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In vitro antioxidant activities from the rhizome of *Elettariopsis wandokthong* Picheans. & Yupparach

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Introduction

Free radicals, atoms or molecules with unpaired electrons, are by-products from mitochondrial electron transport process and they have been reported to involve in several chronic and degenerative diseases such as cardiovascular disease, neurodegenerative disorders, autoimmune disorders and cancer[1-4]. Antioxidants are any substance that delays, prevents or removes oxidative damage to a target molecule[5]. Several essential oils from Zingiberaceous plants such as *Alpinia calcarata*[6], *Curcuma aeruginosa*[7], *Elettariopsis curtisii*[8], *Kaempferia parviflora*[9] and *Zingiber officinale*[10] have been reported for their antioxidant activity. In addition, the crude extracts from the rhizomes of *A. galanga* [11,12], *C. longa*[13], *Hedychium coronarium*[14], *K. rotunda*[12], and *Z. officinale*[13] were also reported for their capacity against free radicals.

E. wandokthong or Wan Dokthong or Wan Maha Saneh (Zingiberaceae) is perennial herb with creeping slender rhizome bearing pseudostem at intervals. From the previous study, we investigated the chemical compositions of the essential oil from the fresh rhizome of *E. wandokthong*[15]. The results showed that monoterpenes, i.e. camphene (22.51 %), fenchyl acetate (9.11 %) and 1,8-cineole (8.12 %), were found as three major components in the fresh rhizome essential oil of *E.* wandokthong[15]. In this study, the antioxidant activities of essential oil and crude ethanolic extract from rhizome of *E. wandokthong* were studied.

Methods

Plant material

The fresh rhizomes of *E. wandokthong* were collected from the botanical garden at Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand in May 2017. They were identified by comparison with the related literature[16] and were also confirmed by Assoc. Prof. Thatree Phadungcharoen and Asst. Prof. Dr. Thaya Jenjittikul.

Isolation of essential oil

The fresh rhizomes of *E. wandokthong* (500 g) were washed with tap water, air dried and then blended into small pieces with the blender. The ground rhizomes were subjected to water distillation using Clevenger apparatus for 4 hrs.

Extraction procedure

The plant material was washed with tap water, air dried and ground into fine powder. The crude ethanolic extract was prepared by maceration of ground sample (250 g) with ethanol (500 ml) on orbital

shaker for 3 days (3 times). The ethanolic extract was filtered and concentrated using rotary evaporator to obtain the crude ethanolic extract.

Preliminary phytochemical screening

Preliminary phytochemical screening of the crude ethanolic extracts was performed by following the methods of Evans[17] and Tiwari[18].

Antioxidant activity

The antioxidant activities of the essential oil and crude ethanolic extract were evaluated by DPPH radical scavenging[19], hydroxyl radical scavenging[19], ferrous ion chelating[19], superoxide anion radical scavenging[20] and ferric reducing power assays[21]. *L*-ascorbic acid and ethylenediaminetetraacetic acid (EDTA) were used as a positive control.

Statistical analysis

All experiments were performed in triplicate. The experimental results were reported as mean ± SD. Data analyses were performed using SPSS software version 18, Duncan multiple range test at $p < 0.05$ probability level.

Results

The essential oil hydrodistillated from the fresh rhizomes of *E. wandokthong* was clear and bright yellow oil with the percent yield of 0.40 % v/w while the crude ethanolic extract was dark yellow viscous liquid with the percent yield of 0.22 %w/w. The preliminary phytochemical screening of the crude ethanolic extract revealed the presence of flavonoids. The DPPH radical scavenging activity, hydroxyl radical scavenging activity and superoxide anion radical scavenging activity of the essential oil and crude ethanolic extract were found to be insignificantly different as compared with those of *L*-ascorbic acid. The EC_{50} values of the essential oil, crude ethanolic extract and positive control were shown in Table 1. The essential oil exhibited strong ferric reducing power with the EC_{50} value of 0.10 ± 0.01 μg/ml while the crude ethanolic extract exhibited weaker ferric reducing power than the essential oil with the EC_{50} value of 0.17 ± 0.02 μg/ml. Moreover, the ferric reducing power of essential oil was found to be insignificantly different as compared with that of *L*-ascorbic acid. The ferrous ion chelating activity of the essential oil and crude ethanolic extract was less than that of EDTA.

Table 1. The EC_{50} values of the essential oil and crude ethanolic extract from the fresh rhizomes of *E. wandokthong*

	EC_{50} (μg/ml)*		
	Essential oil	Crude ethanolic ext.	Positive control**
DPPH radical scavenging assay	14.11 ± 1.70[a]	14.52 ± 1.07[a]	14.77 ± 1.68[a]
OH scavenging assay	18.99 ± 1.43[a]	21.34 ± 1.12[a]	20.05 ± 1.45[a]
Superoxide anion radical	32.41 ± 2.07[a]	34.16 ± 1.78[a]	33.89 ± 1.71[a]
Ferrous ion chelating assay	313.22 ± 8.39[a]	276.20 ± 5.84[b]	161.50 ± 3.56[c]
Ferric reducing power assay	0.10 ± 0.01[a]	0.17 ± 0.02[b]	0.10 ± 0.01[a]

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

** *L*-ascorbic acid was used as a positive control in DPPH radical scavenging assay, OH scavenging assay, Superoxide anion radical scavenging activity 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 ($P < 0.05$).

Discussion

The results obtained from preliminary phytochemical screening of the crude ethanolic extract were in agreement with the works previous reported[8,22]. Chan, Lim and Lim (2007) reported the presence of phenolic compounds from the rhizomes of *E. slahmong*[22] and Chairgulprasert *et al*., 2008 reported the presence of phenolic compounds from the rhizomes of *E. curtisii*[8]. As seen in Table 1. the results indicated that the essential oil and crude ethanolic extract from the fresh rhizomes of *E. wandokthong* exhibited strong antioxidant activity against scavenging DPPH, hydroxyl and superoxide anion radicles. From previously

reports, the essential oil and crude extract from the rhizomes of *E. curtisii* and the crude extract from the rhizomes of *E. slahmong* have been reported for their antioxidant activity[8,22]. In addition, the strong antioxidant activity of the essential oil and crude ethanolic extract may be attributed to the presence of monoterpenoids and flavonoids which have been reported for antioxidant activity, respectively[23-25].

Conclusion

In this study, various antioxidant activities of the essential oil and crude ethanolic extract were investidated. The results showed that the essential oil and crude ethanolic extract possess strong antioxidant activities. It might be concluded that the rhizome of *E. wandokthong* was the potential source for natural antioxidant.

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