Interprof. J. Health Sci. 2021, 19(1): 09-15.
ISSN (online) 2672-9423 ISSN (print) 2672-9628

IN VITRO ANTIOXIDANT ACTIVITY OF ESSENTIAL OIL FROM CURCUMA PETIOLATA ROXB. RHIZOMES

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Received 19 November 2019; Revised 28 April 2021; Accepted 29 April 2021

Abstract: The hydrodistillated essential oil from the fresh rhizomes of *Curcuma petiolata* was investigated for its chemical constituents and antioxidant activity. Its chemical constituents were analyzed by gas chromatography-mass spectroscopy (GC-MS) technique. The major component of the essential oil from the *C. petiolata* fresh rhizomes was found to be β-farnesene (74.80%). DPPH radical scavenging assay, hydroxyl radical scavenging assay, superoxide anion radical scavenging assay, ferrous ion chelating assay and ferric reducing power assays were used for evaluating its antioxidant activity. *L*-ascorbic acid and ethylenediaminetetraacetic acid (EDTA) were used as a positive control. The essential oil showed high capacities of scavenging DPPH radical, hydroxyl radical, superoxide anion radical, and high capacity of ferric reducing power. The ferrous ion chelating activity of the essential oil was significantly lower than that of EDTA.

Keywords: *Curcuma petiolata*, Zingiberaceae, essential oil, antioxidant activity

INTRODUCTION

Reactive oxygen species (ROS), byproducts of cellular redox processes, include free radicals such as superoxide anion radicals (O_2^-), hydroxyl radicals ($OH^\bullet$) and non-free radical species such as hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$) (Hussein 2011). ROS can generate oxidative stress that is a major cause of chronic and degenerative diseases such as cancer, arthritis, aging, autoimmune disorders, cardiovascular disease and neurodegenerative disease (Pham-Huy *et al.*, 2008).

Naturally occurring antioxidant compounds exhibit their antioxidant activity through various mechanisms including chain breaking by donation of hydrogen atoms or electrons that convert free radicals into more stable species and decomposing lipid peroxides into stable final products (Iqbal *et al*., 2013). Therefore, naturally occurring antioxidant compounds may be used as a preventive agent against several chronic diseases.

Essential oil is one important group of natural products that has been reported to have an antioxidant activity. Examples of the plants possessing high antioxidant activity were *Cinnamodendron dinisii* (Andrade *et al*., 2013), *Mentha longifolia* (Iqbal *et al*., 2013), *Nigella sativa* (Burits and Bucar, 2000), *Origanum rotundifolium* (Goze *et al*., 2009), *Siparuna guianensis* (Andrade *et al*., 2013), *Curcuma aromatica* (Al-Reza, *et al*., 2010) and *Pinus massoniana* (Xie *et al*., 2015).

The rhizomes of *Curcuma* species are reported as rich sources of essential oils which are responsible for their pleasant aroma and medicinal values. Several essential oils from the rhizomes of *Curcuma* species have been also reported for their antioxidant activity such as *C. aeruginosa* (George and Britto, 2015; Theanphong *et al.*, 2015), *C. amada* (Vishnupriya *et al*.,

2012; George *et al*., 2015), *C. aromatica* (Al-Reza, *et al*., 2010; Tsai *et al.*, 2011; Shahwar *et al*., 2012), *C. angustifolia* (Theanphong et al., 2017) *C. caesia* (Devi *et al.*, 2015), *C. longa* (Jayaprakasha *et al*., 2002; Singh *et al*., 2010; Liju *et al*., 2011; Tsai *et al*., 2011; Gounder and Lingamallu, 2012; Shahwar *et al*., 2012), *C. phaeocaulis* (Hou *et al*., 2015), *C. sichuanensis* (Tsai *et al*., 2011) and *C. zedoaria* (Mau *et al*., 2003; Lobo *et al*., 2009; Rahman *et al*., 2014).

C. petiolata, Thai cut flowers, has been used in ethnomedicine for treatment of stomach ache and wound healing (Prasad *et al*., 2008; Samuel *et al*., 2010). However, there are no previously reported on chemical constituent and antioxidant activity of essential oil from the fresh rhizomes of *C. petiolata*. Thus, the aims of this study were to investigate chemical composition of essential oil from the fresh rhizomes of *C. petiolata* and their antioxidant activity.

MATERIALS AND METHODS

Plant material

The fresh rhizomes of *C. petiolata* were collected in November-December 2015 from Prachin Buri province, Thailand. The plant sample was identified by Assist. Prof. Dr. Thaya Jenjittikul (Department of Plant Science, Faculty of Science, Mahidol University, Bangkok, Thailand). The voucher specimen of this plant sample was deposited at College of Pharmacy, Rangsit University, Thailand.

Isolation of essential oil

The fresh rhizomes of *C. petiolata* (350 g) were washed with tap water, air dried (24 hr) and then cut into small pieces. The ground materials were subjected to water-distillation using Clevenger apparatus for 3 hr. Its essential oil was collected and stored at 4°C in an air-tight container for further analysis.

GC-MS Analysis

The essential oil was diluted in ethanol (HPLC grade, Merck, Germany) with the oil to ethanol ratio of 1:25 by volume. GC/MS analysis was performed using an Agilent Technologies 7890A GC system equipped with a 5975C inert XL EI/CI MAD with Triple-Axis detector. The DB-5 MS (phenyl: dimethyl arylene siloxane 5: 95) capillary column (30 m in length, 0.25 mm i.d., and 0.25 μm in thickness) was used as a stationary phase. The carrier gas was helium programmed with the flow rate of 1 ml/min. A 1 ml of the diluted oil was injected using Agilent GC sampler 80 autosampler (splitless). The operating conditions of GC oven were started at 60°C for 1 min, ramped at the rate of 3°C/min to 240°C and hold for 5 min. The GC injector and GC-MSD interface temperatures were set at 180°C and 290°C, respectively. Electron impact ionization positive mode at 70 eV was acquired over the mass range of 40-650 m/z at a scan rate of 2.42 amu / second.

Identification of oil components

Essential oil components were identified by comparing the mass fragmentation pattern of each oil component with those that are available in Adams Essential Oil Mass Spectral library and NIST05 Mass Spectral library. The amount of each oil component was determined on the basis of peak area measurement.

Antioxidant activity

DPPH radical scavenging assay

The DPPH radical scavenging activity of the essential oil was performed according to the method of Sudha *et al*. (2011) with few modifications. A 1 ml of various concentrations of

the essential oil in methanol (1-300 µg/ml) was mixed with 6×10^{-5} M DPPH solution in methanol (1 ml). The reaction mixture was shaken vigorously and left to stand in the dark at room temperature for 30 min. The absorbance of the reaction mixture was measured at 517 nm against the blank. *L*-ascorbic acid was used as a positive control. The percentage of DPPH scavenging was calculated as follows:

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

where A_0 is the absorbance of the control and A_1 is the absorbance of the sample.

Hydroxyl radical scavenging assay

Hydroxyl radical scavenging assay of the essential oil was performed according to the method of Sudha *et al*. (2011) with few modifications. A 1 ml of various concentrations of the essential oil in methanol (1-300 µg/ml) was mixed with 1.5 mM $FeSO_4$ (1 ml), 6 mM H_2O_2 (0.7 ml) and 20 mM sodium salicylate (0.3 ml). The reaction mixture was incubated for 1 hr at 37°C. The absorbance of the reaction mixture was measured at 562 nm against the blank. *L*-ascorbic acid was used as a positive control. The percentage of $OH^{\bullet}$ 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, A_1 is the absorbance of the sample and A_2 is the absorbance without sodium salicylate.

Superoxide anion radical scavenging

The superoxide anion radical scavenging activity of the essential oil was performed according to the method of Hussein (2011) with few modifications. A 1 ml of various concentrations of the essential oil in methanol (1-300 µg/ml) was mixed with 50 µM nitroblue tetrazolium (1 ml), 78 µM NADH (1 ml), 16 mM Tris-HCl buffer pH 8.0 (1 ml) and 10 µM phenazine methosulfate (1 ml). 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, A_1 is the absorbance of the sample and A_2 is the absorbance without phenazine methosulfate.

Ferrous ion chelating assay

The ferrous ion chelating assay of the essential oil was performed according to the method of Sudha *et al*. (2011) with few modifications. 2 ml of various concentrations of the essential oil in methanol (1-300 µg/ml) was mixed with 2 mM $FeCl_2$ (0.05 ml) and 5 mM ferrozine (0.2 ml). The reaction mixture was then shaken vigorously and left at room temperature for 10 min. The absorbance of the reaction mixture was measured at 562 nm against the blank. Ethylenediaminetetraacetic acid (EDTA) was used as a positive control. The percentage of ferrous ion chelating was calculated as follows:

Percent chelating = $[1-(A_0-A_1)/A_0] \times 100\%$

where A_0 is the absorbance of the control and A_1 is the absorbance of the sample.

Ferric reducing power assay

The ferric reducing power assay of the essential oil was performed according to the method of Dey *et al*. (2012) with few modifications. 0.5 ml of various concentrations of the essential oil in methanol (0.05-2 µg/ml) was mixed with phosphate buffer pH 6.6 (0.5 ml) and 0.1% $K_3[Fe(CN)_6]$ (0.5 ml). The reaction mixture was incubated at 50°C for 20 min. After incubation, 10% w/v trichloroacetic acid (0.5 ml) were added to the reaction mixture, and then centrifuged at 200 rpm for 10 min. After that, the upper portion (1 ml) was mixed with distilled water (1 ml) and 0.01% $FeCl_3$ (0.1 ml). The reaction mixture was left for 10 min at room temperature. The absorbance of the reaction mixture was measured at 700 nm against the blank. The increasing absorbance of the reaction-mixture indicates the increased reducing power. *L*-ascorbic acid was used as a positive control.

The essential oil concentration at 0.5 of the absorbance (EC_{50}) was obtained from the graph of absorbance at 700 nm against essential oil concentration.

Statistical analysis

All experiments were performed in triplicate. The experimental results were reported as mean ± SD. An unpaired t-test was used for statistical comparisons ($p < 0.05$).

RESULTS AND DISCUSSION

Chemical constituents of the essential oil

The essential oil obtained from the water distillation of the fresh rhizome of *C. petiolata* was clear and pale yellow oil with the percent yields of 0.18 v/w. Fifteen compounds, corresponding to 99.00 % were identified. The chemical constituents of the essential oil, their peak area percentages and Kovats Indices (KIs) are compiled in Table 1, in order of their elution on the DB-5 MS column.

The rhizome essential oil of *C. petiolata* was rich in sesquiterpene hydrocarbons (80.36%), represented by β-farnesene [11]. The three major components were β-farnesene (74.80%) [11], 1,8-cineole (7.15%) [7], and camphene (4.56%) [3].

Antioxidant activity

The antioxidant activities of the rhizome essential oil of *C. petiolata* were investigated by five different methods including DPPH radical scavenging assay, hydroxyl radical scavenging assay, superoxide anion radical scavenging assay, ferrous ion chelating assay, and ferric reducing power assay. Because of different chemical constituents in rhizome essential oil may exhibit the capability of free radical scavenging with different mechanisms such as donating hydrogen atom or electron to DPPH radical, and chelating metal ion (Brewer, 2011). Therefore, in this study five different *in vitro* models were used for evaluating the free radical scavenging activity of the rhizome essential oil of *C. petiolata.*

Table 2 shows that the rhizome essential oil had strong antioxidant activities against DPPH radicals, hydroxyl radicals, and superoxide anion radicals, as well as high ferric reducing power with insignificantly different EC_{50} values as compared with *L*-ascorbic acid. However, the essential oil showed less ferrous ions chelating activity as compared with EDTA. The EC_{50} values of the essential oil and positive control are shown in Table 2.

Table 1. Essential oil constituents of the fresh rhizome of *C. petiolata*

No.	Chemical constituents	KI[a]	Content(%)
Monoterpene hydrocarbons			
1	Tricyclene	926	0.11
2	α-Pinene	939	0.31
3	Camphene	954	4.56
4	β-Pinene	979	0.12
5	Myrcene	990	2.05
6	2-Carene	995	0.29
Oxygenated monoterpenes			
7	1,8-Cineole	1031	7.15
8	Camphor	1146	3.67
Sesquiterpene hydrocarbons			
9	β-Elemene	1390	2.02
10	Caryophyllene	1419	0.59
11	β-Farnesene	1442	**74.80**
12	Germacrene D	1485	1.72
13	γ-Elemene	-	1.23
Oxygenated sesquiterpenes			
14	Germacrone	1693	0.26
Other			
15	2-Undecanone	1294	0.12
	Compound class		
	Terpenoids		
	- Monoterpene hydrocarbons		7.44
	- Oxygenated monoterpenes		10.82
	- Sesquiterpene hydrocarbons		80.36
	- Oxygenated sesquiterpenes		0.26
	Others		0.12
	Total identified		99.00

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

Table 2. The EC_{50} value of the essential oil hydrodistillated from the fresh rhizomes of *C. petiolata* and positive control

Test	EC_{50} (μg/ml)*	
	Essential oil	**Positive control****
DPPH radical scavenging assay	13.07 ± 2.50^a	10.77 ± 1.97^a
$OH^{\bullet}$ radical scavenging assay	20.54 ± 1.74^a	18.27 ± 1.54^a
Superoxide anion radical scavenging assay	37.84 ± 2.32^a	35.21 ± 1.10^a
Ferrous ion chelating assay	247.97 ± 6.06^a	154.19 ± 2.45^b
Ferric reducing power assay	0.10 ± 0.01^a	0.11 ± 0.02^a

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

** *L*-ascorbic acid was used as a positive control in DPPH radical scavenging assay, $OH^{\bullet}$ radical scavenging assay, Superoxide anion radical scavenging assay and Ferric reducing power assay.
EDTA was used as a 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-value = 0.05).

These present results were similar to those previously reported for *C. aeruginosa* (Theanphong *et al.*, 2015), *C. alismatifolia* (Theanphong and Mingvanish, 2017), and *C. leucorrhiza* (Theanphong *et al.*, 2016). The rhizome essential oils of *C. aeruginosa*, *C alismatifolia* and *C. leucorrhiza* were rich in sesquiterpene hydrocarbons and oxygenated sesquiterpenes and have been reported for their antioxidant activities. The rhizome essential oils of *C. aeruginosa*, *C alismatifolia* and *C. leucorrhiza* showed strong antioxidant activities against DPPH radicals and hydroxyl radicals. In addition, the all rhizome essential oils showed high ferric reducing power.

From the results, it might be concluded that the strong antioxidant activity of rhizome essential oil may due to high content of sesquiterpenes (Khan *et al*., 2008)

CONCLUSION

The essential oil hydrodiltillated from the fresh rhizome of *C. petiolata* might be used as a potential source of natural antioxidant. Thus, it is value added for cut flowers in the genus *Curcuma*. Other biological activities and toxicities of the rhizome essential oil of *C. petiolata* should be further evaluated.

ACKNOWLEDGMENTS

The authors would like to acknowledge the Research Institute of Rangsit University for financial support (Grant no. 28/2559).

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