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Chemical constituents and antibacterial activities of crude extract and essential oils of *Alpinia galanga* and *Zingiber officinale*

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Abstract

Crude extract and essential oils from dried rhizome of two Zingiberaceae family plants, *Alpinia galanga* and *Zingiber officinale*, were evaluated for their chemical constituents and antimicrobial activity. The essential oils were analyzed by Gas Chromatography - Mass Spectroscopy (GCMS). The abundant constituents for *A. galanga* were cineole, 4-allylphenyl acetate, α-farnesene, (2,6-dimethylphenyl)borate and α-pinene; and for *Z. officinale* were cineole, 2,2-dimethyl-3-methylenenorbornane, α-curcumenene, β-sesquiphellandrene and rosefuran epoxide. Alpha-pinene, 2,2-dimethyl-3-methylenenorbornane, β-pinene, β-mircene, cineole, β-citral, α-citral, bornyl acetate, α-curcumene, α-zingiberene, β-sesquiphellandrene, and hexadecanoic acid are detected in both galangal and ginger rhizomes. Minimum Inhibitory Concentration (MIC) of essential oils and crude extracts were evaluated by broth dillution method against foodborne bacteria *Bacillus subtilis, Escherichia coli, Staphylococcus aureus, Salmonella typhymurium* and *Vibrio cholera.* MIC of crude extract and essential oils of galangal and ginger against all tested microogranisms were relatively high.

Introduction

Foodborne illness is a growing public health problem in developing as well as developed countries (Jahan, 2012). In Indonesia, paratyphoid is one of common foodborne illness along with food poisoning caused by *Escherichia coli, Vibrio chollera, Bacillus subtilis,* and *Staphylococcus aureus* (Vollaard *et al*., 2004). Plant derived products are often considered to be more natural and safer alternatives to chemicals and thus have became popular in the scope of searching novel antimicrobial agents for food and pharmaceutical applications (Han and Bhat, 2014). Hence, the antimicrobial activities of spices commonly used in Indonesian cullinary against some foodborne illness need to be studied further.

Zingiberaceae is one of the largest families of the plant kingdom. It provides many useful products for food, spices, medicines, dyes and perfumes (Sirirugsa, 1997). Galangal (*Alpinia galanga*; lengkuas in Bahasa Indonesia) and ginger (*Zingiber officinale*; jahe in Bahasa Indonesia) are two members of Zingiberaceae family commonly used as spices in Indonesian culinary. Galangal had been reported possessing antimicrobial activity against *Listeria monocytogenes, Staphylococcus aureus, Shigella* sp., *Bacillus cereus, Escherichia coli, Enterobacter aerogene, E. cloacae, Enterococcus faecalis, Klebsiella pneumoniae, Salmonella typhimurium, Streptococcus epidermis, Aspergillus flavus* and *Mycobacterium tuberculosis* (Oonmetta-aree *et al.*, 2006; Arambewela and Arawwawala, 2007; Natta *et al*., 2008; Srivastava *et al*., 2008; Hsu *et al.*, 2010; Rao *et al*., 2010; Gupta *et al*., 2014). Ginger is one of nine featured Indonesian medicinal plants (Widyawati, 2007) and had been reported having antimicrobial activity against *Bacillus licheniformis, B. spizizenii, B. subtilis, S. aureus, E coli, K. pneumoniae, Pseudomonas stutzeri* and *Candida albicans* (Srivastava *et al*., 2008; Bellik, 2014). The purpose of this study was to compare the antimicrobial activity and chemical constituents of crude extracts and essential oils from galangal and ginger against common foodborne bacteria in Indonesia.

Materials and Methods

Plant materials

Fresh rhizomes of galangal and ginger were purchased from a local market at Purbalingga,

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Table 1. Volatile chemical constituents of galangal rhizome

No	Compound Name	Retention time (min)	Percentage	
			Essential oil	Crude extract
1	α-pinene	6.497	4.974	-
2	2,2-dimethyl-3-methylenenorbornane	7.002	0.305	-
3	(+)-sabinene	8.026	0.332	-
4	β- pinene	8.102	0.830	-
5	β-myrcene	8.882	0.540	-
6	cineole	10.723	45.199	3.613
7	γ-terpinene	12.122	0.367	-
8	β-citral	23.073	0.631	-
9	α-citral	25.021	0.869	-
10	bornyl acetate	25.709	1.176	-
11	4-allylphenyl acetate	29.347	13.718	-
12	geranyl acetate	31.120	2.857	-
13	tetradecane	31.774	-	1.107
14	1,3,4-eugenol methyl ether	31.915	1.559	-
15	β-caryophyllene	32.186	0.359	-
16	α-farnesene	33.745	5.487	2.306
17	α-curcumene	34.429	0.625	-
18	3-furanacetic acid	34.551	1.570	-
19	α-zingiberene	34.826	0.528	-
20	n-pentadecane	35.048	2.064	1.729
21	γ-muurolene	35.285	2.138	-
22	β-sesquiphellandrene	35.651	0.965	-
23	2-methoxy-4-prop-1-enylphenyl acetate	35.858	1.050	-
24	n-hexadecane	37.849	-	1.253
25	(2,6-dimethylphenyl)borate	38.999	5.023	17.756
26	3'-methoxyacetanilide	39.190	1.048	-
27	6,8-heptadecadiene	39.472	0.482	-
28	8-heptadecene	39.656	1.193	1.262
29	n-heptadecane	39.958	-	1.110
30	ethyl (2E)-3-(4-methoxyphenyl)-2-propenoate	41.475	1.418	-
31	decahydro-5,5,8-trimethyl-2-methylene-1-naphthaleneacetaldehyde	42.323	0.213	-
32	farnesyl acetate	43.309	0.781	0.733
33	4,4-dimethyl-6-hydroxy-3,4-dihydrocoumarin	43.454	0.596	-
34	hexadecanoic acid	45.873	-	0.672
35	eicosane	46.484	-	0.691
36	3'-methoxydaidzein	48.696	0.324	-
37	docosane	50.107	-	0.666

Central Java, Indonesia. Plant materials were dried and pulverized to fine powders.

Microbial strains

Five bacterial strains were obtained from the American typeculture collection (ATCC; Rockville, MD, USA). They were *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 8739, *Staphylococcus aureus* ATCC 6538, *Salmonella enterica typhimurium* ATCC 14028 and *Vibrio cholera* ATCC 9027. All microorganisms were stocked in appropriate conditions and regenerated before being used.

Solvent extraction

Solvent extraction of essential oils was carried out by method described previously (Mohamed *et al.* 2013) using hexane as solvent. The extracted essential oils were dehydrated over anhydrous sodium sulfate and stored at 0°C in air-tight glass vials until used for further analysis.

Hydrodistillation

Dried rhyzomes were subjected to hydro-distillation using a Clevenger apparatus for 4 h for the isolation of essential oils according to the recommended method (Guenther, 1961). The volume of the extracted essential oil was recorded. The essential oils yield for galangal and ginger were 0.140 and 0.643% (w/v), respectively. The extracted essential oils were dehydrated over anhydrous sodium sulfate and stored at 0°C in air-tight glass vials until used for further analysis.

Analysis of chemical compositions

The volatile composition of plant extracts were analyzed using GC-MS system (Agilent 6980N GC System coupled to Agilent 5973 inert MSD detector), equipped with a ZB-5 capillary column (30 m x 0.25 mm x 0.25 µm). The carrier gas was helium at flow rate of 1.3 ml/min, and 2 µL of sample was injected. The electron impact technique (70 eV) was used. The injector and detector temperatures were 250°C and 230°C.

Determination of Minimum Inhibitory Concentration (MIC)

The MIC was examined by broth dilution method in nutrien broth using a method described by Al-Reza *et al*. (2010) with a minor modification. Briefly, active cultures for MIC determination were prepared by transferring a loopful of cells from the stock cultures to flasks and inoculated in nutrien broth (NB) medium and incubated at 37°C for 24 h. The cultures were diluted with NB broth to

Table 2. Volatile chemical constituents of ginger rhizome

No	Compound Name	Retention time (min)	Percentage Essential oil	Crude extrac
1	hexanal	3.181	1.281	-
2	α-pinene	6.497	5.784	-
3	2,2-dimethyl-3-methylenenorbornane	7.002	12.203	-
4	β-pinene	8.102	0.777	-
5	6-methyl-5-hepten-2-one	8.729	1.392	-
6	β-mircene	8.882	2.783	-
7	cineole	10.723	23.897	1.858
8	α-terpinolene	13.704	1.278	-
9	α-naginatene	14.399	1.275	-
10	rosefuran epoxide	19.091	4.282	-
11	β-citral	23.073	1.063	-
12	α-citral	25.021	1.460	-
13	geranial	25.148	-	2.067
14	bornyl acetate	25.709	1.017	-
15	2-undecanone	26.557	0.563	-
16	eugenol	29.924	-	2.148
17	2,6-octadien-1-ol	31.127	3.054	-
18	2,4-dimethyl-6-phenyl-3,5-dithioxo-2,3,4,5-tetrahydro-1,2,4-tiazine	32.044	1.171	-
19	(-)-germacrene D	34.230	0.950	-
20	α-curcumene	34.429	8.012	2.786
21	(-)-α-neoclovene	34.589	1.013	-
22	2-isopropyl-5-methyl-9-methylene-bicyclo[4.4.0]dec-1-ene	34.800	1.950	1.299
23	α-zingiberene	34.826	10.073	11.721
24	β-bisabolene	35.239	3.617	-
25	α-farnesene	35.292	3.789	5.002
26	β-sesquiphellandrene	35.651	6.016	5.383
27	n-hexadecane	37.849	-	0.943
28	farnesol	38.606	-	2.519
29	zingerone	39.102	-	5.036
30	hexadecanoic acid	45.873	-	2.164
31	4-(4-hydroxy-3-methoxyphenyl)-2-butanone	50.856	-	3.955
32	cis-[6]-shogaol	51.918	-	2.324
33	bis (p-tolylthio)methane	55.594	-	2.006

achieve an optical density of 10^7 CFU/mL for the test organisms at the wavelenths of 600 nm by UV/Vis Spectrophotometer. Essential oils were diluted to get the final concentration ranging from 0 to 1000 µg/mL in NB medium. Finally, 20 µL inoculums of each bacteria strain (10^7 CFU/mL) was inoculated and the tests were performed at a final volume of 5 mL. The plates were incubated at 37°C for 24 h. The lowest concentration of the test samples, which did not show any visual growth of tested organisms after macroscopic evaluation, was determined as MIC, which was expressed in µg/mL. The experiment was carried out in triplicates.

Statistical analysis

Means separation of MIC was accomplished by Duncan's multiple range tests. Significance was evaluated at $p < 0.05$. Statistical analysis were conducted by the general procedures of SPSS Statistics v.13 (SPSS Inc.).

Result and Discussion

Chemical constituents of galangal are shown in table 1. The most abundant constituents of essential oils are cineole (45.199%), 4-allylphenyl acetate (13,718%), α-farnesene (5.487%), (2,6-dimethylphenyl)borate (5.023%) and α-pinene (5.023%). Cineole is the most common compound of Alpinia essential oil as previously reported (Tewari *et al*., 1999; Kong *et al*., 2000; Islam *et al*., 2014). Galangal essential oil contains α-pinene, β-pinene, β-myrcene, cineole, and bornyl acetate as reported from galangal essential oil from Srilanka (Arambewela and Arawwawala, 2007). The composition of essential oils from a particular species of plant can differ between harvesting seasons and between geographical sources (Burt, 2004).

The most abundant constituent of crude extract of galangal are (2,6-dimethylphenyl)borate (17.756%), cineole (3.613%), α-farnesene (2.306%), n-pentadecane (1.729%) and n-tetradecane (1.107%). The concentration of cineole, α-farnesene,n-pentadecane,(2,6-dimethylphenyl) borate, 8-heptadecene and farnesyl acetate are considered high in galangal rhizome, since those compounds are detected in both crude extract and essential oil of galangal.

Chemical constituents of ginger are shown in table 2. The most abundant constituents of essential oils are cineole (23.897%), 2,2-dimethyl-3-methylenenorbornane (12.203%), α-curcumene (8.012%), β-sesquiphellandrene (6.016%) and rosefuran epoxide (4.282%). The dominant constituent of ginger essential oil obtained from Pakistan was α-terpeneol (El-Ghorab *et al*., 2010) and the main constituent of that from Malaysia was camphene (Sivasothy *et al*., 2011). Both compounds were not detected in our study. Somehow zingiberene, β-myrcene, cineole, and β-farnesene were detected as

Table 3. MIC of crude extracts and essential oils of galangal and ginger (n=3)

Microorganisms	MIC of galangal (µg/mL)		MIC of ginger (µg/mL)	
	Crude extracts	essential oils	Crude extracts	essential oils
B. subtilis	125*	62.5*	500*	1000*
E. coli	>1000	>1000	1000*	500*
S. aureus	1000*	1000*	1000*	1000*
S. typhymurium	1000*	1000*	1000*	1000*
V. cholera	>1000	1000*	>1000	500*

*Mean value of MIC (n=3) was significantly different ($p<0.05$) from negative control

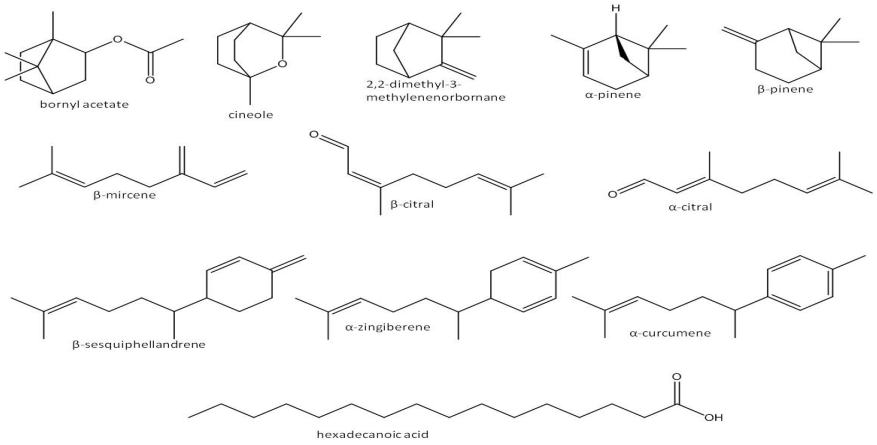

Figure 1. Compounds detected in both galangal and ginger

previously reported.

The most abundant constituents of crude extract of ginger are α-zingiberene (11.721%), β-sesquiphellandrene (5.383%), zingerone (5.036%), β-farnesene (5.002%) and 4-(4-hydroxy-3-methoxyphenyl)-2-butanone (3.955%). From both crude extract and essential oil of galangal are detected the same compounds cineole, α-farnesene, 2-isopropyl-5-methyl-9-methylene-bicyclo[4.4.0] dec-1-ene, α-curcumene, α-zingiberene and β-sesquiphellandrene.

There are 12 compounds detected in both galangal and ginger rhizomes, they are α-pinene, 2,2-dimethyl-3-methylenenorbornane, β-pinene, β-mircene, cineole, β-citral, α-citral, bornyl acetate, α-curcumene, α-zingiberene, β-sesquiphellandrene, and hexadecanoic acid. The structure of compounds detected in both galangal and ginger are shown in figure 1.

MIC of crude extracts and essential oils of galangal and ginger are shown in table 3. Both galangal and ginger possessed relatively low growth inhibition activity against tested bacteria, with the value of MIC 1000 µg/mL or even higher. *B. subtilis* was sensitive to galangal crude extract and essential oil, with MICs 125 and 62.5 µg/mL, respectively. Ginger crude extract's MIC against *B. subtilis* was 500 µg/mL. Ginger essential oil's MIC against *E. coli* and *V. cholera* was 500 µg/mL.

The relatively low antimicrobial activity of crude extract and essential oil of galangal and ginger is related to their chemical constituents. It has been reported that essential oils containing aldehydes or phenols showed the highest antibacterial activity, followed by essential oils containing terpene alcohols. Other essential oils, containing ketones or esters, acetate had much weaker activity. While volatile oils containing terpene hydrocarbons were usually inactive (Bassolé and Juliani, 2012). Galangal essential oils contains high terpene alcohols (47.806%), ketones and esters (26.023%) and terpene hydrocarbons (21.189%), but low aldehydes and phenols (2.42%) constituents. The low antimicrobial activity of ginger is related to small amount of aldehydes and phenols constituents (3.804) and large amount of terpene alcohols (32.508%) and terpene hidrocarbons (59.416%).

Conclusions

Crude extracts and essential oils of galangal and ginger purchased in lokal market at Purbalingga, Central Java, Indonesia possessed a low antimicrobial activity, related to their chemical constituents that mainly contain hydrocarbons.

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