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COMPARISON OF CHEMICAL COMPOSITIONS AND *IN VITRO* ANTIOXIDANT ACTIVITIES OF ESSENTIAL OILS OBTAINED BY STREAM DISTILLATION AND WATER DISTILLATION FROM *CURCUMA ANGUSTIFOLIA* ROXB. ROOTS AND RHIZOMES FROM THAILAND

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Abstract: The chemical compositions of essential oils stream distillated and water distillated from the fresh roots and rhizomes of *C. angustifolia* from Thailand were analyzed by Gas Chromatography-Mass Spectroscopy (GC-MS) technique. The antioxidant activities of essential oils were evaluated by using six various methods including DPPH radical scavenging, hydroxyl radical scavenging, hydrogen peroxide scavenging, ferrous ion chelating, superoxide anion radical scavenging and ferric reducing power assays. *L*-ascorbic acid and ethylenediaminetetraacetic acid were used as a positive control. The major chemical compositions of the essential oils stream distillated and water distillated from the fresh roots and rhizomes were found to be camphor (36.94 and 30.16%) and β-elemenone (64.99 and 54.50%), respectively. The DPPH radical scavenging activity and ferric reducing power of all essential oils were found to be insignificantly different as compared with those of *L*-ascorbic acid. The two rhizome essential oils exhibited significantly higher antioxidant activities against hydroxyl radicals than the two root essential oils and *L*-ascorbic acid. The superoxide anion scavenging activities of the two rhizome essential oils were found to be significantly higher than the two root essential oils. However, the superoxide anion scavenging activities of the two rhizome essential oils were insignificantly different as compared with that of *L*-ascorbic acid. The rhizome essential oil obtained from steam distillation showed the strongest hydrogen peroxide scavenging activity. However, the EC_{50} value of the rhizome essential oil obtained from steam distillation was insignificantly different as compared with that of *L*-ascorbic acid. All essential oils showed less activity against chelating ferrous ions than EDTA.

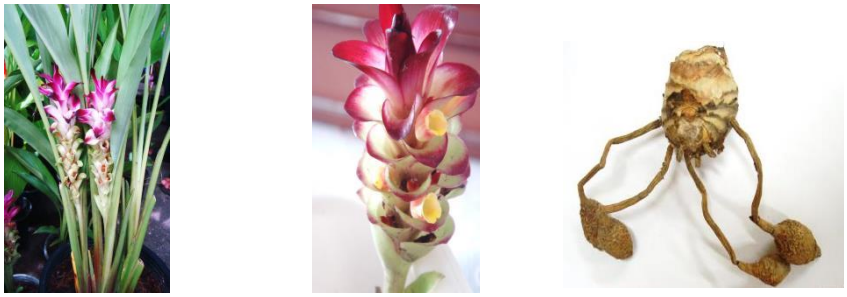
Keywords: *Curcuma angustifolia*, essential oil, antioxidant activity

บทคัดย่อ: องค์ประกอบทางเคมีของน้ำมันระเหยง่ายที่ได้จากการกลั่นด้วยไอน้ำและน้ำจากรากและเหง้าสดของอาวจากประเทศไทยถูกวิเคราะห์ด้วยเทคนิค Gas Chromatography-Mass Spectroscopy (GC-MS) ฤทธิ์ต้านอนุมูลอิสระของน้ำมันระเหยง่ายถูกวิเคราะห์โดยใช้วิธีการที่แตกต่างกัน 6 วิธี ได้แก่ วิธี DPPH radical scavenging, hydroxyl radical scavenging, hydrogen peroxide scavenging, ferrous ion chelating, superoxide anion radical scavenging และ ferric reducing power โดยมี *L*-ascorbic acid และ ethylenediaminetetraacetic acid (EDTA) เป็นสารควบคุมแบบให้ผลบวก ผลการทดลองพบว่า องค์ประกอบทางเคมีหลักในน้ำมันระเหยง่ายจากรากและเหง้าของอาวที่ได้จากการกลั่นด้วยไอน้ำและ น้ำคือ camphor โดยมีปริมาณเท่ากับ 36.94 and 30.16% ตามลำดับ และ β-elemenone โดยมีปริมาณเท่ากับ 64.99 and 54.50% ตามลำดับ ฤทธิ์ต้านอนุมูลอิสระ DPPH radical และ ferric reducing power ของน้ำมันระเหยง่ายทุกชนิดแตกต่างจากฤทธิ์ต้านอนุมูลอิสระ DPPH radical และ ferric reducing power **ของ** *L*-ascorbic acid อย่างไม่มีนัยสำคัญ น้ำมันระเหยง่ายจากเหง้าทั้ง 2 ชนิดมีฤทธิ์ต้านอนุมูลอิสระ hydroxyl radicals สูงกว่าน้ำมันระเหยง่ายจากรากทั้ง 2 ชนิดและ *L*-ascorbic acid ฤทธิ์ต้าน superoxide anions ของน้ำมันระเหยง่ายจากเหง้าทั้ง **2** ชนิดสูงกว่าฤทธิ์ต้าน superoxide anions ของน้ำมันระเหยง่ายจากเหง้าทั้ง 2 ชนิดอย่างมีนัยสำคัญ แต่ไม่แตกต่างจากฤทธิ์ต้าน superoxide anions ของ *L*-ascorbic acid น้ำมันระเหยง่ายจากเหง้าที่ได้จากการกลั่นด้วยไอน้ำมีฤทธิ์ต้าน hydrogen peroxide ดีที่สุด อย่างไรก็ตามพบว่าค่า EC_{50} ของน้ำมันระเหยง่ายจากเหง้าที่ได้จากการกลั่นด้วยไอน้ำไม่แตกต่างจากค่า EC_{50} ของ *L*-ascorbic acid นอกจากนี้ยังพบว่าน้ำมันระเหยง่ายทุกชนิดมีฤทธิ์ ferrous ion chelating น้อยมากเมื่อเทียบกับ EDTA

คำสำคัญ: *Curcuma angustifolia* น้ำมันระเหยง่าย ฤทธิ์ต้านอนุมูลอิสระ

INTRODUCTION

C. angustifolia Roxb. or Ao is a perennial herb with aromatic rhizome and tuberous root that belongs to the family Zingiberaceae (Figure 1). In ethnomecicines, its roots are used to treat dysentery, cough, cold and bone fractures and its rhizomes are used to treat asthma, dysentery, jaundice, pain and stomatitis (Mohanta *et al*., 2006; Kunwar *et al*., 2010; Rokaya, Munzbergova and Timsina, 2010; Tushar *et al*., 2010; Abhyankar and Upadhyay, 2011; Ray, Sheikh and Mishra, 2011; Padal and Sandhyasri, 2013). In Thailand, its young inflorescences are used as food (Khamtang *et al*. 2014).



a b c

Figure 1. Morphological characters of *C. angustifolia* Roxb.
a: Whole plants, b: Inflorescence, c: Rhizome and root

Essential oil, a complex mixture of low molecular weight organic compounds generally widely distributed in higher plants, is obtained from plant materials by various methods such as steam distillation, hydro distillation, turbohydrodistillation, solvent extraction, CO_2 extraction, microwave extraction, ultrasound assisted extraction and mechanical expression (Brewer, 2011; Périno-Issartier *et al*., 2013). However, steam distillation and hydro distillation are two conventional methods that are used for extraction of essential oils. Steam distillation is the most widely used for the production of essential oils in large scale whereas hydro distillation is the routine method for extraction of essential oils, which is recommended by Pharmacopoeias (Wesolowska, Grzeszczuk and Jadczak, 2016).

Reactive oxygen species (ROS), such as superoxide anion (O_2^{-}), hydrogen peroxide (H_2O_2), and hydroxyl radical (HO•) are by products that result from the cellular redox metabolisms (Ray *et al*., 2012; Sisein, 2014). The effects of ROS on health include chronic and degenerative diseases such as cancer, arthritis, aging, autoimmune disorders, cardiovascular and neurodegenerative diseases (Pham-Huy, He and Pham-Huy, 2008).

Antioxidants are molecules that scavenge reactive oxygen species to inhibit chain initiation and/or terminate the chain reaction before vital molecules are damaged (Labo *et al*., 2010). Essential oil is one of the natural products that have been reported to possess antioxidant activities. *C. aeruginosa* (George and Britto, 2015; Theanphong, Mingvanish and Kirdmanee, 2015), *C. amada* (George *et al*., 2015), *C. aromatica* (Al-Reza, *et al*., 2010), *C. leucorrhiza* (Theanphong, Mingvanish and Jenjittikul, 2016), *C. longa* (Shahwar *et al*., 2012), *C. sichuanensis* (Tsai *et al*., 2011) and *C. zedoaria* (Rahman *et al*., 2014) were exemplified as a source of essential oils having strong antioxidant activities. However, there have been no previous reports on chemical compositions and antioxidant activities of essential oils from the fresh roots and rhizomes of *C. angustifolia* from Thailand. Thus, the aims of this study were to compare chemical compositions of essential oils steam distillated and water distillated from the fresh roots and rhizomes of *C. angustifolia* from Thailand and to compare their antioxidant activities by six different methods.

MATERIALS AND METHODS

Plant material

Parts of fresh roots, rhizomes and whole plant of *C. angustifolia* were collected in July, 2016 from Chiang Mai Province, Thailand. The plant sample was identified by comparison with the herbarium specimen collected at the Singapore Herbarium (Singapore Botanic Gardens, Singapore) voucher no. SING 0166477 and also confirmed by Asst. Prof. Dr. Thaya Jenjittikul. Furthermore, the voucher specimen of this plant was deposited at Faculty of Pharmacy, Rangsit University, Patumtani, Thailand.

Preparation of essential oils

A 500 g of each fresh root and rhizome of *C. angustifolia* were steam distillated and water distillated for 4 hrs. The four essential oils were collected and stored at 4 °C in air-tight container before analyzed by GC-MS technique.

GC-MS Analysis

GC/MS analysis was performed using an Agilent Technologies 7890A GC system equipped with a 5975C inert XL EI/CI MAD and Triple-Axis detector using electron impact ionization at 70 eV. The DB-5 MS (phenyl: dimethyl arylene siloxane 5: 95) capillary column (30 m length x 0.25 mm i.d., 0.25 μm film thickness) was used for GC analysis. Helium gas was used as the carrier gas at the flow rate of 1 ml/min. 1 μl of dilute essential oils in ethanol (1:25 v/v) was injected using GC sampler 80 autosampler (splitless mode). The GC oven temperatures for chromatography of essential oil were programmed from being held at 60°C for 1 min, ramped at the rate of 3°C/min to 240°C and held for 5 min. The GC injector and GC-MSD interface temperatures were set at 180°C and 290°C, respectively. The analyzed mass range was 40-650 m/z at the scanning rate of 2.42 amu/second. The total scanning time was 70 min.

Identification of essential oil compositions

Essential oil compositions 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 on the basis of peak area measurement.

Antioxidant activities

DPPH radical scavenging assay

The antioxidant activity of all essential oils was determined in terms of DPPH radical scavenging ability by hydrogen donation (Sudha *et al*., 2011). Briefly, 1 ml of various concentrations of essential oils in methanol (1-500 μg/ml) was mixed with 1 ml of a methanolic solution of DPPH (0.2 mM). The mixture was shaken vigorously and allowed standing in dark condition for 30 min. The absorbance of the reaction mixture was measured at 517 nm. *L*-ascorbic acid was used as a positive control. The percentage of DPPH radical scavenging was calculated as follows:

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

where A_0 is the absorbance of the control (without the sample) and A_1 is the absorbance of the sample.

Hydroxyl radical scavenging assay

The scavenging activity for hydroxyl radicals of all essential oils was determined on the basis of Fenton reaction (Sudha *et al*., 2011). Briefly, 1 ml of various concentrations of essential oils in methanol (1-500 µg/ml) were mixed with 1 ml of $FeSO_4$ (1.5 mM), 0.7 ml of hydrogen peroxide (6 mM) and 0.3 ml of sodium salicylate (20 mM). 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 percentage of OH• 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 without sodium salicylate.

Hydrogen peroxide scavenging assay

The hydrogen peroxide scavenging activity of all essential oils was evaluated according to the method of Keser *et al.* (2012). Briefly, 0.4 ml of various concentrations of essential oils in methanol (1-500 µg/ml) was mixed with 0.6 ml of H_2O_2 in phosphate buffer (pH 7.4, 40 mM). The reaction mixture was left to stand at room temperature for 10 min. The absorbance of the reaction mixture was measured at 230 nm. *L*-ascorbic acid was used as a positive control. The percentage of hydrogen peroxide scavenging was calculated as follows:

Percent scavenging = $[(A_0-A_1)/A_0] \times 100\%$

where A_0 is the absorbance of the control (without the sample) and A_1 is the absorbance of the sample.

Superoxide anion radical scavenging

Superoxide anion radical scavenging of all essential oils was evaluated based on the capacity of the essential oils to inhibit the photochemical reduction of nitro blue tetrazolium (Hussein, 2011). Briefly, 1 ml of various concentrations of essential oils in methanol (1-500 µg/ml) were mixed with 1 ml of nitroblue tetrazolium (50 µM), 1 ml of NADH (78 µM), 1 ml of Tris-HCl buffer (pH 8.0, 16 mM) and 1 ml of phenazine methosulfate (10 µM). The reaction mixture was left to stand at room temperature for 5 min. The absorbance of the reaction mixture was measured at 560 nm. *L*-ascorbic acid was used as a positive control. 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 without phenazine methosulfate.

Ferrous ion chelating assay

The Fe^{2+} chelating activity of all essential oils was determined by inhibition of the formation of Fe^{2+}-ferrozinecomplexes (Sudha *et al*, 2011). Briefly, 2 ml of various concentrations of the essential oils in methanol (1-500 µg/ml) was mixed with 0.05 ml of $FeCl_2$ (2 mM) and 0.2 ml of ferrozine (5 mM). The reaction mixture was left to stand at room temperature for 10 min. The absorbance of the reaction mixture was measured at 562

nm. EDTA was used as a positive control. The percentage of ferrous ion chelating was calculated as follows:

Percent chelating = $[(A_0-A_1)/A_0] \times 100\%$

where A_0 is the absorbance of the control (without the sample) and A_1 is the absorbance of the sample.

Ferric reducing power assay

The reducing power assay of all essential oils was determined based on the electron-donating ability of antioxidants using the potassium hexacyanoferrate reduction method (Dey, *et al*, 2012). Briefly, 0.5 ml of various concentrations of the essential oils in methanol (0.03-10 µg/ml) was mixed with 0.5 ml of phosphate buffer pH 6.6 and 0.5 ml of $K_3Fe(CN)_6$ (0.1%). The reaction mixture was incubated at 50°C for 20 min. After incubation, 0.5 ml of trichloroacetic acid (10% w/v) was added to the reaction mixture and then centrifuged at 200 rpm for 10 min. After that, 1 ml of the supernatant was mixed with 1 ml of distilled water and 0.1 ml of $FeCl_3$ (0.01%). The reaction mixture was left to stand at room temperature for 10 min. The absorbance of the reaction mixture was measured at 700 nm against the blank. The increased absorbance of the reaction mixture indicates the increased reducing power. *L*-ascorbic acid was used as a positive control. The essential oil concentration at the absorbance of 0.5 was used for comparison of ferric reducing power of the sample.

Statistical analysis

All experiments were performed in triplicate. The experimental results were reported as mean ± SD. The EC_{50} value was calculated from the graph of inhibition percentage against essential oil concentration. Data analyses were performed using SPSS software version 18, Duncan multiple range test at $p < 0.05$ probability level. The half maximal effective concentration (EC_{50}) was calculated from the graph obtained by plotting percent scavenging versus concentrations.

RESULTS AND DISCUSSION

Essential oil compositions

The essential oils of the fresh roots and rhizomes of *C. angustifolia* obtained by steam and water distillation were clear and pale yellow oils with the percent yields of 0.30-0.36% v/w. The chemical compositions of all of the essential oils were showed in Table 1.

The root and rhizome essential oils were complex mixtures of monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpenes, phenylpropanoids and hydrocarbon compounds.

With regard to steam distillation, 33 compounds of the root essential oil (95.30 %) were identified while 35 compounds of the rhizome essential oil (96.27%) were identified. Oxygenated sesquiterpenes (67.2%) represented by β-elemenone (64.99%) were major chemical compositions in the root essential oil. In the rhizome essential oil, oxygenated monoterpenes (42.64%) represented by camphor (36.94%) and oxygenated sesquiterpenes (33.18%) represented by germacrone (31.49%) were considered as major chemical compositions.

Thirty-three compounds (96.03%) and forty-tree compounds (97.73%) were identified in the root and rhizome essential oils obtained by water distillation, respectively. The root essential oil was rich in oxygenated sesquiterpenes (59.39%) and β-elemenone (54.50%) was the major chemical composition in the root essential oil. Oxygenated monoterpenes

(35.15%) and oxygenated sesquiterpenes (28.85%) were dominant in the rhizome essential oil and two major chemical compositions were camphor (30.16%) and germacrone (27.70%).

Similar chemical compositions of *C. angustifolia* have been earlier reported by Srivastava, Srivastava and Syamsundar (2006). Camphor and germacrone were rich in the rhizome essential oil of *C. angustifolia* from the Southern India.

The present results showed that the major chemical compositions in the essential oils obtained from steam and water distillation were found to be similar but the quantities of their chemical compositions were different.

Furthermore, the present results were in agreement with the results previously reported by Charles and Simon (1990), Kokoska *et al*. (2008) and Périno-Issartier *et al*. (2013).

Table 1. Essential oil compositions of the fresh roots and rhizomes of *C. angustifolia* obtained by steam distillation and water distillation

Chemical compositions	**KI**[a]	**Content [%]**			
		Steam distillation[b]		Water distillation	
		Root	Rhizome	Root	Rhizome
Monoterpene hydrocarbon					
Tricyclene	926	tr	0.06	0.11	0.18
α-Pinene	939	0.27	0.36	0.64	0.96
Camphene	954	1.14	2.05	3.30	4.68
β-Pinene	979	0.16	0.10	0.59	0.12
Myrcene	990	5.49	4.50	0.27	0.82
α-Phellandrene	1002	0.18	-	-	-
δ-3-Carene	1011	0.17	0.29	0.40	0.47
p-Cymene	1024	-	-	0.06	-
Limonene	1029	0.31	0.63	0.44	0.94
β-Ocimene	1050	0.10	-	0.29	-
γ-Terpinene	1059	0.23	-	0.24	tr
4-Carene	-	-	-	tr	0.08
Oxygenated monoterpene					
1,8-Cineole	1031	0.63	2.02	0.91	2.39
Linalool	1096	0.47	0.52	0.40	0.38
Camphor	1146	9.62	36.94	8.99	30.16
Eucarvone	1150	-	-	-	tr
Isoborneol	1160	0.34	0.47	0.22	0.45
Borneol	1169	0.15	0.71	0.07	0.59
Teppinen-4-ol	1177	0.07	0.28	0.06	0.23
α-Terpineol	1188	0.14	0.42	0.10	0.33
cis Carveol	1229	-	-	-	tr
Carvone	1243	-	0.11	-	0.06
Isobornyl acetate	1285	0.12	0.20	-	0.18
Bornyl acetate	1288	1.70	0.09	1.39	0.05
Myrtenyl acetate	1326	0.43	-	0.35	-
trans-Carveyl acetate	-	0.11	0.17	-	-
7-epi-sesquisabinene hydrate	-	-	0.70	-	0.34

Table 1. Essential oil compositions of the fresh roots and rhizomes of *C. angustifolia* obtained by steam distillation and water distillation (cont.)

Chemical compositions	**KI**[a]	**Content [%]**			
		Steam distillation[b]		Water distillation	
		Root	Rhizome	Root	Rhizome
Sesquiterpene hydrocarbon					
δ-Elemene	1338	0.21	-	-	0.26
β-Elemene	1390	-	-	1.34	1.06
γ-Elemene	1391	-	-	-	1.14
(-)-*α*-Santalene	1417	2.22	2.81	6.47	7.67
α-Bergamotene	1434	-	-	-	0.12
trans-*α*-Bergamotene	1434	0.21	0.42	0.20	1.12
g-Elemene	1436	-	0.60	3.80	-
Alloaromadendrene	1441	1.17	-	0.11	tr
cis β-Farnesene	1442	0.47	0.80	1.47	1.79
Humulene	1454	0.63	-	0.76	0.09
cis-*β*-santalene	1459	0.12	0.08	0.36	0.46
Germacrene D	1485	0.82	0.54	2.66	1.07
α-Zingiberene	1493	-	1.68	-	4.40
Valencene	1496	-	-	0.08	tr
β-Bisabolene	1505	-	1.49	-	3.21
β-Sesquiphellandrene	1522	-	1.02	-	2.56
Oxygenated sesquiterpene					
Curzerene	1499	2.21	-	-	-
β -Elemenone	1589	64.99	0.15	54.50	-
β -Bisabolol	1675	-	1.53	4.89	1.14
Germacrone	1693	-	31.49	-	27.70
Other					
Methyl chavicol	1196	-	0.08	-	tr
2-Heptanol	896	-	0.15	-	0.16
7 hydroxycadalene	-	-	-	0.55	-
2-Heptanol acetate	-	0.10	0.17	-	0.15
Hexanedioic acid, bis (2-ethylhexyl) ester	-	0.14	2.65	-	0.24
9,10-Dimethyl-1,2,3,4,5,6,7,8-octahydroanthracene	-	0.14	-	-	-
Total identified		**95.30**	**96.27**	**96.03**	**97.73**
% Yield (%v/w)		**0.30**	**0.32**	**0.33**	**0.36**

[a]: Kovats index is determined relative to n-alkanes (C6–C24) on a DB-5 MS column
[b]: Data from Thongkhwan *et al*., 2017
tr < 0.05%

Nevertheless, steam distillation seem to be the suitable method for distillating the essential oil from *C. angustifolia* roots and rhizomes, as it afforded high amounts of the major chemical compositions. In addition, the advantage of steam distillation over water distillation are low thermal degradation of chemical compositions in essential oils because the

temperature in distillation generally is not above 100°C and is suitable for the distillation of essential oils in commercial scale.

Antioxidant activities

The antioxidant activities of the root and rhizome essential oils obtained from steam and water distillation were investigated by six different methods including DPPH radical scavenging assay, hydroxyl radical scavenging assay, hydrogen peroxide scavenging assay, superoxide anion radical scavenging, ferrous ion chelating assay and ferric reducing power assay.

The DPPH radical scavenging activity and ferric reducing power of all essential oils were found to be insignificantly different as compared with those of *L*-ascorbic acid. The DPPH radical scavenging activities of all essential oils and *L*-ascorbic acid were in the range of 20.41-99.75% and 29.35-99.96%, respectively (Figure 2). The two rhizome essential oils exhibited significantly higher antioxidant activities against hydroxyl radicals than the root essential oils and *L*-ascorbic acid. The hydroxyl radical scavenging activities of the rhizome essential oils were in the range of 30.02-99.68% while the root essential oils and *L*-ascorbic acid were in the range of 11.41- 89.12% and 31.35-99.78%, respectively (Figure 3). For superoxide anion radical scavenging activity, the two rhizome essential oils showed significantly higher activity than the two root essential oils. As seen in Table 2, the EC_{50} values of the rhizome essential oils were insignificantly different from that of *L*-ascorbic acid. The superoxide anion radical scavenging activities of the rhizome and root essential oils and *L*-ascorbic ranged from 13.99-88.66%, 12.23-85.53% and 16.77-95.43%, respectively (Figure 4). The rhizome essential oil obtained from steam distillation showed the strongest activity against hydrogen peroxide radicals while the root essential oil obtained from water distillation showed the weakest activity. However, the EC_{50} value of the rhizome essential oil obtained from steam distillation was insignificantly different as compared with that of *L*-ascorbic acid. As seen in Figure 5, the hydrogen peroxide radicals scavenging activities of the rhizome and root essential oils and *L*-ascorbic ranged from 20.32-96.42%, 22.54-89.44% and 32.73-98.25%, respectively. All essential oils exhibited weaker activities for chelating ferrous ions, as compared with EDTA (Figure 6). The EC_{50} values that were obtained from the different antioxidant activity tests for all essential oils and positive controls were tabulated in Table 2.

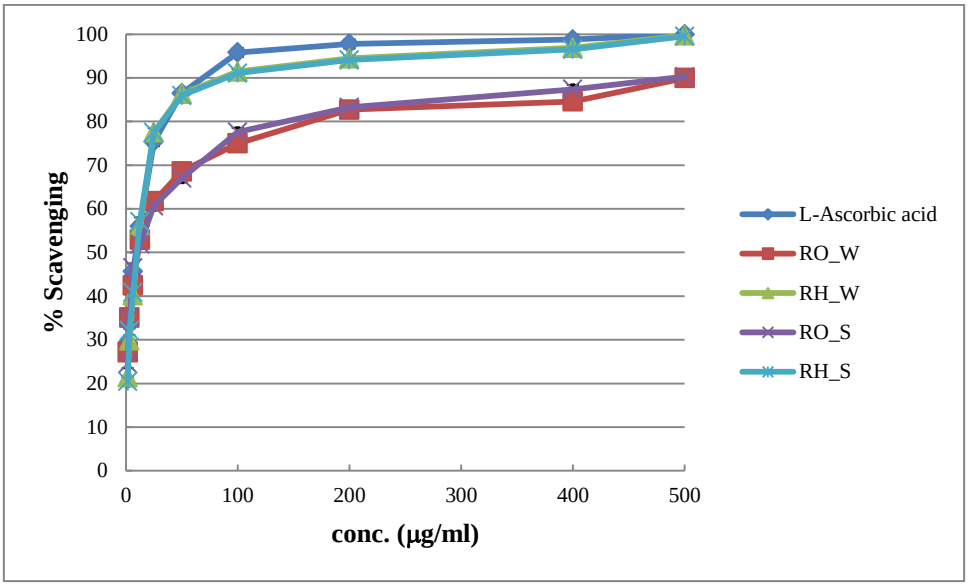


Figure 2. DPPH radical scavenging activity of the essential oils from the fresh roots and rhizomes of *C. angustifolia* and *L*-ascorbic acid.
RO_W: Root essential oil obtained from water distillation
RH_W: Rhizome essential oil obtained from water distillation
RO_S: Root essential oil obtained from steam distillation
RH_S: Rhizome essential oil obtained from steam distillation

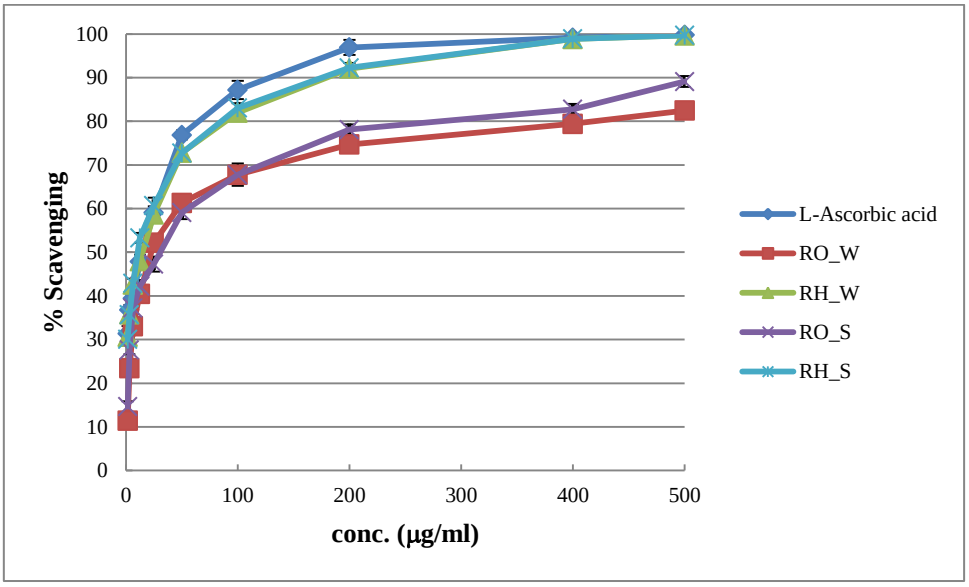


Figure 3. Hydroxyl radical scavenging activity of the essential oils from the fresh roots and rhizomes of *C. angustifolia* and *L*-ascorbic acid.
RO_W: Root essential oil obtained from water distillation
RH_W: Rhizome essential oil obtained from water distillation
RO_S: Root essential oil obtained from steam distillation
RH_S: Rhizome essential oil obtained from steam distillation

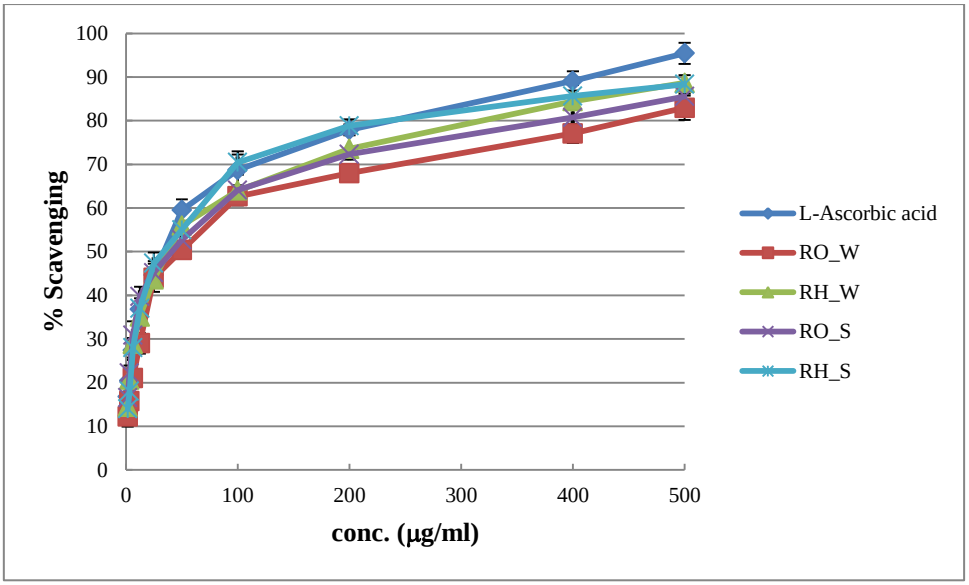


Figure 4. Superoxide anion radical scavenging activity of the essential oils from the fresh roots and rhizomes of *C. angustifolia* and *L*-ascorbic acid.
RO_W: Root essential oil obtained from water distillation
RH_W: Rhizome essential oil obtained from water distillation
RO_S: Root essential oil obtained from steam distillation
RH_S: Rhizome essential oil obtained from steam distillation

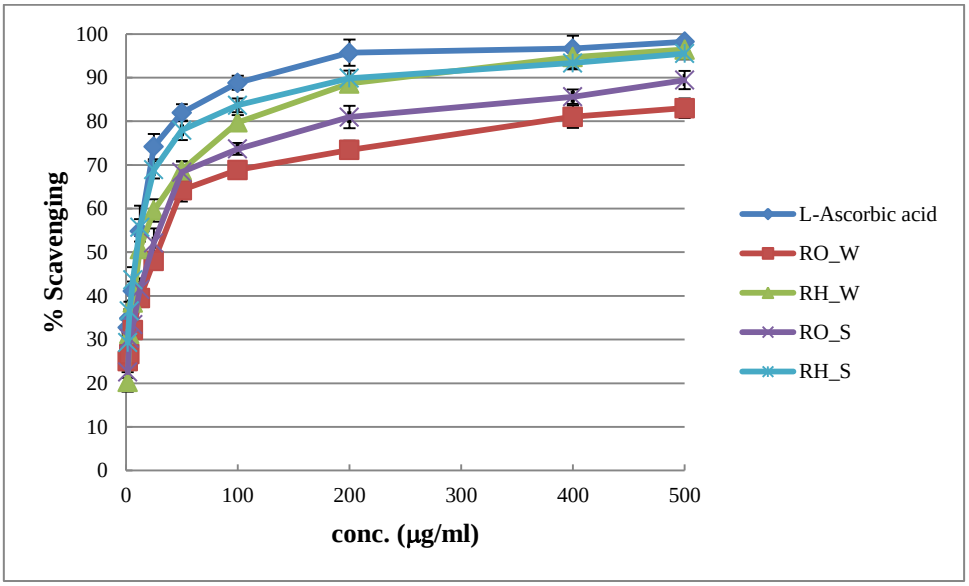


Figure 5. Hydrogen peroxide radical scavenging activity of the essential oils from the fresh roots and rhizomes of *C. angustifolia* and *L*-ascorbic acid.
RO_W: Root essential oil obtained from water distillation
RH_W: Rhizome essential oil obtained from water distillation
RO_S: Root essential oil obtained from steam distillation
RH_S: Rhizome essential oil obtained from steam distillation

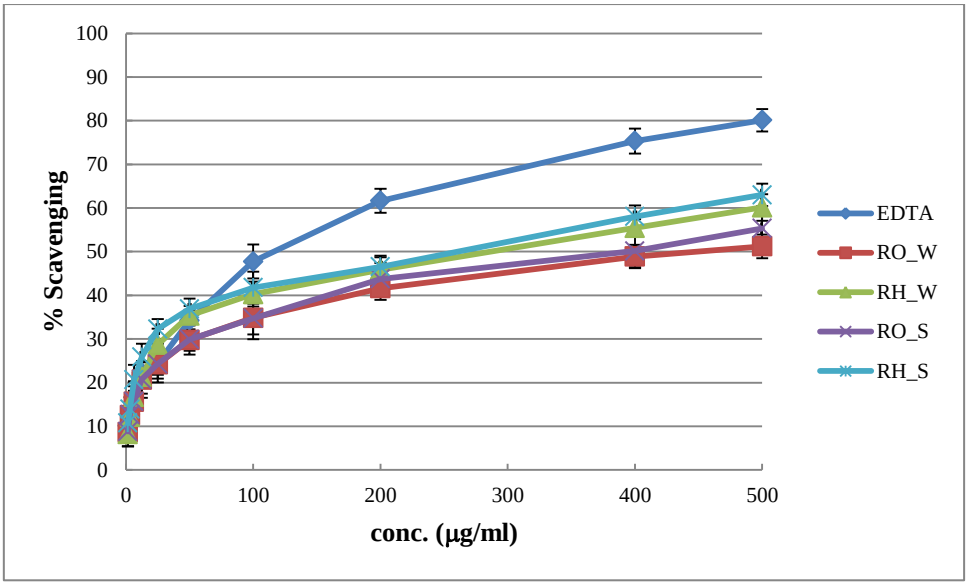


Figure 6. Ferric reducing power of the essential oils from the fresh roots and rhizomes of *C. angustifolia* and EDTA.
RO_W: Root essential oil obtained from water distillation
RH_W: Rhizome essential oil obtained from water distillation
RO_S: Root essential oil obtained from steam distillation
RH_S: Rhizome essential oil obtained from steam distillation

Table 2. The EC_{50} values of the essential oils from the fresh roots and rhizomes of *C. angustifolia* obtained by steam-and water-distillation

Test	EC_{50} (µg/ml)*				
	Essential oil				Positive control**
	Steam distillation		Water distillation		
	Root	Rhizome	Root	Rhizome	
DPPH radical scavenging assay	10.52 ± 1.52[a]	9.81 ± 1.14[a]	10.81 ± 1.93[a]	10.19 ± 1.48[a]	10.77 ± 0.97[a]
OH scavenging assay	23.48 ± 1.59[c]	14.63 ± 1.33[a]	22.63 ± 1.15[c]	15.37 ± 1.59[a]	18.27 ± 1.54[b]
H_2O_2 scavenging activity assay	23.54 ± 2.18[c]	9.59 ± 1.35[a]	28.34 ± 3.93[d]	11.96 ± 2.19[b]	11.04 ± 1.03[a,b]
Superoxide anion radical scavenging activity	42.25 ± 3.03[b]	35.17 ± 2.58[a]	48.89 ± 4.68[c]	38.43 ± 3.59[a]	34.54 ± 2.28[a]
Ferrous ion chelating assay	308.21 ± 10.32[d]	272.74 ± 6.59[b]	319.6 ± 8.29[d]	289.88 ± 12.19[c]	154.2 ± 2.46[a]
Ferric reducing power assay	0.10 ± 0.04[b]	0.09 ± 0.02[a,b]	0.11 ± 0.03[a,b]	0.10 ± 0.03[a]	0.11 ± 0.02[a,b]

* Data are expressed as means ± SD (n = 3)
** *L*-ascorbic acid was use as positive control in DPPH radical scavenging assay, OH scavenging assay, H_2O_2 scavenging activity 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 ($P > 0.05$).

The results showed that the root and rhizome essential oils obtained from steam and water distillation showed the strong antioxidant activities for scavenging DPPH radicals and ferric reducing power. In addition, the two rhizome essential oils showed strong hydroxyl radical and superoxide anion radical scavenging activities.

The antioxidant activities of the root and rhizome essential oils may involve many inhibition mechanisms such as inhibition of chain initiation, decomposition of peroxides, free radical scavenging, reducing capacity and binding of metal ion catalysts (Zeng *et al*., 2012). The present results indicated that the rhizome essential oils have higher antioxidant capacity than the root essential oils. In addition, the results showed that the rhizome essential oil

obtained from steam distillation has higher antioxidant activity than the rhizome oil obtained from water distillation. The strong antioxidant activity of rhizome essential oil may be attributed to the presence of oxygenated monoterpenes in high content (Bayala *et al*., 2014; Barbieri *et al*., 2016).

The results were in agreement with the previous reports. Essential oils of other *Curcuma* species such as *C. aeruginosa* (George and Britto, 2015), *C. amada* (George *et al*., 2015), *C. alismatifolia* (Theanphong and Mingvanish, 2017), *C. aromatica* (Al-Rezaa *et al*., 2010), *C. leucorrhiza* (Theanphong, Mingvanish and Jenjittikul, 2016), *C. longa* (Stanojević *et al*., 2015) and *C. zedoaria* (Mau *et al*., 2003) have been reported for their antioxidant properties.

CONCLUSION

The chemical compositions of the fresh root and rhizome essential oils from *C. angustifolia* obtained from steam and water distillations were similar in quality but different in quantity For their antioxidant activities, the rhizome essential oils exhibited strong activities against DPPH radicals, hydroxyl radicals, hydrogen peroxide radicals, and superoxide anion radicals and had strong reducing power whereas the root essential oils showed strong activities against DPPH radical and had strong reducing power. It can be concluded that the rhizome essential oils of *C. angustifolia* have high potential to be used as natural antioxidants.

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