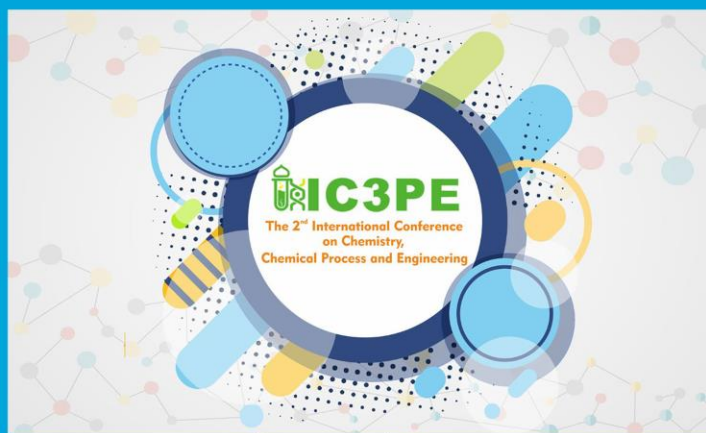


Volume 2026

 **Conference collection**

2nd International Conference on Chemistry, Chemical Process and Engineering (IC3PE)



Yogyakarta, Indonesia

14 August 2018

Editors

Is Fatimah, Hideya Kawasaki, Azlan Kamari, Laemthong Chuenchom,
M. Arsyik Kurniawan S., Imam Sahroni and Miqdam Musawwa

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Location: Yogyakarta, Indonesia

ISBN: 978-0-7354-1746-5

Editors:

Is Fatimah, Hideya Kawasaki, Azlan Kamari, Laemthong Chuenchom, M. Arsyik Kurniawan S., Imam Sahroni and Miqdam Musawwa

Volume number: 2026

Published: Oct 29, 2018

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[Dhina Fitriastuti](#), [Annisa Wahyu Nur Iman](#), [Dea Alvine Lutfiani](#) and [Dian Yuliyanti](#)

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Cite as: AIP Conference Proceedings 2026, 020105 (2018); <https://doi.org/10.1063/1.5065065>
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In vitro antiplasmodial activity of extract and fraction of Temu mangga (*Curcuma mangga*) against Plasmodium falciparum 3D7

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Abstract. *Curcuma mangga*, locally known as “temu mangga” or “kunyit mangga”, is a rhizomatous herb of the *Zingiberaceae* family. The rhizomes of *C. mangga* are used in Java as a seasoning for food and treatment for stomach aches, fever and cancer-related diseases. In the present paper, the antiplasmodial activity of extract and fraction of *C. mangga* rhizome has been conducted. The rhizome was extracted with ethanol by using soxhletation extraction. The ethanol extract was fractioned by using Vacuum Liquid Chromatography (VLC) method with eluent of n-hexane:ethyl acetate (2:1), ethyl acetate and ethanol. The extract and fraction were analyzed by using GC-MS. From the GC-MS data, it is showed that the extract and fraction contained (E)-labda-8 (17), 12-dien-15,16-dial compound which is known have a good anticancer activity. This compound is high percentage contained in ethanol extract and n-hexane:ethyl acetate (2:1) fraction. Thus, the antiplasmodial assay was tested in the both samples. The result of antiplasmodial assay showed that n-hexane:ethyl acetate (2:1) fraction could inhibit the parasitemia growth in 10 µg/mL of dosage with the average inhibition of 8.42% while the ethanol extract in 10 µg/mL of dosage could not inhibit the growth of parasitemia. Ethanol extract and n-hexane:ethyl acetate (2:1) fraction have IC₅₀ values of 62.64 µg/mL and 46.17 µg/mL, respectively. Thus, the n-hexane:ethyl acetate (2:1) fraction displayed better antimalarial activity than ethanol extract.

INTRODUCTION

Malaria continues to threaten the world's people in both the developing and developed countries. Indonesia's geography which is a tropical country makes this disease grow rapidly. Based on The World Malarial Report, there were 209 million malaria cases with 660,000 deaths worldwide in 2010 and Indonesia is one of 104 countries including malaria endemic countries. Recorded in 2010 to 2014, Indonesia ranks fourth in Asian countries with a total of 73% of tropical malaria cases [1].

Malaria is a disease caused by infection with *Plasmodium* parasites that are transmitted through the intermediary bite of a female *Anopheles* mosquito. There are five types of *Plasmodium* which often infect humans. Namely *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae*, *Plasmodium ovale* and *Plasmodium knowlesi*. The five types of *Plasmodium* are found in the tropical regions of the world, such as Africa, America, Eastern Mediterranean, Europe, Southeast Asia and the Western Pacific [2]. *Plasmodium falciparum* (*P. falciparum*) can cause severe acute infections of the kidneys, liver and brain, which can cause death and are a type of parasite that most often infects Indonesia [3].

Malaria prevention efforts have long been carried out but are still not optimal. Many factors become obstacles in the effort to eradicate malaria pests. Among these main factors is the emergence of malaria vectors that are resistant to insecticides and parasites with commercial antimalarials available. *Plasmodium* (especially *P. falciparum*) has been reported by some countries to experience resistance to chloroquine, the current commercial antimalarial [4].

This resistance problem has become a serious and alarming problem because cause many failures in the treatment even lead to death. Besides that WHO targets a reduction in cases and deaths from malaria by 50% or more in 2000-2010 and 75% or more in 2000-2015 [2]. This prompted researchers to look for new effective antimalarial drugs. One of the efforts to find new antimalarials is the isolation of active compounds from natural ingredients that are traditionally used by people in several places to treat malaria. The use of natural ingredients as medicine is very necessary considering that Indonesia has many biological resources that can be utilized.

Curcuma mangga locally known as “temu mangga” or “kunyit mangga”, meaning mango-like turmeric, is a rhizomatous herb of the *Zingiberaceae* family. The rhizomes of *C. mangga* are used in Java as a seasoning for food and treatment for stomach aches, fever and cancer related diseases. Although there have been reports concerning the chemical constituents and biological activities of *C. mangga*, only a few have focused on the antimalarial activity of *C. mangga*. Four reports have shown that *C. mangga* possesses antioxidant, antitumour, antifungal and antiallergic properties [5-9]. In a previous study, it was found that ethanol extract of mango rhizome had an effect on the inhibition of *P. berghei* growth which was infected in male white mice with a dose of 250 mg / Kg BB. The results of this study indicate a barrier to the development of parasitemia by 48.56% [10]. Although *C. mangga* has been studied as an antimalarial drug in *P. berghei* but its activity is still unknown to the inhibition of in vitro assay. The purpose of this study was to determine the antiplasmodial activity of ethanolic extract and n-hexane:ethyl acetate (2:1) fraction of *C. mangga* as antimalarial, as well as to find out secondary metabolites that act as antimalarial active compounds.

EXPERIMENTAL SECTION

Materials and Equipments

Chemicals used in this research were *C. mangga* rhizome, ethanol p.a, ethyl acetate p.a, n-hexane p.a, acetone, TLC, silica gel, chloroquine diphosphate, RPMI, sorbitol and red blood cell (RBC). All chemicals, except *C. mangga* rhizome which was obtained from Center for Research and Development of Medicinal Plants and Traditional Medicines (B2P2TOOT) Indonesia, were purchased from E. Merck with high grade and used without any further purification. Equipment used in this research were laboratory glassware, analytical mass balance (Mettler AT200), centrifuge (Sorvall Biofuge Primo R), Elisa reader (type 680 XR) and LC-MS/MS (Waters, Acquity UPLC I-Class with Xevo G2-XF QTof).

Procedures

Extraction and fractionation

The dried rhizome of *C. mangga* was cut up to ± 0.2 cm. About 100 g of sample was weighed and 400 ml of ethanol was added and extracted in a Soxhlet apparatus at 70 °C. The filtrate was evaporated to dryness at 50°C in a rotary evaporator. And the above process was repeated for several times, until the sufficient amount of extract is produced. The concentrated extract of each plant was stored at 4°C until when required for use. The ethanolic extract was chromatographed via vacuum liquid chromatography on a silicagel column and eluted with n-hexane, ethyl acetate and ethanol to get the fractions. The extract and fractions were evaluated by phytochemical qualitative reactions for usual plant secondary metabolites. The screening was performed for terpenes, alkaloids and phenolic acids.

LC-MS analysis

Samples with concentration of 1 mg/mL in methanol were injected for 20 μ L in the LC-MS column by using mobile phase with gradient eluent system of solvent A (distilled water/-1% of formic acid) and solvent B (acetonitrile/0.1% of formic acid). Condition: RP-18 column, flow rate of mobile phase of 0.6 mL/min, ESI-MS positive ion mode detector.

Antiplasmodium assay

The culture of 3D7 *Plasmodium falciparum* is cultivated by modifying method, i.e. storage on the candle jar in CO₂ incubator at 37 °C. *Plasmodium* is *in vitro* grown using erythrocyte O⁺ with 1-5% of hematocrit in the medium of 1640 RPMI containing 25 mM of HEPES, 30 mM of sodium bicarbonate and 10% human serum. The condition of culture is observed every day. Before doing the assay, *Plasmodium* is synchronized using 5% of sorbitol. The antimalarial assay is conducted in two ways: microscopic and micro radioactive developed by Desjardins *et al* (1979). Into 96 well containing *Plasmodium* culture on trapezoid phase with parasitemia of 2% (hematocrit of 3%), the test sample is added to various concentration. The culture is incubated for 24 and 72 hours. In the former method, the parasitemia was calculated from apusan colored with Giemsa. This value is then used to calculate percentage of inhibitory growth of *Plasmodium*. In the latter method, the parasite growth is calculated based on the taking of [³H]-hypoxanthine by the parasite. As the control, culture of *Plasmodium* without the test compound is used and considered to have the growth of 100%. Antimalarial activity is reported as Inhibitory Concentration 50 (IC₅₀), i.e. concentration needed to inhibit the parasite growth up to 50%.

RESULT AND DISCUSSION

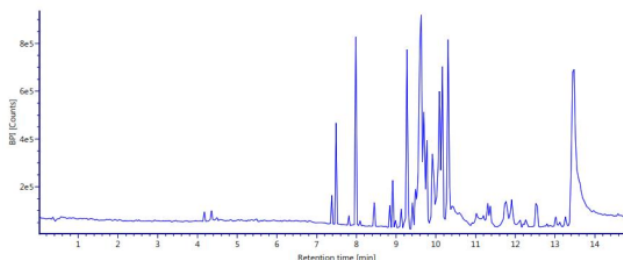
Extraction and Fractination

C. mangga rhizomes that has been prepared was extracted by using soxhlet extraction method and yielded 17.29% of dark brown liquid and thick texture. The obtained crude was then fractionated by VLC (Vacuum Liquid Chromatography) using n-hexane: ethyl acetate (2: 1), ethyl acetate and ethanol eluents. The process produced three fractions according to the eluent used, then each fraction was evaporated and give n-hexane: ethyl acetate (2: 1), ethyl acetate and ethanol fraction with 36.36%, 14.87% and 15, 82% of yield, respectively. Then, the identification was done by using phytochemical screening (Table 1). Phytochemical screening tests are used to identify earlier compounds obtained by their compounds.

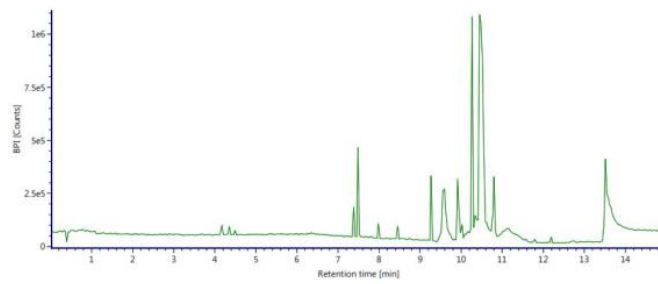
TABLE 1. Phytochemical Screening Results of extract and fraction of *C. mangga*

No.	Compound test	Reagent	Samples		
			Ethanol extract	Ethyl acetate fraction	n-Hexane: ethyl acetate (2: 1) fraction
1.	Alkaloid	Dragendorff	+	+	+
2.	Terpenoid	Vanillin-sulphate	+	+	+
3.	Phenolic	FeCl ₃ 1%	+	+	+

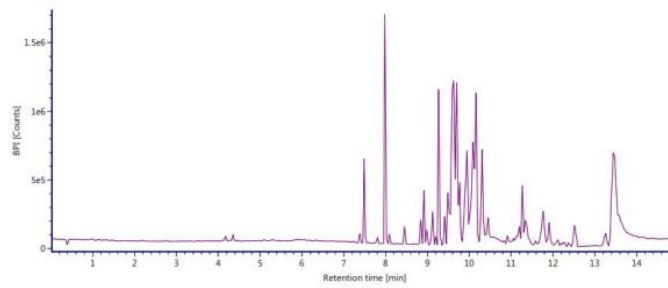
Note: (+) = compound contained in samples



(a)

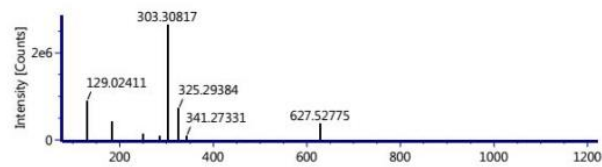


(b)

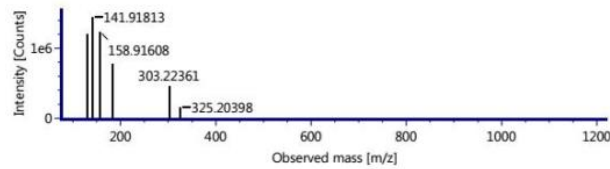


(c)

FIGURE 1. The liquid chromatography–mass spectrometry (LC-MS) chromatograph of (a) ethanol extract, (b) ethyl acetate fraction and (c) n-hexane: ethyl acetate fraction of *C. mangga*



(a)



(b)

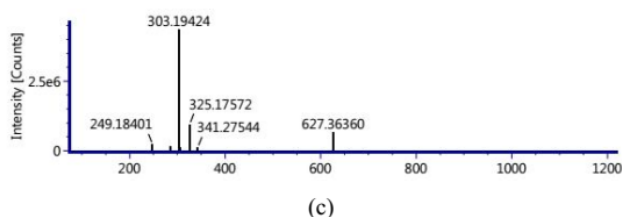


FIGURE 2. Mass spectrometry of major compound in (a) ethanol extract, (b) ethyl acetate fraction and (c) n-hexane: ethyl acetate fraction of *C. mangga*

The results of identification using LC-MS provide information on the existence of several peaks derived from the same compound. The peak with a retention time (t_R) of 7.99 min from each chromatogram was suspected of the target compound given the molecular ion peak on crude extract, ethyl acetate and n-hexane: ethyl acetate fraction of *C. mangga* with $m/z=303,30$ (Fig. 2a), 303.22 (Fig. 2b) and 303.19 (Fig. 2c), respectively. According to Malek et al. (2011), this compound is (E)-labda-8 (17), 12-dien-15,16-dial (Fig. 3). This compound has activity as an anticancer to various cancer cells such as MCF-7, KB, A549, Ca Ski, HCT 116, HT-29, and MRC-5 with IC_{50} value respectively 4.3 ± 1.30 ; 14.5 ± 0.87 ; 19.9 ± 0.38 ; 12.1 ± 0.35 ; 7.6 ± 0.23 ; 6.3 ± 0.26 and $8.9 \pm 0.49 \mu\text{g/mL}$ [11].

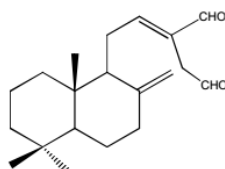


FIGURE 3. Structure of (E)-labda-8 (17), 12-dien-15,16-dial compound

The highest peak intensity of target compound in each chromatogram showed in n-hexane: ethyl acetate fraction. This indicates that the concentration of (E)-labda-8 (17), 12-dien-15,16-dial compound in the n-hexane: ethyl acetate fraction is the highest than the crude extract of ethanol and ethyl acetate fraction. Thus, the antiparasitoidium assay was performed to ethanol extract and n-hexane: ethyl acetate fraction.

Antiplasmodium In Vitro Activity Assay

The average percentage of parasitemia and inhibition of each dose of extract is presented in Table 2. In Table 2, it is shown that the higher the extract dose have the greater the inhibition of growth against *P. falciparum*. The percentage inhibition of growth of *P. falciparum* at the lowest dose of $5 \mu\text{g} / \text{mL}$ for the fraction of n-hexane: ethyl acetate and $10 \mu\text{g} / \text{mL}$ for ethanol extract had negative value. This causes the calculation of IC_{50} value can not be done by using the data. At a dose of $10 \mu\text{g} / \text{mL}$, the n-hexane fraction: ethyl acetate can inhibit the growth of parasitemia with an inhibitory rate of 8.42% while ethanol extract could not inhibit the growth of parasitemia. This shows that the fraction of n-hexane: ethyl acetate has the activity of inhibiting the growth of malaria parasitaemia better than ethanol extract.

TABLE 2. Average percentage of growth of parasitemia and inhibition of *P. falciparum* for ethanol extract and n-hexane: ethyl acetate fraction of *C. mangga* rhizome.

Dose ($\mu\text{g/mL}$)	EtOH extract		n-hex:EtOAc (2:1) fraction	
	% average of parasitemia \pm SD	% average of inhibition	% average of parasitemia \pm SD	% average of inhibition
5	16.61 \pm 2.51	-74.84	10.51 \pm 0.25	-10.68
10	11.31 \pm 0.33	-19.05	8.70 \pm 0.28	8.42
50	7.84 \pm 2.36	17.42	6.55 \pm 0.35	31.00
100	0.24 \pm 0.20	97.42	0.76 \pm 0.01	92.05
200	0 \pm 0	100.00	0 \pm 0	100.00
RPMI (Control -)	9.5	0	9.5	0
IC ₅₀ ($\mu\text{g/mL}$)	62.64		46.17	

Antiplasmodium activity of ethanol extract and n-hexane: ethyl acetate fraction of *C. mangga* rhizome was showed in Figure 4. By using probit analysis, the antiplasmodium activity of ethanol extract and n-hexane: ethyl acetate fraction of *C. mangga* rhizome was determined with IC₅₀ values of 62.64 $\mu\text{g/mL}$ and 46.17 $\mu\text{g/mL}$, respectively. [12] reported that extracts and fractions of medicinal plants were declared to have no antiplasmodium activity when they had IC₅₀ > 50 $\mu\text{g/mL}$, whereas [13] states that if IC₅₀ of an extract is less than 5 $\mu\text{g/mL}$, then its antiplasmodium activity is very good, if the IC₅₀ is 5-10 $\mu\text{g/mL}$, its antiplasmodium activity is good, and if the IC₅₀ > 10 $\mu\text{g/mL}$, its antiplasmodium activity is inactive. Other researchers [14] classified an antiplasmodium-effect plant extract as follows: antiplasmodium activity was excellent when IC₅₀ values were less than 0.1 $\mu\text{g/mL}$; good (active) when IC₅₀ values were 0.1-1 $\mu\text{g/mL}$; moderate if IC₅₀ values were 1.1-10 $\mu\text{g/mL}$; weak if IC₅₀ values were 11-25 $\mu\text{g/mL}$; very weak when IC₅₀ values were 26-50 $\mu\text{g/mL}$, and inactive when IC₅₀ values were more than 100 $\mu\text{g/mL}$. From the various statements of these researchers, it can be categorized that both samples have very weak antiplasmodium activity. However, the antiplasmodium activity of the n-hexane: ethyl acetate fraction is relatively good compared with ethanol extract of *C. mangga* rhizome which is possibly caused by the present of (E)-labda-8 (17), 12-dien-15,16-dial compound in n-hexane: ethyl acetate fraction.

CONCLUSION

Extract and fraction of *C. mangga* contained (E)-labda-8 (17), 12-dien-15,16-dial compound which is known have a good anticancer activity. This compound is high percentage contained in ethanol extract and n-hexane:ethyl acetate (2:1) fraction. The result of antiplasmodial assay showed that n-hexane:ethyl acetate (2:1) fraction could inhibit the parasitemia growth in 10 $\mu\text{g/mL}$ of dosage with the average inhibition of 8.42% while the ethanol extract in 10 $\mu\text{g/mL}$ of dosage could not inhibit the growth of parasitemia. Ethanol extract and n-hexane:ethyl acetate (2:1) fraction have IC₅₀ values of 62.64 $\mu\text{g/mL}$ and 46.17 $\mu\text{g/mL}$, respectively. Thus, the n-hexane:ethyl acetate (2:1) fraction displayed better antimalarial activity than ethanol extract.

ACKNOWLEDGMENT

This research was funded by Direktorat Penelitian dan Pengabdian Masyarakat (DPPM), Universitas Islam Indonesia.

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