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In vitro antimalarial activity of *Syzygium cumini* fruit fraction

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Abstract

Background: Malaria is still one of the most severe public health problems worldwide. The development of treatment, prevention, and control of malaria is one of the substantial problems in the world.

Aims: To investigate the *in vitro* antimalarial activity of *Syzygium cumini* methanol fruit fraction.

Methods: *Syzygium cumini* L fruit powder was macerated with methanol (PA) and the extract obtained was fractionated using the liquid–liquid partition method with n-hexane, ethyl acetate, butanol, chloroform, methanol, and water solvents. *In vitro* antimalarial assay was conducted using the culture of *Plasmodium falciparum* 3D7 strain culture that had reached >5% growth and was examined for IC_{50} values using a 24-well microplate in duplicate. Each treatment and control well contained 1,080 µl of complete media. Well, number 1 was added with 120 µl fraction, and then the solution was diluted until it reached 0.01, 0.1, 1, 10, and 100 µg/ml the final concentration in the microtiter well. The control only contained complete media and infected erythrocytes without the addition of anti-malarial drugs. The microplate was incubated for 48 hours. After 48 hours, a thin blood smear was made fixed with methanol and stained with 20% Giemsa for 20 minutes to determine the IC_{50} value by plotting sample concentrations and percentage of parasitemia in Excel.

Results: The IC_{50} values of ethyl acetate fraction, n.hexane fraction, butanol fraction, and water fraction were 1.189, 76.996, 1,769, and 15.058 µg/ml, respectively. Whereases the IC_{50} values of C1 fraction (mix fraction from chloroform: methanol 100:0 and 90:10) and C4 fraction (mix fraction from chloroform: methanol 20:80, 10:90, and 0:100) were 100.126 and 1.015 µg/ml, respectively. The results showed that the IC_{50} value of ethyl acetate, butanol, and C4 fraction were lower than 10µg/ml and were considered as good activity (strong antimalarial activity).

Conclusion: The ethyl acetate, butanol and C4 subfraction from *S. cumini* fruit have the potential to be developed as an antimalarial agent.

Keywords: Infectious disease, Malaria, IC_{50}, *Syzygium cumini* L fruit.

Introduction

Malaria is still one of the most severe public health problems worldwide. The development of treatment, prevention, and control of malaria is one of the substantial problems in the world. The use of common antimalarial drugs has been challenged variously due to the development of parasite resistance to some antimalarial drugs. There is also no effective vaccine to control malaria infection due to the complex life cycle of the *Plasmodium* parasite (Batista *et al.*, 2009).

The problem of antimalarial drug resistance increases and there are difficulties in accessing effective antimalarial drugs, due to the spread of malaria-resistant multidrug parasites, so it is urgently needed to develop new antimalarial drugs from natural materials. One of the many medicinal plants found in Indonesia is juwet (*Syzygium cumini*). The results of a study showed that *S. cumini* has strong radical scavenging and antioxidant activity (Zhang and Lin, 2009). Phenolic compounds and flavonoids contained in *S. cumini* have antioxidant and anti-inflammatory activities (Borges *et al.*, 2017). The anti-malarial activities of flavonoids inhibit the fatty acid biosynthesis (FASII) of the parasite as well as inhibiting the influx of L-glutamine and myoinositol into infected erythrocytes (Rudrapal and Chetia, 2016). The use of some parts of the *S. cumini* plant as an antimalarial showed that *S. cumini* fruit as a therapeutic adjuvant has a better inhibitory effect on *Plasmodium* as compared to the leaves and bark (Maslachah and Sugihartuti, 2018; Maslachah *et al.*, 2020). Determining the antimalarial activity of compounds in *S. cumini* L fruit can be made by *in vitro*. *In vitro* antimalarial testing is a test that does not involve the intervention of the host physiological factor, so the results obtained are the result

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of a parasite that is directly exposed to the test material and can be examined for inhibition of growth and maturation into schizont (Maji, 2018). The result of *in vitro* testing on malarial material can be determined based on the value of 50 concentration inhibitors (IC_{50}), so this research was conducted with the fraction of *S. cumini* L fruit as *in vitro* antimalarial assay against *Plasmodium falciparum* 3D7 strain to determine the IC_{50} value.

Materials and Methods

Materials

The solvents used to make *S. cumini* fractionation were n-hexane (Merck), chloroform (Merck), Methanol (Merck), Butanol (Merck), ethyl acetate (Merck), and aquadest. Materials used to make *P. falciparum* culture media and to make *in vitro* test solutions were aquadest, HEPES buffer (Sigma), RPMI 1640 (Gibco BRL, USA), sodium bicarbonate, gentamicin, plasma, and human erythrocytes, DMSO (Sigma-Aldrich), Giemsa dyes, methanol, and emersion oil.

Plasmodium isolate

Isolate of *P. falciparum* 3D7 (Chloroquine-sensitive) strain used in this study from the University Faculty of Pharmacy Airlangga Surabaya Indonesia.

Plant material

Syzygium cumini L fruit was obtained from Lumajang city of East Java, Indonesia, and was identified in the Herbal laboratory of Materia Medica, Batu with letter number 067/1873/102.20/2023.

Preparation of fruit S. cumini L extract

The cleaned fruit is dried by aerating it in the open air. After drying, it is ground to a fine powder. *Syzygium cumini* L fruit powder was macerated with 96% methanol (PA) for 3 × 24 hours then the filtrate was evaporated with rotavapor at 50°C and then dried using a freeze dryer to produce a thick extract.

Preparation of Syzygium fruit cumini L fraction

Extract fractionation uses the solid-liquid method with n-hexane, ethyl acetate, butanol, and water solvents. The filtrate results are evaporated. After drying, all fractions were weighed to find out each weight. Extract fractionation with chloroform and methanol was carried out by vacuum liquid chromatography, methanol extract of 5 g, was put in a porcelain cup. Silica gel 60 GF254 weighs as much as 20 g and takes a third part to be mixed into the extract and crushed until silica and extract form a homogeneous powder. The elution process uses eluents with a series of motion phases as follows: $CHCl_{3:}$ MeOH (100:0), $CHCl_{3:}$ MeOH (90:10), $CHCl_{3:}$ MeOH (80:20), $CHCl_{3:}$ MeOH (70:30) and $CHCl_{3:}$ MeOH (60:40), $CHCl_{3:}$ MeOH (50: 50), $CHCl_{3:}$ MeOH (40:60), $CHCl_{3:}$ MeOH (30:70), $CHCl_{3:}$ MeOH (20:80), $CHCl_{3:}$ MeOH (10:90), and $CHCl_{3:}$ MeOH (0:100). The fractions obtained were evaporated and conducted for thin layer chromatography (TLC) investigation using mobile phase chloroform: methanol 7:3. Fractions that have the same Rf value and spot color are combined into one mixture.

In vitro testing procedure

In vitro activity antimalarial test was conducted using the culture of *P. falciparum* 3D7 strain. *Plasmodium falciparum* 3D7 strain which had been stored in liquid nitrogen was thawed using the Rowe method. *In vitro* culture using the Trager and Jensen method. O-type human red blood cells, hematocrit 5% in RPMI 1640 medium (GIBCO BRL, USA), supplemented with 22.3 mM HEPES (sigma), Hypoxanthine sodium bicarbonate with 10% plasma from human O-blood type. *In vitro* tests were carried out in good culture 24 with 1% parasitemia (1 ml suspension/well). For the analysis of sample concentrations starting from 0.01, 0.1, 1, 10, and 100 µg/ml. The culture was incubated at 37°C for 48 hours, a thin blood smear was made fixed with methanol and stained with Giemsa staining 20% for 20 minutes. Then washed with water and dried. After that, the percentage of parasitemia *P. falciparum* 3D7 strain was calculated by counting the number of infected erythrocyte cells per 1,000 erythrocyte cells under a light microscope at 1,000× magnification.

Plasmodium falciparum 3D7 strain culture that had reached >5% growth was examined for IC_{50} values using a 24-well microplate in duplicate. The serial concentration of fractions that we tested for antimalarial activity were 0.01, 0.1, 1, 10, and 100 µg/ml. The final concentration in the microtiter well with two repetitions. The control only contained complete media and infected erythrocytes without the addition of anti-malarial drugs. The microplate was incubated for 48 hours. After 48 hours a thin blood smear was fixed with methanol, stained with 20% Giemsa for 20 minutes, washed with water, and dried.

Calculation percentage of parasitemia and percentage of growth inhibition

The percentage of parasitemia is calculated by the following (Ljungstrom *et al.*, 2004; Garcia *et al.*, 2008):

$$\text{\% Parasitemia} = \frac{\text{The number of infected erythrocytes}}{\text{1,000 erythrocytes}} \times 100\%.$$

The calculation of parasitemia percentage was made in 1,000 erythrocytes using a light microscope with 1,000× magnification, the growth and the percentage of inhibition were done with the following equation (Inbaneson *et al.*, 2013):

$$\text{\% Inhibition} = 100\% - \frac{\text{(\% Parasitemia treatment} \times 100\%)}{\text{\% Control parasitaemia}}.$$

Then, the SPSS 15 probit analysis program is used to determine the IC_{50} value.

Results

Results of activity test of antimalarial *in vitro* with the culture of *P. falciparum* strain 3D7 using the Trager and Jensen (1976) methods. The results of the percentage of parasitemia, percentage of growth, percentage of

inhibition, and IC_{50} *P. falciparum* 3D7 are shown in Tables 1 and 2.

Results extract fractionated with technique partition liquid–liquid. The results of the fractionation process graded from the fraction test are shown in Table 3.

Table 1. The average percent of parasitemia of *P. falciparum* 3D7 gives a fraction of *S. cumini* L extract *in vitro*.

Fractions	Dosage (µg/ml)	% Parasitemia	
		0 hours	48 hours
Ethyl acetate	-	0.70	2.58
	100	0.70	0.48
	10	0.70	1.29
	1	0.70	1.66
	0.1	0.70	2.05
	0.01	0.70	2.33
n-Hexane	-	0.70	2.23
	100	0.70	0.51
	10	0.70	1.82
	1	0.70	1.88
	0.1	0.70	2.12
	0.01	0.70	2.35
Butanol	-	0.70	2.80
	100	0.70	1.21
	10	0.70	1.49
	1	0.70	1.86
	0.1	0.70	2.22
	0.01	0.70	2.32
Water	-	0.48	1.62
	100	0.48	0.42
	10	0.48	1.12
	1	0.48	1.32
	0.1	0.48	1.40
	0.01	0.48	1.61
C1	-	0.92	4.13
	100	0.92	0.70
	10	0.92	3.22
	1	0.92	3.61
	0.1	0.92	3.81
	0.01	0.92	4.26
C4	-	0.70	2.26
	100	0.70	0.76
	10	0.70	1.34
	1	0.70	1.54
	0.1	0.70	1.74
	0.01	0.70	2.05

Results TLC observations and Wavenumber (cm^{-1}) of several extract fractions of *S. cumini* L are shown in Tables 4 and 5.

Results profile TLC chromatogram with eluent ethyl acetate: chloroform (7:3). Results identification to spots that appear were done with sighting stain citric borate, and then checked with UV lamp on Lambda 254 and 366 nm. The results show (A). Rf 0.70, colorless, (B) Rf 0.81, colorless, (C) Rf 0.88, colorless, (D) Rf 0.96, green, and E) Rf 0.96 orange color (Fig. 1). The results of Fourier transform infra red (FTIR) ethyl acetate were 18 peaks, N-hexane 19 peaks, butanol 14 peaks, water 14 peaks, C1 16 peaks, C4 14 peaks. FTIR spectra of several extract fractions of *S. cumini* L (Fig. 2).

Discussion

The results of the antimalarial activity test showed that the C4 fraction (chloroform: methanol with a ratio of 20:80, 10:90, and 0:1,000 had the highest antimalarial activity with IC_{50} value of 1.015 µg/ml, then ethyl acetate with IC_{50} value 1.189 µg/ml, Butanol with IC_{50} value 1.769 µg/ml, water with IC_{50} value 15.058 µg/ml, n-hexane with IC_{50} value of 76.996, fraction C1 (chloroform: methanol with a ratio of 100:0 and 90:10) with IC_{50} value 100.126 µg/ml and C2 (chloroform: methanol; 80:20,70:30) and C3 (chloroform: methanol; 60:40, 50:5, 40:60, and 30:70) fraction not detected. A testing sample is considered to be a strong antimalarial inhibition if it has an IC_{50} value less than 10 µg/ml, while moderate if the IC_{50} value is 10–50 µg /ml and low activity if the IC_{50} ranges from 50 to 100 µg/ml and if the IC_{50} is above 100 µg/ml, is declared less active (inactive). From these data, it is shown that the antimalarial activity of the C4, ethyl acetate, and butanol fractions has strong antimalarial activity while the water fraction was moderate and the n-hexane fraction was low activity and the C1 fraction was less active (inactive) (Dolabela *et al.*, 2008).

Fractionation was carried out using the different polarity of solvents such as n-hexane, ethyl acetate, butanol, and water as well as chloroform and methanol to extract the active ingredients and to separate the active compounds from *S. cumini* fruit extract. *Syzygium cumini* contains a variety of compounds that have different polarities, such as triterpenoids, polyphenols, flavonoids, and anthocyanins. A previous study reported that *in vitro* antimalarial activity test on clove extract showed an association of antioxidant effects of phenol compounds and their derivatives such as flavonoids and tannins produced from clove plants with the ability to inhibit *P. falciparum* growth) (Brahmam *et al.*, 2018; Dharmawati, 2019). Methanol extract of *S. cumini* contains alkaloids, amino acids, flavonoids, glycosides, phytosterols, saponins, steroids, tannins, and triterpenoids. The results of chemical studies reported that there is a relationship between the form of chemical conformation the antimalarial activity against *Plasmodium vivax* and *P. falciparum*

Table 2. Percent growth, percent inhibition, and IC_{50} *P. falciparum* 3D7 administration of *S. cumini* L extract fraction *in vitro*.

Fractions	Dosage (µg/ml)	Average percent growth (%)	Average percent inhibition (%)	IC_{50} µg/ml
Ethyl acetate	-	1.88	-	1,189
	100	0.00	100.00	
	10	0.59	68.62	
	1	0.96	48.94	
	0.1	1.35	28.19	
	0.01	1.63	13.30	
n-Hexane	-	1.53	-	76,996
	100	0.00	100.00	
	10	1.12	26.80	
	1	1.18	22.88	
	0.1	1.42	7.19	
	0.01	1.65	0.00	
Butanol	-	2.10	-	1,769
	100	0.51	75.71	
	10	0.79	62.38	
	1	1.16	44.76	
	0.1	1.52	27.62	
	0.01	1.62	22.86	
Water	-	1.14	-	15,058
	100	0.00	100.00	
	10	0.64	43.86	
	1	0.84	26.32	
	0.1	0.92	19.30	
	0.01	1.13	0.88	
C1	-	3.21	-	100,126
	100	0.00	100.00	
	10	2.30	28.35	
	1	2.69	16.20	
	0.1	2.89	9.97	
	0.01	3.34	0.00	
C4	-	1.56	-	1,015
	100	0.06	96.15	
	10	0.64	58.97	
	1	0.84	46.15	
	0.1	1.04	33.33	
	0.01	1.35	13.46	

(Kumar *et al.*, 2009; Oliveira *et al.*, 2009; Onguéné *et al.*, 2013). The alkaloid component of *Strychnos malacoclados* bark also has antimalarial activity (Tchinda *et al.* 2011). Elfita *et al.* (2011) stated that 7-hydroxypiranopiridin-4-one alkaloid compound ($C_8H_7NO_2$) as antimalarial potential, isolated from the microbial endophytic microbial (*Andographis paniculata* Nees) IC_{50} value of 0.201 µM. Several

Table 3. Fractionation results graded extract methanol fruit *S. cumini* L.

Fraction test	Added solvent volume (ml)	Weight fraction (g)
n-Hexane	900	5
Ethyl acetate	800	11.5
Butanol	800	34
Water	300	137

Table 4. Results TLC observations.

Code	Color	Rf
A	No colored	0.70
B	No colored	0.81
C	No colored	0.88
D	Green	0.96
E	Orange	0.96

nonalkaloid compounds that act as antimalarials have been reported. Batista *et al.* (2009) suggested that antimalarial compounds are found in terpene, limonoid, flavonoid, chromon, xanton, antraquinone, and miscellaneous groups. Sutthivaiyakit *et al.* (2009) suggested that the compound components of diterpene, sesquiterpenes, and sesquiterpenicumarin from Jatropha integerrima plants have antimalarial activity *in vitro*. Uchôa *et al.* (2010) group of triterpenoid compounds

Table 5. Wavenumber (cm^{-1}) of several extract fractions of *S. cumini* L.

Fraction								
Etyl acetat	**n-Hexane**	**Butanol**	**Air**	**C1**	**C2**	**C3**	**C4**	**Bond**
3,373	3,419	3,388	3,390	3,774	3,401	3,393	3,388	NH, CH OH
	3,010	2,938	2,937	3,390	2,929	2,938	2,935	
	2,925			2,927	2,856			
	2,854			2,855				
			2,103			2,098		Triple bond C=C (Nitril)
1,667	1,732	1,728	1,731	1,738	1,732	1,726	1,728	C=O, C=N, and C=C
1,615	1,698	1,634	1,633	1,652	1,667	1,638	1,634	
1,523	1,633				1,523			
	1,517							
1,448	1,464	1,403	1,415	1,455	1,399	1,415	1,416	NO_2, C=O C–N C–H
1,397	1,377	1,346	1,249	1,378	1,384	1,384	1,244	
1,340	1,189	1,231	1,057	1,258	1,280	1,347	1,190	
1,195	1,059	1,058	919	1,078	1,194	1,243	1,059	
1,110	922	919	867	922	1,074	1,188	920	
1,025	869	867	818	868	1,027	1,077	868	
966	814	817	778	817	968	1,059	818	
923	774	778	631	780	816	918	779	
876	758	631	596	702	780	866	703	
812	721	522		626	596	818	630	
776	577				521	778		
698						630		
633						593		
577						520		
18 peaks	19 peaks	14 peaks	14 peaks	16 peaks	18 peaks	19 peaks	14 peaks	

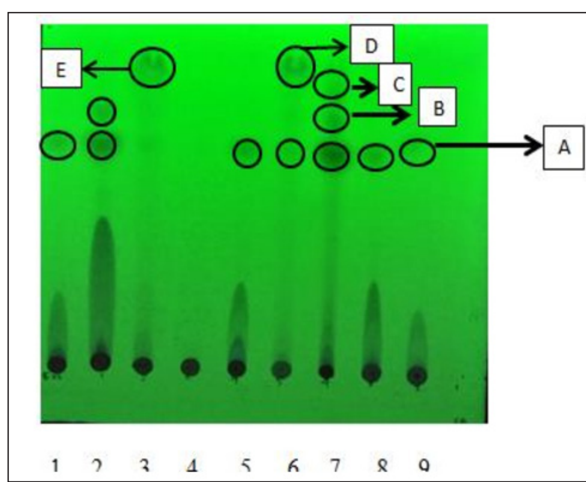

Fig. 1. Chromatogram of TLC results with UV lamp on Lambda 254 and 366 nm. (1) Methanol extract; (2) ethyl fraction acetate; (3) fraction n-hexane; (4) water fraction; (5) fraction butanol; (6) fraction C1; (7) C2 fraction; (8) C3 faction; and (9) C4 fraction.

from the *Cecropia pachystachya* plant are antimalarial. Antimalarial activity of lactone sesquiterpenes *in vitro* against *P. falciparum* was reported by Sulsen *et al.* (2011) on *Ambrosia tenuifolia* plant and Toyang *et al.* (2013) on *Vernonia guineensis* (Asteraceae). *Artemicia annua* plant has an antimalarial active compound namely artemisinin which belongs to the group of sesquiterpenes compounds (Guo, 2016). Some other compounds are proven to be antimalarial β-karyofilen (Kamaraj *et al.*, 2017). Gallic acid derivatives (octyl gallate) with hydroxyl groups are found in the aromatic ring (Arsianti *et al.*, 2017). Methyl galat (Arsianti *et al.*, 2018), coumarin (Pingaew *et al.*, 2014; Tanjung *et al.*, 2016; Beaufay *et al.*, 2017).

The antimalarial activity of these antioxidant potential compounds which have low molecular weight, and can interfere with the redox metabolism of malaria parasites, acts as an inhibitor of the formation of antioxidant enzymes and can interfere with the formation of hemozoin. Also, the nature of a compound determines its activity. The active compound contained

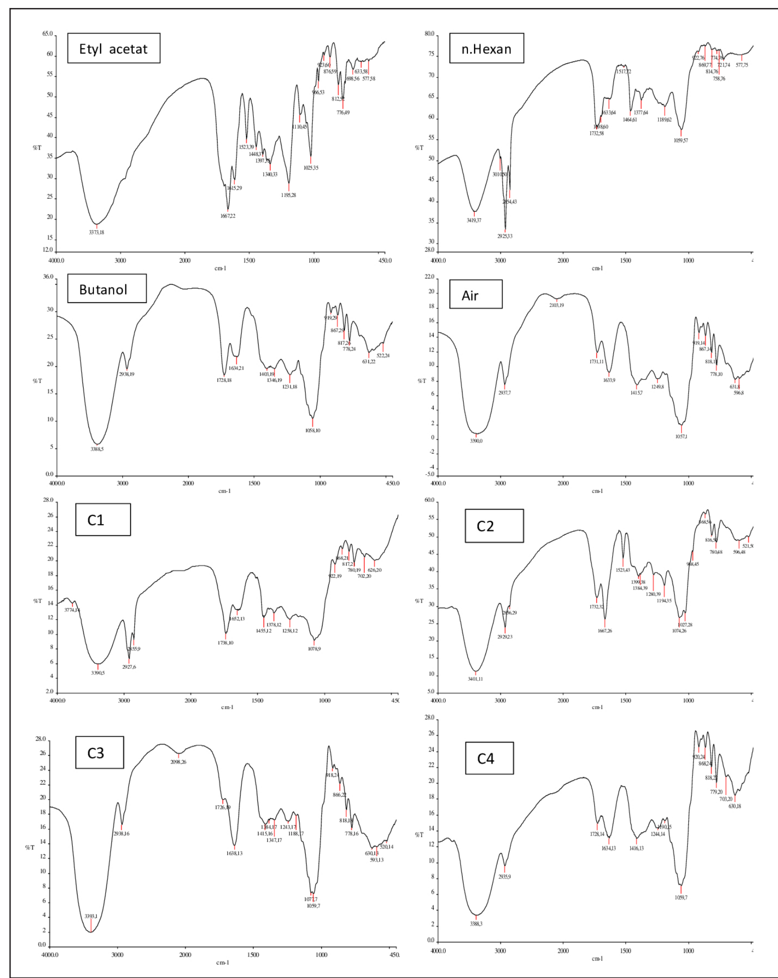

Fig. 2. FTIR spectra of several extract fractions of *S. cumini L.*

in the test fraction (supposed as flavonoid) has semipolar properties so that it is easier to penetrate the semipolar layer than the nonpolar lipid layer which makes it more difficult for the compound to enter the cell membrane (Becker *et al.*, 2004). The activity of flavonoids as an antimalarial with the mechanism of action by inhibiting the biosynthesis of fatty acids (FAS II) pathway, which is in the parasite's apicoplast such as luteolin-7-O-β-d- glucopyranoside, reported as the first natural product to target the plasmodial FAB I enzyme which regulates the FAS II pathway (Waller *et al.*, 2003). Some flavonoids also show the ability to inhibit l-glutamine and myoinositol influx in infected erythrocytes or work with interference with hemin degradation (Elford, 1986).

Conclusion

The ethyl acetate, butanol and C4 fraction from *S. cumini* fruit has the potential to be developed as an antimalarial agent. Further identification of the active compounds of these fractions is to be investigated.

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Conflict of interest

The authors declare that there is no conflict of interest.

Authors contributions

Research project leader and coordinating research, designed study, analyzed data, drafted the paper and corresponding author by Lilik Maslachah. Processing, examination, and analysis of *in vitro* data and reading the final manuscript by Neny Purwitasari. All authors contributed to the manuscript's reading, reviewing, revising, and approving the final version.

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Data availability

All data supporting the findings of this study are available within the manuscript.

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