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Research Article

Chemical constituents and antioxidant activities of essential oils from roots and rhizomes of *Curcuma alismatifolia* Gagnap. from Thailand

Orawan Theanphong[1*] and Withawat Mingvanish[2]

[1]Department of Pharmacognosy, Faculty of Pharmacy, Rangsit University, Pathumthani, Thailand
[2]Department of Chemistry, Faculty of Science, King Mongkut's University of Technology Thonburi, Bangkok, Thailand
***E-mail**: theanphong@gmail.com

Abstract

The essential oils hydrodistilled from roots and rhizomes of *Curcuma alismatifolia* Gagnap. from Thailand were analyzed by GC-MS technique. (-)-Xanthorrhizol (52.37%) and β-curcumene (42.00%) were found to be the major components in the root and rhizome essential oils, respectively. Antioxidant activities of both essential oils were evaluated using five different methods including DPPH radical scavenging assay, hydroxyl radical scavenging assay, hydrogen peroxide scavenging assay, ferrous ion chelating assay and ferric reducing power assay. *L*-ascorbic acid and EDTA were used as a positive control. The DPPH radical scavenging activity and ferric reducing power of the root and rhizome essential oils were insignificantly different as compared with *L*-ascorbic acid while the hydroxyl radical scavenging activity of the rhizome essential oil was significantly higher than those for the root essential oil and *L*-ascorbic acid. The root and rhizome essential oils exhibited weaker activities for scavenging hydrogen peroxide molecules and chelating ferrous ions, as compared with *L*-ascorbic acid and EDTA, respectively.

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

Introduction

Free radicals, molecules or molecular fragments containing one or more unpaired electrons, were by-products from mitochondrial electron transportation processes that have been reported for the major cause of chronic and degenerative diseases including cancer, arthritis, aging, autoimmune disorders, cardiovascular and neurodegenerative diseases (Pham-Huy et al., 2008; Lone et al., 2013; Sisein, 2014). To eliminate free radicals, many synthetic substances and naturally-occurring substances are used. Essential oils, one group of naturally-occurrinng substances, have been found to have antioxidative properties. Essentail oils extracted from *Cananga odorata* (Sacchetti et al., 2005), *Eugenia caryophyllata* (Chaieb et al., 2007), *Nigella sativa* (Burits & Bucar, 2000), *Origanum rotundifolium* (Goze et al., 2009), *Thymus vulgaris* (Sacchetti et al., 2005), *Curcuma longa* (Sacchetti et al., 2005; Singh, et al., 2010) and *Zingiber officinale* (Sacchetti et al., 2005) have been exemplified as naturaly-occurring substances having strong antioxidant activity.

C. alismatifolia or Siam tulip (Figure 1.) is an ornamental plant that is cultivated for cut flower industry. However, there has been no report on the antioxidant activities of the essential oils from the fresh roots and rhizomes of *C. alismatifolia*. Thus, the aims of this study were to investigate chemical compositions of the essential oils from the fresh roots and rhizomes of *C. alismatifolia* and to evaluate antioxidant activities of essential oils by five different methods.

Figure 1. Morphological characteristics of *C. alismatifolia*
A: Inflorescence; B: Root and rhizome

Materials and methods

Plant materials

The fresh roots and rhizomes of *C. alismatifolia* were collected from Prachin Buri Province, Thailand in December, 2014. The plant samples were identified by Asst. Prof. Dr. Thaya Jenjittikul, Faculty of Science, Mahidol University, Thailand, and the voucher specimens (RSU 0027) were deposited at Faculty of Pharmacy, Rangsit University, Thailand.

Preparation of essential oil extracts

The essential oils of the fresh roots and rhizomes of *C. alismatifolia* (300 g) were obtained by water-distillation using Clevenger apparatus for 3 h. The obtained essential oils were collected and stored at 4 ˚C in air-tight container prior to being analyzed by GC-MS technique.

GC-MS Analysis

The chemical constituents of the essential oils were analyzed by GC-MS, using an Agilent Technologies 7890A GC system equipped with a 5975C inert XL EI/CI MAD with Triple-Axis detector. The GC column was a DB-5 MS capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness). The carrier gas was helium with the flow rate of 1 ml/min. The GC oven temperatures were programmed from being held at 60 ˚C for 1 min, ramped at the heating rate of 3 ˚C/min to 240 ˚C and held for 5 min at the end of GC-MS analysis. 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 *m/z* 40-650 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 on the basis of peak area measurement.

Antioxidant activities

DPPH radical scavenging assay

The DPPH radical scavenging activity of the essential oils was evaluated according to the method of Sudha et al. (2011) with few modifications. The essential oil in methanol (1 ml) was mixed with 0.2 mM 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. *L*-ascorbic acid was used as a positive control. The percentage of DPPH 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 DPPH.

Hydroxyl radical scavenging assay

Hydroxyl radical scavenging activity of the essential oils was evaluated according to the method of Sudha et al. (2011) with few modifications. The essential oil in methanol (1 ml) was mixed with 1.5 mM $FeSO_4$ (1 ml), 6 mM hydrogen peroxide (0.7 ml) and 20 mM sodium salicylate (0.3 ml). The reaction mixture was incubated at 37 °C for 1 h. The absorbance of the reaction mixture was measured at 562 nm. *L*-ascorbic acid was used as a positive control. 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 without sodium salicylate.

Hydrogen peroxide scavenging assay

Hydrogen peroxide scavenging activity of the essential oils was evaluated according to the method of Keser et al. (2012) with few modifications. The essential oil in methanol (0.4 ml) was mixed with 40 mM hydrogen peroxide in phosphate buffer pH 7.4 (0.6 ml). 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 H_2O_2 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 hydrogen peroxide.

Ferrous ion chelating assay

Ferrous ion chelating activity of the essential oils was evaluated according to the method of Sudha et al. (2011) with few modifications. The essential oil in methanol (2 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

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mixture was measured at 562 nm. Ethylenediaminetetraacetic acid (EDTA) was used as a positive control. 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 without ferrozine.

Ferric reducing power assay

Ferric reducing power activity of the essential oils was evaluated according to the method of Dey et al. (2012) with few modifications. The essential oil in methanol (0.5 ml) was mixed with phosphate buffer pH 6.6 (0.5 ml) and 0.1% $K_4[Fe(CN)_6]$ (0.5 ml). The reaction mixture was incubated at 50 °C for 20 min. After incubation, 10% w/v trichloroacetic acid (0.5 ml) was added to the reaction mixture and centrifuged at 200 rpm for 10 min. After that, the supernatant (1 ml) was mixed with distilled water (1 ml) and 0.01% $FeCl_3$ (0.1 ml). 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 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 numeric results were reported as mean ± SD. The Duncan test and a one-way analysis of variance (ANOVA) were used for multiple comparisons (SPSS Statistics version 18).

Results and discussion

Chemical components of the essential oils

Hydrodistillation of the fresh roots and rhizomes of *C. alismatifolia* yielded clear and bright yellow essential oils with the percent yields of 0.10 and 0.12% v/w, respectively. Twenty-three compounds, corresponding to 98.84%, were identified in the root essential oil while eighteen compounds, corresponding to 98.77%, were identified for the rhizome essential oil. The three major components of the root essential oil were (-)-xanthorrhizol (52.37%), *ar*-curcumene (27.44%) and α-cedrene (4.08%) whereas the three major components of the rhizome essential oil were β-curcumene (42.00%), (-)-xanthorrhixol (36.59%) and α-curcumene (7.49%).

The experimental results were in agreement with the research works previously reported. (-)-Xanthorrhixol and β-curcumene were reported as the major components in essential oils from *C. xanthorrhiza* (Jantan et al., 1999) and *C. aromatica* (Kojima et al., 1998), respectively. In addition, the results showed that the root and rhizome essential oils of *C. alismatifolia* are a promising source of (-)-xanthorrhixol, that has various biological activities including antioxidant, anti-inflammatory, antibacterial, neuroprotective, nephroprotective, hepatoprotective and estrogenic activities (Oon et al., 2015).

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Antioxidant activities

The antioxidative capabilities of the essential oils of the fresh roots and rhizomes of *C. alismatifolia* were investigated by five different methods including DPPH radical scavenging assay, hydroxyl radical scavenging assay, hydrogen peroxide scavenging assay, ferrous ion chelating assay and ferric reducing power assay. The root and rhizome essential oils showed DPPH radical scavenging and ferric reducing power activities insignificantly different with *L*-ascorbic acid. The hydroxyl radicals scavenging activity of the rhizome essential oil was significantly higher than those of the root essential oil and *L*-ascorbic acid. It was found that the effectiveness of scavenging hydroxyl radicals was in the decreasing order: rhizome essential oil > root essential oil = *L*-ascorbic acid. The root and rhizome essential oils exhibited weaker activities for scavenging hydrogen peroxide molecules and chelating ferrous ions, as compared with *L*-ascorbic acid and EDTA, respectively. The EC_{50} values of the root and rhizome essential oils and positive control were shown in Table 1. The results were similar to those previously reported. Antioxidant activities of some essential oils from rhizomes of *Curcuma* species such as *C. aeruginosa* (George & Britto, 2015), *C. amada* (Tamta et al., 2016), *C. aromatic* (Al-Reza et al., 2010), *C. longa* (Sacchetti et al., 2005; Singh et al., 2010) and *C. zedoaria* (Rahman et al., 2014) have been reported.

Table 1. The EC_{50} values of the essential oils from the fresh roots and rhizomes of *C. alismatifolia* and positive controls.

Test	EC_{50} (μg/ml)*		
	Root essential oil	**Rhizome essential oil**	**Positive control****
DPPH radical scavenging assay	10.20 ± 0.94^a	11.48 ± 1.02^a	10.77 ± 0.97^a
OH Scavenging assay	18.04 ± 1.72^a	11.39 ± 1.44^b	18.27 ± 1.54^a
H_2O_2 Scavenging activity assay	31.82 ± 1.68^a	39.92 ± 1.49^b	11.04 ± 1.03^c
Ferrous ion chelating assay	180.28 ± 3.26^a	182.99 ± 5.49^a	154.19 ± 2.45^b
Ferric reducing power assay	0.12 ± 0.03^a	0.12 ± 0.02^a	0.11 ± 0.01^a

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

** *L*-ascorbic acid was use as a positive control for DPPH radical scavenging assay, OH Scavenging assay, H_2O_2 Scavenging activity assay and Ferric reducing power assay. EDTA was use as a positive control for 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

This is the first report on the chemical compositions of the essential oils from the fresh roots and rhizomes of *C. alismatifolia*. It was found that the root and rhizome essential oils of *C. alismatifolia* obtained from hydrodistillation were rich in (-)-xanthorrhixol and β-curcumene, respectively. As a result, these data might be used as additional information for phytochemical and chemotaxonomic studies of the genus *Curcuma*. In addition, it might be used as an alternative source of xanthorrhixol as well as a potential source of natural antioxidant, food supplements and pharmaceutical applications. Thus, it is value added for this plant.

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