Effect of *Alpinia galangal* essential oil on bacteria spoilage

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Abstract

The use of natural antimicrobial agent is garnering attention due to consumer and producer awareness of food safety. This study aimed to study an effect of *Alpinia galangal* essential oil on bacteria spoilage. The essential oil from *Alpinia galangal* was extracted by hydro distillation and its chemical composition was analyzed by gas chromatography/mass spectrometry (GC-MS). The major compositions in the obtained essential oil were 1, 8-cineole (53.482%), 5-t-butyl-3-hexa-3, 5-dien-2-one (13.485%) and dl-limonene (4.849%). The antibacterial activity of essential oil against food-borne bacteria in seafood, such as *Escherichia coli* TISTR 887, *Staphylococcus aureus* TISTR 517 and *Salmonella typhimurium* TISTR 292, was tested by disc diffusion method. The various concentrations of essential oil 0, 15, 30, and 45 µl/ml. were applied to paper discs on the Mueller Hinton Agar (MHA) containing the testing bacteria. The antibacterial activity was determined by measuring clear zone around the disc. The *Alpinia galangal* essential oil showed antimicrobial activity against all bacteria tested at high concentration (45%). The minimum inhibitory concentrations (MIC) of *Alpinia galangal* essential oil against *Escherichia coli* TISTR 887, *Staphylococcus aureus* TISTR 517 and *Salmonella typhimurium* TISTR 292 were 0.78±0.00, 1.56±0.00 and 0.78±0.00 µl/ml. The research results indicated that the essential oil from *Alpinia galangal* has a great potential to be applied as a natural antimicrobial agent for using as a food preservative.

Keywords: *Alpinia galangal*, antibacterial, spoilage, essential oil

Introduction

Bacteria contamination in frozen seafood may results from raw materials, waste, food additives, production methods, workers including equipments or during keeping and transportation sample. *E. coli*, *S. aureus* and *S. typhimurium* is founded in frozen seafood which cause food poisoning whether contamination (Chudiwal et al. 2010). At the present time, antibacterial which was synthesized by chemical processing lead to health impact (Geanne et al. 2012). Galangal (*Alpinia galangal*) or “Kha” in Thai has readitionally been used as spice in Thai foods. This spice is, like other spices, rich in phenolic compounds such as flavonoids and phenolic acids. In Thailand, galangal is used for medical purposes such as carminative, stomachic, antispasmodic, antiphlogistic and antibacterial drugs (Pornpimon and Devahastin 2008). *A.galanga* is a good quality of aphrodisiac drug as well as it also possess antiulcer activity, anthelmintic activity, positive anti-inflammatory activity and essential oils it showed antimicrobial activity against Gram positive bacteria (Akhtar et al. 2010). Hence, the using *Galanga* essential oils (Zingiberaceae) which is natural herbs, are among developed bacteria and mold inhibition to replace chemical herbs (Khantha et al. 2007). In general Journal found that *Galanga* essential oil found can inhibit free radical and bacteria (Okazaki and Oshima 1952). The amount of *galanga* essential oil has effect on bacteria inhibition.

Among *galanga* essential oil studied here, 1,8-cinede camphor and 13-pinenes are the best antibacterial potential (Oonmetta-aree et al. 2006).

Methodology

Extraction of *Alpinia galangal* essential oils

Rhizomes of *A. galanga* (6-12 months age) cultured in the Samut Sakhon of Thailand were collected in August 2012. Fresh rhizomes were used for extraction of the essential oil. Rhizome was prepared by slicing the fresh rhizomes into small pieces. The fresh rhizomes were chopped and subjected to hydro-distillation for 8 h; a Clevenger apparatus was used to obtain the essential oil fraction. The oil was stored in dark vials at 4 ^{o}C before analyzing.

Determination of essential oil profiles

The chemical composition of the essential oil was analyzed using GC-MS technique. The GC-MS analysis was performed on an Agilent 6890 gas chromatograph operating in electron impact (EI, 70 eV)mode. The gas chromatograph was equipped with and HP 5973 mass selective detector and fitted with a fused silica capillary column (HP-5MS) supplied by HP, USA (30.0 m × 250 µm i.d., 0.25 µm film thickness). The oven temperature was programmed to increase from 100 to 280°C at a rate of 3°C/min and finally stay isothermal for 10 min. The carrier gas was helium introduced at a rate of 1.0 mL/min. A diluted sample of 1.0 µL was injected manually and the split ratio was adjusted to 40:1. GCMS analyses were performed using a Thermo Finnigan-TRACE GC (Waltham, Massachusetts, USA) coupled with a TRACE MS plus (EI, 70 eV) from the same company.

Antibacterial activity assay

Microbial strains

The food-borne microorganisms used in this study consisted of *Escherichia coli* (TISTR 887), *Staphylococcus aureus* (TISTR 517) and *Salmonella typhimurium* (TISTR 292). Mueller Hinton broth (MHB) (Himedia, India) was used to culture the bacteria. All strains were stored at −20°C in glycerol and sub cultured twice in MHB at 37°C 24 h before testing.

Antimicrobial activity

Bacterial activity of essential oil was tested by disc diffusion method using Muller-Hinton agar (MHA) (Himedia, India) as a culture medium. *Escherichia coli* (TISTR 887), *Staphylococcus aureus* (TISTR 517) and *Salmonella typhimurium* (TISTR 292) were spreaded on MHA. Sterile paper disc (Whatman No. 1, diameter 6.0 mm) was impregnated with essential oil at different concentrations (0, 15, 30, and 45 µl/ml.). And control discs were similarly prepared using distilled water before putting on the MHA. Incubation was made at 37 ° C for 24 h. Diameters of inhibition zones were measured and recorded.

Determination of minimum inhibitory concentrations(MICs)

The MIC of *A.galanga* essential oil were determined by broth dilution method Tween 20 was used to solubilize the extracts. All tests were performed in Mueller Hinton broth. Serial two-fold dilutions of the oil ranging from 100 to 0.097 µl/ml. Tubes were incubated for 24 h at 37 ºC. The lowest concentration of essential oil that completely inhibits visual growth of bacteria (no turbidity) is recorded as MIC.

Statistical analysis

Antimicrobial experiments were conducted in triplicate. Data points were expressed as the mean clear zone and MIC ± standard deviation. Duncan's multiple range tests were used to determine the significant difference of mean values. Statistical significance was determined at 95 % confidence ($p \leq 0.05$).

Results

Chemical composition of the essential oil

The relative quantitative values of *A. galangal* essential oil were presented in Table 1. The most important constituents of the were 1,8-cineole (53.482%), 5-t-butyl-3-hexa-3,5-dien-2-one (13.485%) and dl-limonene (4.849%). Table 1 shows the identified compound and percentage obtained by GC/MS.

Antioxidant activity

The inhibit on essential oil on bacteria spoilage such as *Escherichia coli* TISTR 887, *Staphylococcus aureus* TISTR 517 and *Salmonella typhimurium* TISTR 292. The various concentrations of essential oil 0, 15, 30, and 45 µl/ml. were applied to paper discs on the Mueller Hinton Agar. According to the results of the antibacterial activity of essential oil at concentrations at 45 µl/ml showed that interesting antibacterial effect against *E. coli*, *S. aureus* and *S. Typhimurium* with zones of inhabitation diameter 0.24, 0.2 and 0.1 mm, respectively. While the essential oil at concentrations at 30 and 15 µl/ml showed interesting antibacterial effect against only *E. coli* with inhibition zones diameter of 0.12 mm. The distilled deionized water did not show antibacterial activity against the tested bacteria (negative control). The essential oil showed antimicrobial activity against all bacteria tested at high concentration (45%) (Table 2). These results are in substantial agreement with a previous study [6] that report reveal that *A. galangal* essential oil had the strongest inhibition against *E. coli*, *S. aureus* and *S. typhimurium*.

Minimum Inhibitory Concentrations (MIC) of the essential oil was determined by using a broth dilution method (2-fold serial dilution). As the MIC was found to be strong bacterial activity against *E. coli*, *S. aureus* and *S. Typhimurium*, a finding that was echoed by the oil MIC against each strain of 0.78±0.00, 1.56±0.00, and 0.78±0.00 µl/ml, respectively. A point worth mentioning is that *A. galangal* essential oil had strong activity against Gram-negative bacteria, which are known for their insensitivity to many antibacterial agents [7]. Previous studies on the antimicrobial activity of *Alpinia* species, especially *A. galangal*, showed inhibitory activity against a wide spectrum of microorganisms [8]. The current results also indicated the considerable potential of *A. galangal* essential oil to inhibit bacteria spoilage, which trend to be resistant to antibiotics.

Table 1 Essential oil composition of *A. galangal* identified by GC-MS

Compound	Retention time(min)	%Area
Alpha.-Terpinolene	3.198	0.75
Benzene	3.279	1.01
dl-Limonene	3.338	4.849
1,8-Cineole	3.409	53.483
gamma.-Terpinene	3.657	1.384
Alpha.-Terpinolene	4.067	0.586
1,6-Octadien	4.132	0.354
p-Mentha-1	5.373	0.958
3-Cyclohexen-1-ol	5.615	4.789
Para-Cymen-8-OL	5.707	0.141
.ALPHA. TERPIRNEOL	5.853	2.357
2-Cyclohexen-1-ol	6.063	0.078
TRANS-(+)-CARVEOL	6.43	0.153
Chavicol	7.121	0.189
Acetic acid	8.108	0.309
verbenene	8.475	0.21
Alpha.-Terpinolene	9.403	0.127
5-t-Butyl-hexa-3	9.651	13.485
Phenol	9.732	3.202
cis-2,6-Dimethyl-2,6-octadiene	9.883	0.132
Thymyl acetate	9.975	0.288
CIS-Limonene Oxide	10.121	0.293
2-Oxabicyclo	10.293	0.249
Carvacryl Acetate	10.531	0.261
2,6-Octadien-1-ol	10.822	0.605
.Beta.Elemene	11.227	0.489
1,2-dimethoxy-4	11.507	0.814
Trans (.Beta.)-Caryophyllene	12.155	1.688
.alpha.-Humulene	13.239	1.672
.beta.-Selinene	14.335	1.122
.alpha.-selinene	14.626	0.811
.beta.-Bisabolene	15.015	0.99
7-epi-.alpha.-selinene	15.387	0.094
.beta.-Sesquiphellandrene	15.543	0.122
Phenol,2-methoxy-4	15.63	0.308
Germacrene B	16.72	0.498
2,4A,8,8-Tetramethyl-1	20.043	0.819
junipercamphor	21.462	0.192
Nerolidol	26.378	0.108
Pentadecanoic acid	28.924	0.033

Table 2 Bacterial inhibitory zone after exposure to the *A. galangal* essential oil using the disc diffusion method.

Concentrations	Inhibition Zone (mm)*		
	E. coli	*S. aureus*	*S. Typhimurium*
15	0.12±0.04	ND	ND
30	012±0.04	ND	ND
45	0.24±0.05	0.2±0.10	0.1±0.00

ND: not detected. Data were expressed as mean±S.D.

Discussion

In this study, we use rhizomes of *A. galanga* that is between 6-12 months. Since the age of *A. galanga* the amount of influence it. Natta et al.(2008) reported that the Zingiberacease extracts 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 generally a function of three factors: genetically determined properties, the age of the plant and the environment.

Conclusion

Essential oil obtained by hydro distillation from *Alpinia galangal*. Volatile components of all extracts were analyzed by gas chromatograhymass spectrometry (GC-MS). The major components of 1,8-cineole (53.482%), 5-t-butyl-3-hexa-3,5-dien-2-one (13.485%) and dl-limonene (4.849%). Their antibacterial effects towards *E. coli*, *S. aureus* and *S. Typhimurium* were tested by a disc diffusion method. The essential oil showed antimicrobial activity against all bacteria tested at high concentration (45%). The minimum inhibitory concentrations (MIC) of *Alpinia galangal* essential oil against to *Escherichia coli* TISTR 887, *Staphylococcus aureus* TISTR 517 and *Salmonella typhimurium* TISTR 292 were 0.78±0.00, 1.56±0.00, and 0.78±0.00 µl/ml.

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