

EVALUATION OF ANTIRADICAL SCAVENGER ACTIVITY OF EXTRACT AND COMPOUNDS FROM
ETLINGERA CALOPHRYS STEMSSAHIDIN I^{1*}, WAHYUNI¹, MUH. HAJRUL MALAKA¹, JABBAR A¹, IMRAN², MARIANTI A MANGGAU³

¹Department of Pharmacy, Faculty of Pharmacy, Universitas Halu Oleo, Kendari 93232, Indonesia. ²Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Halu Oleo, Kendari 93232, Indonesia. ³Department of Pharmacy, Faculty of Pharmacy, Universitas Hasanuddin, Makassar 90245, Indonesia. Email: sahidin02@yahoo.com

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ABSTRACT

Objective: This study was aimed to isolate and identify the chemical compounds from the methanol extract of the stems of *Etingera calophrys* as well as to evaluate their potential as free radical scavengers.

Methods: The isolated compounds were obtained using vacuum liquid chromatography (VLC) and radial chromatography techniques. The structures of these compounds were determined on the basis of their measured spectroscopic evidence (infrared, one dimensional (1D), and 2D nuclear magnetic resonance) and by comparison with literature data. The free radical scavenging activity of the methanol extract of the stems and the isolated compounds were measured using qualitative and quantitative 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays.

Results: Yakuchinone A (1), *p*-hydroxybenzoic acid (2), and stigmasterol (3) were successfully isolated and identified from the stems of *E. calophrys*. The methanol extract and compounds 1-3 showed free radical scavenging activity with half maximal inhibitory concentration (IC₅₀) values of 38.36, 39.07, 46.60, and 153.4 µg/mL, respectively, as compared to ascorbic acid with an IC₅₀ value of 10.53 µg/mL.

Conclusions: Yakuchinone A (1) and *p*-hydroxybenzoic acid (2) are first reported from the genus *Etingera*. The presence of these compounds may contribute to the free radical scavenging activity of the methanol extract of the stems of *E. calophrys*. This finding suggested that *E. calophrys* may serve as a source of natural antioxidant compounds and could be used in the development of antioxidant herbal preparation.

Keywords: *Etingera calophrys*, Zingiberaceae, Stem extract, Compounds, 2,2-diphenyl-1-picrylhydrazyl.

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INTRODUCTION

As part of continuing study on traditional medicinal plants, we investigated the chemistry and pharmacological aspects of *Etingera* species. Our previous study included plants species from Dipterocarpaceae [1-4], *Jatropha* [5-7], Annonaceae [8], *Pongamia* [9], *Imperata* [10], *Polygonum* [11,12], and *Dillenia* [13].

Zingiberaceae is one of the common plants in Indonesia used as traditional medicines [14]. The genus *Etingera* belongs to the Zingiberaceae family and contains approximately 150-200 species of worldwide distribution. Of these, as many as 54 species of this genus have been recorded in Indonesia, including 48 species from Sulawesi, and 6 species from Java [15]. Species of this genus have been used in medicinal folklore to treat various ailments, and the presence of the volatile and non-volatile entities in these species has gained research interests among scientists.

Previous studies revealed the presence of phenylpropanoids, flavonoids, and phytosterols in the species of *Etingera*. The leaves of *Etingera elatior* produced quinic acid-containing cinnamic acid derivatives, including 3-*O*-caffeoylquinic acid, 5-*O*-caffeoylquinic acid (chlorogenic acid), and 5-*O*-caffeoylquinic acid methyl ester [16]. In addition, its leaves also contained kaempferol-3-glucuronide, quercetin-3-glucuronide, quercetin-3-glucoside, and quercetin-3-rhamnoside [17]. Moreover, leaves and rhizomes of *Etingera brevilabrum* and *Etingera sphaerocephala* var. *grandiflora* produced β -sitosterol and stigmasterol [18,19]. The latter species also yielded a simple phenolic paeonol [19].

Different parts of *Etingera* species also have proven to have promising biological activities. Leaves and stems of *E. brevilabrum* exhibited anticholesterol activity [18], while the leaves and rhizomes of *E. elatior* performed antioxidant, antibacterial, and tyrosinase inhibitory activity [17,20-25]. Antibacterial and antioxidant activities also exhibited by the leaves extract of *E. fulgens* [20]. Other studies revealed the potency of *E. littoralis* rhizomes and *E. maingayi* leaves as antibacterial agents [26]. Moreover, essential oil of *Etingera fenzlii* (Kurz) K. Schaum was safe for repellent source [27].

Species *Etingera calophrys* (K. Schum) A. D. Poulsen is an endemic plant species from Sulawesi Tenggara [15]. To our knowledge, the chemistry and pharmacology aspects of this plant have not been investigated thus far, requiring more research. Hence, the present work will facilitate and report the isolation and identification of chemical compounds from the methanol extract of *E. calophrys* stems, as well as their antioxidant activity based on the capacity to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals.

MATERIALS AND METHODS

General procedures

Instruments were used Cary Varian 100 Conc. UV spectrophotometer, PerkinElmer Spectrum One fourier transform-IR spectrophotometer, and JEOL ECP 500 nuclear magnetic resonance (NMR) spectrometer (500 MHz for ¹H and 125 MHz for ¹³C). Chromatography techniques were performed using Kieselgel 60 F₂₅₄, 0,25 mm, silica gel 60 GF₂₅₄, and silica 60 g (Merck, Darmstadt, Germany). Thin-layer chromatography (TLC) plates were derivatized using a cerium sulfate reagent (Merck,

Darmstadt, Germany). DPPH was purchased from Merck (Darmstadt, Germany).

Sample

Stems of *E. calophrys* were collected from the Wolasi Forest, South Konawe, Sulawesi Tenggara, in April 2016. The plant specimen was identified and stored in the Herbarium Bogoriense, Indonesia.

Extraction and isolation

The dried powdered stems of *E. calophrys* (3kg, 230–270 mesh) were macerated with methanol (MeOH, 3 × 7.5 L, 24 h each time) at room temperature and yielded a dried methanol extract as a dark green gum (150 g). This extract was further fractionated using a silica gel vacuum liquid chromatography (VLC) (10 × 5 cm, 150 g), eluted with *n*-hexane-ethyl acetate (from 8:2 to 0:10) followed by pure MeOH, and gave 5 main fractions (F1-F5) with weight of 1.1, 5.0, 10.3, 10.2, and 58.4 g, respectively. Main fraction F2 was re-fractionated using a silica gel VLC (5 × 5 cm, 50 g) and gradient eluted with *n*-hexane-ethylacetate (from 7:3 to 0:10) and MeOH as mobile phases, to yield subfractions F21 (0.2 g), F22 (1.0 g), F23 (0.8 g), and F24 (1.2 g). Subfraction F23 was chromatographed using a silica gel radial chromatography (RC) with chloroform–MeOH (95:5) and pure MeOH as mobile phases, to produce pure compound 1 (0.2 g). Furthermore, the main fraction F3 was subjected to a silica gel VLC (10 × 5 cm, 150 g), separated gradient with *n*-hexane-ethyl acetate (from 6:4 to 0.10) followed by pure MeOH, and afforded subfractions F31 (0.3 g), F32 (1.2 g), F33 (1.8 g), F34 (1.2 g), and F35 (3.4 g). Subfraction F33 was further purified using silica gel RC with elution of *n*-hexane-ethyl acetate (85:15) followed by pure MeOH, to give pure compound 2 (0.1 g). Subfraction F34 was also purified using silica gel RC, separated using a mixture of *n*-hexane-ethyl acetate (75:25) and MeOH, and yielded pure compound 3 (0.05 g).

Radical scavenger activity

The antioxidant activity of methanol extract and isolated compounds from *E. calophrys* was evaluated using qualitative and quantitative DPPH methods [28,29] with slight modifications. For qualitative analysis, sample was applied on a TLC plate within the concentration range of 0.1–100 µg, developed using a suitable mobile phase and dried at room temperature. The dried plate was then sprayed with DPPH solution (0.2% in MeOH) and incubated for 30 min in the dark at room temperature. The observation of active samples was made based on the intensity of yellow spots against the purple background on the plate. Furthermore, for quantitative analysis, triplicate 1 mL aliquots of samples were mixed with 1 mL of DPPH solution (500 µM; 0.2 mg/mL) and incubated for 30 min in the dark at room temperature. The absorption was measured spectrophotometrically at 517 nm against methanol as a blank. The amount of sample at which the absorbance at 517 nm decreased a half from the initial value was determined as the half maximal inhibitory concentration (IC₅₀) value, expressed in µg/mL. Ascorbic acid was used as positive control.

RESULTS

Physicochemical property and spectroscopic data of the isolated compounds from *E. calophrys*

Three chemical compounds (1–3) were successfully isolated and identified from the methanol extract of *E. calophrys* stems. Structures of these compounds were determined based on their physicochemical property and spectroscopic spectra of IR and NMR. These values were also compared with those reported in the previous studies.

Yakuchinone A (1); yellow oil; ¹HNMR (500MHz, CDCl₃) δ_H (ppm): 7.28 (m, 2H, H-2"/H-6"), 7.18 (m, 3H, H-3"/H-4"/H-5"), 6.84 (m, 3H, H-5"), 6.67 (dd, *J* = 4.06 Hz, 2H, H-2"/H-6"), 5.62 (s, 1H, OH), 3.84 (s, 3H, OCH₃), 2.83 (t, *J* = 7.45 Hz, 2H, H-1), 2.69 (t, *J* = 7.45 Hz, 2H, H-2), 2.61 (t, *J* = 7.1 Hz, 2H, H-7), 2.41 (t, *J* = 6.83 Hz, 2H, H-4), 1.61 (t, *J* = 3.58 Hz, 4H, H-5/H-6); ¹³CNMR (125MHz, CDCl₃) δ_C (ppm): 210.5 (C-3), 146.5 (C-3'), 144.0 (C-4'), 142.3 (C-1"), 133.8 (C-1'), 128.5 (C-3"/C-5"), 125.9 (C-4"), 128.4 (C-2"/C-6"), 120.9 (C-6'), 114.5 (C-5'), 111.2 (C-2'), 55.9 (OCH₃),

44.7 (C-2), 43.0 (C-4), 35.8 (C-7), 31.1 (C-6), 29.6 (C-1), 23.5 (C-5). NMR spectral data were identical to those given in Chacha *et al.* [28].

p-Hydroxybenzoic acid (2); white amorphous powder; ¹HNMR (500MHz, CDCl₃)δ_H (ppm): 7.91 (d, *J* = 8.4 Hz, 2H, H-2/H-6), 6.91 (d, *J* = 8.4 Hz, 2H, H-3/H-5); ¹³CNMR (125MHz, CDCl₃) δ_C (ppm): 167.6 (C-7), 162.7 (C-4), 132.6 (C-2/C-6), 122.6 (C-1), 115.9 (C-3/C-6). NMR spectral data were identical to those given in Fidrianni *et al.* [29].

Stigmasterol (3); white needle crystal; mp: 169–171; ¹HNMR (500MHz, CDCl₃)δ_H (ppm): 5.35 (br d, 1H, H-6), 5.15 (dd, *J* = 15 Hz, H-22), 5.02 (dd, *J* = 15Hz, 1H, H-23), 3.35 (m, 1H, H-3), 2.27 (m, 1H, H-4a), 2.22 (m, 1H, H-4b), 2.02 (m, 1H, H-12), 1.97 (m, 1H, H-20), 1.95 (m, 1H, H-2a), 1.93 (m, 2H, H-7), 1.85 (m, 1H, H-2b), 1.82 (m, 1H, H-1^a), 1.66 (m, 1H, H-25), 1.54 (m, 2H, H-15), 1.49 (m, 1H, H-8), 1.47 (m, 2H, H-11), 1.44 (m, 2H, H-28), 1.27 (m, 1H, H-16), 1.15 (m, 1H, H-1b), 1.08 (m, 1H, H-17), 1.00 (br s, 3H, H-21), 1.00 (br s, 3H, H-19), 1.00 (br s, 1H, H-26a), 0.97 (m, 1H, H-14), 0.91 (m, 1H, H-24), 0.91 (br d, 1H, H-9), 0.91 (br d, 1H, H-27a), 0.84 (br d, 1H, H-29a), 0.84 (br d, 1H, H-18a), 0.81 (br d, 2H, H-26b), 0.81 (br d, 1H, H-27b), 0.79 (br d, 1H, H-29b), 0.79 (br d, 1H, H-18b), 0.69 (br s, 1H, H-27c), 0.67 (br s, 1H, H-29c), 0.67 (br s, 1H, H-18c); ¹³CNMR (125MHz, CDCl₃) δ_C (ppm): 141.9 (C-5), 138.5 (C-22), 129.4 (C-23), 121.9 (C-6), 71.9 (C-3), 56.9 (C-14), 56.2 (C-17), 51.4 (C-24), 50.3 (C-9), 42.5 (C-4/C-13), 40.7 (C-20), 39.9 (C-12), 37.4 (C-1), 36.7 (C-10), 32.1 (C-7/C-8), 31.8 (C-2), 31.1 (C-25), 28.4 (C-16), 26.3 (C-28), 24.4 (C-15), 21.3 (C-19/C-21), 21.2 (C-11), 19.2 (C-26), 19.0 (C-27), 12.2 (C-29), 12.0 (C-18). NMR spectral data were identical to those given in Yahya *et al.* [19].

Free radical scavenging activity

Table 1 summarizes the DPPH free radical scavenging activity of methanol extract and isolated compounds from *E. calophrys* stems. The lowest IC₅₀ was achieved by the methanol extract followed by yakuchinone A (1), *p*-hydroxybenzoic acid (2), and stigmasterol (3).

DISCUSSION

Purification of the methanol extract of *E. calophrys* stems using silica gel chromatography techniques resulted in the isolation of yakuchinone A (1), *p*-hydroxybenzoic acid (2), and stigmasterol (3). To our knowledge, the presence of the three compounds in *E. calophrys* was reported for the first time in the present work. Compound 1, classified as a diarylheptanoid was first reported from the fruits of *Alpinia oxyphylla* (*Zingiberaceae*) [30]. The presence of this compound in *E. calophrys* is considered unique since its occurrence in other investigated *Etlingera* species has not been recorded. Meanwhile, compound 2 is one of the common phenolic acids that can be found in most plant species. This compound is present in lignins structures that are components of plant cell walls [31]. Similarly, compound 3 is a common steroid found in plants and has been reported from *E. brevilabrum* leaves and rhizomes [18] and *E. sphaerocephala* var. *Glandiflora* rhizomes [19]. Structures of these compounds are displayed in Fig. 1.

Methanol extract of *E. calophrys* stems exhibited significant free radical scavenging activity as compared to ascorbic acid as positive control. Compounds 1–3 isolated from this extract also performed scavenging activity toward DPPH radicals. However, their activities

Table 1: IC₅₀ values of methanol extract and compounds 1–3 toward DPPH radical

Sample	IC ₅₀ (µg/mL)
Methanol extract	38.36
Yakuchinone A (1)	39.07
<i>p</i> -Hydroxyl benzoic acid (2)	46.60
Stigmasterol (3)	153.4
Ascorbic acid (positive control)	10.53

IC₅₀: Half maximal inhibitory concentration, DPPH: 2,2-diphenyl-1-picrylhydrazyl

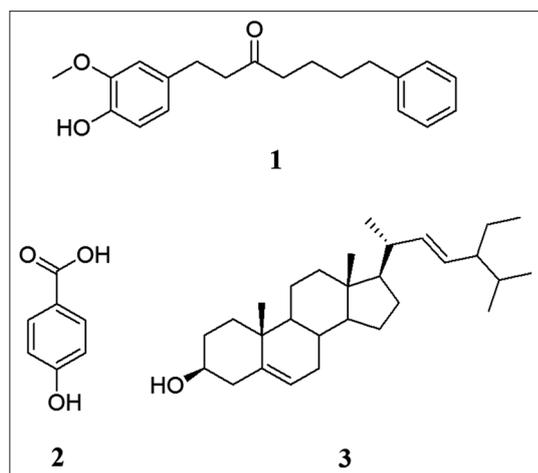


Fig. 1: Structures of compounds 1-3

were considered lower than the methanol extract, indicating by high IC_{50} values (Table 1). This also indicated that there may be other entities in the methanol extract that synergistically enhanced the scavenging activity. Nevertheless, yakuchinone A (1) showed potent scavenging activity among the isolated compounds, in agreement with the previous study [32]. Hence, this compound could be developed as natural antioxidant agent. Structurally, compound 1 possessed two aromatic units that may play important role to scavenge DPPH radicals by producing DPPH-H as by-product [33]. Meanwhile, compound 2 has one aromatic unit in which its scavenging activity was lower than compound 1. In contrast, compound 3 showed very weak free radical scavenging activity, in agreement with our previous study [11]. The presence of this compound in the methanol extract of *E. calophrys* stems may contribute antagonistically in reducing the scavenging activity.

CONCLUSION

Three secondary metabolites were isolated from the methanol extract of *E. calophrys* stems, including yakuchinone A, *p*-hydroxybenzoic acid, and stigmasterol. Of these, yakuchinone A and *p*-hydroxybenzoic acid are first isolated from the genus *Etingera*. On biological activities, potency of the crude methanol extracts as an antiradical agent was supported by the activity of isolated compounds. The methanol extract and isolated compounds from this plant, in particular, yakuchinone A and *p*-hydroxybenzoic acid could be used in the development of natural antioxidant agents.

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AUTHORS CONTRIBUTION

Sahidin I: Isolation and structure determination of all compounds; Manuscript writing, Wahyuni: Radical scavenger evaluation, Muh. Hajrul Malaka: Sample collection; Structure determination, Jabbar A: Radical scavenger evaluation; Sample collection, Imran: Sample determination; Isolation of compounds, Marianti A. Manggau: Manuscript writing; Radical scavenger evaluation.

CONFLICT OF INTEREST

I certify that I have participated sufficiently in the conception and design of this work entitled "EVALUATION OF ANTIRADICAL SCAVENGER ACTIVITY OF EXTRACT AND COMPOUNDS FROM ETLINGERA CALOPHRYS STEMS" and the analysis of the data, as well as writing of this manuscript, to take public responsibility for it. I believe the manuscript represents valid work. I have reviewed the final version

of manuscript and approve it for publication. The manuscript is not published elsewhere, in any language, and is not under simultaneous consideration by any other journal. All Authors agree that the contents of the manuscript are confidential and will not be copyrighted, submitted, or published elsewhere (including the Internet), in any language, while acceptance by the Journal is under consideration. Furthermore I attest that I shall produce the data upon which the manuscript is based for examination by the editors or their assignees, if requested.

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