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Standardization of *Cassia spectabilis* DC leaves and antimalarial activities of ethyl acetate extract

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ABSTRACT

One of the Indonesian medicinal plants, *Cassia spectabilis* is traditionally used to treat several diseases, including malaria. The quality of raw materials influences the quality of drug derived from a plant. Standardizing the raw materials followed by the antimalarial activity test are needed to azure the quality of product prepared from plants. The aim of this study was to standardize the quality of *C. spectabilis* leaves and to evaluate the antimalarial activities of ethyl acetate extract. The fresh material of *C. spectabilis* leaves was observed in its specific and non-specific parameters. *In vitro* test was done by using *Plasmodium falciparum* 3D7. *In vivo* test was done using a 4-day suppressive test method against mice infected with *P. berghei* for four consecutive days. Heme detoxification inhibitory activity test was carried out using the modified Basilico method. The leaves of *C. spectabilis* meet the quality requirement for raw materials of traditional medicine. The ethyl acetate extract showed *in vitro* antiplasmodial activity against *P. falciparum* 3D7 and in vivo antimalarial activity against *P. berghei* infection with an IC_{50} value of 27.28 µg/mL and ED_{50} value of 1.74 mg/kg, respectively. The extract also showed heme detoxification inhibitory activity with an IC_{50} value of 0.33±0.01 mg/mL. The leaves of *C. spectabilis* meet the quality requirement, and the ethyl acetate extract from standardized *C. spectabilis* leaves possessed a potential antimalarial activity that deserves to be further developed.

Keywords: malaria, *Cassia spectabilis*, heme detoxification, *Plasmodium berghei*, *Plasmodium falciparum*, standardization

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INTRODUCTION

Malaria is an infectious disease caused by parasite from the genus of *Plasmodium*, and transmitted by the bite of female *Anopheles* mosquito. Malaria remains the leading cause of death worldwide, most commonly in Africa and some Asian countries. Meanwhile, malaria in developed country caused by the imported cases from endemic areas (Talapko et al., 2019). The reduction in mortality and morbidity by at least 40% in 2020, as the important goals of the Global Technical Strategy for malaria is difficult to achieve (World Health Organization, 2018). Since 2010, a significant reduction in the burden of malaria has occurred, however, a slowdown and an increase in the number of cases between 2015 and 2017 have been observed. Reducing the number of cases in countries with the highest-burden is the most critical step in the global eradication of malaria (Talapko et al., 2019).

Drug resistance has been reported as severe global problem, even to all antimalarial drugs, however, artemisinin-based combination therapy (ACT) remains an effective therapy (Alonso & Noor, 2017). New drugs are needed to overcome the problem, where a number of new antimalarial discoveries and developments are ongoing, and some of them come from medicinal plants.

The species of *Cassia* (Caesalpiniaceae) is a well-known medicinal plant found in India and other tropical countries. One of the known species for the treatment of malaria is *Cassia siamea* which traditionally is consumed by drinking the decoction of leaves or flowers. The decoction of the flowers is also used as a body bath to treat malaria and liver disorders (Kamagaté et al., 2014). Several scientific studies have also proven the antimalarial activity of *C. siamea* along with its active compounds, either *in vitro* or *in vivo* (Ekasari et al., 2009). Apart from that, several other species of *Cassia* with clear antimalarial properties functioned throughout Africa, such as *C. occidentalis*, *C. africana*, *C. floribunda*, and *C. hirsuta*. Some of them have been shown to have *in vivo* antimalarial activity (Grace et al., 2012).

Previously, an *in vitro* antimalarial activity of methanolic extracts of leaves from *C. spectabilis* has been reported to show the highest inhibition against *P. falciparum* with an IC_{50} value of 2.66 µg/mL (Ekasari et al., 2018). The ethanol extract of the leaves also showed antimalarial activity, both in the *in vitro* and *in vivo* test, with an IC_{50} value of 12.52 µg/mL, and an ED_{50} value of 131.5 mg/kg, respectively (Ekasari et al., 2018). The results proved the *C. spectabilis* leaves extract can be further potentially developed into antimalarial drug.

Medicinal plants play an important role in promoting health. They are widely spread all over the world but mostly grow in tropical countries. Recently, 25% of modern medicines, directly or indirectly were derived from plants (Patwekar et al., 2015). The quality of herbal drugs is highly affected by the quality of the raw materials. Further, the various factors such as cultivation, harvesting, and production influence the quality of raw materials. Quality assurance has to meet the specified standard. Raw materials of poor quality can affect the pharmacological activity of the product, therefore standardization of raw materials is the main key in obtaining a qualified drug (Yadav & Prajapati, 2011), because standardization is an essential step in obtaining a safe, effective, and qualified pharmaceutical product (Purwantiningsih et al., 2011). The determination of the specific and non-specific standard parameters of *C. spectabilis* leaves are reported herein. The improvement of the results will make these Indonesian plants as the material for traditional medicine, especially for antimalarial medicament.

Further investigation showed active antimalarial alkaloid compounds that reveals a structural pattern identical to (–)-7-hydroxycassine (Ekasari et al., 2021). The first antimalarial drug which contains alkaloid is quinine isolated from the *Cinchona* bark and is still used to treat malaria that is resistant to several drugs. Alkaloids of *C. spectabilis* leaves present in higher amounts in ethanol, methanol, and ethyl acetate extracts (Veerachari & Bopaiah, 2011). Antimalarial activity tests of methanol and ethanol extract of *C. spectabilis* leaves have been done before. Therefore, this study used ethyl acetate leaf extract of *C. spectabilis* to determine its antimalarial activity.

Assessment of antimalarial activity can be done *in vitro* to *P. falciparum* and in vivo to rodent plasmodia (Fidock et al., 2004). *In vitro* test includes IC_{50} determination against *P. falciparum* type, both drug-resistant and drug-sensitive strains. *P. falciparum* culture can be used to study how parasites enter erythrocytes, screen for new drugs, isolate and characterize strains and clones, and identify immunogenic

antigens and parasite genomes (Kaira et al., 2006). As for the advantages of the *in vitro* method, there are precise and efficient; fast; a large number of compounds can be evaluated at the same time; synergism or antagonism with drug combinations can be studied; and a better assessment of the intrinsic activity of a drug.

In vivo test includes a four-day primary test for suppression of parasitaemia or inhibition of parasite growth in mice. This is the most widely used preliminary test, in which the efficacy of a compound is assessed by comparing blood parasitaemia and survival time of mice in treated and untreated mice (Kaira et al., 2006).

In addition to testing for *in vitro* and *in vivo* antimalarial activities, the identification of compounds that inhibit β-hematin formation is an approach for detecting antimalarial drugs (Mosaddegh et al., 2018). Malaria parasites digest hemoglobin in vacuoles into amino acids and heme, where the free heme formed can be toxic to the parasites themselves. To protect the body from the poisonous heme, *Plasmodium* is known to have several detoxification mechanisms such as the formation of hemozoin, heme-binding protein, and degradation of free heme by H_2O_2 (Slater & Cerami, 1992). Some drugs show an antimalarial effect through the inhibition of hemozoin formation, such as quinoline and xanthones and their derivatives, which are the most important mechanism for detoxification (Fong & Wright, 2013). For *in vitro* antimalarial assessment, a synthetic polymer that is identical to hemozoin, namely β-hematin, is used.

Based on the description above, the aim of this study is to observe the antimalarial activity of ethyl acetate extract of *C. spectabilis* leaves, both *in vitro* against *P. falciparum* and *in vivo* against *P. berghei* infected mice, as well as its heme detoxification inhibitory activity.

MATERIALS AND METHODS

Plant Materials

Fresh *C. spectabilis* leaves were obtained and determined at Purwodadi Botanical Garden – Indonesian Institute of Sciences (LIPI), Pasuruan, Indonesia (B-160/IPH.06/KS.02/III/2019). The dried leaves were produced using a standard guideline *How to make simplicia* (Ministry of Health Republic of Indonesia, 1985) and were ground into powder using a pollinating machine.

Standardization of *C. spectabilis* Leaves

Standardization of *C. spectabilis* leaves were carried out based on standard guideline *Parameter Standar Umum Ekstrak* (Ministry of Health Republic of Indonesia, 2000) by determining the specific and non-specific parameters. The specific parameters including identification, macroscopic, microscopic, organoleptic, water- and ethanol-soluble extract content, as well as chemical content such as essential oils and total flavonoids were determined. Essential oil content was determined using xylol distillation method, while total flavonoid content was determined using aluminum chloride spectrophotometric method.

The non-specific parameters including loss on drying, total water content, total ash, total acid insoluble ash, as well as residue of pesticides, heavy metals, and microbial contamination were determined. Determination of total water content was carried out using toluene distillation method, while total ash and total acid insoluble ash were determined using thermogravimetric analysis method.

Preparation of ethyl acetate crude extracts

C. spectabilis leaves were extracted at room temperature in the research room of the Department of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia. The extract was made by macerating 500 g of dry leaf powder in 2500 mL of ethyl acetate for 3x24 h. The extract was then evaporated on a rotary evaporator.

Experimental animals

Adult BALB/c male mice (20–30 g) were obtained from the Faculty of Veterinary Medicine, Universitas Airlangga. Animals were acclimatized to the laboratory environment (room temperature

24±1°C, humidity 55±5%, 12 h light/dark cycle) for two weeks before the test and provided free access to food and water sources. The research protocol was approved by the Animal Use and Care Committee of Faculty of Veterinary Medicine, Universitas Airlangga (2.KE.181.10.2018).

In Vitro antimalarial activity

The stock solution of extract was prepared in dimethyl sulfoxide (DMSO) and diluted with complete RPMI 1640 culture media until the final concentrations of the solutions were ranging from 0.001 to 100 µg/mL. The test was carried out in duplicate. The plates were incubated in a candle jar for 48 h under CO_2 conditions at 37°C. The contents of the well were harvested by making a thin smear of blood on clean microscopic slides after the incubation process. The blood smear was then fixed using methanol and stained for 10 min in 10% Giemsa solution (pH 7.3). Each IC_{50} values was calculated using probit analysis based on the percentage of *P. falciparum* growth inhibition.

In Vivo antimalarial activity

In vivo testing of ethyl acetate leaf extract of *C. spectabilis* was carried out on rodent malaria parasites (*Plasmodium berghei*) using a 4-day suppressive test method after the inoculation of $2x10^7$ parasites/mice intraperitoneally (Fidock et al., 2004). A group of eight mice (BALB/c, male, eight weeks old) was used for each dose. Five other mice were kept as donors who were inoculated but untreated. The negative and positive control groups each received a suspension of 0.5% sodium carboxymethylcellulose (Na CMC) solution and dihydroartemisinin + piperaquine (20.8 + 166.4 mg/kg/day, p.o.) (standard drug). Four doses of ethyl acetate leaf extract of *C. spectabilis* tested 1, 10, 100, and 200 mg/kg/day. Each mouse was given a 0.5% Na CMC solution, plant extracts, and standard drugs orally. The first administration was started after three hours of parasite inoculation (D_0) and then continued at the same time for three additional days (D_1–D_3). The percentage of parasitaemia was determined on D_4 with a thin blood smear using the formula described by Fidock et al. (2004). ED_{50} was rated as a dose causing 50% inhibition of parasite growth compared to growth in negative controls.

Heme detoxification inhibitory activity

Heme detoxification inhibitory activity was carried out on ethyl acetate extract of *C. spectabilis* leaves based on the modified Basilico method (Basilico et al., 1998). The test materials were plant extracts and positive control (chloroquine diphosphate) with various levels, which were 4; 2; 1; 0.5; 0.25; and 0.1 mg/mL. Each level was replicated three times. The dissolution process of the test material used 10% DMSO and was carried out by dissolving the test material in 100 µL of DMSO and added with distilled water to a concentration of 10% DMSO. Each microtube was added as much as 50 µL of the test material, 100 µL of 1 mM hematin solution in 0.2 M NaOH, and 50 µL of glacial acetic acid and incubated for 24 h at 37°C. Then the solution was centrifuged at 8000 rpm for 10 min, the supernatant was removed, and the precipitate was washed with 200 µL of DMSO. Washing was performed three times. At the final stage of washing, the precipitate was dissolved in 200 µL of 0.1 M NaOH as much as 100 µL of the solution was distributed into 96-well microplates to measure the absorbance using ELISA reader at a wavelength of 405 nm. Inhibition percentage was calculated, and IC_{50} (the content of the test compound which was able to inhibit the formation of β-hematin by up to 50%) was calculated using probit analysis.

Statistical analysis

The data were expressed as mean ± standard error of the mean (S.E.M). Statistical significance of means was determined by one-way analysis of variance (ANOVA), followed by Dunnett's (posthoc test) to compare the measured parameters (parasitemia and level of β-hematin) with negative controls. The analysis was performed with a 95% confidence interval, and *p*-values less than 0.05 was considered to be statistically significant.

RESULT AND DISCUSSION

Determining specific standard parameters of *C. spectabilis* includes macroscopic and microscopic observation, organoleptic test, water-soluble essence, ethanol-soluble essence, volatile oil, as well as total flavonoid level. Macroscopic observation results are shown in Figure 1 and Table 1.

Figure 1. *Cassia spectabilis* leaves and flowers

Table 1. Morphological observation of *Cassia spectabilis* leaves

Characteristic	Result
Leaf	
Shape	Oval-oblong
Leaf tip	Blunt with a shallow notch at the tip
Leaf base	Blunt or quite rounded
Surface	Upper surface: glabrous, quite glossy
	Lower surface: Pubescent
Edge	Even
Leaf vein	Pinnate
Size	
Length	3 – 7.5 cm
Width	1 – 2.5 cm
Color	Green to brownish-green
Stipule	Long

Microscopic observations, including fragments of mesophyll, crystal fibers, mesophyll with calcium oxalate crystal, and trichome, are shown in Figure 2.

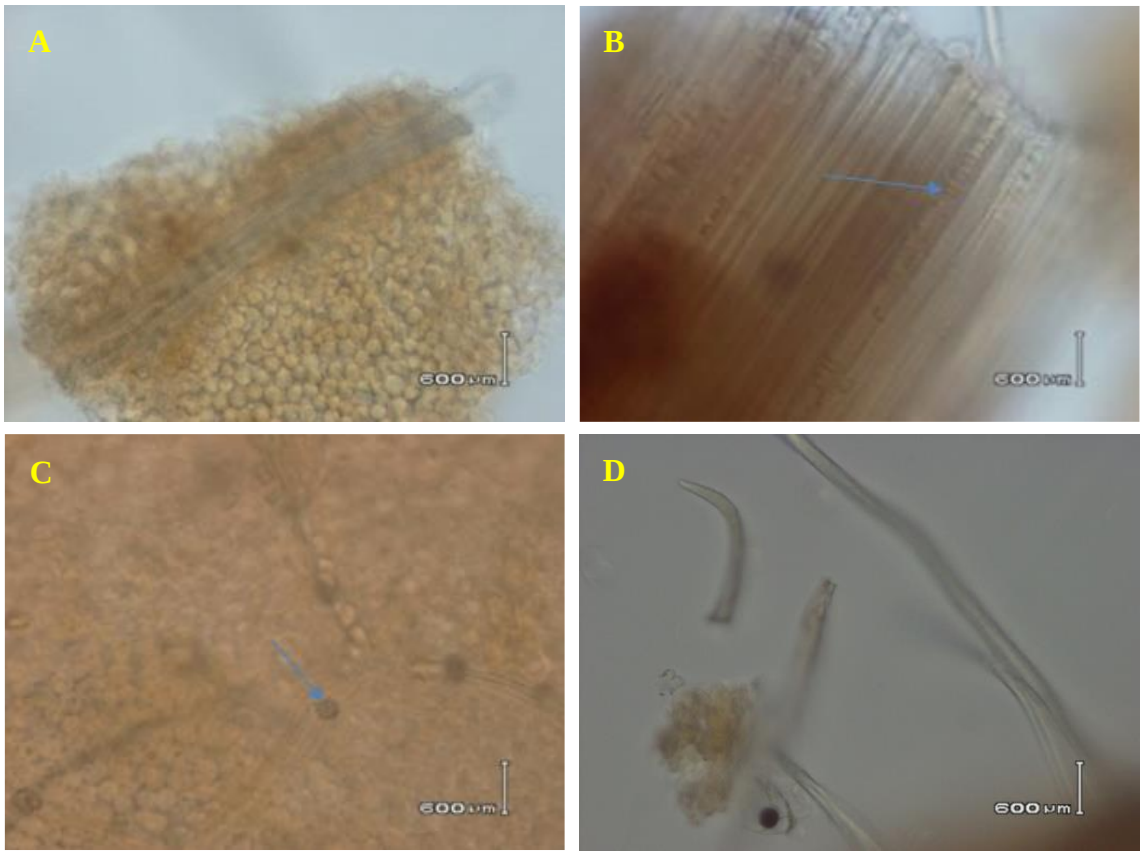

Figure 2. Fragments of (A) mesophyll; (B) crystal fibers (prism shape, blue arrow); (C) mesophyll with calcium oxalate crystal (blue arrow); and (D) trichome

Other results of the specific and non-specific parameters were summarized in Table 2. For determination of non-specific parameters, such as loss on drying, water level, total ash, and acid-insoluble ash of dried leaves powder of *C. spectabilis* it meets the quality required to be used as the raw material of traditional medicine.

In the determination of microbes, the dried leaves powder of *C. spectabilis* already met the requirements regulated by the National Agency of Drug and Food Control (2014), as well as the determination of residue of pesticides and heavy metals Table 2. Pesticide is a chemical substance still used in agriculture, whereas it can cause damage to health because of its carcinogenic, mutagenic, and teratogenic effects (El-Nahhal & Radwan, 2013). Heavy metal is a chemical element with high molecular weight. It is in solid form at room temperature. Heavy metal is essential for living creatures in small amounts, but in large amounts, it causes a dangerous effect on the human.

In vitro antimalarial test results from ethyl acetate leaf extract of *C. spectabilis* are shown in Table 3, where the IC_{50} value obtained was 27.28 µg/mL.

Table 2. Summary of results of determination of specific and non-specific parameters of dried leaves powder of *C. spectabilis*

Parameter	Result	Limit allowed for dried herbal raw materials
Specific parameters:		
Organoleptic		
Color	Slightly brownish	
Taste	Fresh	
Smell	Weak	
Water-soluble essence	3.72 ± 0.11 % w/w	-
Ethanol soluble essence	1.61 ± 0.09 % w/w	-
Volatile oil	0.17 % v/w	-
Total flavonoid content	2.22 ± 0.20 % w/w	-
Non-specific parameters:		
Loss on drying	7.81 ± 0.11 % w/w	-
Water level	7.54 ± 0.38 % w/w	-
Total ash	8.56 ± 0.09 % w/w	-
Acid-insoluble ash	2.26 ± 1.14 % w/w	-
Microbial contamination:		
Mold	60 colonies/g	≤ 104 colonies/g
Yeast	< 10 colonies/g	≤ 104 colonies/g
APM *Coliform*	7.4 colonies/g	≤ 106 colonies/g
Escherichia coli	Negative	Negative/g
Salmonella sp.	Negative	Negative/g
Staphylococcus aureus	Negative	Negative/g
Pseudomonas aeruginosa	Negative	Negative/g
Pesticide residues:		
Organochlorine	Negative	
Carbamate	Negative	
Heavy metal residues		
Lead (Pb)	0.250 mg/kg	≤ 10 mg/kg
Cadmium (Cd)	0.199 mg/kg	≤ 0.3 mg/kg
Zinc (Zn)	4.679 mg/kg	-
Copper (Cu)	0.346 mg/kg	-

Note: Data are expressed as mean ± S.E.M. (n = 3)

Based on the classification according to Gessler et al. (1994), the antimalarial activity of extracts with an IC_{50} value of <10 µg/mL is considered very good; 10–50 µg/mL is moderate, and >50 µg/mL is low. This suggests that *in vitro* antimalarial activity from ethyl acetate leaf extract of *C. spectabilis* was moderate because the IC_{50} value was in the range of 10 to 50 µg/mL.

In vivo antimalarial activity test results from ethyl acetate leaf extract of *C. spectabilis* against *P. berghei* infected mice can be seen in Figure 3 and Table 4. Figure 3 shows that each mouse has a different parasitic growth profile. This difference is due to variations in the immune system of each mouse.

Table 3. The average inhibition percentage of ethyl acetate extract of *C. spectabillis* leaves against *P. falciparum* 3D7 strain *in vitro*

Sample	Concentration (µg/mL)	Parasitemia (%)	Inhibition (%)	IC_{50} (µg/mL)
EACS	100	0.36 ± 0.03	100.00 ± 0.00	27.28
	10	2.54 ± 0.05	41.18 ± 6.00	
	1	2.69 ± 0.07	35.66 ± 6.88	
	0.1	3.20 ± 0.11	16.91 ± 8.95	
	0.01	4.44 ± 0.30	0.00 ± 0.00	
Negative control	-	3.66 ± 0.13	-	-

Note: Data are expressed as mean ± S.E.M. (n = 2). No significantly different in the percentage of parasitemia and inhibition compared to the negative control ($p > 0.05$). EACS = ethyl acetate extract of *C. spectabilis* leaves. DMSO was used for solubilizing the extracts and as a negative control

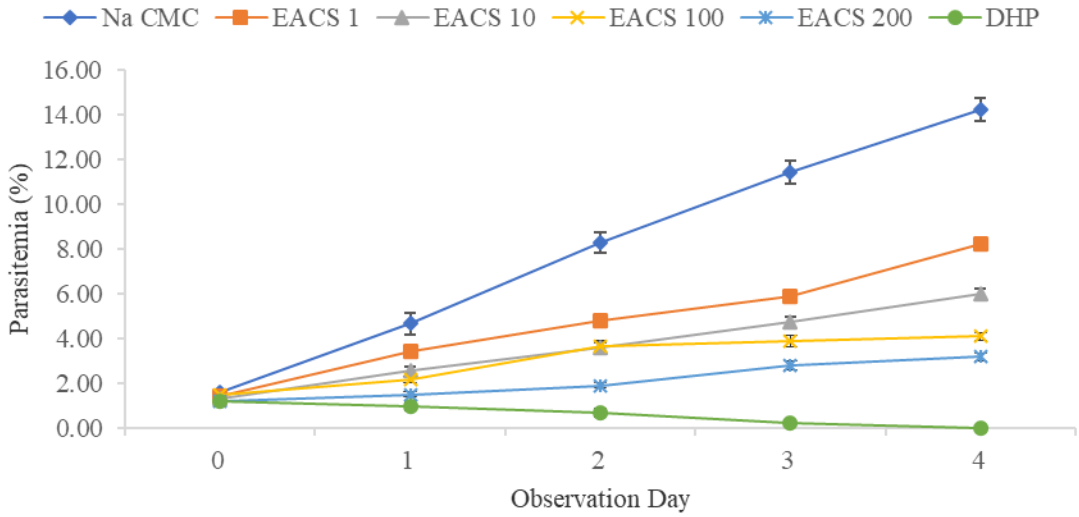

Figure 3. The effect of ethyl acetate extract of *C. spectabilis* leaves on parasitemia percentage of *P. berghei* infected mice on 4-day suppression test; data are mean ± S.E.M. (n = 8); Na CMC = sodium carboxymethylcellulose, DHP = dihydroartemisinin + piperaquine, EACS = ethyl acetate extract of *C. spectabilis* leaves; numbers refer to doses in mg/kg/day

Table 4. Percentage of parasitemia result treated *in vivo* of negative control, positive control, and ethyl acetate extract of *C. spectabilis* leaves

Treatment	Dose (mg/kg/day, p.o.)	Parasitemia (%)		Suppression (%)	ED_{50} (mg/kg)
		Day 0	Day 4		
EACS	1	1.46 ± 0.11	8.24 ± 0.20*	46.23	1.74
	10	1.32 ± 0.14	6.01 ± 0.21*	62.81	
	100	1.52 ± 0.11	4.09 ± 0.19*	79.62	
	200	1.22 ± 0.10	3.20 ± 0.17*	84.30	
DHP	20.8 + 166.4	1.23 ± 0.22	0.00 ± 0.00*	100.00	ND
Negative control	-	1.63 ± 0.07	14.24 ± 0.51	-	-

Note: Data are expressed as mean ± S.E.M. (n = 8). *Significantly different compared to negative control ($p < 0.05$). EACS = ethyl acetate extract of *C. spectabilis* leaves; DHP = dihydroartemisinin + piperaquine. Na CMC were used as a negative control. ND = Not determined

In vivo antimalarial activity test of ethyl acetate leaf extract of *C. spectabilis* against *P. berghei*-infected mice resulted in the inhibition of parasite growth was a dose-dependent manner, with the greatest inhibition of 84.30% at a dose of 200 mg/kg. *In vivo* antimalarial activity is calculated from the ED_{50} value, which was based on the probit analysis that revealed the dose which inhibits the growth of parasites by 50% of the total population. The analysis resulted in an ED_{50} value of 1.74 mg/kg, which revealed the ethyl acetate leaf extract of *C. spectabilis* to be a very active antimalarial (Muñoz et al., 2000). This value was lower than that of 90% ethanol extract of *C. spectabilis* leaves, which was 131.5 mg/kg (Ekasari et al., 2018). On the other hand, ethanol extract, chloroform extract, and aqueous leaf extract of *C. siamea* have an ED_{50} of 34.70 mg/kg, 19.59 mg/kg, and 83.77 mg/kg, respectively (Ekasari et al., 2009). Ethyl acetate extract from other plant species, such as *Garcinia husor* (Clusiaceae) bark, showed antimalarial activity against *P. berghei*-infected mice with an ED_{50} value of 22.30 mg/kg (Kainama et al., 2019), which was much higher than that of ethyl acetate leaf extract of *C. spectabilis* in this current study.

The heme detoxification inhibitory activity test of ethyl acetate leaf extract of *C. spectabilis* using Basilico method and read by ELISA reader at a wavelength of 405 nm resulted in heme α-chlorohemin reacted with acetate in a 24 h incubation formed β-hematin. The use of DMSO solution was aimed to remove the residual hematin that was still mixed with β-hematin crystals that were insoluble in DMSO washing solution. The application of NaOH to the formed-hematin precipitate would convert it to alkaline hematin, which could be measured by ELISA reader. The heme detoxification inhibitory activity was expressed in IC_{50}, which was the level of the test compound capable of inhibiting β-hematin formation by up to 50%. IC_{50} calculation results using probit analysis can be seen in Table 5.

Table 5. IC_{50} values of chloroquine diphosphate and ethyl acetate extract of *C. spectabilis* leaves

Sample	Concentration (mg/mL)	Level of β-hematin (mM)	Inhibition (%)	IC_{50} (mg/mL)
EACS	4.00	15.71 ± 4.01*	88.92 ± 1.78	0.33 ± 0.01
	2.00	29.51 ± 2.92*	78.54 ± 0.36	
	1.00	45.26 ± 5.60*	67.25 ± 1.10	
	0.50	61.06 ± 7.04*	55.75 ± 0.62	
	0.25	74.34 ± 7.71*	46.01 ± 0.67	
	0.10	95.93 ± 7.87*	30.02 ± 1.70	
CQ	4.00	32.19 ± 9.51*	78.24 ± 4.20	0.56 ± 0.01
	2.00	43.98 ± 8.62*	68.64 ± 2.86	
	1.00	54.89 ± 8.55*	60.54 ± 1.90	
	0.50	69.17 ± 7.77*	49.85 ± 0.47	
	0.25	88.02 ± 7.03*	35.76 ± 1.65	
	0.10	102.62 ± 5.85*	24.78 ± 3.35	
Negative control	-	137.72 ± 14.48	-	-

Note: Data are expressed as mean ± S.E.M. (n = 3). *Significantly different compared to negative control ($p < 0.05$). EACS = ethyl acetate extract of *C. spectabilis* leaves; CQ = chloroquine diphosphate. DMSO was used as a negative control

The mechanism of action of antimalarial compounds in inhibiting heme detoxification consists of two mechanisms, the first interaction occurs between terpenoid compounds, phenols, and sterols with the heme electronic system, and the second mechanism is the interaction between the test compound (extract) which has a hydroxyl group that can bind heme iron ions (Turalely et al., 2011). Based on these facts, the mechanism of action of ethyl acetate leaf extract of *C. spectabilis* inhibiting heme detoxification probably by interacting with the alkaloid hydroxyl group in the extracted compound, which is bound to the heme iron ion.

According to Frölich et al. (2005), the compounds that have a formation resistance in inhibiting β-hematin formation greater than 60% was a good potential as inhibitors of β-hematin formation, while smaller than 40% was as weak inhibitors in inhibitory effect of ethyl acetate leaf extract of *C. spectabilis* in β-hematin formation was a good potential inhibitor, where was greater than 60% at the concentrations of 4; 2; and 1 mg/mL. In the result of inhibition percentage in β-hematin extract and positive control formation, it was shown that higher levels in the test material would increase the percentage inhibition in β-hematin formation.

The IC_{50} value of heme detoxification inhibitory activity from ethyl acetate leaf extract of *C. spectabilis* was 0.33 mg/mL. It is lower than the IC_{50} value of positive control with a value of 0.56 mg/mL, indicating that ethyl acetate leaf extract of *C. spectabilis* was an active extract in inhibiting heme detoxification (Baelmans et al., 2000). Based on this result, heme detoxification inhibitory activity could be a mechanism of action of ethyl acetate leaf extract of *C. spectabilis* as an antiplasmodial.

CONCLUSION

Overall, this study was concluded that the dried leaves powder of *C. spectabilis* meets the quality requirements and recommended to be used as the raw material of traditional medicine. The results of antiplasmodial activity showed that the ethyl acetate leaf extract of *C. spectabilis* has good activity as an antiplasmodial, either *in vitro* or *in vivo*, and showed heme detoxification inhibitory activity. Further research is needed on the compounds that play a role in antimalarial activity and their mechanism of action so that they can be used as an alternative as anti-malarial drugs or as a combination drug with other antimalarials.

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