Society of Medical Education & Research

2023, Volume 3, Issue 2, Page No: 96-106
ISSN: 3108-4826

Journal of Medical Sciences and Interdisciplinary Research

Exploring the Anti-Malarial and Anti-Protozoan Capabilities of Indonesian Plant Extracts for Neglected Tropical Disease Treatment

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Abstract

Neglected tropical diseases (NTDs) affect approximately 2 billion people globally and are caused by a range of pathogens, including protozoa, bacteria, and trypanosomes, primarily in tropical and subtropical regions. Among the 17 NTDs listed by the World Health Organization (WHO), protozoan infections such as those caused by Plasmodium, Entamoeba, Leishmania, and Trypanosoma represent a major public health concern. Indonesia, with its exceptional biodiversity resulting from a tropical climate, offers a wealth of plant species with promising bioactivities, making them potential sources for therapeutic development. This has led to an increased focus on exploring Indonesian plant extracts and their isolated compounds for anti-protozoal effects. A total of 48 plant extracts from the genera Cratoxylum, Diospyros, and Artocarpus were tested for antiprotozoal activity. Their effects were evaluated in vitro against Plasmodium falciparum (Pf), Entamoeba histolytica (Eh), Leishmania donovani (Ld), Trypanosoma brucei rhodesiense (Tbr), and Trypanosoma cruzi (Tc).
Dichloromethane extracts derived from Cratoxylum arborescens roots showed pronounced inhibitory activity, with IC50 values between 0.1 and 8.2 µg/mL. Cochinchinone C, a compound isolated from these extracts, displayed potent activity against Pf, Eh, Ld, Tbr, Tc trypomastigotes, and Tc epimastigotes, with IC50 values of 5.8 µM, 6.1 µM, 0.2 µM, 0.1 µM, 0.7 µM, and 0.07 µM, respectively. Remarkably, this is the first report of cochinchinone C demonstrating antiprotozoal activity. Cytotoxicity testing revealed low toxicity and high selectivity (selectivity index >10) against both cancerous and normal human cell lines, underscoring its potential as a lead compound for drug development. Cochinchinone C represents a promising candidate for antiprotozoal drug development and highlights Indonesia's untapped potential as a source of bioactive natural products for combating neglected tropical diseases.

Keywords: Cochinchinone C, Antiprotozoal, Neglected tropical disease, Natural products

Introduction

Neglected tropical diseases (NTDs) are infections that disproportionately affect populations in low-resource tropical and subtropical regions, with nearly 2 billion people affected worldwide. These diseases are caused by diverse pathogens, including protozoa and helminths, and pose significant challenges for public health management. Protozoal NTDs—such as Chagas disease, leishmaniasis, and African trypanosomiasis—remain particularly problematic due to their complex life cycles and limited treatment options [1, 2]. Effective management often requires preventive chemotherapy and continuous research into new therapeutics, yet resource constraints frequently slow drug development. To address this, the WHO introduced a roadmap for 2021–2030 to accelerate the discovery and deployment of treatments for NTDs, emphasizing the role of natural products in drug innovation [2, 3].

Access this article online https://smerpub.com/

Received: 04 May 2023; **Accepted:** 16 August 2023

How to cite this article: Kim SH, Park JH, Lee MC, Choi DW, Kwon HJ, Yoon ST. Exploring the Anti-Malarial and Anti-Protozoan Capabilities of Indonesian Plant Extracts for Neglected Tropical Disease Treatment. J Med Sci Interdiscip Res. 2023;3(2):96-106. https://doi.org/10.51847/nl8eyt1Efx

Protozoan parasites—including Entamoeba histolytica, Giardia lamblia, Leishmania donovani, and Trypanosoma brucei—pose major risks, especially to immunocompromised individuals, causing diseases such as amebiasis, leishmaniasis, African trypanosomiasis, and Chagas disease [4, 5]. Malaria, caused by Plasmodium falciparum, continues to contribute to millions of severe cases and hundreds of thousands of deaths annually [6]. Despite this burden, the discovery of new antiprotozoal compounds remains limited, while current therapies face challenges such as resistance and adverse effects [7].

Historically, ethnopharmacology has guided the identification of plant-derived antiprotozoal drugs. Notable examples include quinine from Cinchona bark and artemisinin from Artemisia annua, both of which have shaped modern antimalarial therapy [8]. Indonesia, ranked as the second most biodiverse country globally after Brazil according to the 2022 Global Biodiversity Index, offers a rich repository of plant species suitable for therapeutic exploration [9].

Several Indonesian genera demonstrate pharmacological promise. Cratoxylum (Hypericaceae) produces xanthone derivatives with antibacterial, anti-inflammatory, antiparasitic, and antioxidant properties [10–12]. Diospyros (Ebenaceae) exhibits a wide range of activities, including neuroprotection, antimicrobial, antiprotozoal, antifungal, anti-inflammatory, analgesic, and antipyretic effects [13]. Species of Artocarpus (Moraceae) have traditional applications in treating inflammation, malaria, diarrhea, diabetes, and helminth infections [14].

This study systematically investigated 48 extracts from Cratoxylum, Diospyros, and Artocarpus for activity against major protozoan pathogens of public health relevance, including Pf, Eh, Ld, Tbr, and Tc. Among the compounds identified, cochinchinone C from Cratoxylum arborescens roots demonstrated potent antiprotozoal activity while maintaining minimal cytotoxicity against human cell lines, marking it as a promising candidate for further drug development.

Materials and Methods

Plant material

Samples from different parts of plants belonging to the Cratoxylum genus, including leaves, stems, twigs, and roots, were gathered in the Muara Teweh area of Central Kalimantan, Indonesia. In contrast, leaf samples from the Diospyros and Artocarpus genera were obtained from Purwodadi, located in Pasuruan, East Java, Indonesia. Authentication of the plants was performed by the botanist Mr. Rony Irawanto, S.Si., M.T., at the Purwodadi Botanical Garden, part of the Indonesian Institute of Sciences in East Java, Indonesia, under determination letter No: B-108/IPH.06/AP.01/II/2020. The specimens, as listed in **Table 1**, were preserved in the Laboratory of Natural Product Medicine and Development (NPMRD) at the Institute of Tropical Disease, Universitas Airlangga. The selection of Cratoxylum, Diospyros, and Artocarpus for this investigation was based on prior published reports and their documented ethnopharmacological applications [8, 10, 13, 14].

Table 1. List of plant species extracted for antiprotozoal test and antiprotozoal activity

No.	Species	Family	Plant Part	Extract Code	Pf IC_{50} (µg/mL)	Ld IC_{50} (µg/mL)	Tbr IC_{50} (µg/mL)	Eh IC_{50} (µg/mL)
1	Cratoxylum rigidum (Cr)	Hypericaceae	Stem	Cr.SH	>10	20.1	9.2	69.7
2	Cratoxylum rigidum (Cr)	Hypericaceae	Stem	Cr.SD	>10	>100	38.1	>100
3	Cratoxylum rigidum (Cr)	Hypericaceae	Stem	Cr.SM	>10	>100	>100	>100
4	Cratoxylum cochinchinense (Cc)	Hypericaceae	Stem	Cc.SH	>10	66.6	21.9	66.2
5	Cratoxylum cochinchinense (Cc)	Hypericaceae	Stem	Cc.SD	>10	5.3	3.1	>100
6	Cratoxylum cochinchinense (Cc)	Hypericaceae	Stem	Cc.SM	>10	>100	28.7	>100
7	Cratoxylum cochinchinense (Cc)	Hypericaceae	Twig	Cc.TH	>10	20.9	7.0	>100
8	Cratoxylum cochinchinense (Cc)	Hypericaceae	Twig	Cc.TD	>10	45.9	18.7	>100

9	Cratoxylum cochinchinense (Cc)	Hypericaceae	Twig	Cc.TM	>10	>100	30.4	>100
10	Cratoxylum formosum (Cf)	Hypericaceae	Stem	Cf.SH	>10	38.8	29.3	>100
11	Cratoxylum formosum (Cf)	Hypericaceae	Stem	Cf.SD	>10	38.2	14.1	>100
12	Cratoxylum formosum (Cf)	Hypericaceae	Stem	Cf.SM	>10	>100	30.5	>100
13	Cratoxylum arborescens (Ca)	Hypericaceae	Root	Ca.RH	>10	6.5	3.7	49.4
14	Cratoxylum arborescens (Ca)	Hypericaceae	Root	Ca.RD	0.1	2.8	2.9	22.0
15	Cratoxylum arborescens (Ca)	Hypericaceae	Root	Ca.RM	>10	69.8	37.3	>100
16	Cratoxylum arborescens (Ca)	Hypericaceae	Twig	Ca.TH	>10	9.9	10.7	>100
17	Cratoxylum arborescens (Ca)	Hypericaceae	Twig	Ca.TD	6.0	1.3	6.4	>100
18	Cratoxylum arborescens (Ca)	Hypericaceae	Twig	Ca.TM	>10	58.7	44.6	>100
19	Cratoxylum glaucum (Cg)	Hypericaceae	Stem	Cg.SH	>10	>100	26.1	>100
20	Cratoxylum glaucum (Cg)	Hypericaceae	Stem	Cg.SD	>10	43.2	32.1	>100
21	Cratoxylum glaucum (Cg)	Hypericaceae	Stem	Cg.SM	>10	25.6	25.9	>100
22	Cratoxylum glaucum (Cg)	Hypericaceae	Leaves	Cg.LH	6.1	>100	18.2	>100
23	Cratoxylum glaucum (Cg)	Hypericaceae	Leaves	Cg.LD	2.1	8.1	0.7	>100
24	Cratoxylum glaucum (Cg)	Hypericaceae	Leaves	Cg.LM	4.5	24.4	1.3	>100
25	Cratoxylum glaucum (Cg)	Hypericaceae	Root	Cg.RH	>10	>100	16.1	>100
26	Cratoxylum glaucum (Cg)	Hypericaceae	Root	Cg.RD	>10	>100	23.5	>100
27	Cratoxylum glaucum (Cg)	Hypericaceae	Root	Cg.RM	4.4	>100	34.7	>100
28	Cratoxylum glaucum (Cg)	Hypericaceae	Twig	Cg.TH	>10	18.5	6.6	>100
29	Cratoxylum glaucum (Cg)	Hypericaceae	Twig	Cg.TD	>10	>100	6.5	>100
30	Cratoxylum glaucum (Cg)	Hypericaceae	Twig	Cg.TM	>10	48.5	6.8	>100
31	Cratoxylum sumatranum (Cs)	Hypericaceae	Twig	Cs.TH	>10	24.6	11.0	>100
32	Cratoxylum sumatranum (Cs)	Hypericaceae	Twig	Cs.TD	0.3	1.2	3.2	75.3
33	Cratoxylum sumatranum (Cs)	Hypericaceae	Twig	Cs.TM	>10	23.1	5.9	>100
34	Diospyros malabarica (Dm)	Ebenaceae	Leaves	Dm.LH	>10	>100	16.2	>100
35	Diospyros malabarica (Dm)	Ebenaceae	Leaves	Dm.LD	>10	>100	>100	>100
36	Diospyros malabarica (Dm)	Ebenaceae	Leaves	Dm.LM	>10	>100	>100	>100
37	Diospyros celebica (Dc)	Ebenaceae	Leaves	Dc.LH	>10	>100	>100	>100
38	Diospyros celebica (Dc)	Ebenaceae	Leaves	Dc.LD	>10	>100	>100	>100
39	Diospyros celebica (Dc)	Ebenaceae	Leaves	Dc.LM	>10	>100	>100	>100
40	Artocarpus heterophyllus (Ah)	Moraceae	Leaves	Ah.LH	>10	>100	>100	>100
41	Artocarpus heterophyllus (Ah)	Moraceae	Leaves	Ah.LD	>10	47.2	19.1	>100
42	Artocarpus heterophyllus (Ah)	Moraceae	Leaves	Ah.LM	>10	>100	>100	>100
43	Artocarpus altilis (Aa)	Moraceae	Leaves	Aa.LH	>10	>100	14.6	>100

44	Artocarpus altilis (Aa)	Moraceae	Leaves	Aa.LD	8.6	18.2	6.1	>100
45	Artocarpus altilis (Aa)	Moraceae	Leaves	Aa.LM	>10	36.1	6.3	>100
46	Artocarpus camansi (Ac)	Moraceae	Leaves	Ac.LH	>10	>100	42.9	>100
47	Artocarpus camansi (Ac)	Moraceae	Leaves	Ac.LD	8.6	>100	11.1	>100
48	Artocarpus camansi (Ac)	Moraceae	Leaves	Ac.LM	>10	27.6	6.2	>100

Note: S: stem; T: twig; R: root; L: leaves; H: n-hexane extract; D: dichloromethane extract; M: methanol extract. Pf: *Plasmodium falciparum*; Eh: *Entamoeba histolytica*; Ld: *Leishmania donovani*; Tbr: *Trypanosoma brucei rhodesiense*.

Extraction and fractionation procedures

A sequential extraction methodology, moving from non-polar to polar solvents, was applied to the plant material. About 250 g of crushed plant parts were consecutively treated with n-hexane, dichloromethane, and methanol to generate extracts **(Table 1)** for antiprotozoal screening. Extracts showing notable bioactivity in preliminary assays were subjected to a larger-scale extraction, focusing on the dichloromethane root extract of Cratoxylum arborescens (Ca.RD). For this, 1 kg of homogenized root material underwent the same sequential solvent process to yield sufficient extract for downstream purification. Bioactive fractions were separated using Vacuum Liquid Chromatography (VLC) on Silica gel 60 with an elution gradient of n-hexane and dichloromethane (100–0%). This yielded ten fractions (Ca.RD-D1 through Ca.RD-D10), which were then assayed for antiparasitic activity, with their respective yields listed in **Table 2**. Further purification of fraction Ca.RD-D7 through crystallization produced Compound (1). The progress of fractionation and purity of compounds were monitored using thin-layer chromatography (TLC), visualized under 254 nm and 366 nm UV light, with 10% H2SO4 spray for detection.

Compound characterization

Structural determination of the isolated compound was performed via nuclear magnetic resonance spectroscopy (NMR), including ^{1}H and ^{13}C NMR, and compared to literature values. Spectra were recorded in CDCl3 at 400 MHz (^{1}H) and 100 MHz (^{13}C) using a JEOL ECS-400 spectrometer.

Organism and culture

The P. falciparum 3D7 strain was maintained in RPMI-1640 medium at 37 °C under a mixed gas atmosphere (5% O_2, 5% CO_2, 90% N_2). E. histolytica clonal strain HM-1:IMSS cl6 [15] trophozoites were grown axenically in Diamond's BI-S-33 medium at 35.5 °C. Axenic amastigote clumps of L. donovani were cultured in complete SM medium [16], whereas T. b. rhodesiense strain IL-1501 was propagated in HMI-9 medium [17]. T. cruzi strain TcLuc2 was cultured in monolayers of mouse embryonic fibroblast (3T3) cells using DMEM without phenol red at 37 °C under 5% CO_2, while the epimastigote stage of T. cruzi was grown in liver infusion/tryptone (LIT) medium at 28 °C [18]. Various cell lines, including human hepatoma cells (Huh7 [19] and HepG2 [20]), baby hamster kidney-21 (BHK-21) [21], and African green monkey kidney (Vero) cells [22], were maintained in DMEM with phenol red at 37 °C in a humidified 5% CO_2 atmosphere.

In vitro antiprotozoal assays

Evaluation against plasmodium falciparum

The antimalarial activity of plant extracts, fractions, and purified compounds was determined against synchronized P. falciparum 3D7 using a modified Plasmodium lactate dehydrogenase (pLDH) assay according to Makler [23]. Parasites were synchronized with 5% (w/v) D-sorbitol, and parasitemia was quantified by light microscopy. Cultures were adjusted to 2% hematocrit and 0.3% parasitemia in RPMI medium supplemented with 50% RBCs. Test samples were dissolved in DMSO, while chloroquine diphosphate served as the positive control in distilled water. Initial screening used 10 µg/mL, and serial dilutions ranging from 0.01 to 50 µg/mL were prepared for IC50 determination. Cultures (100 µL) with varying concentrations were placed in 96-well plates, keeping DMSO ≤0.5%. Plates were incubated at 37 °C for 72 h under 5% CO2, 5% O2, and 90% N2, then frozen at -30 °C to lyse RBCs. After thawing for 1 h at room temperature, pLDH activity was measured using a reagent mixture of 50 mM sodium L-lactate, 0.25% Triton X-100, 100 mM Tris-HCl (pH 8.0), 50 µM APAD, 240 µM NBT, and 1 U/mL diaphorase. Ninety microliters of this mixture were added per well, plates were shaken for uniformity, incubated 30 min in the dark at room temperature, and absorbance recorded at 650 nm with a Promega plate reader. Experiments were performed in triplicate (n = 3).

Evaluation against entamoeba histolytica

For E. histolytica HM-1:IMSS cl6, 5×10^3 trophozoites per well were plated in 96-well plates with extracts dissolved in 50% DMSO. Metronidazole was used as a reference drug. Serially diluted test extracts (2 µL) were added to 200 µL of parasite culture and incubated anaerobically at 35.5 °C for 48 h. Following incubation, 10% WST-1 in OPTI-MEM was added after removing 100 µL of media [24], incubated for 30 min, and absorbance measured at 450 nm using a Victor Nivo reader. The percentage inhibition was calculated using the formula:

$$\%Inhibition = \left\{\frac{(Control\ cells - Control\ media) - (Abs.score - Control\ media)}{(Control\ cells - Control\ media)}\right\} \times 100 \quad (1)$$

The concentration at which parasite growth was reduced by 50% (IC50) was calculated using GraphPad Prism software, version 8.3.1 (332), with values below 10 µg/mL considered indicative of strong inhibitory potential of the extract against the parasite.

Assessment of anti-leishmania activity

Axenic amastigotes of Leishmania donovani were harvested and maintained at 37 °C in a humidified 5% CO2 environment [16]. The cultures were spun at 1,200 rpm for 10 minutes, supernatants removed, and pellets resuspended in 6 mL of complete SM medium. To prevent clumping, cells were repeatedly pipetted before transferring into 96-well plates. Each well received 1×10^6 cells/mL, except the control wells, and plates were gently agitated using a Multidrop combi. Serial dilutions of plant extracts were applied, and cultures were incubated for 68 h at 37 °C under CO2. Afterward, 10 µL of 0.5 mM resazurin was added per well, followed by a 4 h incubation, and fluorescence was measured (excitation 528 nm, emission 590 nm) with a SpectraMax Gemini XS microplate reader. IC50 values were calculated using GraphPad Prism v8.3.1 (332).

Assessment of anti-trypanosoma activity

Trypanosoma brucei rhodesiense

The IL-1501 strain of T. b. rhodesiense [17] was grown in HMI-9 medium supplemented with heat-inactivated FBS under 5% CO2. Extracts, fractions, and isolated compounds were dissolved in DMSO (final concentration 1%) and diluted in culture medium. Serial concentrations were tested to determine IC50 using the AlamarBlue assay [25]. Parasites (2×10^4 cells/mL) were exposed to compounds or pentamidine (positive control) in 96-well plates for 69 h at 37 °C with 5% CO2. After incubation, 10 µL of 0.5 mM resazurin in PBS was added, followed by a 3 h incubation. Fluorescence was measured at 536 nm excitation and 588 nm emission. All experiments were performed in duplicate.

Activity against trypanosoma cruzi – trypomastigote

The T. cruzi Tulahuen strain (TcLuc2) was maintained in DMEM without phenol red, and the trypomastigote stage was propagated in 3T3 cell monolayers, which were cultured in DMEM with phenol red supplemented with inactivated FBS [18]. Serial dilutions of test compounds were evaluated using a modified luciferase assay to determine IC_{50} values [26]. Initially, TcLuc2 cells at a final density of 1×10^6 cells/mL were added to each well and incubated for 24 h, followed by the addition of 3T3 cells at 2×10^5 cells/mL. After four days of incubation with the test compounds, 30 µL of a luciferase reaction buffer—comprising 0.5 µM Tris-HCl (pH 7.8), 25 mM $MgCl_2$, 1 mg/mL D-Luciferin, 125 µM ATP, 125 µM CoA, 1% (w/v) Triton X-100, and water—was added to each well. Luminescence was then measured at 560 nm.

Trypanosoma cruzi – epimastigote stage

Epimastigotes were maintained in LIT medium with FBS [16] at 5×10^3 cells/mL. Serially diluted compounds were added to 96-well plates and incubated under anaerobic conditions at 28 °C for four days. Following incubation, luciferase buffer was applied, and luminescence measured at 560 nm. IC50 values were determined using GraphPad Prism v8.3.1 (332), with concentrations below 10 µg/mL considered indicative of strong anti-parasitic activity.

Cytotoxicity evaluation in mammalian cells

Human hepatoma (Huh7, HepG2), BHK-21, and Vero cells were cultured in DMEM supplemented with 3.7 g/L NaHCO3, 10% heat-inactivated FBS, and 1% penicillin-streptomycin at 37 °C with 5% CO2. Cells were seeded into 96-well plates at densities of 3×10^4 (Huh7), 1×10^4 (HepG2), 5×10^3 (BHK-21), and 1×10^4 (Vero). Test compounds were added in serial dilutions following modifications of Coatti *et al*. [27]. Plates were incubated for 44 h, then 10 µL of 0.5 mM resazurin was added and

incubated for 4 h. Fluorescence was read using a Nivo microplate reader (excitation 530 nm, emission 595 nm). CC50 values, representing the concentration causing 50% cell death, were calculated using GraphPad Prism v8.3.1 (332). Substances with CC50 above 20 µg/mL were considered non-toxic. Selectivity index (SI) was calculated as CC50/IC50, with higher values indicating a better balance of efficacy and safety.

Results and Discussion

In vitro antiprotozoal screening of plant extracts

The antiprotozoal potential of plant-derived extracts was evaluated using a stratified solvent extraction approach, sequentially employing n-hexane, dichloromethane, and methanol on dried plant material. A total of six species from the genus Cratoxylum, two species from Diospyros, and three species from Artocarpus were processed, resulting in 48 distinct extracts, summarized in **Table 1**. Extracts from Cratoxylum displayed a wide spectrum of activity, with several demonstrating IC50 values below 10 µg/mL, indicating strong inhibition of protozoal growth. In contrast, most extracts from Diospyros and Artocarpus exhibited IC50 values above 10 µg/mL, suggesting comparatively lower potency.

Among all samples tested, the dichloromethane extracts from the root of Cratoxylum arborescens (Ca.RD) and the twig of Cratoxylum sumatranum (Cs.TD) showed the most pronounced activity across all protozoan species. Given its superior performance relative to Cs.TD, the Ca.RD extract was selected for further fractionation and compound isolation.

Fractionation and isolation of active compounds from Ca.RD

Fractionation of 2.5 g of Ca.RD using vacuum liquid chromatography (VLC) generated ten fractions, labeled Ca.RD-D1 through D10. Subsequent antiprotozoal testing revealed that all fractions except D1 exhibited measurable activity against the panel of protozoa. In the Plasmodium falciparum assay, fraction D6 was the most potent, achieving an IC50 of 0.8 µg/mL. Conversely, fractions D7 showed the strongest inhibition against E. histolytica, L. donovani, and T. b. rhodesiense, with IC50 values of 1.6 µg/mL, 0.2 µg/mL, and 0.1 µg/mL, respectively.

Further purification of fraction D7 by crystallization yielded yellow crystals, designated as compound (1), with a yield of 32.6 mg (0.005%). When tested against the protozoan panel, compound (1) exhibited IC50 values of 2.3 µg/mL (P. falciparum), 0.3 µg/mL (E. histolytica), 0.1 µg/mL (L. donovani), and 0.1 µg/mL (T. b. rhodesiense), as detailed in **Table 2**.

Table 2. Antiprotozoal activities of selected extract, fractions, compound (1), and standard drugs

Extract/Fractions Code	Yield (%)	E. histolytica (IC_{50} µg/mL)	P. falciparum (IC_{50} µg/mL)	T. brucei rhodesiense (IC_{50} µg/mL)	L. donovani (IC_{50} µg/mL)
Ca.RD	0.357	8.2	0.1	0.9	0.6
Ca.RD-D1	0.007	inactive	inactive	inactive	inactive
Ca.RD-D2	0.003	inactive	inactive	10.3	inactive
Ca.RD-D3	0.004	inactive	6.2	1.7	9.5
Ca.RD-D4	0.008	inactive	6.6	3.5	inactive
Ca.RD-D5	0.009	inactive	6.0	1.3	9.6
Ca.RD-D6	0.008	4.4	0.8	0.1	0.4
Ca.RD-D7	0.009	1.6	5.8	0.1	0.2
Ca.RD-D8	0.003	1.6	1.7	0.6	1.2
Ca.RD-D9	0.010	inactive	3.3	inactive	inactive
Ca.RD-D10	0.242	6.8	1.9	inactive	0.7
Compound (1)	0.005	0.3	2.3	0.1	0.1
Standard Drugs					
Chloroquine	-	-	1.8	-	-
Metronidazole	-	2.6	-	-	-
Amphotericin B	-	-	-	-	0.3

Pentamidine	-	-	-	0.06	-

Data presented as mean of triplicates. "Inactive" indicates IC_{50} > 10 μg/mL. "-" indicates not tested.

Structure identification of compound (1)

The elucidation of compound (1) as Cochinchinone C (molecular formula $C_{24}H_{26}O_6$) relied on comparison of its ^1H and ^{13}C NMR spectra with literature values. Analysis of the ^{13}C NMR spectrum **(Table 3)** indicated a total of 24 carbon signals, including carbonyl resonances at δ 180.80 (assigned to C-9) and δ 201.28 (assigned to C-6), which supported the existence of both a conjugated and an unconjugated ketone functionality. In the ^1H NMR spectrum, a downfield singlet at δ 12.00 was attributed to a chelated hydroxyl group (1-OH), while three aromatic signals appeared as an ABM spin system: δ 6.54 (dd, J = 9 Hz, H-2), δ 7.40 (t, J = 8.3 Hz, H-3), and δ 6.50 (dd, J = 8.3 Hz, H-4). Additional key features included an olefinic signal at δ 7.50 (H-8), a singlet methoxyl at δ 3.63 (7-OCH_3), geminally coupled methylene protons at δ 1.57 (dd, Hb-10) and δ 2.39 (d, Ha-10), a methine at δ 2.52 (d, H-11), and signals characteristic of a prenyl substituent: δ 4.38 (t, H-16), δ 2.63 (d, H-15), δ 1.35 (s, H-18), and δ 0.99 (s, H-19) [28, 29]. The proposed structure for Cochinchinone C is depicted in **Figure 1**.

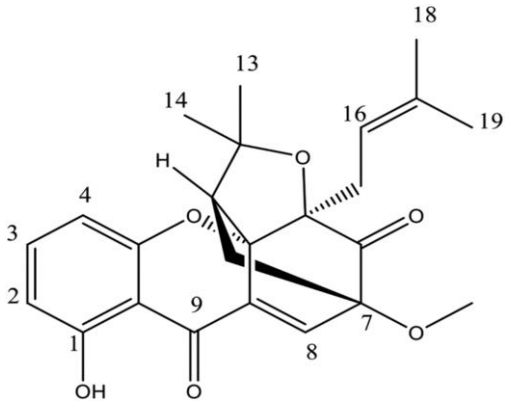

Figure 1. Molecular structure of cochinchinone C

Table 3. 1H (400 MHz) and 13C (100 MHz) NMR data for compound (1) in CdCl3

Position	Reference δC (ppm)	Reference δH (J, Hz)	Compound (1) δC (ppm)	Compound (1) δH (J, Hz)
1-OH	162.9 C	12.10 s	162.93	12.00 s, 1 H
2	109.5 CH	6.55 dd (8.4)	109.63	6.54 dd, 1 H (9)
3	138.9 CH	7.41 t (8.4)	139.09	7.40 t, 1 H (8.3)
4	107.4 CH	6.52 dd (8.4)	107.52	6.50 dd, 1 H (8.3)
4a	159.4 C	–	159.51	–
5a	88.8 C	–	88.79	–
5	84.1 C	–	84.24	–
6	201.1 C	–	201.28	–
7	84.8 C	–	84.93	–
8	135.3 CH	7.51 d (1.2)	135.29	7.50 d, 1 H (1.4)
9	180.7 C	–	180.80	–
8a	132.1 C	–	132.20	–
9a	106.1 C	–	106.20	–
10a	29.7 CH_2	2.39 d (13.2)	29.78	2.39 d, 1 H (12.8)
10b	–	1.58 dd (13.2)	–	1.57 dd, 1 H (11.4)
11	49.4 CH	2.53 d (9.6)	49.47	2.52 d, 1 H (9.8)
12	83.9 C	–	84.07	–
13	30.4 CH_3	1.68 s	30.45	1.67 s, 3 H
14	29.0 CH_3	1.32 s	29.12	1.31 s, 3 H
15	29.2 CH_2	2.64 d (7.8)	29.28	2.63 d, 2 H (8.3)
16	118.4 CH	4.41 t (7.8)	118.55	4.38 t, 1 H (8.8)
17	135.7 C	–	135.84	–

18	25.5 CH_3	1.37 s	25.61	1.35 s, 3 H
19	16.7 CH_3	1.01 s	16.76	0.99 s, 3 H
7-OCH_3	54.1 CH_3	3.64 s	54.18	3.63 s, 3 H

Antiprotozoal activity, cytotoxic effects, and selectivity of cochinchinone C

The potential of cochinchinone C against T. cruzi was assessed to explore its role as a therapeutic agent. The compound demonstrated inhibitory effects on both amastigote and trypomastigote stages of T. cruzi, with IC_{50} values indicating higher potency against this parasite compared to other protozoa. Cytotoxicity was evaluated across several mammalian cell lines, and the corresponding selectivity index (SI) was determined **(Table 4)**.

Table 4. In vitro antiprotozoal, cytotoxicity and selectivity index of cochinchinone C

Compound	Inhibition (IC_{50} µM)					Cytotoxicity (CC_{50} µM)				Selectivity Index (SI)
	Pf	Eh	Ld	Tbr	Tc-t	Tc-e	HepG2	Huh7	BHK-21	
Cochinchinone C	5.8	6.1	0.2	0.1	0.7	0.07	13.1	22.7	13.9	13.4

SI = CC_{50}/IC_{50}. Pf: *P. falciparum*, Eh: *E. histolytica*, Ld: *L. donovani*, Tbr: *T. brucei rhodesiense*, Tc-t: *T. cruzi* trypomastigote, Tc-e: *T. cruzi* epimastigote

The inhibitory concentrations (IC50) for cochinchinone C toward different protozoan parasites varied between 0.07 and 5.8 µM. As per World Health Organization (WHO) guidelines, such concentrations qualify as highly active in terms of antiparasitic inhibition, suggesting strong prospects for developing new treatments against these parasites. Moreover, evaluations of cytotoxicity in cell lines including Huh7, HepG2, BHK-21, and Vero revealed selectivity indices (SI) greater than 10 against E. histolytica, L. donovani, T. b. rhodesiense, and T. cruzi, while exceeding 2 against P. falciparum. This indicates that cochinchinone C has limited harmful effects on both cancerous and healthy mammalian cells [30].

Advancing medications for neglected tropical diseases (NTDs), such as those caused by E. histolytica, L. donovani, T. brucei rhodesiense, and T. cruzi, involves numerous obstacles but also substantial potential. Key difficulties encompass insufficient funding, complex approval processes, and weak economic motivations for investment [31]. Pharmaceutical firms often overlook these conditions due to their prevalence among low-income communities, leading to reduced research efforts. Strict oversight standards and scarce resources further complicate the progression of viable candidates from research stages to patient use. Nevertheless, these issues are countered by emerging possibilities through joint efforts and inventive approaches. Initiatives like partnerships between public and private sectors, along with support from international health bodies, have boosted investigative work and secured additional resources for NTD-focused programs. Technological progress, including rapid screening techniques and computer-based design, provides novel methods for detecting and refining candidate molecules. Multidisciplinary alliances among academic institutions, commercial entities, governmental agencies, and charitable groups offer pathways to surmount these barriers and enhance health results for at-risk groups [32].

This investigation positions Cratoxylum arborescens as a valuable source of compounds effective against protozoans, enriching the understanding of how Indonesian botanical materials can combat parasitic infections. As part of the Hypericaceae family and common in Southeast Asia—including regions in Indonesia and Thailand—this species has been noted earlier for yielding xanthones and anthraquinones with diverse bioactivities [33], encompassing anti-HIV effects [34], anticancer properties [35], and antimicrobial actions [36].

The work centered on the root extract obtained via dichloromethane from C. arborescens, which displayed notable in vitro efficacy against protozoans, resulting in the purification of cochinchinone C as the primary active agent. This known substance had been previously separated from roots of C. cochinchinense and C. formosum ssp. pruniflorum, where it showed modest antioxidant effects and cytotoxic action against the MCF-7 breast cancer line (IC50 of 0.36 µg/mL) [28, 29]. Additional reports indicated that cochinchinone C from C. cochinchinense roots had cytotoxicity toward the NCI-H187 lung cancer line (IC50 of 2.3 µg/mL) alongside

antimalarial effects on P. falciparum (IC50 of 6.3 μM) [37]. In the present work, cochinchinone C derived from C. arborescens exhibited comparable antimalarial potency against P. falciparum (IC50 of 5.8 μM), aligning closely with prior results from C. cochinchinense (IC50 of 6.3 μM). Thus, the antimalarial data here do not represent a new discovery.

Although prior studies documented antimalarial effects of cochinchinone C on P. falciparum—a pathogen not classified as an NTD—no reports existed on its actions against the NTD-related protozoans E. histolytica, L. donovani, T. brucei rhodesiense, and T. cruzi. The current isolation of cochinchinone C from C. arborescens roots marks the initial documentation of its efficacy against these four parasites. Related cage-prenylated xanthones, such as cochinchinoxanthone and cochinchinone D from Cratoxylum sumatranum stem bark, have been proposed as effective options against E. histolytica via interference with the NAD kinase pathway [19]. These results reinforce earlier observations, underscoring the value of cage-prenylated xanthones like cochinchinone C in antiparasitic applications. The enhanced potency of certain xanthones may stem from a hydroxyl at the C1 position on the A-ring, facilitating internal hydrogen bonds that heighten the reactivity of the C8 enone system [38]. Such structural traits likely underlie the antiparasitic mechanisms of xanthones.

Cochinchinone C shows marked potency against L. donovani, T. b. rhodesiense, and Trypanosoma cruzi, making it a viable candidate for advanced therapeutic exploration. Its molecular attributes—incorporating prenyl, hydroxyl, and methoxyl moieties—support its effectiveness against parasites, reflected in favorable low IC50 figures combined with reduced cytotoxicity, establishing it as a potentially secure antiparasitic option. Additionally, the discovery of cochinchinone C as an effective agent from Cratoxylum arborescens emphasizes the role of naturally derived substances in yielding innovative treatment leads. The wide structural variety and intricate chemistry of plant-based compounds provide an extensive pool of molecules with varied therapeutic actions. Ongoing investigations into Indonesian flora and similar biodiverse areas could uncover additional remedies for parasitic illnesses and broader medical needs. Natural compounds frequently offer distinct modes of action and precise targeting, providing benefits like lower toxicity and decreased likelihood of resistance compared to fully synthetic alternatives. To maximize this potential in therapeutic development, coordinated efforts among experts in chemistry, pharmacology, biology, and clinical medicine are essential to thoroughly assess and advance promising substances into practical therapies [39].

Conclusion

Results from this investigation highlight Cratoxylum arborescens as an important resource for antiparasitic agents, with cochinchinone C standing out as a strong contender for future therapeutic advancement. Additional examinations could explore the compound's antiparasitic mechanisms or potential combinations with existing medications. The work also underscores the value of Indonesia's ecological diversity in supplying plant-derived treatments. Furthermore, the effective separation and analysis of cochinchinone C illustrate the advantages of integrated scientific strategies that blend indigenous knowledge with contemporary techniques. Looking ahead, sustained research into natural compound variety and partnerships with local populations offer great potential for tackling worldwide health issues. By utilizing botanical resources and respecting ancestral practices, progress can be made toward creating reliable, efficient, and appropriate therapies for parasitic conditions and related concerns.

Acknowledgments: None

Conflict of Interest: None

Financial Support: None

Ethics Statement: None

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