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Chemical Composition and Antibacterial Activity of Essential Oils from Three Aromatic Plants of the Zingiberaceae Family in Malaysia

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Abstract: *The essential oils of Boesenbergia rotunda (Temu Kunci), Curcuma mangga (Temu Pauh) and Kaempferia galanga (Cekur) were extracted using steam distillation, and the main constituents of the essential oils were analysed using gas chromatography-mass spectrometry (GC-MS). More than 10 constituents were identified in each essential oil. The main compounds in B. rotunda were nerol (39.6%) and L-camphor (36.0%), whereas ethyl-(E)-3-(4-methoxyphenyl)prop-2-enoate (57.2%) and ethyl cinnamate (39.1%) were identified in K. galanga. C. mangga contained mainly L-beta-pinene (95.6%). Antibacterial activity was assessed using the disc diffusion method and the minimum inhibitory concentration (MIC) was determined. The most active essential oil for all selected Gram-positive (Staphylococcus aureus and Bacillus cereus) and Gram-negative bacteria (Pseudomonas aeruginosa and Escherichia coli) was B. rotunda (inhibition zone of 10.3–16.0 mm), followed by C. mangga (inhibition zone of 7.33–12.3 mm). The essential oil extracted from K. galanga exhibited no antibacterial activity against any of the bacteria tested. B. rotunda showed higher antibacterial activity than C. manga, with MIC values of 1.3 × 10^{-2} μl ml^{-1} (S. aureus), 2.6 × 10^{-2} μl ml^{-1} (P. aeruginosa and E. coli) and 0.66 × 10^{-2} μl ml^{-1} (B. cereus) compared to MIC values of 2.6 × 10^{-2} μl ml^{-1} (S. aureus and B. cereus) and 5.3 × 10^{-2} μl ml^{-1} (P. aeruginosa and E. coli) for C. mangga.*

Keywords: Zingiberaceae, essential oil, *Boesenbergia rotunda*, *Curcuma mangga*, *Kaempferia galanga*

1. INTRODUCTION

Traditional medicine has long been accepted as an alternative to western medicinal practice in many countries. Traditional medicine was once regarded as the sole source of treatment, making it a focus in the search for solution to increasing drug resistance among pathogenic microorganisms. The World Health Organization (WHO) has reported that over 80% of the world's population relies on traditional medicine, which is largely plant based, for their primary healthcare needs.[1]

Most essential oils extracted from medicinal plants are collected from the wild.[2] Evaluation of the biological activities of the essential oils from several medicinal plant species has revealed that some exhibited interesting characteristics, such as insecticidal, antibacterial and antifungal activities.[3] Due to the growing concern on the impact of using synthetic chemicals as medicines and food preservatives, researchers are increasingly turning their attention to natural products to develop better drugs against viral and microbial infections.[4]

Zingiberaceae, the family of plants that includes ginger, is predominantly found in tropical Asia, and it is primarily used in traditional medicines, spices and perfumes.[5] Peninsular Malaysia contains approximately 160 Zingiberaceae species. The genus *Curcuma* is widely distributed in tropical Asia and Australia. *Curcuma mangga* is mainly used as a condiment, as it is an edible substance, and its rhizome yields aromatic oils. Turmeric, another member of this genus, was used in ancient times as a dye, medicine and magical symbol.[6] Another genus, *Kaempferia* is broadly distributed throughout Australia, Asia and Africa. This species has a very fragrant rhizome, and it is traditionally used to treat many diseases, including ascariasis, abdominal pain in women and rheumatism.[7] *Boesenbergia rotunda*, another Zingiberaceae species known as Chinese ginger, is found in China and Southeast Asia. It is also called "Fingerroot" due to its physical appearance. Its rhizome is used as a traditional remedy for swelling, tumours, wounds and colic.[8]

In this study, the essential oils of 3 aromatic medicinal plants from the *Zingiberaceae* family, *Kaempferia galanga*, *Boesenbergia rotunda* and *Curcuma mangga* (also known as *Cekur*, *Temu Kunci* and *Temu Pauh*, respectively in Malaysia), were extracted to investigate and compare their chemical compositions and screen for antibacterial activity against select Gram-negative (*Pseudomonas aeruginosa* and *Escherichia coli*) and Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus cereus*). These plants were chosen due to their potential as medicinal plants, and the evaluated Gram-negative and Gram-positive bacteria were chosen based on their pathogenic effects. Steam distillation was used to extract the essential oils, which were then analysed by gas chromatography-mass spectrometry (GC-MS). The antibacterial activity was assessed using the wet disc diffusion method. Gentamycin (10 µg) and tetracycline (10 µg) susceptibility discs were used as positive controls, and blank discs served as negative controls. Each plant extract was tested in triplicate.

2. EXPERIMENTAL

2.1 Plant Materials

Fresh plant rhizomes were purchased from local suppliers in Kuantan, Pahang, Malaysia. Samples were stored at room temperature to keep them fresh until the extraction process. The rhizomes of *B. rotunda*, *C. mangga* and *K. galanga* were identified by Dr. Shamsul Khamis, a botanist from Universiti Putra Malaysia, Malaysia. Voucher specimens (PIIUM 0216, PIIUM 0217 and PIIUM 0218, respectively) were deposited in the Herbarium, Kulliyyah of Pharmacy, International Islamic University Malaysia.

2.2 Extraction of Essential Oils

The essential oils from the rhizomes of the selected aromatic medicinal plants were extracted by steam distillation. Each sample (1.5 kg) was washed and chopped into small pieces. The water was boiled at a high temperature for 5 to 10 h until no more essence exuded from the plant samples. The oil collected was assembled into a Bijou bottle, and the distillate product was mixed with dichloromethane (DCM) and separated using a separating funnel. The product was dehydrated by anhydrous sodium sulphate, and the organic solvent was removed in vacuo. The total amounts of essential oils collected were 4.5 g (0.3%, *C. mangga*), 4.0 g (0.27%, *B. rotunda*) and 5.5 g (0.37%, *K. galanga*). The obtained essential oils were weighed and stored at low temperature (4°C) for analysis.

2.3 Analysis of Essential Oils

The analyses of the essential oils of *B. rotunda*, *C. mangga* and *K. galanga* were performed using the PerkinElmer (PerkinElmer Inc., Connecticut, U.S.) AutoSystem XL gas chromatography, equipped with an Elite-5 fused-silica capillary column (inner diameter 30 m × 0.32 m, 0.25 µm film thickness) directly coupled to a TurboMass Gold GC-MS. The carrier gas was helium at a flow rate of 5 ml min^{-1}. The initial temperature was set to 50°C, and it was increased by 4°C min^{-1} until reaching a final temperature of 250°C. The essential oil (5 ml) was dissolved in 495 ml of dichloromethane (1:100 v/v) and injected into the column. The essential oil constituents were identified by comparing their mass spectra with the national Institute of Standards and Technology (NIST) mass spectral database library, and they were confirmed by comparison with data published in literature when possible. The composition was reported as the relative percentage of the total peak area using the following calculation:

Relative % of peak area = (Area of the peak/Total peak area) × 100

2.4 Bacterial Strains

2 Gram-negative (*P. aeruginosa* ATCC 27852 and *E. coli* ATCC 35218) and 2 Gram-positive (*S. aureus* IMR S 1386/07 A and *B. cereus* ATCC 11778) bacterial strains were used in this study. The strains were purchased from American Type Culture Collection (Manassas, Virginia, U.S.) and the Institute for Medical Research (Kuala Lumpur, Malaysia). All bacteria tested were cultivated at 37°C and cultured on Mueller-Hinton agar.

2.5 Initial Screening

The disc-diffusion method was used to screen the antibacterial activity of the essential oils following the standard methods described by the National Community for Clinical Laboratory Standards (NCCLS). Broth containing the tested microorganisms was uniformly swabbed on Mueller Hinton agar plates using sterile cotton swabs. Sterile blank discs were individually impregnated with pure essential oil (5 and 10 µl) and placed onto the inoculated agar plates. The distance between the discs was wide enough to allow for the reading of inhibition zones. The plates were inverted and incubated at 37°C for 18 to 24 h. For positive controls, a commercial disc containing gentamycin (10 µg) was used for the plates with *S. aureus*, *E. coli* and *B. cereus*, and a tetracycline (30 µg) disc was used for *P. aeruginosa*. A blank disc was used as a negative control. Antibacterial activity was evaluated by measuring the diameter of inhibition zones (mm) against the tested microorganisms. All tests were performed in triplicate.

3. RESULTS AND DISCUSSION

In this study, essential oils from the rhizomes of 3 aromatic medicinal plants from the Zingiberaceae family (*B. rotunda*, *C. mangga* and *K. galanga*) were extracted to identify their chemical compositions and screen for antibacterial activity. 2 Gram-positive (*S. aureus* and *B. cereus*) and 2 Gram-negative bacteria species (*P. aeruginosa* and *E. coli*) that are related to food-borne diseases were tested. These strains are also broadly used in screening tests due to their significance as human pathogens that can cause disease.[9]

Antibacterial activity may be related to the major chemical constituents in the essential oils. The chemical components of essential oil are separated into 5 main classes: monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpenes and others, such as diverse functions and esters.[10] In our study, the greatest amount of essential oils

was obtained from the fresh rhizomes of *K. galanga* (5.5 g), followed by *C. mangga* (4.5 g) and *B. rotunda* (4.0 g). Chemical composition analysis of the essential oils by GC-MS identified the presence 19 components in *B. rotunda*, 12 components in *C. mangga* and 15 components in *K. galanga*.

The GC-MS data tabulated in Table 1 summarises the main chemical composition of the essential oil of *B. rotunda*. The main constituents detected were nerol (39.56%) and *L*-camphor (36.01%).

Table 1: Chemical composition of the essential oil of *B. rotunda.*

Retention Time (R_t)	Compound	Molecular weight (MW)	% composition
16.55	Nerol	154	39.56
12.95	*L*-camphor	152	36.01
9.14	Cineole	154	9.47
20.88	*Trans*-methyl cinnamate	162	6.84
9.60	Fenchene	136	2.01
17.11	*Cis*-p-mentha -2, 8-dien-1-ol	152	1.55
14.62	Cyclohexene, 1-methyl-5-(1-methylethenyl)-, (*R*) -	136	1.09
13.24	*Z,Z,Z* -4,6,9-Nonadecatriene	262	0.63
18.11	Octanal, (2,4-dinitrophenyl) hydrazine	308	0.54
13.84	Ethanol, 2-(9,12- octadecadienyloxy)- (*Z,Z)*	310	0.53
46.42	Pinostrobin chalcone	270	0.42
9.04	Limonene	136	0.38
11.52	2-(7-hydroxymethyl-3,11-dimethyl-dodeca-2,6,10–trienyl)-	328	0.31
9.26	dl-α-(Methylaminomethyl) benzyl alcohol	151	0.22
10.52	Chloromethyl 2-chloroundeconoate	268	0.18
11.23	8- tetradecen-1-ol acetate	254	0.12
11.74	Furan-3-carboxanide,2-methyl-*N*-(4-morpholyl)-	210	0.11
46.36	Hemanthidine	317	0.03
		Total	100

The main chemical constituents found in the essential oil of *C. mangga* are listed in Table 2. Of the 12 constituents identified, the first 2 components, both *L*-β-pinene (95.57%), made up the major component found. The next main component, α-pinene (1.84%), is another terpene isomer but with different stereochemistry.

Table 2: Chemical composition of the essential oil of *C. mangga.*

Retention Time (R_t)	Compound	Molecular Weight (MW)	% composition
7.82	*L*-β-pinene	136	80.01
7.46	*L*-β-pinene	136	15.56
6.23	α-pinene	136	1.84
9.61	Ocimene	136	0.86
9.05	Limonene	136	0.58
11.33	Perillene	150	0.57
11.09	1-Pentanol, 5-cyclopropylidene	126	0.23
8.89	Methyl *N*-(*N*-benzyloxycarbonyl- β-1-aspartyl)-beta- glucosaminide	442	0.10
9.26	Galactonic phenylhydrazide	286	0.09
11.80	4-Methoxyphenoxyformide,*N*-methyl-*N*-[4-(1-pyrrolidinyl-butynyl]	302	0.08
9.14	Corynan-17-ol,18,19-didehydro-10-methoxy-, acetate (ester)	368	0.06
12.02	Propionamide,3-methoxy carbonyl-*N*-methyl-*N*-[4-(1-pyrrolidinyl-butynyl]	266	0.02
		Total	100

A total of 15 chemical components were identified in *K. galanga* by GC-MS (Table 3). Ethyl-(*E*)-3-(4-methoxyphenyl)prop-2-enoate (57.16%) and ethyl cinnamate (39.09%) were the main constituents.

The essential oils (5 and 10 µl per disc) were screened for their antibacterial activity using the disc-diffusion method. Essential oils that exhibited antibacterial activity towards a microorganism produced a clear zone on the agar around the disc.[11] However, the essential oil of *K. galanga* exhibited no zone of inhibition toward either Gram-positive or Gram-negative bacteria.

The results of the MIC tests for *B. rotunda* and *C. manga* revealed that *B. rotunda* inhibited the bacterial growth at 0.66×10^{-2} µl ml^{-1} for *B. cereus* (Table 4), whereas the lowest concentration of *C. mangga* that inhibited bacterial growth was 2.6×10^{-2} µl ml^{-1} toward *S. aureus* and *B. cereus* (Table 5).

The essential oil of *B. rotunda* also had higher antibacterial activity (inhibition zone of 10.3–16.0 mm) compared to *C. mangga* (inhibition zone of 7.33–12.3 mm) for the selected Gram-positive and Gram-negative bacteria (Table 6).

Table 3: Chemical composition of the essential oil of *K. galanga.*

Retention Time (R_t)	Compound	Molecular Weight (MW)	% composition
31.75	Ethyl-(*E*)-3-(4-methoxyphenyl)prop-2-enoate	206	57.16
23.51	Ethyl cinnamate	176	39.09
7.46	*L*-β-pinene	136	1.18
13.83	Borneol	154	0.81
6.23	α-pinene	136	0.43
24.55	Nonadecane	268	0.37
9.05	2-[1-(Adamantan-1-ylamino)-2,2,2-trifluoro-etthlidene] –malononitrile	295	0.28
7.80	Mycrene	136	0.24
9.14	Cyclohexane,(2-nitro-2-propenyl)-	169	0.09
7.31	2-[1-(Adamantan-1-ylamino)-2,2,2-trifluoro-etthlidene] –malononitrile	295	0.08
8.31	3-Benzylsulfanyl-3-fluro-2-trifluoromethyl-acrylic acid methyl ester	294	0.08
5.94	9-Borabicyclo [3.3.1] nonane,9-(1-methyl propyl)	178	0.07
8.89	*m*-Cymene	134	0.07
14.12	Codlelure	182	0.05
		Total	100

Table 4: Minimum inhibitory concentrations of *B. rotunda.*

Bacteria	Concentration(µl ml^{-1}) (10^{-2})								Controls	
	5.3	2.6	1.3	0.66	0.33	0.16	0.082	0.04	Ant (+ve)	Cult bac (–ve)
S. aureus	X	X	X	√	√	√	√	√	X	√
P. aeruginosa	X	X	√	√	√	√	√	√	X	√
B. cereus	X	X	X	X	√	√	√	√	X	√
E. coli	X	X	√	√	√	√	√	√	X	√

Notes: X = no bacteria growth observed; √ = bacteria growth observed; Ant = antibiotic; Cult bac = Culture bacteria; +ve = positive; –ve = negative.

Table 5: Minimum inhibitory concentrations of *C. mangga*.

Bacteria	Concentration (µl ml^{-1}) (10^{-2})								Controls	
	5.3	2.6	1.3	0.66	0.33	0.16	0.082	0.04	Ant (+ve)	Cult bac (–ve)
S. aureus	X	X	√	√	√	√	√	√	X	√
P. aeruginosa	X	√	√	√	√	√	√	√	X	√
B. cereus	X	X	√	√	√	√	√	√	X	√
E. coli	X	√	√	√	√	√	√	√	X	√

Notes: X = no bacteria growth observed; √ = bacteria growth observed; Ant = antibiotic; Cult bac = Culture bacteria; +ve = positive; –ve = negative.

Table 6: Diameter of inhibition zone produced by essential oils.

Bacteria	Volume (µl)	*B. rotunda*[*]	*C. mangga*[*]	*K. galanga*
S. aureus[a]	5	13.00 ± 4.58	9.00 ± 2.65	–
	10	14.67 ± 2.08	12.30 ± 2.52	–
B. cereus[b]	5	13.33 ± 0.58	8.67 ± 1.15	–
	10	16.00 ± 3.00	8.67 ± 0.58	–
E. coli[c]	5	13.67 ± 0.58	8.33 ± 0.58	–
	10	14.67 ± 1.53	10.33 ± 0.58	–
P. aeruginosa[d]	5	10.33 ± 0.58	7.33 ± 0.58	–
	10	12.00 ± 3.00	7.67 ± 0.58	–

Notes:
1. [*] *millimetres*
2. *Minus sign (–) indicates no antibacterial activity*
3. [a] *Gentamycin (10 µg) produced a zone of 18.67 mm;* [b] *Gentamycin (10 µg) produced a zone of 19.83 mm;* [c] *Gentamycin (10 µg) produced a zone of 19.17 mm;* [d] *Tetracycline (30 µg) produced a zone of 14.55 mm.*

Essential oils containing terpenes, especially mono- and sesquiterpenes, also possess antimicrobial activity.[12] Although there are many published articles on the chemical composition of the plant extracts of *B. rotunda*, very few publications have investigated the essential oils of its rhizome. Nerol and *L*-camphor, the main constituents of the rhizome of *B. rotunda*, are an oxygenated monoterpene and a bicyclic ketone terpene, respectively. Both compounds exhibit antibacterial activity.[3] Previous research on the antimicrobial actions of monoterpenes suggests that they diffuse into and damage cell membrane structures.[13] In addition, the presence of an oxygen in the framework of a ketone, such as *L*-camphor, increases the antimicrobial properties.[12] The presence of large amounts of these 2 compounds is believed to be responsible for the antibacterial activity observed here.

Several studies have investigated the chemical composition of *C. mangga*. Wong et al.[14] found the major constituents of its essential oil were myrcene (78.6%) and β-pinene (3.7%), while a recent report by Kamazeri et al.[15] identified the main constituents as caryophyllene oxide (18.71%) and caryophyllene (12.69%). Our studies found that the composition of *C. mangga* is dominated by pinene-type monoterpene hydrocarbons. α-Pinene and β-pinene possess antimicrobial properties.[10] Differences in stereochemistry influence their bioactivity; α-isomers are relatively inactive compared to β-isomers.[12] Furthermore, ocimene (isomer of myrcene), which is found in considerable amounts in *C. mangga* essential oil, also has antibacterial activity.[16] The remaining compounds were only present in small or trace amount.

In agreement with our results, several studies have reported that camphor exhibits more potent antibacterial activity than β-pinene, particularly against Gram-positive bacteria.[17,18] The vast number of chemical components found in essential oils may be related to the mechanism of action underlying their antibacterial properties. Most reports investigating the mechanism of action underlying the activity of essential oils against common food-borne bacteria agree that, in general, Gram-positive bacteria are more sensitive than Gram-negative bacteria.[3]

Among all the plants tested, only *K. galanga* failed to show any antibacterial property. Ethyl-(*E*)-3-(4-methoxyphenyl)prop-2-enoate (57.16%) was identified as the main compound, followed by ethyl cinnamate (37.39%). Few studies have focused on the antibacterial activities of these 2 constituents. Ethyl cinnamate is one of the main constituents of the essential oil of *Lippia chevalieri* flowers (30.3%), but it does not have significant antimicrobial activity.[19] To the best of our knowledge, *K. galanga* is not reported to have antibacterial activity, although it has selective toxicity against *Aspergillus fumigatus*.[20] Other chemical compounds, such as *L*-β-pinene (1.18%), α-pinene (1.41%, oxygenated monoterpenes) and borneol (0.81%, monoterpene hydrocarbons), have antibacterial activities, but the proportions of these compounds were too small to effect the bacteria tested.

4. CONCLUSION

B. rotunda, *C. mangga* and *K. galanga* are aromatic medicinal plants with antibacterial activity toward *Staphylococcus aureus* (IMR S 1386/07 A), *Bacillus cereus* (ATCC 11778), *Pseudomonas aeruginosa* (ATCC 27852) and *Escherichia coli* (ATCC 35218). The growth of both Gram-positive bacteria and Gram-negative bacteria was inhibited by the essential oils tested, except for the oil derived from *K. galanga*. These results indicate that each essential oil has its

own chemical composition, which may be correlated with its antibacterial activity. The essential oils from the three rhizomes of the Zingiberaceae family displayed antibacterial properties, and their activities could be attributed to qualitative and quantitative differences in the chemical constituents of the individual essential oils. These inexpensive natural remedies hold promise as alternatives to current antibiotics against pathogens, although further evaluations regarding the toxicity of these oils are needed.

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