

# Antimalarial activity of Cratoxyarborenone E, a prenylated xanthone, isolated from the leaves of *Cratoxylum glaucum* Korth

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## Abstract

This research aims to discover active compounds from the leaf extract from *Cratoxylum glaucum* using the bioassay-guided isolation method. The multistage extraction of *C. glaucum* leaves was conducted using n-hexane, dichloromethane, and methanol. The LDH assay was employed to analyze antimalarial activity, and the Resazurin assay was used to measure cytotoxicity values. The structure of the active isolates was determined using spectroscopic techniques. The compound 1 was successfully isolated from the dichloromethane extract of *C. glaucum* leaves (Cg.FD) and confirmed as a prenylated xanthone namely cratoxyarborenone E. The in vitro antimalarial activity showed an  $IC_{50}$  value of  $5.82 \pm 0.04 \mu\text{M}$ , the cytotoxicity assay exhibited a  $CC_{50}$  value of  $20.74 \pm 0.04 \mu\text{M}$ , and the SI value was determined to be 3.56. Our research demonstrates that cratoxyarborenone E was first reported from Cg.FD and shows promise as a prospective candidate for new antimalarial drugs.

## Keywords

antimalarial, *Cratoxylum glaucum*, prenylated xanthone, LDH assay, *Plasmodium falciparum*

## Introduction

Malaria, a disease caused by parasites of the *Plasmodium* species, is spread to humans through the sting of an infected female *Anopheles* mosquito (Adedeji et al. 2020). *Plasmodium* has 172 species, with five of them capable of infecting humans: *Plasmodium falciparum*, *P. knowlesi*, *P. malariae*, *P. ovale*, and *P. vivax* (Walker et al. 2014; Ashley et al. 2018). *P. falciparum* is one of the most dangerous species of malaria parasite because it almost always results in the most severe

and potentially fatal forms of the disease. *P. falciparum* infections can cause cerebral malaria, adult respiratory distress syndrome, acute malarial hepatitis, blackwater fever, hyperpyrexia, adrenal insufficiency-like syndrome, hypoglycemia, cardiac arrhythmias, hyperparasitemia, gastrointestinal syndromes, and noncardiogenic pulmonary edema (Mangal et al. 2017). According to the World Malaria Report (WMR) data for the year 2023, the global prevalence of malaria is significant, with 249 million people experiencing malaria cases across 85 countries (World Health Organization

2023). Malaria has become one of the infectious diseases that must be addressed by 2030 under the Sustainable Development Goals (SDGs) program. This initiative will be incorporated into the WHO's comprehensive global malaria strategy from 2016 to 2030. The primary goals are to achieve a 90% decrease in the number of reported cases and deaths by the year 2030, along with the elimination of malaria in no fewer than 35 nations and the prevention of malaria resurgence in countries where it has been eradicated (Raviglione and Maher 2017; Hasyim et al. 2024).

Drug resistance and side effect reports are two challenges in treating malaria. Resistance against antimalarial medications obstructs control endeavors and heightens the susceptibility to sickness and death resulting from malaria (Pandey et al. 2023). One strategy to tackle this problem is to look for new candidates derived from natural sources. In recent decades, it has been possible to successfully identify and synthesize compounds from natural sources, including plants, which has led to the development of lead compounds for numerous infectious diseases, including malaria (Ebob et al. 2021). However, only two plants have successfully been used as malaria drugs, namely quinine (stem bark of *Cinchona succirubra*) and artemisinin (leaves of *Artemisia annua*) (Dolabela et al. 2012; Tajuddeen and Van Heerden 2019).

According to reports, the *Cratoxylum* genus could be investigated as a viable plant candidate for discovering novel antimalarial compounds. Major categories of polyphenolic compounds with antimalarial action have been identified in *Cratoxylum*, including xanthone, flavonoids, quinones, anthraquinones, and phenols (Lian Ee et al. 2013; Juanda et al. 2019; Yin Bok et al. 2023). Extracts of bark from *n*-hexane of *C. maingayi* and ethyl acetate of *C. cochinchinense* have been successfully identified for their bioactive antimalarial compounds such as vismione B ( $IC_{50}$  0.66  $\mu\text{g/mL}$ ), formoxanthone C ( $IC_{50}$  1.19  $\mu\text{g/mL}$ ), macluraxanthone ( $IC_{50}$  1.35  $\mu\text{g/mL}$ ), gerontoxanthone I ( $IC_{50}$  1.68  $\mu\text{g/mL}$ ), and fuscaxanthone E ( $IC_{50}$  3.02  $\mu\text{g/mL}$ ) (Laphookhieo et al. 2009). Dichloromethane extracts from the bark of *Cratoxylum sumatranum* have shown a strong decrease in *P. falciparum* using an  $IC_{50}$  value of  $0.44 \pm 0.05$   $\mu\text{g/mL}$  and no toxicity with a  $CC_{50}$  value of  $29.09 \pm 0.05$   $\mu\text{g/mL}$  (Tumewu et al. 2021).

Therefore, based on the chemotaxonomy approach, *Cratoxylum glaucum* Korth holds promise as a candidate for discovering antimalarial compounds. This plant is traditionally known as gerunggang merah. Nearly every part of the plant, especially the leaves, is used as a traditional medicine to promote breast milk production and treat fever, cough, and diarrhea (Yingngam et al. 2014; Mahardika and Roanisca 2018). The bioactivity of this plant has been reported as an antioxidant, antibacterial, xanthine oxidase inhibitor, and antidiabetic agent (Sim et al. 2011; Juanda et al. 2019; Roanisca et al. 2021).

Studies regarding the antimalarial properties of this plant have yet to be reported. Considering the potential content of its compounds, research regarding the efficacy of this plant as a potential antimalarial can be carried out.

The present study aims to investigate the antimalarial properties of the *C. glaucum* leaf extract, which has the

potential for further research in searching for possible antimalarial compounds. In this context, bioassay-guided isolation will isolate active compounds that inhibited *P. falciparum* growth on a lactate dehydrogenase (LDH) assay.

## Materials and methods

### Plant materials

The fresh leaves of *C. glaucum* were obtained from the Balikpapan Botanical Garden, East Kalimantan, Indonesia. Dr. Ratih Damayanti, S.Hut. M. Si., Directorate of Scientific Collection Management at BRIN Cibinong, Jakarta, Indonesia, determined the plants for identification and authentication. A voucher specimen has been issued (B-847/II.6.2/IR.01.02/5/2023) by the Directorate of Scientific Collection Management at BRIN Cibinong, Jakarta, Indonesia.

### Bioassay-guided isolation

The leaves of *C. glaucum* (Cg.F) were subjected to a multi-stage extraction process. Initially, a powder of Cg.F (1 kg) was extracted with solvents in order of polarity, such as *n*-hexane (4.5 L), dichloromethane (3 L), and methanol (3 L). The solvents were subsequently removed from each extract under pressure to yield *n*-hexane extract (Cg.FH, 22.56 g, 2.26% w/w), dichloromethane extract (Cg.FD, 46.82 g, 4.68% w/w), and methanol extract (Cg.FM, 46 g, 4.6% w/w). After the initial antimalarial screening and determining the  $IC_{50}$  value, Cg.FD exhibited the strongest activity and chose to separate further. The extract (Cg.FD, 2.5 g) was fractionated under vacuum liquid chromatography (VLC) with hexane-EtOAc gradient elution (100:0–0:100) and produced 12 fractions (Cg.FD-F1–F12). Only 5 fractions (Cg.FD-F3; F4; F5; F6; and F8) showed more than 50% inhibition on antimalarial screening. Subsequently, the  $IC_{50}$  value of the active fraction is determined; Cg.FD-F5 showed the strongest activity and was carried out to the next separation. The fraction (Cg.FD-F5, 1.042 mg) was separated using Sephadex LH-20 with a chloroform-methanol isocratic elution (2:8 v/v) and obtained into nine fractions (Cg.FD-F5.1–F5.9). Fraction Cg.FD-F5.5 was identified as compound 1 (102 mg).

### Structure determination of a compound

The structure of compound 1 was checked for purity level using HPLC reverse phase column C-18 with methanol-water isocratic elution (8.5:1.5 v/v) with a flow rate of 1.5 mL/min and characterized using a UV absorbance detector in HPLC (Shimadzu, Kyoto, Japan). Mass spectra were utilized with the UPLC-Q-TOF-MS system (Shimadzu, Kyoto, Japan). NMR spectroscopy methods (JEOL 400 MHz and 100 MHz), including 1D NMR ( $^1\text{H-NMR}$  and  $^{13}\text{C-NMR}$ ) and 2D NMR (HMBC) techniques. The obtained spectral data were compared with previously reported studies.

## Culture of *Plasmodium falciparum*

The *Plasmodium falciparum* chloroquine-sensitive (3D7 strain) was obtained from the Center for Natural Product Medicine and Research Development (NPMRD), the Institute of Tropical Disease (ITD), Universitas Airlangga, Indonesia. The O type red blood cells (RBC) was acquired from the Indonesian Red Cross of Surabaya, Indonesia. The parasites of *P. falciparum* were grown using red blood cells (type O) at a hematocrit of 2% with RPMI-1640 media (Gibco, Waltham, USA), albumax 10% (v/v), and 50 µg/mL hypoxanthine (Sigma) under 5% O<sub>2</sub>, 5% CO<sub>2</sub>, and 90% NO<sub>2</sub> at a temperature of 37 °C.

## Lactate dehydrogenase (LDH) assay

Extracts, fractions, and compound 1 of *C. glaucum* were determined for their antimalarial activity. The culture of parasites was synchronized using sorbitol 5% w/v to obtain the ring stage. The sample with a variant concentration of 0.01, 0.05, 0.1, 0.5, 1, 5, 10, and 50 µg/mL was put into each well plate in one microliter and replicated three times. Ninety-nine microliters of parasite (ring-stage) were added. Subsequently, it was incubated for 72 hours at 37 °C in a mixture of gases (5% O<sub>2</sub>, 5% CO<sub>2</sub>, and 90% NO<sub>2</sub>). After that, the well plate was stored at -30 °C overnight. The original LDH-buffer solution, which contained 10 mL of Tris-HCl, Triton X-100, sodium L-lactate, and deionized water, was enhanced with APAD stock solution (10 mg/mL, Oriental Yeast Co., Ltd.), 2 mg of NBT (10 mg/mL, Sigma), and 200 µL of diaphorase stock solution (50 units/mL, Sigma). Then, carefully mix the ingredients of the LDH buffer solution and store it in the absence of light. Subsequently, 90 µL of the prepared substrate was put into each well plate. This well plate was covered with aluminum foil, placed in a flatbed shaker set at 650 rpm, and maintained at room temperature. Afterward, the plate was subjected to incubation for 30 minutes. The absorbance of each well was measured using the multi-scan sky-high microplate spectrophotometer (Thermo Fisher Scientific) at a wavelength of 650 nm (Wang et al. 2019; Wijayanti et al. 2021). The categorization for antimalarial activity in drug discovery is as follows: very potent (<5 µg/mL), moderately potent (>5–50 µg/mL), weakly potent (>50–100 µg/mL), and inactive (>100 µg/mL) (Chinchilla et al. 2012).

## Cytotoxicity assay

The cytotoxicity assay of compound 1 was investigated using the resazurin assay. Vero cell lines were cultured in high glucose (D-MEM) media supplemented with L-Glutamine, Phenol Red (Wako, Fujifilm), NaHCO<sub>3</sub>, fetal bovine serum 10% (v/v) (Gibco), and Penicillium streptomycin 1% (v/v) (Sigma). One hundred microliters cultured (1×10<sup>6</sup>) were seeded into a micro-96 well plate and incubated for 24 hours at a temperature of 37 °C. Then, discarded the medium and added serial dilutions of compound 1 with the following series of concentrations: 100, 50, 25, 12.5, and 6.25 µg/mL. Then, the well plate was subsequently incubated for 2 days

in a CO<sub>2</sub> 5% incubator at 37 °C. After incubation, a well plate was filled with 10 µL of resazurin solution (0.5 mM) and incubated for 4 hours. After the incubation, fluorescence measurements were performed using a Nivo plate reader (PerkinElmer) at a wavelength for excitation of 530 nm and a wavelength for emission of 595 nm (Larayetan et al. 2019).

## Data analysis

The average of three repeat experiments ± standard deviation shows all the data. The antimalarial activity (IC<sub>50</sub>) and cytotoxicity (CC<sub>50</sub>) values were calculated using GraphPad Prism 9.3.0 edition for Windows (GraphPad Software Inc., USA). The selectivity index (SI) is determined by dividing the CC<sub>50</sub> value by the IC<sub>50</sub> value.

## Results and discussion

Currently, drug discovery for new antimalarials is highly necessary because of the increasing drug resistance in malaria and the expanding cases of malaria. Medicinal plants are known to have great potential due to their secondary metabolite content for discovering a new drug candidate, particularly for malaria. Bioassay-guided isolation is now widely used to sift through extensive collections of plant extracts and fractions to obtain the active compounds. These assays isolate bioactive compounds from intricate mixtures, utilizing separation and analytical methods. The process entails sequentially testing fractionated material for its activity in a bioassay and assessing its purity through analytical techniques (Weller 2012; Perera et al. 2019; Phukhatmuen et al. 2020; Afsar et al. 2022). Therefore, the chemotaxonomic approach identifies the polyphenol content with the antimalarial activity of the genus *Cratoxylum* (Lian Ee et al. 2013; Juanda et al. 2019; Yin Bok et al. 2023). Consequently, developing new antimalarial drugs, particularly from *Cratoxylum glaucum* leaves, is crucial.

## Antimalarial extract and fractions of *C. glaucum*

The antimalarial activity of three extracts of *C. glaucum* leaves (Cg.FH, Cg.FD, and Cg.FM) has been evaluated. The dichloromethane extract (Cg.FD) has the strongest activity inhibiting *P. falciparum*, as shown by an IC<sub>50</sub> value of 2.12 ± 0.04 µg/mL (Table 1). Therefore, further isolation of compounds with antimalarial properties from Cg.FD was pursued.

**Table 1.** Antimalarial activity of *C. glaucum* leaf extract.

Sample	IC <sub>50</sub> (µg/mL)
Cg.FH	6.10 ± 0.02
Cg.FD	2.12 ± 0.04
Cg.FM	4.51 ± 0.03

All data are represented as the average ± SD of triplicates. Cg.FH: *Cratoxylum glaucum* Folium n-hexane; Cg.FD: *Cratoxylum glaucum* Folium Dichloromethane; Cg.FM: *Cratoxylum glaucum* Folium Metanol.

Fractionation on Cg.FD resulted in five active fractions (F3; F4; F5; F6; and F8), and the fraction Cg.FD-F5 exhibited the strongest activity with an  $IC_{50}$  value of  $1.5 \pm 2.91$   $\mu\text{g/mL}$  (Table 2). Then, the separation of the fraction of Cg.FD-F5 produces compound 1 (Cg.FD-F5.5), forming yellow amorphous crystals. According to the criteria for antimalarial drug discovery activities, all active extracts and fractions of *C. glaucum* leaves can be classified as having very potent activity because they have an  $IC_{50}$  value  $<5$   $\mu\text{g/mL}$  (Chinchilla et al. 2012).

**Table 2.** Antimalarial activity of active fractions of *C. glaucum*.

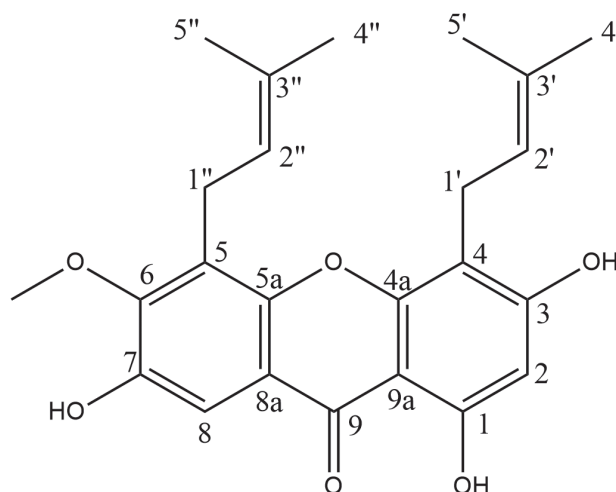
Sample	$IC_{50}$ ( $\mu\text{g/mL}$ )
Cg.FD-F3	$2.56 \pm 3.42$
Cg.FD-F4	$2.42 \pm 0.72$
Cg.FD-F5	$1.5 \pm 2.91$
Cg.FD-F6	$1.69 \pm 0.93$
Cg.FD-F8	$2.07 \pm 3.78$

All data are represented as the average  $\pm$  SD of triplicates.

## Identification of compound 1

Compound (1): Yellow crystal, UV absorbance 240, 262, 318, 378 nm.  $m/z$  410.1801  $[M+H]^+$  (calcd for  $C_{24}H_{26}O_6$ , 410.1808).  $^1\text{H}$  NMR (400 MHz, acetone- $d_6$ ): 6.29 (s, 1H, H-2, ArH), 7.50 (s, 1H, H-8, ArH), 5.25 (q,  $J$  6.8 Hz, 1H, H-2'), 3.48 (d,  $J$  6.8 Hz, 2H, H-1'), 1.63–1.76 (s, 3H, H-4' and H-5'), 3.64 (d, 2H,  $J$  6.8 Hz, H-1''), 5.31 (q,  $J$  6.8 Hz, 1H, H-2''), 1.67–1.80 (s, 3H, H-4'' and H-5''), 12.98 (1H, s, OH-1), 3.96 (3H, s, -OCH<sub>3</sub>-6).  $^{13}\text{C}$  NMR (100 MHz, acetone- $d_6$ ):  $\delta$ C 161.34 (C-1), 97.43 (C-2), 162.42 (C-3), 106.41 (C-4), 124.24 (C-5), 152.54 (C-6), 147.43 (C-7), 107.56 (C-8), 180.37 (C-9), 155.30 (C-4a), 148.95 (C-5a), 116.32 (C-8a), 102.49 (C-9a), 21.45 (C-1'), 122.18 (C-2'), 131.09 (C-3'), 25.04 (C-4'), 17.18 (C-5'), 22.97 (C-1''), 122.92 (C-2''), 132.00 (C-3''), 25.04 (C-4''), 17.27 (C-5''), and 60.38 (C-OCH<sub>3</sub>-6). HMBC H-2/ C-1, C-4, C-9a; H-8/ C-6, C-8a, C-9; H-1'/C-3, C-4, C-4a, C-2', C-8'; H-5'/C-4'; H-1''/C-5, C-6, C-3'', C-5''; H-2''/C-4'', C-5''; H-4''/C-3'', C-5''; H-5''/C-2''; OH-1/C-1, C-2, C-9a. Purity level on HPLC 254 nm: 98.301%; 210 nm: 97.785%; 365 nm: 98.658% (Suppl. material 1: figs S1–S6).

The  $^1\text{H}$ -NMR and  $^{13}\text{C}$ -NMR spectra on compound 1 indicated a prenylated xanthone group, including the xanthone skeleton, hydroxyl, and two prenyl groups (Suppl. material 1: figs S1, S2). The xanthone skeleton features a C6-C3-C6 (ABC) ring structure. Rings A and B form a conjugated aromatic ring system composed of two rings. Rings A and B are typically attached to hydroxyl, methoxyl, and isoprene groups. Ring C is arranged with oxygen atoms and carbonyl groups (Remali et al. 2022; Yahia et al. 2023). An AB system on compound 1 was identified with two aromatic protons at  $\delta$ H 6.29 (1H, s, H-2) and 7.50 (1H, s, H-8), respectively. Six oxygenated aromatic carbon signals appear at  $\delta$ C 161.34 (C-1), 162.42 (C-3), 124.24 (C-5), 147.43 (C-7), 155.30 (C-4a), and 148.95 (C-5a). The Ring C region has a carbonyl group at  $\delta$ C 180.37 (C-9). One hydroxyl group appears at  $\delta$ H 12.98 (1H, s, OH-1). Prenylated xanthone was found on the  $^1\text{H}$  and  $^{13}\text{C}$  NMR signals assigned



**Figure 1.** Structure of compound 1 (cratoxyarborenone E) from the leaves of *C. glaucum*.

to the prenyl units of an isolated compound to be split into two distinct sets. The first prenyl group is at  $\delta$ H 3.48 (2H, d, H-1'), 5.25 (1H, m, H-2'), 1.63 (3H, s, H-4'), 1.76 (3H, s, H-5'), and  $\delta$ C 21.45 (C-1'), 122.18 (C-2'), 131.09 (C-3'), 25.04 (C-4'), and 17.18 (C-5'). The second prenyl is at  $\delta$ H 3.64 (2H, d, H-1''), 5.31 (1H, m, H-2''), 1.67 (3H, s, H-4''), 1.80 (3H, s, H-5''), and  $\delta$ C 22.97 (C-1''), 122.92 (C-2''), 132.00 (C-3''), 25.04 (C-4''), and 17.27 (C-5''). The prenyl substituents on the xanthone skeleton can be easily identified in the  $^1\text{H}$  NMR signal, namely two methyl signals ( $\text{CH}_3$ ) at  $\delta$ H 1.4–1.7, one methine signal ( $=\text{CH}$ ) at  $\delta$ H 5.00–5.40 ppm, and one methylene signal ( $\text{CH}_2$ ) at  $\delta$ H 3.2–3.6 ppm (Huang et al. 2021; Kurniawan et al. 2021).

The HMBC of 2D NMR was used to confirm the locations of the two side chains in the isolated compound. The first prenyl protons at  $\delta$ H 3.48 (2H, d, H-1') were assigned two bond correlations to  $\delta$ C 106.41 (C-4), three bond correlations to 162.42 (C-3), and 155.30 (C-4a). The second prenyl proton in  $\delta$ H 3.64 (2H, d, H-1'') was positioned at  $\delta$ C 124.24 (C-5) through two bond correlations and three bonds at 152.54 (C-6) (Suppl. material 1: fig. S3). After comparing the data with the literature (Seo et al. (2002)), compound 1 from the leaves of *C. glaucum* was confirmed as cratoxyarborenone E (Fig. 1). This compound was previously isolated from the leaves of *C. sumatranum* and demonstrated cytotoxic activity against the human oral epidermoid carcinoma (KB) cell line. In this study, cratoxyarborenone E was first reported from the leaves of *C. glaucum* and reported as antimalarial against *P. falciparum* 3D7.

## The antimalarial and cytotoxicity assays of compound 1

Compound 1 has antimalarial activity with  $IC_{50}$  values of  $2.13 \pm 0.04$   $\mu\text{g/mL}$  (5.82  $\mu\text{M}$ ) and is categorized as having good antimalarial activity because it has an  $IC_{50}$  between 1 and 20  $\mu\text{M}$  (Badisa et al. 2007). The antimalarial activity of compound 1 decreased compared to its active fraction (Cg.FD-F5). The biological activity is frequently lost once the pure compound is isolated because fractions



**Table 3.** Antimalarial (IC<sub>50</sub>), cytotoxicity (CC<sub>50</sub>), and selectivity index (SI) of compound 1.

Compound	IC <sub>50</sub> (μM)	CC <sub>50</sub> (μM)	SI
Compound 1	5.82 ± 0.04	20.74 ± 0.05	3.56
Chloroquine diphosphate	0.015 ± 0.05	193.80 ± 0.01	>100

All data are represented as the average ± SD of triplicates.

consist of multiple compounds (Dietz et al. 2016; Tumewu et al. 2023). Therefore, the activity of the fractions might not be solely the responsibility of one or two compounds, but multi-compounds might be involved. The cytotoxicity of compound 1 on Vero cells was evaluated using a resazurin-based assay. A drug is idealized when it has no adverse effects on normal cells (Badisa et al. 2007; Adepu and Ramakrishna 2021; Indrayanto et al. 2021). Based on the plant screening program of the United States National Cancer Institute, it is classified as having in vitro cytotoxicity if the CC<sub>50</sub> is < 30 μg/ml for the crude extract and the CC<sub>50</sub> is < 10 μM for compounds (Kaharudin et al. 2020). Compound 1 has a CC<sub>50</sub> value of 20.74 μM or is classified as non-toxic. Furthermore, this compound selectivity index (SI) is 3.56, indicating potential for development as an antimalarial drug (Table 3). The selectivity index measures how effective an investigational drug is at halting cell division compared to its ability to induce cell death. Higher selectivity indices indicate maximum activity with the least cellular damage and are preferred (Sinha et al. 2019). A compound with an SI value < 1 suggests the presence of toxic components and should not be used as an herbal drug (Indrayanto et al. 2021). In antimalarial research, the SI value enables a more qualitative evaluation of substances as possible new therapeutic possibilities (Teng et al. 2019; Alves et al. 2021). Therefore, further research is needed to determine the compound mechanisms of action and in vivo antimalarials.

## Conclusion

Cratoxyarborenone E was first reported in the extract of *C. glaucum* leaf extract. Based on the IC<sub>50</sub> value, it showed

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good antimalarial activity and was relatively non-toxic at the CC<sub>50</sub> value. This compound has the potential to be used as a new antimalarial drug.

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## Ethical statement

This research does not involve using any human subjects or animals.

## Credit authorship contribution statement

Suryanto Suryanto: Phytochemistry and bioassay work, data analysis, and writing the original manuscript. Lidya Tumewu: Compound identification, review, and editing. Hilkatul Ilmi: Bioassay work, data analysis, review, and editing. Suciati Suciati: Compound identification and review. Achmad Fuad Hafid: Conceptualization, methodology, and review. Aty Widyawaruyanti: Conceptualization, methodology, and review.

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## Supplementary material 1

### Supplementary data

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Data type: pdf

Explanation note: fig. S1: <sup>1</sup>H-NMR of compound 1 (cratoxyarborenone E); fig. S2: <sup>13</sup>C-NMR of compound 1 (cratoxyarborenone E); fig. S3: HMBC of compound 1 (cratoxyarborenone E); fig. S4: UV absorbance of compound 1 (cratoxyarborenone E); fig. S5: Mass spectrometry of compound 1 (cratoxyarborenone E); fig. S6. Purity level of compound 1 (cratoxyarborenone E) on HPLC.

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