ORIGINAL ARTICLE

Chemical components and biological activities of volatile oil of *Kaempferia galanga* Linn.

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Abstract

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Volatile oil of dried rhizome of *Kaempferia galanga* obtained by water distillation was determined for its chemical components using gas chromatography and mass spectrometry (GC-MS). The major chemical constituents were identified as ethyl-*p*-methoxycinnamate (31.77%), methylcinnamate (23.23%), carvone (11.13%), eucalyptol (9.59%) and pentadecane (6.41%), respectively. Antimicrobial activity of the volatile oil was tested against various microbes using agar disc diffusion method with the inhibition zones from 8.0-31.0 mm. Brine shrimp toxicity of volatile oil exhibited an EC_{50} value of 26.84 µg/ml; whereas the volatile oil was inactive for antioxidant activity (IC_{50} >100 µg/ml).

Key words : *Kaempferia galanga*, volatile oil components, bioactivity

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บทคัดย่อ

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การศึกษาองค์ประกอบทางเคมีและฤทธิ์ทางชีวภาพของน้ำมันหอมระเหยจากเหง้าเปราะหอม

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การศึกษาองค์ประกอบทางเคมีของน้ำมันหอมระเหยจากเหง้าเปราะหอมที่กลั่นด้วยน้ำ โดยวิธีแก๊สโครมาโตกราฟี พบสารสำคัญหลักได้แก่ ethyl-*p*-methoxycinnamate (31.77%), methylcinnamate (23.23%), carvone (11.13%), eucalyptol (9.59%) และ pentadecane (6.41%) ตามลำดับ ในการทดสอบฤทธิ์ทางชีวภาพพบว่า น้ำมันหอมระเหยจากเหง้าเปราะหอมมีฤทธิ์ต้านจุลินทรีย์หลายชนิด โดยให้ค่า inhibition zone ในขนาด 8.0-31.0 มม. ด้วยวิธี agar disc diffusion method สำหรับการทดสอบ brine shrimp toxicity test ให้ค่า EC_{50} เท่ากับ 26.84 μg/ml ในขณะที่น้ำมันหอมระเหยไม่มีฤทธิ์ antioxidant (IC_{50} > 100 μg/ml).

ภาควิชาเภสัชเวทและเภสัชพฤกษศาสตร์ คณะเภสัชศาสตร์ มหาวิทยาลัยสงขลานครินทร์ อำเภอหาดใหญ่ จังหวัดสงขลา 90112

Kaempferia galanga Linn. is one of the plants in Zingiberaceae family. The rhizome extract has been potently active against bacterial infections. Indigenous medical practitionists use these rhizomes for treatment of scariasis, bacterial infections, tumor and it is also applied externally for abdominal pain in women and used topically for treatment of rheumatism (Hirschhornn, 1983). In Thailand, the dried rhizome has been used as cardiotonic and CNS stimulant (Mokkhasmit *et al.,* 1971), whereas an acetone extract has an effect on monoamine oxidase inhibition (Noro *et al*., 1983). The 95% ethanol extract of this plant possessed antibacterial activity against *Staphylococcus aureus* and hot water extract against *Escherichia coli* (George and Pandalai,1949). The rhizome of *K. galanga* has been used for treatment of fungal derived-skin diseases as well as eczema (Tungtrongjit, 1978). The present study is aimed to determine the volatile oil components of *K. galanga* and its biological activities.

Materials and Methods

1. Plant material

The rhizomes of *Kaempferia galanga* were collected from Amphur Chana, Songkhla province, Thailand, and were identified by Assist. Prof. Supreeya Yuenyongsawad. The dried rhizome powder (100 g) was extracted by water distillation for volatile oil content determination. The volatile oil obtained was then collected and stored at 4ºC.

2. Determination of volatile oil components

The chemical components of volatile oil was determined by a Hewlett-Packard 5890 series II plus gas chromatography Hewlett-Packard 5972 series mass selective detector. The operating parameters were as follows:Column: HP 5 MS 30 m. x 0.25 mm. x 0.25 (m; inlet temperature 250ºC; detector temperature 280ºC; inject volume 1μl; column temperature 80ºC-280ºC; rate 15ºC/min. The spectra were recorded and compared with the terpene library.

3. Bioactivity tests

3.1 Antioxidant activity

Volatile oil was studied for the ability to scavenge the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical by comparison with a well known synthetic antioxidant, butylated hydroxytoluene (BHT). Briefly, a portion of sample solution was mixed with the same volume of 6 x 10^{-5} M DPPH in ethanol and allowed to stand at room temperature for 30 minutes. The absorbance were then measured at 520 nm (Hatano *et al*., 1989).

3.2 Antibacterial activity

Antibacterial activity of an essential oil

was assayed by the agar disc diffusion method (Barry and Thornsberry, 1991) against three Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923, *Streptococcus faecalis* and *Bacillus subtilis*); three Gram-negative bacteria (*Salmonella typhi*, *Shigella flexneri, Escherichia coli* ATCC 25922) and a certain fungus, *Candida albicans*. These bacteria and fungus were kindly provided by the Department of Pathology, Faculty of Medicine, Prince of Songkla University.

Briefly, a sterile paper disc (6 mm diameter) impregnated with 10 µl of an essential oil was placed on the surface of each plate previously inoculated with baterial/fungal inoculum and incubated for 24 h at 37ºC. The results of agar disc diffusion assay were evaluated by measuring the inhibition zone diameters (in mm).

3.3 Brine shrimp lethality assay

Volatile oil of different concentrations of 80, 60, 40, 20 and 10 µg/ml of *K. galanga* were prepared by dilution the volatile oil with DMSO (dimethylsulfoxide), alcohol and sea water. Three replications of each concentration of sample were tested for brine shrimp lethality bioassay as described by Solis 1993. LD_{50} values were determined using the probit analysis method (Finney 1971).

Results and Discussion

The volatile oil content obtained from the rhizomes of *K. galanga* was 1.11% v/w, and it exhibited yellow color with a characteristic odor. As shown in Table 1, the components of *Kaempferia galanga* oil were found to be α-pinene (1.28%), camphene (2.47%), carvone (11.13%), benzene (1.33%), eucalyptol (9.59%), borneol (2.87%), methyl cinnamate (23.23%), pentadecane (6.41%) and ethyl-*p*-methoxycinnamate (31.77%). Among them, the compound that exhibited the highest content was ethyl-*p*-methoxycinnamate. This component has been reported to show many biological activities, such as anticancer (Zheng *et al*., 1993) and anti-monoamine oxidase activities (Noro *et al*., 1983). Puthan and coworkers (Puthan *et al*., 1926) reported that the oil of *K. galanga* from India possessed ethylcinnamate and ethyl-*p*-methoxycinnamate as the main components, together with paraffin hydrocarbon; whereas those of the present study were methylcinnamate and ethyl-*p*-methoxycinnamate. Another report indicated that the main components of *K. galanga* oil were found to be *β*-phyllandrene, *α*-terpineol, ethylcinnamate and dihydro *β*-sesquiphyllandrene (Sudibyo, 2000). These results are somewhat

Table 1. Volatile oil components, retention time and peak area (%) of *Kaemferia galanga* oil.

Peak number	Components	Retention time (min.)	Peak area %
1	pinene	3.32	1.28
2	camphene	3.50	2.47
3	carvone	4.19	11.13
4	benzene	4.32	1.33
5	eucalyptol	4.45	9.59
6	borneol	5.94	2.87
7	methyl cinnamate	8.89	23.23
8	pentadecane	9.00	6.41
9	ethyl-*p*-methoxycinnamate	11.21	31.77
	unidentified		9.94
	total		100.00

Table 2. Antimicrobial activity of *K. galanga* essential oil and standard antibiotics by agar disc diffusion assay.

Microbes	Diameter of inhibition zone (mm)	
	Essential oil of *K. galanga*	**Tetracycline (30 μg/disc)**
A) *Gram-positive bacteria*		
1. *Staphylococcus aureus* ATCC 25923	+ve, ϕ 12 mm.	+ve, ϕ 31 mm.
2. *Streptococcus faecalis*	+ve, ϕ 14 mm.	+ve, ϕ 15 mm.
3. *Bacillus subtilis*	+ve, ϕ 16 mm.	+ve, ϕ 18 mm.
	Essential oil of *K. galanga*	**Tetracycline (30 μg/disc)**
B) *Gram-negative bacteria*		
4. *Salmonella typhi*	+ve, ϕ 9 mm.	+ve, ϕ 21 mm.
5. *Shigella flexneri*	+ve, ϕ 12 mm.	+ve, ϕ 10 mm.
6. *Escherichia coli ATCC 25922*	+ve, ϕ 8 mm.	+ve, ϕ 23 mm.
	Essential oil of *K. galanga*	**Clotrimazole (2.5 μg/disc)**
C) *Fungi*		
7. *Candida albicans*	+ve, ϕ 31 mm.	+ve, ϕ 25 mm.

different from our result which might imply that the climatic and geographic conditions in different areas may affect the production of essential oil components.

For antioxidant activity (DPPH assay), the volatile oil of *K. galanga* was inactive at concentration 100 μg/ml. Regarding antimicrobial activity, the volatile oil of *K. galanga* exhibited marked activity against Gram-positive and Gram-negative bacteria; and also against a fungus, *C. albicans*, by using agar disc diffusion method (Table 2). The result revealed that the oil of this plant possessed marked antimicrobial activity against Gram-positive bacteria with the inhibition zones from 12.0-16.0 mm., and 8.0-12.0 mm. against Gram-negative bacteria; whereas it potently inhibited *C. albicans* with an inhibition zone of 31.0 mm., which was stronger than that of standard antifungal Clotrimazole (diameter = 25.0 mm.). It is suggested that the essential oil of this plant may be useful for treatment of the diseases caused by these bacteria and fungi, such as skin diseases and diarrhea.

For brine shrimp lethality assay, the volatile oil of *K. galanga* give appreciable activity against brine shrimp lethality test with LD_{50} of 26.84 μg/ml. The result indicated that essential oil of *K. galanga* might possess some physiological activities since this oil was toxic to brine shrimp.

In conclusion, the main components, especially ethyl-*p*-methoxycinnamate, could be used as a biomarker for standardization of this plant and the results of bioactivities suggest that the essential oil of *K. galanga* could to be used for treatment of some microbial infections, which also agrees with the traditional use of this plant in treatment of those fungal- and bacterial-derived skin diseases (Tungtrongjit, 1978). Moreover, *K. galanga* should also be subjected to more elaborated bioassay for specific pharmacological activities.

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