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Short Communication

Primary study on the components and main physico-chemical as well as biological properties of the oil of *Alpinia galanga* (L.) Wild in Phu Tho-Vietnam

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The oil of *Alpinia galanga* (L.) Willd in Phu Tho was obtained by steam distillation and dried with Na_2SO_4. By Gas chromatography-mass spectrometry (GC-MS) method, 29 components in the oil were predicted by comparing their retention times and molecular weights with the standards'. In particular, there were 10 hydrocarbons such as monoterpenes: 29.15%, sesquiterpenes: 21.06%, and 19 oxygenated components like aldehydes (7.29%), alcohols (32.43%), ketones (1.09%), and esters (7.57%). Physico-chemical properties, antioxidant activities as well as antimicrobial activities of the oils were also analyzed. The density (at 20°C), acid index and ester index of the oil were 0.812 g/ml; 0.653 mg KOH/g and 0.728 mg KOH/g, respectively. The antioxidant activity was determined by using 1,1-diphenyl-2-picrylhydrazol (DPPH) radical percentage inhibition and it was 47.15±0.34%. Antimicrobial activity against *Salmonella typhi, Bacillus cereus, Staphylococcus aureus* and *Escherichia coli* of the oil was identified by agar diffusion method.

Key words: Oil of *Alpinia galangal* (L.) Willd in Phu Tho-Vietnam, components, physico-chemical, biological activity.

INTRODUCTION

The *Alpinia galanga* (L.) Willd is planted in mountainous areas of the Doan Hung, Ha Hoa, Lam Thao, Phu Ninh district of the Phu Tho province. *A. galanga* (L.) Willd shows effects in medical field. It has been used to weld and increase digestion, reduce swelling pain and fever. In particular, in South East Asia, *A.galanga* (L.) Willd is used to treat skin diseases, dyspepsia, some symptoms of digestive tract, flu, malaria, rheumatoid arthritis and some other kinds of infections. *A. galanga* (L.) Willd is also used to produce medicines to treat stomach cancer and throat cancer (Moi et al., 2002).

The components of different varieties of this plant have

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shown variability (Scheffer et al., 1981). The components and their bioactivities of some *A. galanga* (L.) Willd have been reported (Loi et al., 2015). However, the components as well as their main physico-chemical and biological properties of *A. galanga* (L.) Willd in PhuTho, Vietnam have not been evaluated yet. Therefore, the aim of this study is to primarily analyze the components, physico-chemical indexes and antibacterial activity of the oil of *A. galanga* (L.) Willd in Phu Tho.

MATERIALS AND METHODS

The *A. galanga* (L.) Willd, identified by Assoc. Prof. Dr. Tran Huy Thai, Institute of Ecology and Biological Resources, was harvested from Phu Tho province in 2015. The essential oil was obtained by steam distillation after drying with Na_2SO_4. The sample was stored in the Department of Biotechnology and Food Processing, Hanoi University of Industry, Vietnam. The sample has been stored at the Department of Biotechnology and Food, Hanoi University of Industry.

The tested bacterial strains (*Staphylococcus aureus*, *Escherichia coli, Salmonella typhi* and *Bacillus cereus*) were obtained from School of Biotechnology and Food Technology, Hanoi University of Science and Technology. All chemicals and media were purchased from Sigma.

Oil extraction

The oil was extracted by using rhizome of the plant with water in oil distillation equipment Clevenger (Germany) in the ratio of 1:4 (w/v) respectively for 180 min.

Gas chromatography mass spectrometry (GC-MS)

The sample and standards were run parallelly in the *GC-MS* experiment. Gas chromatography (GC) analysis was performed by using Agilent Technologies HP 6890 Plus Gas chromatograph system equipped with Flame Ionization Dectector (FID) and fitted with HP-5MS columns (30 m × 0.25 mm, film thickness 0.25 µm). The temperature was programmed as follows: The column temperature was programmed from 80 to 150°C in 23.3 min at a rate of 3°C/min and then from 150 to 220°C in 8.75 min at a rate of 8°C/min. The used injector temperature was 230°C. The *MS* conditions were as follows: Ionization voltage was 70 eV, transfer temperature was 250°C, the carrier gas was helium used at a flow rate of 0.5 ml/min, and the split ratio of the injector was 1:5 (Loi et al., 2015; Charles et al., 1992; Thang and Loi, 2016; Chau et al., 2014). The *MS* fragmentation patterns were compared with known patterns of other oils and with those in the literature by using Wiley (Wiley 9thVersion), NIST 08Libraries (on ChemStation HP). The percentage of each component was calculated by the percentage of its peak area.

Determination of physico-chemical properties of oil

The density, angle of rotation, refraction index, acid index and ester index of the oil were determined by using ISO 8444: 2010, ISO 8446: 2010, ISO 8445: 2010, ISO 8450: 2010 and ISO 8451: 2010, respectively (Anthology of the National Standards for essential oil - testing methods, 2010).

Determination of antioxidant activity using free radical scavenging activity

The free radical scavenging activity of the oil was measured by using 1,1-diphenyl-2-picrylhydrazol (DPPH) *(*Molyneux, 2004; Matook and Fumio, 2006; Shyu and Hwang, 2002*)*. A 0.5 mM solution of DPPH in methanol and 0.005 M acetate buffer (pH 5.5) were prepared. An aliquot of 0.1 ml of the sample solution was added to the tube containing 2 ml of acetate buffer, 1.9 ml of methanol and 1 ml of DPPH solution. In the blank tube, DPPH was removed; in the control tube, 1 ml of DPPH was added to the tube containing 2 ml acetate buffer and 2 ml methanol. The mixture was shaken immediately after adding DPPH and allowed to stand at room temperature in the dark. The decrease in absorbance at 517 nm was measured after 30 min until the reaction reached plateau. Vitamin C with the concentration of 0.5 mM was used as a positive control and its free radical scavenging activity was performed in parallel in the same experiment. These experiments were run in duplicate.

The inhibitory percentage of DPPH was calculated as follows:

$$\text{Scavenging effect (\%)} = [(A_o - (A - A_b)) / A_o] \times 100\%.$$

Wherein A_o is the value of absorbance of the control at the wavelength of 517 nm; A is the value of absorbance of the sample at the wavelength of 517 nm; and A_b is the value of absorbance of the blank at the wavelength of 517 nm.

Determination of antibacterial activity using agar diffusion method

Antibacterial activity was roughly determined by agar diffusion method. 50 µl of the oil was put into wells on the plates containing tested bacterial strains. The activity was roughly estimated by the diameter of the antibacterial round (mm), which was calculated by the formula D- d (mm), wherein D was the diameter of the antibacterial round (mm) and d was the hole diameter (mm) (Perez et al., 1990).

RESULTS AND DISCUSSION

The components of the oil

GC-MS of the sample was performed in order to roughly determine the components of the oil. Based on the retention times and molecular weights of the sample and the standards (the GC profile was not shown here), 29 components and their percentages in the oil were evaluated and shown in the Table 1. The table showed that 29 components were predicted in the oil of *A. galanga* (L.) Willd in Phu Tho-Vietnam. Ten out of them were hydrocarbons (such as monoterpenes: 29.15% and sesquiterpenes: 21.06%) and the rest were oxygenated ones (like aldehydes: 7.29%, alcohols: 32.43%, ketones: 1.09% and esters: 7.57%). The results provided additional evidence to show varied percentages of the components of the oils of *A. galanga* (L.) Willd. Notably, the amounts of aldehydes and alcohols in the oil were higher than those of the oil in Malaysia (De Pooter et al., 1985). Probably, the differences were due to the geographical conditions such as the soil factors, weather,

Table 1. The components of the oil of *Alpinia galanga*(L.) Willd in Phu Tho-Viet Nam.

S/N	Components	Retention time (min)	Proportion (%)
Monoterpenes			**29.15**
1	α-Pinene	3.456	2.14
2	Camphene	4.177	5.98
3	β-Pinene	4.643	1.93
4	α-Terpinene	4.868	6.72
5	Limonene	8.054	8.32
6	p-Cymene	8.370	2.17
7	Terpinolene	14.368	1.89
Sesquiterpenes			**21.06**
8	β-cubebene	12.114	2.21
9	α-Humulene	15.658	5.62
10	Valencene	15.714	3.67
11	α-Farnesene	15.857	3.89
12	δ-Cadinene	16.183	5.67
Aldehydes			**7.29**
13	Octanal	6.015	3.18
14	Nonanal	13.718	1.15
15	Citronellal	14.104	1.89
16	Neral	14.816	1.07
Alcohols			**32.43**
17	α-Farnesol	5.335	3.16
18	β-Farnesol	7.116	5.59
19	Citronellol	7.532	2.17
20	Geranyllinelol	7.601	6.37
21	Borneol	7.843	7.09
22	Ascaridol	8.875	1.97
23	Terpinen-4-ol	9.925	3.04
24	α-Terpineol	13.043	1.92
25	Nerolidol	14.906	1.12
Ketones			**1.09**
26	α-Thujone	15.443	1.09
Esters			**7.57**
27	Linalyl acetate	6.856	4.12
28	Neryl acetate	13.945	1.67
29	Genaryl acetate	15.515	1.78
	Total		**98.59**

% was calculated by the percentage of chromatographic peak area.

climate, growing conditions and harvesting time (Charles et al., 1992).

The physical-chemical indexes of the oil of *A.galanga* (L.) Willd in Phu Tho-Vietnam

The density, angle of rotation, refraction index, acid index and ester index of the oil were presented in Table 2.

These results were consistent with those of the oils of *A. galanga* (L.) Willd from Malaysia (De Pooter et al., 1985). In particular, the oil had a density (0.821), which was smaller than 0.9 and refractive index (1.415), which was smaller than 1.47. However, no significant differences were observed in these values. The value of the angle rotation of the sample showed that the oil was

Table 2. Physico-chemical indexes of the oil of *A. galangal* (L.) Willd in Phu Tho-Viet Nam

S/N	Physical-chemical indexes	Result
1	Density at 20°C	0.812
2	Anglerotationα^{20}_{D}	84° 35’
3	Refractive indexn^{20}_{D}	1.458
4	Acid index (mg KOH/g)	0.653
5	Ester index (mg KOH/g)	0.728

Table 3. The diameters of antibacterial activity rounds of the oil *of A. galangal* (L.) Willd in Phu Tho-Viet Nam

S/N	Tested microorganisms	Diameter of antibacterial round (mm)
1	*Salmonella typhi*	29.17
2	*Bacillus cereus*	31.42
3	*Staphylococcus aureus*	26.25
4	*Escherichia coli*	27.12

capable of being dissolved in both polar organic and nonpolar organic solvents. The acid index of the sample showed that the oil could be less of an oxidation. This result was coincident with the percentage of the components of the oil. In particular, the total oxygenated components determined in this research were less than 50% (Table 1).

The biological activities of the oil of *A. galanga* (L.) Willd in Phu Tho-Vietnam

The free radical scavenging activity DPPH of the oil

The DPPH free radical scavenging activity of the oil of *A. galanga* (L.) Willd was 47.15±0.34% and this value was a bit higher than that of 0.5 mM vitamin C (39.65±0.42%). These activities of the oils of the leaves of *Liquidambar formosana* Hance in Bac Giang and *Citrus sinensis* peel were found to be 41.13 ± 0.22% and 45.32 ± 0,18%, respectively (Loi et al., 2015; Matook and Fumio, 2006). Therefore, we could say that The DPPH free radical scavenging activity of the oil of *A. galanga* (L.) Willd is higher than that of the leaves of *L. formosana* Hance in Bac Giang and *C. sinensis* peel.

Antibacterial activity of the oil of Alpinia galangal (L.) *Willd in Phu Tho*-Vietnam

In order to estimate the antibacterial potentials of the oil of *A. galanga* (L.) Willd in Phu Tho-Vietnam, agar diffusion method was used in this experiment. Tested microorganisms used in this experiment were *S. aureus, E. coli, S. typhi* and *B. cereus.* The diameters of antibacterial activity rounds of the oil against these bacteria were shown in Table 3. The results showed that the oil of *A. galangal* (L.) Willd in Phu Tho–Vietnam possessed antibacterial activity against all of the four microorganisms tested. Among them, the antibacterial activity against *B. cereus* was the highest one. The activity of the oil of *A. galanga* (L.) Willd in this research is similar to that of the oils of the leaves of *L. formosana* Hance in Bac Giang as these oils were found to possess antibacterial acitivities against all of the four tested microorganisms (Loi et al., 2015).

Conflict of Interests

The authors have not declared any conflict of interests.

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