by sterile forceps. After incubation at appropriate conditions, the diameters of inhibition zones around each test disc were measured.

Determination of physical and chemical properties of five selected essential oils

Extraction of essential oils from five selected plants by distillation technique

Approximately 100 g of each selected plant, i.e. clove, cinnamon, mint, lemon grass and ginger were chopped or cut into small pieces. Each aromatic plant was hydrodistilled for 5 hours in a Clevenger - type apparatus. The isolated oils were dehydrated by addition of anhydrous sodium sulphate and kept in an amber color glass bottle.

Chemical analysis of five selected essential oils

GC analysis was performed using a Fisons gas chromatograph model 8000 series equipped with a FID detector and a DB-5 capillary column (30m×0.25µm; film thickness 0.25 µm). The operating conditions were as follows: carrier gas: helium with a flow rate of 2 ml/min; column

temperature: 50-220^{o}C at 4^{o}C/min; injector and detector temperatures : 230^{o}C. In addition, the GC/MS analysis was performed on a mass spectrometer operating at 70 eV ionization energy, equipped with a HP-5MS column (30 m x 0.3 mm x 0.25 µm). The oven temperature was programmed from 50^{o}C to 220^{o}C at 4^{o}C/min.

The identification of the oil components was accomplished by comparing their GC retention indices as well as their mass spectra with corresponding data of authentic compounds or published spectra in the library such as Wiley 7 database and Adams database.

Physical properties of five selected essential oils

Physical properties of five selected essential oils were carried out as follows:

- ISO 280-1976 Essential oils- Determination of refractive index [15].
- ISO 592-1981 Essential oils- Determination of optical rotation [16].
- ISO 279-1981 Essential oils- Determination of relative density at 20°C [17].

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) [3]

Preparation of test solution

Stock solution of each essential oil was prepared in sterile 40% ethanol at the concentration of 100 mg/ml and kept at 4-10^{o}C till required.

Preparation of test media

Various amounts of essential oil stock solution were incorporated in molten solid media, cooled to 50^{o}C to obtain different concentrations of the essential oil ranging from 50 µg/ml to 5 mg/ml in final volume of 20 ml media.

Assay

Pieces of 10 mm -Millipore membrane were placed on the surface of solidified test media (2 pieces/bacterial strain). Five microliters of the inoculum were loaded onto the center of Millipore membranes. All plates were then incubated at appropriate conditions. MIC values were defined as the lowest concentration of each essential oil which completely inhibited the bacterial growth on the Millipore membrane.

growth into each tube containing 10 ml of fresh liquid medium (1 piece/tube). After incubation at the appropriate conditions, the MBC was regarded as the lowest concentration of essential oil showing no growth of bacteria in liquid medium.

Results and Discussion

Determination of antimicrobial activity of essential oil

Figure 1 presents antimicrobial activities of some plants. According to their inhibitory effects, 28 tested essential oils could be classified into five groups as shown in Table 1.

(1) The essential oils able to inhibit 7 strains; *Cinnamomum zeylanicum* Nees*, Zingiber cassumunar* Roxb., *Mentha arvensis* L., *Syzygium aromaticum* and *Piper betel* L.

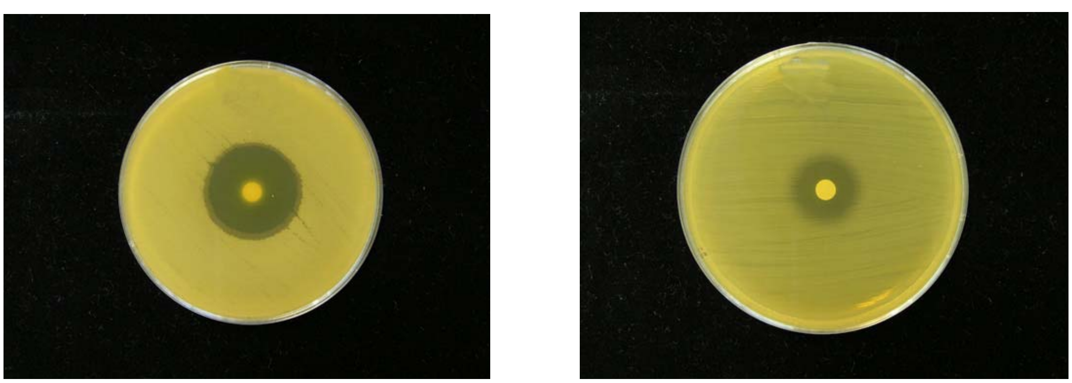
(2) The essential oils able to inhibit 5 strains; *Boesenbergia rotunda* (L.) Mansf., *Zingiber offinale Roscoc* (Chantaburi originated), *Cymbopogon citratus* (De Can dolle) Stapf), Cymbopogon nardus Rendle and *Ocimum basilicum* L. var. citratum

(3) The essential oils able to inhibit 4 strains; *Ocimum.tenuiflorum* L., *Alpinia conchigera* Griff.*, Zingiber ottensii* Veleton, *Litse elliptica* Blume and *Citrus hystrix* DC.

(4) The essential oil able to inhibit 3 strains; *Citrus auranitfolia* Swingle and *Alpinia galangal* (L.) wild

(5) The essential oil able to inhibit 2 strains or having no inhibitory activity e.g. *Curcuma aromatica* Salisb, *Ocimum basilicum* L. etc.

According to the inhibition zones obtained, it was found that *C. jejuni* DMST 15190 and *C. perfringens* DMST 15191 were the most sensitive strains to the essential oil tested, followed by *B. cereus* DMST 5040. In contrast, most of the essential oils tested showed very weak or no inhibitory activity against *P. aeruginosa* ATCC 27893 (Table 1).



a) cinnamon oil against *B. cereus* b) clove bud oil against *S*. Enteritidis DMST 17368

Figure 1. Clear zones show antimicrobial activities of essential oils obtained from cinnamon(*Cinnamomum zeylanicum* Nees) *and clove* (*Syzygium aromaticum*)

Table 1. Antimicrobial activities of 28 essential oils.

Plant	Inhibition zone (mm)*							
	S. T	S. En	E. co	P. ae	S. au	B. cer	C. jeju	C. per
Alpinia conchigera Griff.	14.25	14R	12.75	8.5	22.75	22.75	25	23.5
Alpinia galangal (L.) wild	15	11.5	10.5	6	9	9	28	23
Apium graveolens L.	8.5	6	6	6	6	6	6	6
Boesenbergia rotunda (L.) Mansf.	14.5	19	11	6	13.25	13.25	11.5	18
Cinnamomum bejolghota Sweet.	21.5	16.63	19.25	12	32	27	59.5	90
Citrus aurantifolia Swingle	16.5	10R	10.25	6	10.5	12.83 R	20.5	20.5
Citrus hystrix DC.	18.25	6	12	6	90	90	90	6
Curcuma aromatica Salisb.	6	6	6	6	24R	12.5	19.25	11.5
Curcuma dosmestica Valeton.	6	6	6	6	10	10	6	6
Cymbopogon citrates stapf.	24	11	18.5	7.5	90	40	90	80
Cymbopogon nardus Rendle.	20.63	12.75	10.5	6	44	23.5	16.5	35.5
Kampferia galangal L.	11.5	8	6	8	10.5	8.5	6	6
Litsea elliptica Blume	11	6	6	6	22	13.5	31.5	14
Mentha arvensis L.var.*piperascens* Malinv	27	10.5	14.5	9	50	34.25	90	90
Ocimum basillicium L.	16	9R	9.5	6	6	9	14	13
Ocimum basilicum L.var.*citratum*	32.5	12.5	12	6	44	41	80	80

Table 1. Antimicrobial activities of 28 essential oils (continued………..).

Plant	Inhibition zone (mm)*							
	S. T	S. En	E. co	P. ae	S. au	B. cer	C. jeju	C. per
Ocinum gratissimum. L.	11.5	11	9	8.5	20	10	12	19
Ocinum tenuiflorum L	13.5	20.25	11	9	16.75	16.75	11	20
Piper betel L.	16	18.5	15	10	27.5	14.5	16	34
Piper nigrum L.	6	6	6	6	16.5R	10.75 R	8	30
Polygonnium odoratum Lour.	8	6	6	6	12R	8	6	6
Psidium guajava L.	6	6	6	6	39.5R	12R	25.5	13.5
Psidium guajava L. Sali var.	6	6	6	6	39R	12R	32.5	16
Syzygium aromaticum	19.5	14.25	16.5	12	32	17.5	20.5	22.5
Zingiber cassumuma Roxb.	27.5	24.5	28.5	16.5	30R	18.25	21	23
Zingiber offinale Roscoc	12	10	12	6	*39*	*23*	7	7
Zingiber offinale Roscoc (Chantaburi originated)	14	14.25	14	6	12.2	12.2	14	22
Z.ottensii veleton	16	10.5	11	6	52R	15.5	42.5	57.5
amoxycillin	26.4	18.3	21.85	6	35.6	12.13	48.43	49.75
imipenen	ND	ND	ND	18.83	ND	ND	ND	ND

S.T = *Salmonella* Typhimurium TISTR 292; S. En = *Salmonella* Enteritidis DMST 17368; E.co = *Escherichia coli* TISTR 292; P. ae = *P. aeruginosa*; S.au = *S. aureaus* ; B.cer = *B. cereus*; C.jeju = *Campylobacter jejuni*; Cl. per = *Clostridium perfringens* DMST 1591

* Including the diameter of the filter disc (6 mm), 15 µl of essential oil in a disc.

To obtain more data concerning antimicrobial activities of these essential oils against food spoilage bacteria, the inhibitory assay was also performed using *Aeromonas hydrophilla*, *Enterococcus* sp., *Alcaligenes* sp., *Micrococcus luteus* and *leuconostoc* sp. as the indicator strains. The results showed that the essential oils obtained from ginger mint, clove and cinnamon possessed inhibitory activity against these bacteria (Table 2).

Table 2. Antimicrobial effects of five essential oils against some food spoilage bacteria.

Bacteria type	Inhibition zone (mm)*					
	Ginger	Lemon grass	Mint	Clove	Cinnamon	Amoxycillin
Aeromonas hydrophilla	19.5	32.5	14.8	25	26.8	16
Enterococcus spp.	13.5	24.5	17	15	14	30
Alcaligenes sp.	90	90	90	35	39	37
M. luteus	22	38	24	27	19	53
Leuconostoc sp.	10	12	17.5	20	15	27

* Including diameter of paper disc (6 mm)

Chemical analysis of five selected essential oils

Table 3 presents yield obtained from distillation of the selective plants. As shown, each plant contained a little amount of the essential oil. The results showed that clove, cinnamon, mint, lemon grass and ginger produced 4.0, 1.53, 1.0, 0.25 and 0.2% yield, respectively. Thus, the comparison between the five selective essential oils showed that the clove provided the highest yield and the ginger provided the lowest yield.

Table 3. Yield of five selected essential oils.

No.	Plants	Yield, %
1	Clove	4.0
2	Cinnamon	1.53
3	Mint	1.0
4	Lemon grass	0.25
5	Ginger	0.20

Chemical analyses of five selected essential oils are presented in Table 4-8. As shown, each essential oil contained different ingredients at different levels, thus having different antimicrobial activities against different bacteria. For example, the major constituent of the essential oil obtained from cinnamon are methyl cinnamate, and E-Cinnamal dehyde ,while the major constituent of the essential oil obtained from lemon grass are neral (citral b) and geranial (citral a).

Table 4. Chemical analysis of clove bud oil.

Chemical constituents	Quantity, %
eugenol	87.66
trans-caryophyllene	5.27

Table 5. Chemical analysis of cinnamon oil.

Chemical constituents	Quantity, %
α- pinene	0.61
camphene	0.59
limonene	0.30
1,8-cineol	1.89
e -cinnamaldehyde	21.60
methyl cinnamate	57.44

Table 6. Chemical analysis of lemon grass oil.

Chemical constituents	Quantity, %
6-methyl-5-hepten-2-one	0.99
trans–linalool oxide	0.13
linalool	0.57
neral (citral b)	18.49
trans–geraniol	0.45
geranial (citral a)	28.93
geranic Acid	9.48
neral diethyl acetal	4.44
3-hexen-1-ol	0.35
caryophyllene oxide	0.79
t-muurolol	2.37

Table 7. Chemical analysis of mint oil.

Chemical constituents	Quantity, %
α- pinene	0.12
β - pinene	0.18
limonene	0.72
menthone	3.72
isomenthone	2.14
menthol	87.55

Table 8. Chemical analysis of ginger oil.

Chemical constituents	Quantity, %
β - pinene	3.2
camphene	1.02
β- myrcene	4.26
limonene	7.90
1,8-cineol	9.46
linalool	0.69
neral (citral b)	15.36
geraniol	0.65
geranial (citral a)	21.57
ar-curcumene	3.55
zingiberene	5.27
nerolidol	3.44
β -sesquiphellandrene	2.55

Physical properties of five selected essential oils

Physical properties i.e. refractive index, optical rotation and relative density of five selected essential oils are presented in Table 9. As shown, the refractive indices of the five essential oils are almost similar. The essential oil extracted from cinnamon has the highest value of refractive index (1.576). The optical rotation indicated that the essential oils obtained from cinnamon and lemon grass have different angles (negative polarized) when compared to the essential oils obtained from ginger, clove, and mint. The relative density of the five essential oils shows that, at 20°C, the essential oils obtained from cinnamon and clove have higher density or are heavier than water, while the essential oils obtained from lemon grass, ginger and mint have lower density or are lighter than water.

Table 9. Physical properties of five selected essential oils.

Essential oil	Refractive index	Optical rotation	Relative density at 20°C
Cinnamon	1.576	-0.35	1.0519
Lemon grass	1.482	-0.23	0.8863
Ginger	1.487	18.45	0.8879
Clove	1.535	0.85	1.0676
Mint	1.459	23.43	0.9089

MIC and MBC values against tested bacteria

Except for *P. aeruginosa* ATCC 27853, most of tested bacteria were susceptible to lemongrass oil, mint oil, cinnamon oil and clove oil with MIC values ranging from 0.4 -2 mg/ml (Table 10). Ginger oil was found to have higher MIC values (4-6 mg/ml) for tested bacteria. In addition, *S. aureaus* ATCC 6538 was shown to be the most susceptible strain.

It should be noted that mode of action of lemongrass oil, mint oil and clove oil agianst *S.* Typhimurium DMST 17424, *S.* Enteritidis 17368, *E. coli* O157, and *S. aureaus* ATCC 6538 seemed to have bactericidal activity since their MIC values were similar or equal to the MBC values. On the contrary, the MBC:MIC ratios of cinnamon oil for *S.* Typhimurium DMST 17424, *S.* Enteritidis 17368 and *E. coli* O157 were higher than 2, indicating that the mode of action of cinnamon oil against these bacteria was only inhibitory action.

In the cases of *C. jejuni* DMST 15190 and *C. perfringens* DMST 15191, it could be seen that the MIC values of five essential oils were in the range of 0.4-1 mg/ml, which were lower than those for the other tested bacteria. However, the MBC values of these essential oils against both bacteria were higher when compared to those obtained for the remaining tested bacteria. The results indicated that ginger oil, lemongrass oil, mint oil, clove oil and cinnamon oil could only inhibit the growth of *C. jejuni* DMST 15190 and *C. perfringens* DMST 15191.

Table 10. MIC and MBC values of five essential oils against tested bacteria.

Bacteria	**Ginger** mg/ml		**Lemon grass** mg/ml		**Mint** mg/ml		**Clove** mg/ml		**Cinnamon** mg/ml	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
*S.*Typhimurium DMST 17424	5	5	1.5	1.5	1.5	1.5	2	2	1.5	3.5
S. Enteritidis DMST 17368	4.6	5	1.5	1.5	1.5	1.5	2	2	0.8	2.5
E. coli O157	2	2.5	1.5	1.5	1	1	2	2	0.7	2.5
P. aeruginosa ATCC 27853	>9	>9	>9	>9	>9	>9	5	>9	2	2
S. aureaus ATCC 6538	5	>9	1	1	1	1	1	1	1	2
B. cereus DMST 5040	6	>9	4	5	2	>9	2	5	1	2
C. jejunii DMST 15190	1	>9	1	>9	1	>9	2	>9	1	>9
C. perfringens DMST 15191	0.4	3.5	1	>9	0.4	3.5	2	>9	0.4	>9

Conclusion

Twenty-eight essential oils extracted from Thai herbs and spices have shown some inhibitory effects against the tested bacteria. However, different essential oils have different antibacterial effects against the tested bacteria since each essential oil possesses different major constituents.

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