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ESSENTIAL OIL COMPOSITION AND ANTIOXIDANT ACTIVITY FROM *ALPINIA SIAMENSIS* K. SCHUM. RHIZOME

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Abstract: The chemical compositions of hydrodistilled essential oils from different ages of fresh rhizome of *A. siamemsis* (6 and 9 month) were analysed by GC-MS technique. 1, 8-Cineole was obtained as the main component in both essential oils (20.49 and 29.68%, respectively). The antioxidant activities of two essential oils were evaluated by DPPH radical scavenging, hydroxyl radical scavenging, ferrous ion chelating, superoxide anion radical scavenging and ferric reducing power assays. *L*-ascorbic acid and ethylenediaminetetraacetic acid (EDTA) were used as a positive control. The DPPH radical scavenging activity and ferric reducing power of both of essential oils and *L*-ascorbic acid were found to be insignificantly different whereas the hydroxyl radical scavenging activity of the 6-month rhizome essential oil was found to be significantly higher than the 9-month rhizome essential oil and *L*-ascorbic acid. The superoxide anion radical scavenging activities of the 6- and 9-month rhizome essential oils were found to be insignificantly different. However, the 9-month rhizome essential oil had much higher superoxide anion radical scavenging activity than *L*-ascorbic acid. Both of the essential oils showed less chelating activity for ferrous ions as compared with EDTA.

Keywords: *Alpinia siamensis*, Zingiberaceae, essential oil, antioxidant activity

บทคัดย่อ: การศึกษาองค์ประกอบทางเคมีของน้ำมันระเหยง่ายที่ได้จากการกลั่นด้วยน้ำของเหง้าสดของข่าบ้านที่มีอายุ 6 และ 9 เดือน ด้วยเทคนิค GC-MS พบ 1, 8-cineole เป็นองค์ประกอบหลักในน้ำมันระเหยง่ายของเหง้าข่าบ้านทั้ง 2 อายุ โดยมีปริมาณ 20.49 และ 29.68% ตามลำดับ การประเมินฤทธิ์ต้านอนุมูลอิสระของน้ำมันระเหยง่ายด้วยวิธี DPPH radical scavenging, hydroxyl radical scavenging, ferrous ion chelating, superoxide anion radical scavenging and ferric reducing power assays โดยมี *L*-ascorbic acid และ ethylenediaminetetraacetic acid (EDTA) เป็นสารควบคุมแบบให้ผลบวก พบว่าน้ำมันระเหยง่ายจากเหง้าทั้ง 2 อายุมีฤทธิ์ต้านอนุมูลอิสระ DPPH radical และ ferric reducing power แตกต่างจาก *L*-ascorbic acid อย่างไม่มีนัยสำคัญ ในขณะที่น้ำมันระเหยง่ายจากเหง้าที่มีอายุ 6 เดือน มีฤทธิ์ต้านอนุมูลอิสระ hydroxyl radical สูงกว่าน้ำมันระเหยง่ายจากเหง้าที่มีอายุ 9 เดือน และ *L*-ascorbic acid อย่างมีนัยสำคัญ ฤทธิ์ต้านอนุมูลอิสระ superoxide anion radical ของน้ำมันระเหยง่ายจากเหง้าทั้ง 2 อายุไม่แตกต่างกันอย่างมีนัยสำคัญ โดยน้ำมันระเหยง่ายจากเหง้าที่มีอายุ 9 เดือน มีฤทธิ์ต้านอนุมูลอิสระ superoxide anion radical สูงกว่า *L*-ascorbic acid อย่างมีนัยสำคัญ นอกจากนี้ยังพบว่า น้ำมันระเหยง่ายจากเหง้าทั้ง 2 อายุมีฤทธิ์ ferrous ion chelating น้อยมากเมื่อเทียบกับ EDTA

คำสำคัญ: ข่าบ้าน น้ำมันระเหยง่าย ฤทธิ์ต้านอนุมูลอิสระ Zingiberaceae

INTRODUCTION

Free radicals, molecules containing an unpaired electron, are generated in the body by various endogenous and exogenous mechanisms (Lone *et al*., 2013; Kabel, 2014; Dhaliwal and Singh, 2015). Hydroxyl radical, superoxide anion radical, hydrogen peroxide, singlet oxygen, hypochlorite, nitric oxide radical, and peroxynitrite radical are the most important free radicals that cause chronic and degenerative diseases such as cancer, arthritis, cardiovascular and neurodegenerative diseases (Lobo *et al*., 2010; Pham-Huy *et al*., 2008).

Antioxidants are compounds that can delay or inhibit autoxidation either by inhibiting the formation of free radicals or by inhibiting the initiation or propagation of oxidation chain reactions on the basis of different mechanisms such as scavenging, chelating and breaking chain reaction (Brewer, 2011; Marín *et al*., 2016).

Essential oils are one of the natural products and some of them have been reported to possess antioxidant activity. Examples of Zingiberaceous plants reported that their essential oils exhibited antioxidant activity were *Alpinia calcarata* (Arambewela *et al*., 2010), *C. aeruginosa* (George and Britto, 2015; Theanphong *et al.*, 2015), *C. longa* (Shahwar *et al*., 2012), *Hedychium forrestii* var. *palaniense* (Thomas and Mani, 2016), *K. parviflora* (Wungsintaweekul *et al*., 2010), *Zingiber officinale* (Singh *et al*., 2005) and *Z. zerumbet* (Singh *et al*., 2014).

A. siamemsis, commonly known as Kha Ban in Thailand, is perennial herb in the family Zingiberaceae. The rhizome of this plant has been used in traditional medicines for treatment of stomachache and headache (Nontasit *et al*., 2015). In Thailand, its young rhizome was used as food and spices. Although *A. siamensis* and *A. galangal* have very similar morphology, both of them can be distinguished by their characteristics of bracteole (Saensouk *et al*., 2017). *A. siamensis* has tube bracteole while *A. galanga* has flat bracteole.

To the best of our knowledge, there have been no previous reports on chemical compositions and antioxidant activities of essential oils from the rhizomes of *A. siamemsis* from Thailand. Thus, the aims of this study were to compare chemical compositions of essential oils hydrodistillated from the fresh rhizome of *A. siamensis* at the age of 6 and 9 months and to evaluate their antioxidant activities.

MATERIALS AND METHODS

Chemicals

L-Ascorbic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), ethylenediaminetetraacetic acid (EDTA), ferric chloride, ferrozine, nitroblue tetrazolium, reduced nicotinamide adenine dinucleotide (NADH), phenazine methosulfate, phosphate buffer pH 6.6 and sodium salicylate were purchased from Sigma Aldrich (USA); Tris-HCl buffer pH 8.0 from Invitrogen (USA); methanol, potassium hexacyanoferate and trichloroacetic acid from Merck (Germany); ferrous sulfate from Farmitalia Carlo Erba (Italy) and hydrogen peroxide from Chem supply (Australia). All chemicals were of reagent grade.

Plant material

The whole parts of *A. siamensis* at different ages (6 and 9 months) were collected from Chaiyaphum Province, Thailand in July and October, 2016, respectively. The plant sample was identified by Asst. Prof. Thaya Jenjittikul (Department of Plant Science, Faculty of Science, Mahidol University, Bangkok, Thailand). The voucher specimen of this plant was deposited at College of Pharmacy, Rangsit University, Thailand.

Isolation of essential oils

The fresh rhizomes of *A. siamensis* (350 g) were washed with tap water, air dried and then blended into small pieces with the blender. The ground rhizomes were subjected to hydrodistillation using Clevenger apparatus for 3 hr. The oils were collected and stored at 4 °C in air-tight containers before being analyzed by GC-MS technique.

GC-MS Analysis

Gas chromatography-mass spectroscopy (GC-MS) analysis for each oil sample was performed using an Agilent Technologies 7890A GC system equipped with a 5975C inert XL EI/CI MAD and Triple-Axis detector. The DB-5 MS capillary column (30 m x 0.25 mm i.d., and 0.25 μm film thickness) was used for GC analysis. Helium was used as the carrier gas (1 ml/min). One μl of an essential oil diluted in ethanol (1:20 by volume) was injected using a GC sampler 80 autosampler in the split mode with the split ratio of 1:20 by volume. The GC oven temperatures were started at 60°C for 1 min, increased at a rate of 3°C / min up to a final temperature of 240°C, where it was then held constant for 5 min. The GC injector temperature was set at 180°C and the GC-MSD interface temperature was set at 290°C. Electron impact ionization positive mode at 70 eV was acquired over the mass range of 40-650 m/z at the scanning rate of 2.42 amu /second. The total scanning time was 70 min.

Identification of essential oil components

Essential oil components were identified by comparing their mass fragmentation patterns with Adams Essential Oil Mass Spectral Library and NIST 05 Mass Spectral Library. The amount of each oil component was determined based on peak area measurement.

Antioxidant activities

DPPH radical scavenging assay

The DPPH radical scavenging activity of essential oils was determined according to the method described by Sudha *et al*. (2011) with few modifications. Each 3 ml of the reaction mixture contained 1.5 ml of an essential oil solution with a known concentration (1-500 μg/ml) and 1.5 ml of 0.2 mM DPPH. The reaction mixture was left to stand for 30 min at room temperature under light protection. The absorbance of the reaction mixture was measured at 517 nm. *L*-ascorbic acid was used as a positive control. All solutions used in this experiment were prepared in methanol. The percentage of DPPH radical scavenging was calculated as follows:

$$\text{percent scavenging} = [1-(A_1-A_2)/A_0] \times 100\%$$

where A_0 is the absorbance of the control (without the sample), A_1 is the absorbance of the sample and A_2 is the absorbance of the reaction mixture without DPPH.

Hydroxyl radical scavenging assay

Hydroxyl radical scavenging activity was evaluated according to the method described by Sudha *et al*. (2011) with few modifications. Each 3 ml of the reaction mixture contained 1 ml of an essential oil solution with a known concentration (1-500 μg/ml), 1 ml of 1.5 mM $FeSO_4$, 0.7 ml of 6 mM H_2O_2 and 0.3 ml of 20 mM methanolic sodium salicylate. The reaction mixture was incubated at 37°C for 1 hr. The absorbance of the reaction mixture was measured at 562 nm. *L*-ascorbic acid was used as a positive control. The essential oil solutions used in this experiment were prepared in methanol. The percentage of $OH^{\bullet}$ radical scavenging was calculated as follows:

percent scavenging = $[1-(A_1-A_2)/A_0] \times 100\%$

where A_0 is the absorbance of the control (without the sample), A_1 is the absorbance of the sample and A_2 is the absorbance of the reaction mixture without sodium salicylate.

Ferrous ion chelating assay

Ferrous ion chelating activity was evaluated according to the method described by Sudha *et al*. (2011) with few modifications. Each 2.25 ml of the reaction mixture contained 2 ml of an essential oil solution with a known concentration (1-500 µg/ml), 0.05 ml of 2 mM $FeCl_2$ and 0.2 ml of 5 mM ferrozine. The reaction mixture was left to stand for 10 min at room temperature. The absorbance of the reaction mixture was measured at 562 nm. EDTA was used as a positive control. The essential oil solutions used in this experiment were prepared in methanol. The percentage of ferrous ion chelating was calculated as follows:

percent scavenging = $[1-(A_1-A_2)/A_0] \times 100\%$

where A_0 is the absorbance of the control (without the sample), A_1 is the absorbance of the sample and A_2 is the absorbance of the reaction mixture without ferrozine.

Superoxide anion radical scavenging

Superoxide anion radical scavenging was evaluated according to the method described by Hussein (2011) with few modifications. Each 5 ml of reaction mixture contained 1 ml of an essential oil solution with a known concentration (1-500 µg/ml), 1 ml of 50 µM nitroblue tetrazolium, 1 ml of 78 µM NADH, 1 ml of 16 mM Tris-HCl buffer pH 8.0 and 1 ml of 10 µM phenazine methosulfate. The reaction mixture was left to stand for 5 minutes at room temperature. The absorbance of the reaction mixture was measured at 560 nm. *L*-ascorbic acid was used as a positive control. The essential oil solutions used in this experiment were prepared in methanol. The percentage of superoxide anion radical scavenging was calculated as follows:

percent scavenging = $[1-(A_1-A_2)/A_0] \times 100\%$

where A_0 is the absorbance of the control (without the sample), A_1 is the absorbance of the sample and A_2 is the absorbance of the reaction mixture without phenazine methosulfate.

Ferric reducing power assay

Ferric reducing power activity was evaluated according to the method described by Dey *et al*. (2012) with few modifications. Each 3 ml of reaction mixture contained 1 ml of an essential oil solution with a known concentration (0.03-10 µg/ml), 1 ml of phosphate buffer pH 6.6 and 1 ml of 0.1% potassium hexacyanoferate. The reaction mixture was incubated at 50°C for 20 min. After incubation, 1 ml of 10%trichloroacetic acid was added to the reaction mixture and the reaction mixture was centrifuged at 200 rpm for 10 min. After that, 1 ml of supernatant was mixed with 1 ml of deionized water and 1 ml of 0.01% $FeCl_3$. The reaction mixture was left to stand for 10 min at room temperature. The absorbance of the reaction mixture was measured at 700 nm. The increased absorbance of the reaction mixture indicates the increased reducing power. *L*-ascorbic acid was used as a positive control. The essential oil solutions used in this experiment were prepared in methanol. The essential oil concentration at the absorbance of 0.5 was used for comparison of ferric reducing power of the samples.

Statistical analysis

All experiments were performed in triplicate. The experimental results were reported as mean ± SD. The EC_{50} value was estimated from the graph of percent scavenging against essential oil concentration. Data analyses were performed using SPSS software version 18, Duncan multiple range test at $p < 0.05$ probability level.

RESULTS AND DISCUSSION

Essential oil components

Eighty-four compounds comprising 98.34-99.13% were identified from the essential oils hydrodistillated from the fresh 6- and 9-month of *A. siamemsis* rhizomes (Table 1). Sesquiterpene hydrocarbons (46.71%) were found to be the main components in the 6-month rhizome essential oil and were followed by oxygenated monoterpenes (38.56%) and monoterpene hydrocarbons (8.85%) whereas oxygenated monoterpenes (42.81%) were found to be the main components in the 9-month rhizome essential oil and were followed by sesquiterpene hydrocarbons (33.60%) and monoterpene hydrocarbons (8.15%). 1,8-Cineole, an oxygenated monoterpene, was the main component in both of the essential oils (20.49 and 29.68%, respectively).

1,8-Cineole was also reported as the main component in the essential oils from the rhizome of *A. calcarata* (Arambewela *et al*., 2010), *A. galanga* (Raina *et al*., 2002; Wu *et al*., 2014), *A. officinarum* (Ly *et al*., 2001; Zhang *et al*., 2010) and *A. nigra* (Kanjilal *et al*., 2010). In contrast, β-pinene was reported as the main component in the essential oils from the fresh rhizomes of several *Alpinia* species such as *A. allughas* (Prakash *et al*., 2007), A. *malaccencis* (Muchtaridi *et al*., 2014) and *A. mutica* (Salim *et al*., 2016). However, the chemical compositions of essential oils may be influenced by the stage of plant growth, harvesting stage, extraction method, geographical source and/or adaptive metabolism of the plants (Al-Reza *et al*., 2010; Saeb and Gholamrezaee, 2012; Khalid and El-Gohary, 2014; Theanphong *et al*., 2017).

Table 1. Essential oil components of the fresh rhizomes of *A. siamensis* aged 6 and 9 months

Compound name	KI[a]	6-Month rhizome	9-Month rhizome
Monoterpene hydrocarbons			
α-Pinene	939	1.32	1.56
Camphene	954	tr	-
Sabinene	975	3.09	0.24
β-Pinene	979	0.64	2.53
Myrcene	990	0.62	0.46
β-Phellandrene	1002	-	0.35
p-Cymene	1024	0.24	0.42
Limonene	1029	2.07	1.59
β-Ocimene	1050	tr	-
γ-Terpinene	1059	0.54	0.63
Terpinolene	1088	0.08	0.27
4-Carene	-	0.24	0.11
Oxygenated monoterpenes			
2,3-Dehydro-1,8-cineole	991	tr	-
1,8-Cineole	1031	**20.49**	**29.68**
Linalool	1096	0.06	-
trans-*p*-Menth-2-en-1-ol	1140	tr	-
trans-Pinocamphone	1162	tr	-
Terpinen-4-ol	1177	0.65	1.46
p-Cymene-8-ol	1182	tr	-
α-Terpineol	1188	0.58	0.70
Bornyl acetate	1288	0.53	-
Carvacrol	1299	0.16	-
Myrtenyl acetate	1326	tr	-
trans-Carvyl acetate	1342	0.15	-
Citronellyl acetate	1352	3.26	-
Eugenol	1359	-	0.19
Chavicol acetate	1372	4.63	9.58
Geranyl acetate	1381	0.38	0.24
Eugenyl acetate	1522	-	0.96
Cuminyl acetate	-	7.68	-
Sesquiterpene hydrocarbons			
α-Copaene	1376	0.07	0.09
β-Elemene	1390	0.78	-
α-Santalene	1417	-	0.30
Caryophyllene	1419	0.36	0.29
β-Copaene	1432	0.08	1.63
trans-α-Bergamotene	1434	0.44	9.69
γ-Elemene	1436	0.07	-
α-Guainene	1439	0.17	-
Alloaromadendrene	1441	3.28	0.20
cis-β-Farnessene	1442	0.12	0.82
Humulene	1454	11.23	1.06
γ-Gurjunene	1477	0.44	-
γ-Muurolene	1479	-	0.25
Germacrene D	1485	10.40	0.31
α-Zingiberene	1493	-	0.74

Table 1. Essential oil components of the fresh rhizomes of *A. siamensis* aged 6 and 9 months (Cont.)

Compound name	KI[a]	6-Month rhizome	9-Month rhizome
α-Selinene	1498	0.18	-
α-Farnesene	1505	4.29	2.65
β-Bisabolene	1505	1.04	9.38
cis-β-Bisabolene	1505	4.10	-
γ-Cadinene	1513	tr	-
β-Sesquiphellandrene	1522	1.48	5.67
Epizonarene	1529	0.16	-
trans-Cadina-1,4-diene	1534	0.07	-
Germacrene B	1559	-	0.52
Cedrene-V6	-	1.63	-
γ-Selinene	-	0.18	-
δ-Guaiene	-	5.20	-
α-Panasinsen	-	0.88	-
Neoisolongifolene	-	0.05	-
Oxygenated sesquiterpenes			
Caryophyllene oxide	1583	0.05	-
α-Cadinol	1654	0.23	0.36
α-Bisabolol	1675	0.11	0.63
Z-trans-α-Bergamotol	1690	-	0.47
E,E-Farnesal	1741	0.07	-
Z,E-Nerolidol	-	0.06	-
Farnesol acetate	-	0.08	0.23
Phenylpropanoids			
Chavicol	1250	tr	0.52
Methyleugenol	1403	0.11	0.16
Hydrocarbon and other compounds			
Tetradecane	1400	0.86	-
Pentadecane	1500	-	2.78
Hexadecane	1600	tr	-
Heptadecane	1700	0.06	-
Nonadecane	1900	tr	5.09
Eicosane	2000	0.18	-
Heneicosane	2100	0.27	-
Docosane	2200	tr	-
Tricosane	2300	0.44	2.74
Tetracosane	2400	tr	-
Pentacosane	2500	0.36	-
Hexacosane	2600	0.18	-
Cyclodecene	-	0.78	-
8-Heptadecene	-	0.13	1.10
6,9-Heptadecaiene	-	-	0.49
2,4-Dimethyl benzoic acid	-	0.26	-
Total identified		98.34	99.13

[a]: Kovats index is determined relative to n-alkanes (C6–C24) on a DB-5 MS column
tr: < 0.05%

Antioxidant activities

In this study, antioxidant activities of the essential oils of the fresh 6 and 9 month aged *A. siamensis* rhizomes were assessed by DPPH radical scavenging assay, hydroxyl radical scavenging assay, ferrous ion chelating assay, superoxide anion radical scavenging assay and ferric reducing power assay. Five selected antioxidant activity tests were caused by different chemical constituents in essential oils. These substances may exhibit the capability of free radical scavenging with different mechanisms such as donating hydrogen atom or electron to DPPH radical (Brewer, 2011).

The both of essential oils showed strong DPPH radical scavenging activity and ferric reducing power with insignificantly different EC_{50} values as compared with *L*-ascorbic acid (Table 2). The essential oil of the rhizomes aged 6 months exhibited higher antioxidant activities against $OH^{\cdot}$ radicals than the essential oil of the rhizomes aged 9 months and *L*-ascorbic acid. It was found that the effectiveness of scavenging hydroxyl radical radicals was in the decreasing order from essential oil of the rhizomes aged 6 months > essential oil of the rhizomes aged 9 months, *L*-ascorbic acid. Table 2 showed that the essential oil of the rhizomes aged 9 months exhibited significantly higher superoxide anion radical scavenging activity than *L*-ascorbic acid while the essential oil of the rhizomes aged 6 months exhibited insignificantly different superoxide anion radical scavenging activity as compared with *L*-ascorbic acid. However, the both of essential oils showed less ferrous ions chelating activity as compared with EDTA. The EC_{50} values of the essential oils studied and positive control are shown in Table 2.

The results showed that the essential oils of the fresh 6- and 9-month *A. siamensis* rhizomes had strong antioxidant activities for scavenging DPPH radicals, hydroxyl radical, superoxide anion radical scavenging activities and ferric reducing power with percent scavenging in the range of 19.28-98.74%.

The results were similar with the previous reports. Arambewela *et al*. (2010) and Zhang *et al*. (2010) reported that the essential oils of *A. calcarata* and *A. officinarum* rhizomes possessed high antioxidant properties.

Table 2. The EC_{50} values of essential oils from the fresh rhizomes of *A. siamensis* aged 6 and 9 months

Test	EC_{50} (μg/ml)*		
	6-Month rhizome	**9-Month rhizome**	**Positive control****
DPPH radical scavenging assay	12.13 ± 1.68^{a}	11.69 ± 1.24^{a}	11.50 ± 1.06^{a}
OH scavenging assay	13.76 ± 1.02^{a}	15.68 ± 1.44^{b}	15.13 ± 1.73^{b}
Ferrous ion chelating assay	192.87 ± 7.06^{a}	204.28 ± 9.52^{a}	146.36 ± 6.47^{b}
Superoxide anion radical scavenging activity	$32.83 \pm 1.44^{a,b}$	30.10 ± 2.32^{a}	34.24 ± 2.09^{b}
Ferric reducing power assay	0.11 ± 0.01^{a}	0.10 ± 0.01^{a}	0.11 ± 0.01^{a}

* Data are expressed as means ± SD (n = 3)

** *L*-ascorbic acid was use as positive control in DPPH radical scavenging assay, OH scavenging assay, Superoxide anion radical scavenging activity and Ferric reducing power assay. EDTA was use as positive control in Ferrous ion chelating assay

Means ± SD followed by the same letter for each experiment, within a row, are not significantly different ($P < 0.05$).

CONCLUSION

Chemical components and antioxidant activities of the essential oils of the fresh *A. siamensis* rhizomes with the age of 6 and 9 months were investigated. The results showed that 1, 8-cineole was the main composition in both rhizome essential oils. Both of the rhizome essential oils showed strong degree of antioxidant potentials towards DPPH, OH and O_2 radical scavenging and radical reducing power. Thus, it can be concluded that the essential oils of the rhizome of *A. siamensis* may be a new potential source of natural antioxidants.

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