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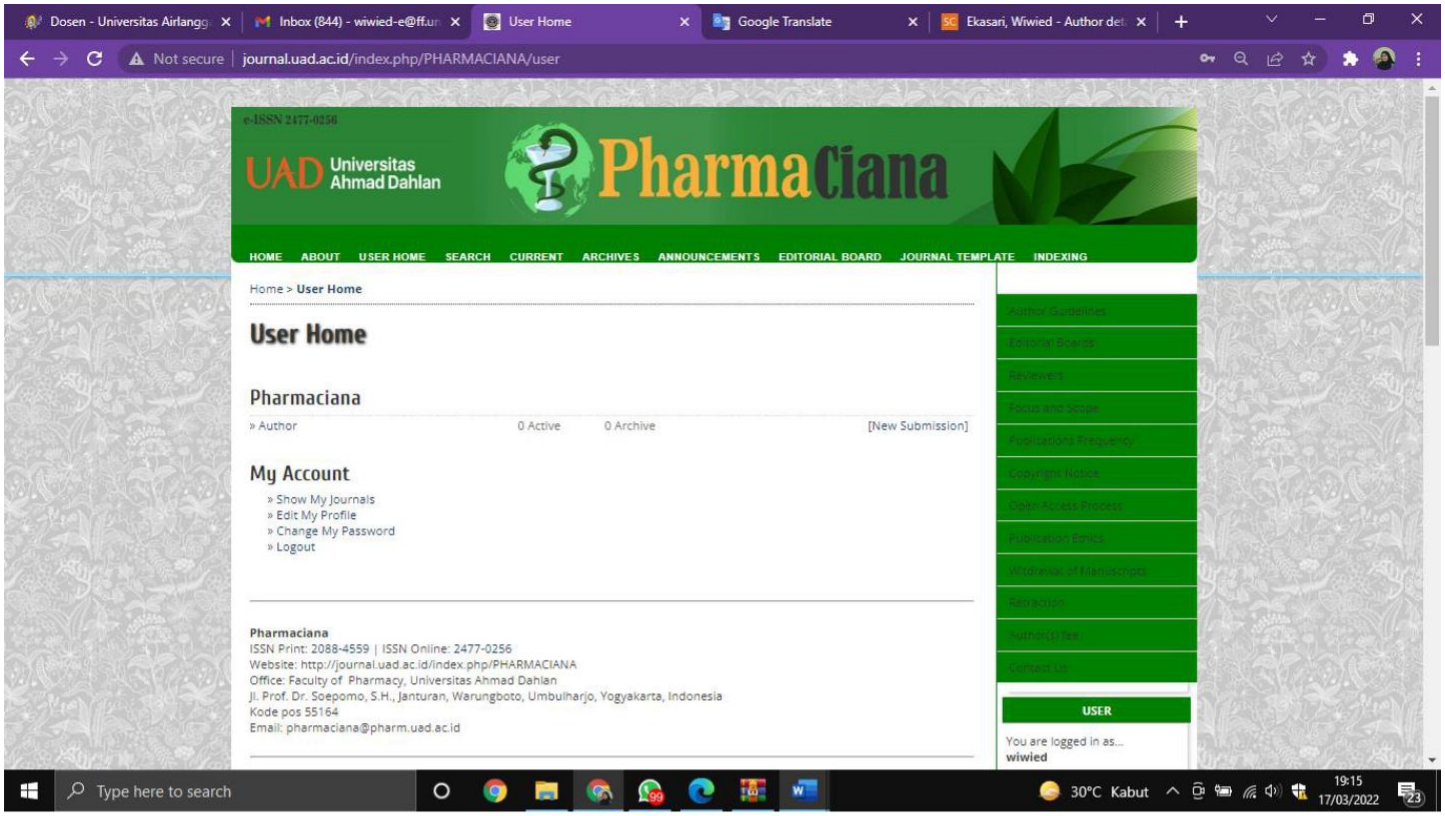

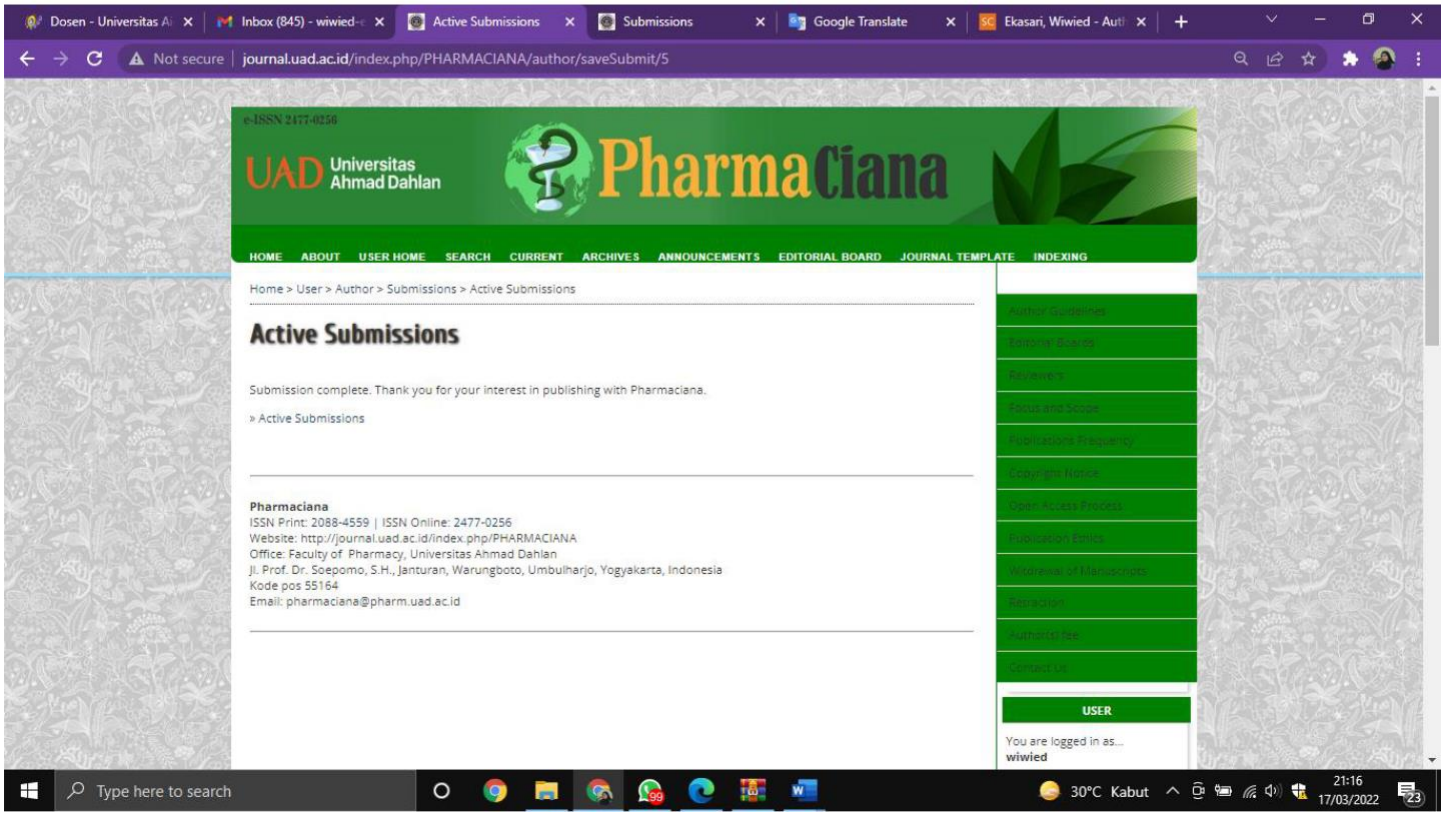

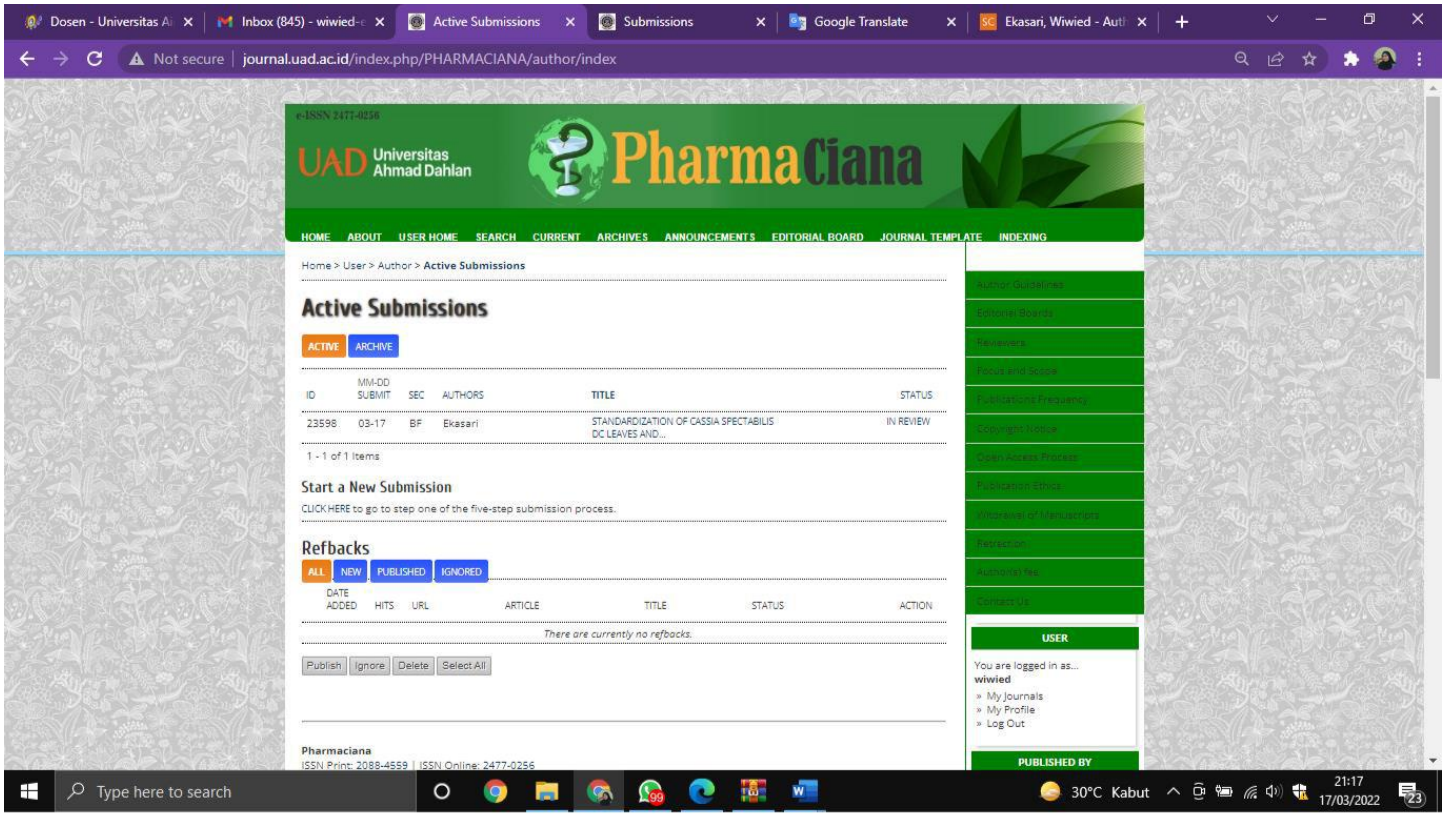
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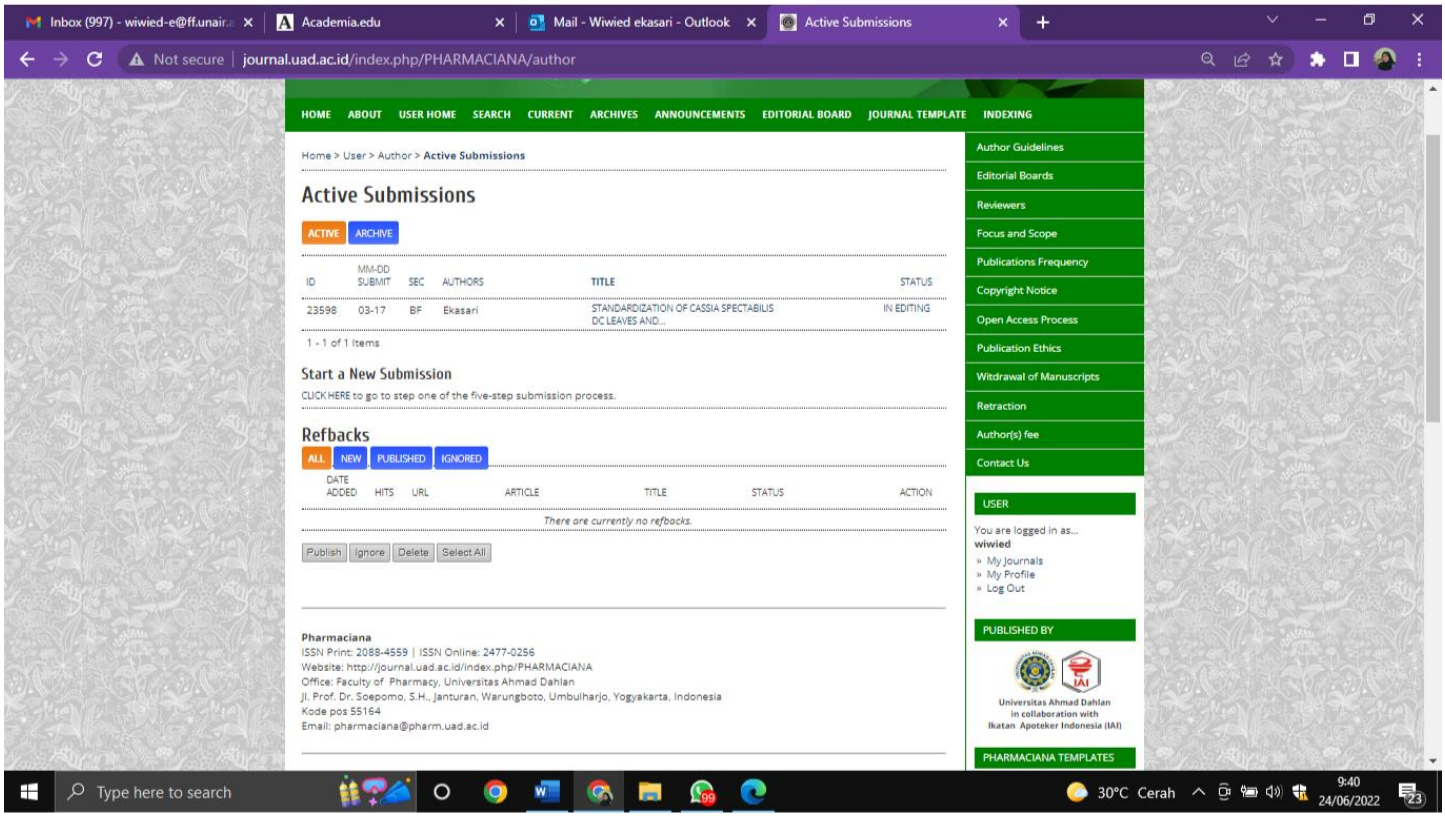
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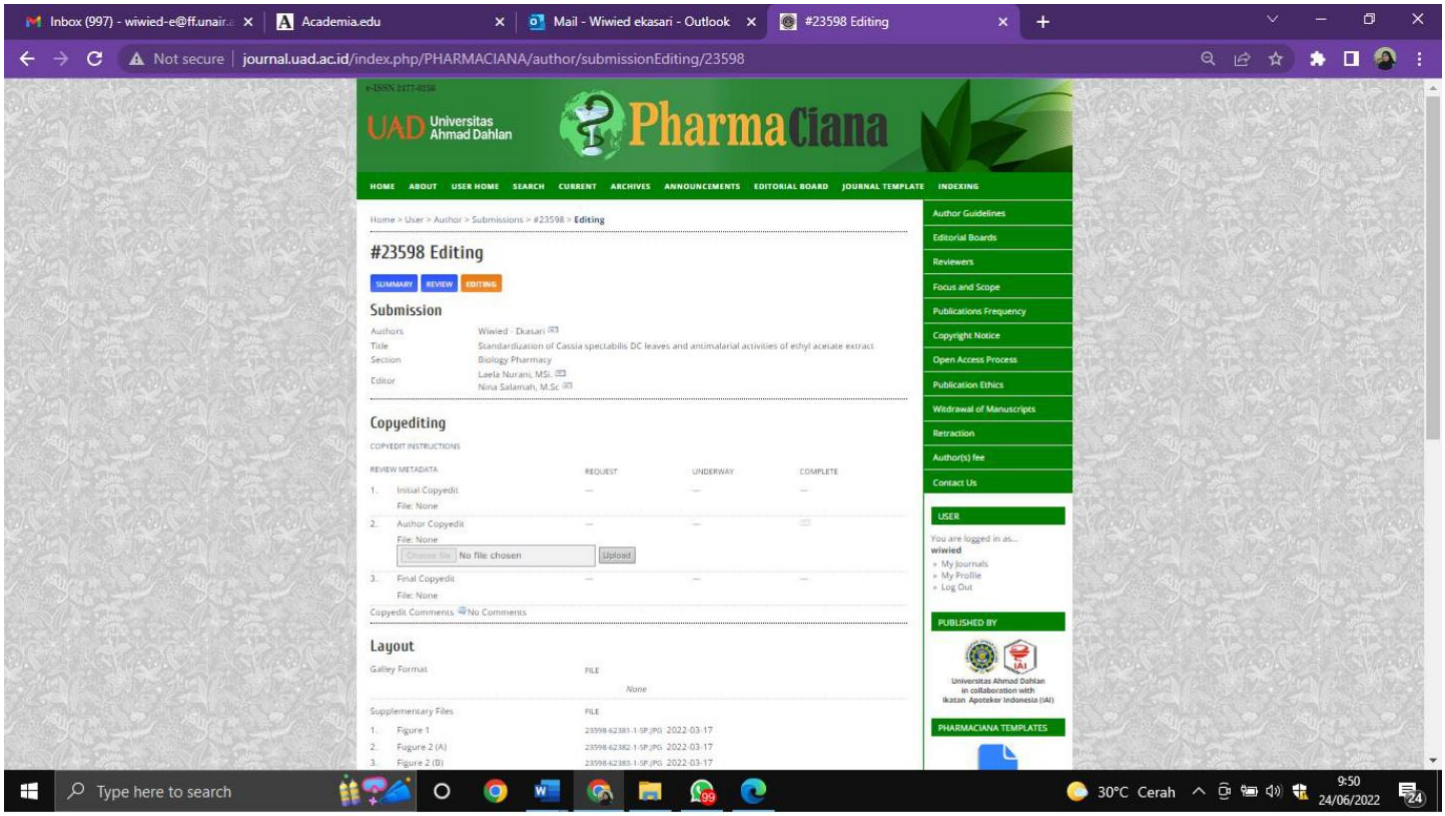

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4 messages

Prof.Dr. Nurkhasanah Mahfudh,M.Si.,Apt <nurkhasanah@pharm.uad.ac.id> Thu, Mar 17, 2022 at 9:16 PM
To: "Dr. Wiwied - Ekasari" <wiwied-e@ff.unair.ac.id>

Dr. Wiwied - Ekasari:

Thank you for submitting the manuscript, "Standardization of Cassia spectabilis DC leaves and antimalarial activities of ethyl acetate extract" to Pharmaciana. With the online journal management system that we are using, you will be able to track its progress through the editorial process by logging in to the journal web site:

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2. PROSES REVIEW

wiwied ekasari <wiwied-e@ff.unair.ac.id> Wed, May 11, 2022 at 12:41 PM
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Kepada Yth.
Prof.Dr. Nurkhasanah Mahfudh,M.Si.,Apt
Pharmaciana

Bersama ini saya informasikan bahwa manuscript saya yang berjudul "**Standardization of Cassia spectabilis DC leaves and antimalarial activities of ethyl acetate extract**" telah submit pad jurnal Pharmaciana tanggal 17 Maret 2022 dan mendapat ID 23598.

Sudah mendapat respon dari 2 reviewer untuk direvisi pada tanggal 4 april 2022. Manuskript hasil revisi sudah saya kirim kembali melalui system on line pada pertengahan April 2022. Namun sayangnya setelah itu saya mengalami kesulitan untuk memantau perkembangan perjalanan manuskipt karena sudah tidak bisa lagi membuka website PHARMACIANA sampai saat ini.

Mohon informasi perkembangan manuscript saya di jurnal Pharmaciana, dan apakah ada website lain untuk bisa membuka jurnal ini sehingga bisa memantau perjalanan manuscript tersebut.

Demikian, terima kasih ata kerjasamanya,

Dr. Wiwied Ekasari., MSi., Apt
Departemen Ilmu Kefarmasian
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Nurkhasanah Mahfudh <nurkhasanah@pharm.uad.ac.id> Wed, May 11, 2022 at 10:56 PM
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wiwied ekasari <wiwied-e@ff.unair.ac.id> Thu, Jun 9, 2022 at 7:28 PM
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Terimakasih atas informasinya tentang website Pharmaciana. Sekarang kami telah dapat membuka website tersebut.

Menginformasikan juga bahwa manuscript kami yang berjudul "**Standardization of Cassia spectabilis DC leaves and antimalarial activities of ethyl acetate extract**" telah kami kirimkan revisinya tercatat tgl 21 April 2022 dengan kode 23598-63163-1-ED di system website..
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Demikian, terima kasih atas kerjasamanya, kami menunggu kabar baik tentang manuskript tersebut.

Dr. Wiwied Ekasari., MSi., Apt
Departemen Ilmu Kefarmasian
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Standardization of *Cassia spectabilis* DC leaves and antimalarial activities of ethyl acetate extract

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ABSTRACT

Cassia spectabilis is one of the Indonesian medicinal plants traditionally used to treat different diseases, including malaria. Quality of a drug derived from plants is also influenced by the quality of its raw materials. Thus, in order to assure the quality of products made from plants, it is necessary to standardize the raw materials and following with the antimalarial activity test. The aim of this study was to standardize the quality of *C. spectabilis* leaves and to evaluate its antimalarial activities of ethyl acetate extract. The fresh material of *C. spectabilis* leaves were observed its specific and non-specific parameters. In vitro test was done by using *Plasmodium falciparum* 3D7. In vivo test was done using 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 material 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 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 IC_{50} value of 0.33±0.01 mg/mL. The leaves of *C. spectabilis* leaves meet the quality requirement and the ethyl acetate extract from standardized *C. spectabilis* leaves possessed a potential an antimalarial activity which deserves to be further developed.

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

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INTRODUCTION

Malaria is a severe disease caused by parasites of the *Plasmodium* genus, which is transmitted to humans through the bite of infected female *Anopheles* mosquito. Malaria remains the leading cause of death worldwide, most commonly in Africa and some Asian countries. Meanwhile, in developed countries malaria occurs because it is imported from endemic areas (Talapko et al., 2019). The World Health Organization (2018) reports that it is difficult to achieve the two important goals of Global Technical Strategy for Malaria, which is a reduction in mortality and morbidity by at least 40% by 2020. Since 2010, there has been a significant reduction in the burden of malaria, but analysis shows a slowdown, and even an increase in the number of cases between 2015 and 2017. The most critical step in the global eradication of malaria is reducing the number of cases in countries with the highest burden (Talapko et al., 2019).

Drug resistance is a severe global problem and has been reported in all antimalarial drugs although in most of malaria endemic countries, artemisinin-based combination therapy (ACT) remains an effective therapy (Alonso & Noor, 2017). To overcome the problem of resistance, new drugs are needed, 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 are 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 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 IC_{50} value of 12.52 µg/mL, and ED_{50} value of 131.5 mg/kg, respectively (Ekasari et al., 2018). Based on this result, it is proved that *C. spectabilis* leaves can be potentially developed in further research as antimalarial plants.

Medicinal plants play an important role in promoting health. They are widely spread all over the world, but mostly grow in tropical countries. Until now, it is reported that 25% of modern medicines, directly or indirectly, are derived from plants (Patwekar et al., 2015). The quality of herbal drugs is highly affected by the quality of the raw materials. And the quality of the raw materials can be influenced by factors, including cultivation, harvesting, and production. Quality assurance cannot be achieved unless meeting the specified standard. Standardization of raw materials holds the main key in obtaining a qualified drug because raw materials of poor quality can affect the pharmacological activity of the product (Yadav & Prajapati, 2011).Therefore, standardization is an essential step to obtain a safe, effective, and qualified pharmaceutical product (Purwantiningsih et al., 2011). In this research, the specific and non-specific standard parameters of *C. spectabilis* leaves were determined and the results can be improved as it is one of the Indonesian plants which can be used as the material for traditional medicine, especially for antimalarial medicament.

Further investigation on compounds that might contribute to antimalarial activity reveals that *C. spectabilis* leaves is proven to have active antimalarial alkaloid compounds, which had a structural pattern that was 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).

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Antimalarial activity test of methanol and ethanol extract of *C. spectabilis* leaves have been done before. Therefore, this study used ethyl acetate extract of *C. spectabilis* leaves 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, 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.

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MATERIALS AND METHOD

Plant Materials

Fresh *C. spectabilis* leaves 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 *Cara Pembuatan Simplisia* (Ministry of Health Republic of Indonesia, 1985), and were ground into powder using a pollinating machine.

Determination of Specific Parameter

Identity

Description of the botanical nomenclature, including the scientific name of the plant, part of the plant used, and local name of the plant.

Macroscopic test

Macroscopic test was undertaken by morphological observation of fresh leaves with or without magnifying glass.

Microscopic test

Fresh leaves sections of *C. spectabilis* including transverse section of the costa, transverse section of the mesophyll, longitudinal section of upper epidermis, and longitudinal section of lower epidermis, along with the dried leaves powder were treated with a drop of water in a slide and observed under a microscope. The next observation was continued in a similar way by replacing water with chloral hydrate (heated) and staining with phloroglucinol-HCl reagent.

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Organoleptic

Organoleptic test was undertaken by sensory observation including appearance, colour, smell, and taste of dried leaves powder.

Determination of water-soluble essence

Five grams of dried leaves powder were macerated in 100 mL water-chloroform in a plugged flask for 24 h while occasionally shaken for the first 6 h. Let it stand for 18 h then filtered. 20 mL of the filtrate was evaporated to dryness in a heated porcelain cup and the remainder filtered and heated at 105°C until it reached constant weight. Water soluble essence was obtained by calculation of the air-dried materials (Ministry of Health Republic of Indonesia, 2000).

Determination of ethanol-soluble essence

Five grams of dried leaves powder were macerated in 100 mL 95% ethanol in a plugged flask for 24 h while occasionally shaken for the first 6 h. Let it stand for 18 h then filtered. 20 mL of the filtrate was evaporated to dryness in a heated porcelain cup and the remainder filtered and heated at 105°C until it reached constant weight. Ethanol soluble essence was obtained by calculation of the air-dried materials (Ministry of Health Republic of Indonesia, 2000).

Determination of volatile oil

10-50 grams of dried leaves powder were put into a dried flask and added with boiling stones and 450 mL of water. The flask was set in the distillation apparatus and biuret was filled with water. 0.2 mL of xylol was added in the biuret. Flask was heated in the water bath. Therefore, the distillation was streamed slowly yet systematically. Distillation was stopped about 1-6 h. The volume of volatile oil and xylol was achieved in this step. Blank experiment of xylol was undertaken with the same method above without using extract. The aim of this blank experiment is to detect whether there is evaporation of xylol or not. The result can be used as reduction factor (Ministry of Health Republic of Indonesia, 2000).

Determination of total flavonoids level

In this method, rutin was used to make the standard calibration curve. 25 mg of rutin was dissolved in ethanol and then diluted to 125, 250, 500, and 1000 ppm. Stock solution of dried leaves powder were made from one gram of powder were transferred to a flask and added to 25 mL ethanol and centrifuged at 200 rpm for an hour. Samples were then filtered to a 25 mL volumetric flask and the volume was made up with ethanol. Test solution was made from 0.5 mL of dried leaves powder stock solutions and rutin solution were separately added to 1.5 mL ethanol, 0.1 mL aluminium chloride 10%, 0.1 mL potassium acetate 1 M and 2.8 mL distilled water. Each of the solutions was then mixed well and allowed to stand for 30 min at room temperature. Sample blank was prepared in a similar way by replacing aluminium chloride with distilled water. Their absorbance was then measured (Ministry of Health Republic of Indonesia, 2000).

Determination of Non-Specific Parameter

Determination of loss on drying

One gram of dried leaves powder was weighed and put into a weighing bottle heated at 105°C for 30 min. The dried leaves powder was put into the weighing bottle evenly and dried to the constant weight at 105°C.

Determination of water level

A quantity of *C. spectabilis* dried leaves powder expected to give 1-4 mL water was weighed respectively, transferred to a dried flask and then added with a few pieces of boiling stones. 200 mL of water-saturated toluene was added into the flask and the apparatus was assembled. Toluene was filled through the condenser to the receiving tube, followed by heating the flask gently for 15 min. Once the toluene began to boil, the temperature was adjusted to allow the distillation to continue at a rate of 2 drops per second. After the water was completely distilled, the inside of the condenser was rinsed with water-saturated toluene. The distillation was continued for 5 min. The receiving tube was allowed to cool to room temperature. The water and toluene were left to stand a

while until completely separated before the volume of water was recorded (Ministry of Health Republic of Indonesia, 2000).

Determination of total ash level

Two grams of dried leaves powder were weighed and put evenly into a preheated porcelain crucible. The porcelain crucibles were heated slowly to 600°C for an hour, then cooled and weighed to the constant weight. Ash level was obtained by calculation of the air-dried material (Ministry of Health Republic of Indonesia, 2000).

Determination of acid-insoluble ash level

Two grams of dried leaves powder and extract were respectively weighed and put evenly into preheated porcelain crucibles. The porcelain crucibles were heated slowly to 600°C for an hour and then cooled. The collected ash was boiled with 25 mL chloride acid for 5 min. The acid-insoluble part was filtered using ash-free filter paper, heated slowly to 600°C for an hour, then cooled and weighed to the constant weight. Ash level was obtained by calculation of the air-dried material (Ministry of Health Republic of Indonesia, 2000).

Determination of pesticide residue, heavy metal and microbial contamination

Residue of pesticide, heavy metal and microbial contamination was undertaken using the standard methodology in Indonesia and the extract common standard parameters of herbal drug (Ministry of Health Republic of Indonesia, 2000).

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

Experimental animal used in this study was BALB/c strain male mice obtained from Faculty of Veterinary Medicine, Universitas Airlangga, with a weight of ±20-30 grams. Mice were acclimatized for two weeks at a temperature of 24±1°C and humidity of 55±5% prior to in vivo test. In all in vivo experiments, the animals were kept in cages with raised, wide-mesh floors to prevent coprophagy. The ethical certificate was obtained from the Ethic Commission of Faculty of Veterinary Medicine of Universitas Airlangga (2.KE.181.10.2018). At the end of the tests, all animals were followed the euthanasia procedure. The mice were sacrificed by cervical dislocation after anaesthesia by intraperitoneal injection of 100 mg/kg ketamine (Carbone et al., 2012). The dead animals were then buried.

***In Vitro* Antimalarial Activity**

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The stock sample solution was prepared in dimethyl sulfoxide (DMSO) and diluted to the required concentration with complete media (RPMI 1640, 10% human plasma, 25 mM HEPES, and 25 mM $NaHCO_3$) until the final concentrations of the sample on the well culture plate were: 10; 1; 0.1; 0.01; 0.001 µg/mL. The test was carried out in duplicate. The plates were incubated under CO_2 condition at 37°C in a candle jar for 48 h. After the incubation, the contents of the well were harvested by making a thin smear of blood on clean microscopic slides. The blood smear was then fixed using methanol and stained for 10 min in 10% Giemsa solution (pH 7.3). Each 50% inhibitory concentration (IC_{50}) was calculated based on the inhibition percentage towards *P. falciparum* using probit analysis.

***In Vivo* Antimalarial Activity**

In vivo testing of ethyl acetate extract of *C. spectabilis* leaves was carried out on rodent malaria parasites (*Plasmodium berghei*) using 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, 8 weeks old) were used for each dose. Five other mice were kept as donors who were inoculated but untreated. The two controls (negative and positive) 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 extract of *C. spectabilis* leaves tested were 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 3 h of parasite inoculation (D_0), and then continued at the same time for 3 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 3 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 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 was expressed as mean ± standard error of mean (S.E.M). Statistical significance of means was determined by one-way analysis of variance (ANOVA), followed by Dunnett's (post-hoc test) to compare the measured parameters (parasitemia and level of β-hematin) with negative controls. The analysis was performed with 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 flavonoids 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 observation including fragments of mesophyll, crystal fibers, mesophyll with calcium oxalate crystal, and trichome are shown in Figure 2.

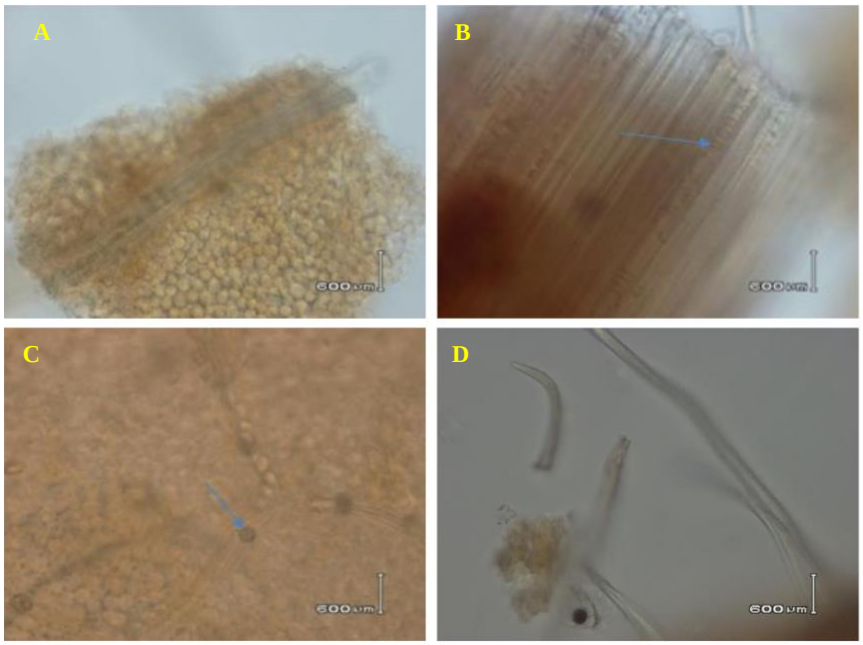

Figure 2. Fragments of (A) mesophyll; (B) crystal fibers (prism shape, blue arrow); (C) mesophyll with calcium oxalate crystal (blue arrow); and (D) trichome

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 requirement to be used as raw material of traditional medicine.

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	-

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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).

In determination of microbes, the dried leaves powder of *C. spectabilis* already met the requirements regulated by National Agency of Drug and Food Control (2014), as well as the determination of residue of pesticide and heavy metal (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 effect (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 little amounts, but in large amounts it causes the dangerous effect to human.

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

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 percentage of parasitemia and inhibition compared to negative control (p > 0.05). EACS = ethyl acetate extract of *C. spectabilis* leaves. DMSO was used for solubilizing the extracts and as negative control.

Based on the classification according to Gessler et al. (1994), the antimalarial activity of extracts with IC_{50} value less than 10 µg/mL is considered very good; 10 to 50 µg/mL is considered moderate; and more than 50 µg/mL is considered to have low activity. This suggests that in vitro

antimalarial activity from ethyl acetate extract of *C. spectabilis* leaves was moderate because of the IC_{50} value was in the range 10 to 50 µg/mL.

In vivo antimalarial activity test results from ethyl acetate extract of *C. spectabilis* leaves 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.

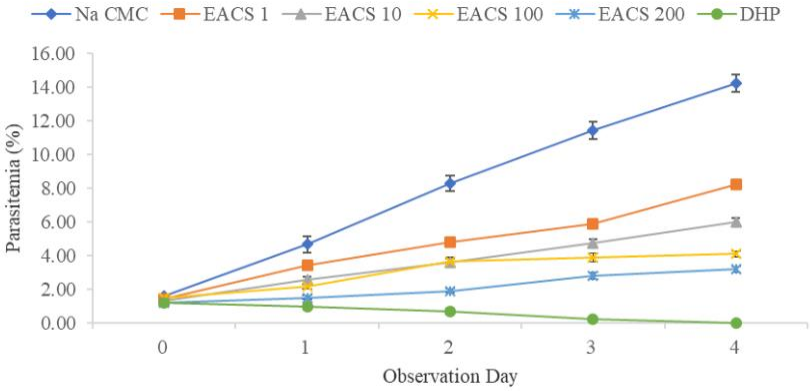

Figure 3. The effect of ethyl acetate extract of *C. spectabilis* leaves on parasitemia percentage of *P. berghei* infected mice on 4-day suppression test; data are mean ± S.E.M. (*n* = 8); Na CMC = sodium carboxymethylcellulose, DHP = dihydroartemisinin + piperaquine, EACS = ethyl acetate extract of *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 extract of *C. spectabilis* leaves 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 inhibit the growth of parasites by 50% of total population. The analysis resulted in an ED_{50} value of 1.74 mg/kg, which revealed the ethyl acetate extract of *C. spectabilis* leaves 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 leaf aqueous extract of *C. siamea* has 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 ED_{50} value of 22.30 mg/kg (Kainama et al., 2019), which was much higher than that of ethyl acetate extract of *C. spectabilis* leaves in this current study.

The heme detoxification inhibitory activity test of ethyl acetate extract of *C. spectabilis* leaves 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 were used as a negative control.

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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 extract *C. spectabilis* leaves in inhibiting heme detoxification probably by interacting with the alkaloid hydroxyl group in the extract compound which 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 extract of *C. spectabilis* leaves 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.

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

CONCLUSION

Overall, this study was concluded that the dried leaves powder of *C. spectabilis* meet the quality requirements and recommended to be used as raw material of traditional medicine. The results of antiplasmodial activity showed that the ethyl acetate extract of *C. spectabilis* leaves have 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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ACKNOWLEDGEMENT

We would like to thank The Ministry of Research, Technology, and Higher Education of the Republic of Indonesia for financial support.

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REFERENCES

Alonso, P., & Noor, A. M. (2017). The global fight against malaria is at crossroads. *The Lancet*, *390*(10112), 2532–2534. https://doi.org/10.1016/S0140-6736(17)33080-5

Baelmans, R., Deharo, E., Muoz, V., Sauvain, M., & Ginsburg, H. (2000). Experimental conditions for testing the inhibitory activity of chloroquine on the formation of β-hematin. *Experimental Parasitology*, *96*(4), 243–248. https://doi.org/10.1006/expr.2000.4558

Basilico, N., Pagani, E., Monti, D., Olliaro, P., & Taramelli, D. (1998). A microtitre-based method for measuring the haem polymerization inhibitory activity (HPIA) of antimalarial drugs. *Journal of Antimicrobial Chemotherapy*, *42*(1), 55–60. https://doi.org/10.1093/jac/42.1.55

Carbone, L., Carbone, E. T., Yi, E. M., Bauer, D. B., Lindstrom, K. A., Parker, J. M., Austin, J. A., Seo, Y., Gandhi, A. D., & Wilkerson, J. D. (2012). Assessing cervical dislocation as a humane euthanasia method in mice. *Journal of the American Association for Laboratory Animal Science*, *51*(3), 352–356.

Ekasari, W., Basuki, D. R., Arwati, H., & Wahyuni, T. S. (2021). Antiplasmodial activity of Ethanolic extract of Cassia spectabilis DC leaf and its inhibition effect in heme detoxification. *BMC Complementary Medicine and Therapies*, *21*(1), 1–12. https://doi.org/10.1186/s12906-021-03239-9

Ekasari, W., Wahyuni, T. S., Arwaty, H., & Putri, N. T. (2018). Determination of effective dose of antimalarial from Cassia spectabilis leaf ethanol extract in Plasmodium berghei-infected mice. *African Journal of Infectious Diseases*, *12*(Special Issue 1), 110–115. https://doi.org/10.2101/Ajid.12v1S.16

Ekasari, W., Widyawaruyanti, A., Zaini, N. C., Syafruddin, D., Honda, T., & Morita, H. (2009). Antimalarial activity of cassiarin A from the leaves of Cassia siamea. *Heterocycles*, *78*(7), 1831–1836. https://doi.org/10.3987/COM-09-11680

El-Nahhal, Y., & Radwan, A. A. (2013). Human health risks: Impact of pesticide application. *Journal of Environment and Earth Science*, *3*(7), 199–210.

Fidock, D. A., Rosenthal, P. J., Croft, S. L., Brun, R., & Nwaka, S. (2004). Antimalarial drug discovery: efficacy models for compound screening. *Nature Reviews. Drug Discovery*, *3*(6), 509–520. https://doi.org/10.1038/nrd1416

Fong, K. Y., & Wright, D. W. (2013). Hemozoin and antimalarial drug discovery. *Future*

Medicinal Chemistry, *5*(12), 1437–1450. https://doi.org/10.4155/fmc.13.113

Frölich, S., Schubert, C., Bienzle, U., & Jenett-Siems, K. (2005). In vitro antiplasmodial activity of prenylated chalcone derivatives of hops (Humulus lupulus) and their interaction with haemin. *Journal of Antimicrobial Chemotherapy*, *55*(6), 883–887. https://doi.org/10.1093/jac/dki099

Gessler, M. C., Nkunya, M. H. H., Mwasumbi, L. B., Heinrich, M., & Tanner, M. (1994). Screening Tanzanian medicinal plants for antimalarial activity. *Acta Tropica*, *56*(1), 65–77. https://doi.org/10.1016/0001-706x(94)90041-8

Grace, M. H., Lategan, C., Graziose, R., Smith, P. J., Raskin, I., & Lila, M. A. (2012). Antiplasmodial activity of the ethnobotanical plant Cassia fistula. *Natural Product Communications*, *7*(10), 1263–1266. https://doi.org/10.1177/1934578x1200701002

Kainama, H., Fatmawati, S., Santoso, M., Kakisina, P., & Ersam, T. (2019). In vitro and in vivo antiplasmodial of stem bark extract of Garcinia husor. *HAYATI Journal of Biosciences*, *26*(2), 81–89. https://doi.org/10.4308/hjb.26.2.81

Kaira, B. S., Chawla, S., Gupta, P., & Valecha, N. (2006). Screening of antimalarial drugs: An overview. *Indian Journal of Pharmacology*, *38*(1), 5–12. https://doi.org/10.4103/0253-7613.19846

Kamagaté, M., Koffi, C., Kouamé, N. M., Akoubet, A., Yao, N. A. R., & Die-Kakou, H. M. (2014). Ethnobotany, phytochemistry, pharmacology and toxicology profiles of Cassia siamea Lam. *The Journal of Phytopharmacology*, *3*(1), 57–76.

Ministry of Health Republic of Indonesia. (1985). *How to make simplicia*. Ministry of Health Republic of Indonesia.

Ministry of Health Republic of Indonesia. (2000). *General standard parameters of m medicinal plant extract*. Ministry of Health Republic of Indonesia.

Mosaddegh, M., Irani, M., & Esmaeili, S. (2018). Inhibition test of heme detoxification (ITHD) as an approach for detecting antimalarial agents in medicinal plants. *Research Journal of Pharmacognosy*, *5*(1), 5–11.

Muñoz, V., Sauvain, M., Bourdy, G., Callapa, J., Bergeron, S., Rojas, I., Bravo, J. A., Balderrama, L., Ortiz, B., Gimenez, A., & Deharo, E. (2000). A search for natural bioactive compounds in Bolivia through a multidisciplinary approach. Part I. Evaluation of the antimalarial activity of plants used by the Chacobo Indians. *Journal of Ethnopharmacology*, *69*(2), 127–137. https://doi.org/10.1016/S0378-8741(99)00148-8

National Agency of Drug and Food Control. (2014). *Regulation of Head of National Agency of Drug and Food Control Republic Indonesia Number 12 about Quality Requirements of Traditional Medicine*. National Agency of Drug and Food Control.

Patwekar, S. L., Suryawanshi, A. B., Gaikwad, M. S., Pedewad, S. R., & Potulwar, A. P. (2015). Standardization of herbal drugs: An overview. *The Pharma Innovation Journal*, *4*(9), 100–104.

Purwantiningsih, Purwantini, I., & Santoso, D. (2011). Identification of standard parameters of Kepel leaves [Stelechocarpus Burahol (BL.) Hook. F. & TH.] and the extract as raw material for anti-hyperuricemic medicaments. *Asian Journal of Pharmaceutical and Clinical Research*, *4*(Suppl. 1), 149–153.

Slater, A. F. G., & Cerami, A. (1992). Inhibition by chloroquine of a novel haem polymerase enzyme activity in malaria trophozoites. *Letters to Nature*, *355*(6356), 167–169. https://doi.org/10.1038/355167a0

Talapko, J., Škrlec, I., Alebić, T., Jukić, M., & Včev, A. (2019). Malaria: The past and the present. *Microorganisms*, *7*(6), 179. https://doi.org/10.3390/microorganisms7060179

Turalely, R., Susidarti, R. A., & Wijayanti, M. A. (2011). In vivo antiplasmodial of the most active fraction and its compound of Kapur leaves (Harmsiopanax aculeatus Harms) extract against Plasmodium berghei. *Tropical Medicine Journal*, *1*(2), 131–140. https://doi.org/10.22146/tmj.4575

Veerachari, U., & Bopaiah, A. K. (2011). Preliminary phyto-chemical evaluation of the leaf extract of five Cassia species. *Journal of Chemical and Pharmaceutical Research*, *3*(5), 574–583.
World Health Organization. (2018). *World malaria report 2018*. WHO Press.
Yadav, P., & Prajapati, P. K. (2011). Quality control parameters for medicinal plants, an overview. *Asian Journal of Biomedical and Pharmaceutical Sciences*, *1*(5), 12–16.

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

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ABSTRACT

Cassia spectabilis is one of the Indonesian medicinal plants traditionally used to treat different diseases, including malaria. Quality of a drug derived from plants is also influenced by the quality of its raw materials. Thus, in order to assure the quality of products made from plants, it is necessary to standardize the raw materials and following with the antimalarial activity test. The aim of this study was to standardize the quality of *C. spectabilis* leaves and to evaluate its antimalarial activities of ethyl acetate extract. The fresh material of *C. spectabilis* leaves were observed its specific and non-specific parameters. In vitro test was done by using *Plasmodium falciparum* 3D7. In vivo test was done using 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 material 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 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 IC_{50} value of 0.33±0.01 mg/mL. The leaves of *C. spectabilis* leaves meet the quality requirement and the ethyl acetate extract from standardized *C. spectabilis* leaves possessed a potential an antimalarial activity which deserves to be further developed.

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

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INTRODUCTION

Malaria is a severe disease caused by parasites of the *Plasmodium* genus, which is transmitted to humans through the bite of infected female *Anopheles* mosquito. Malaria remains the leading cause of death worldwide, most commonly in Africa and some Asian countries. Meanwhile, in developed countries malaria occurs because it is imported from endemic areas (Talapko et al., 2019). The World Health Organization (2018) reports that it is difficult to achieve the two important goals of Global Technical Strategy for Malaria, which is a reduction in mortality and morbidity by at least 40% by 2020. Since 2010, there has been a significant reduction in the burden of malaria, but analysis shows a slowdown, and even an increase in the number of cases between 2015 and 2017. The most critical step in the global eradication of malaria is reducing the number of cases in countries with the highest burden (Talapko et al., 2019).

Drug resistance is a severe global problem and has been reported in all antimalarial drugs although in most of malaria endemic countries, artemisinin-based combination therapy (ACT) remains an effective therapy (Alonso & Noor, 2017). To overcome the problem of resistance, new drugs are needed, 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 are 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).

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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 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 IC_{50} value of 12.52 µg/mL, and ED_{50} value of 131.5 mg/kg, respectively (Ekasari et al., 2018). Based on this result, it is proved that *C. spectabilis* leaves can be potentially developed in further research as antimalarial plants.

Medicinal plants play an important role in promoting health. They are widely spread all over the world, but mostly grow in tropical countries. Until now, it is reported that 25% of modern medicines, directly or indirectly, are derived from plants (Patwekar et al., 2015). The quality of herbal drugs is highly affected by the quality of the raw materials. And the quality of the raw materials can be influenced by factors, including cultivation, harvesting, and production. Quality assurance cannot be achieved unless meeting the specified standard. Standardization of raw materials holds the main key in obtaining a qualified drug because raw materials of poor quality can affect the pharmacological activity of the product (Yadav & Prajapati, 2011). Therefore, standardization is an essential step to obtain a safe, effective, and qualified pharmaceutical product (Purwantiningsih et al., 2011). In this research, the specific and non-specific standard parameters of *C. spectabilis* leaves were determined and the results can be improved as it is one of the Indonesian plants which can be used as the material for traditional medicine, especially for antimalarial medicament.

Further investigation on compounds that might contribute to antimalarial activity reveals that *C. spectabilis* leaves is proven to have active antimalarial alkaloid compounds, which had a structural pattern that was 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

test of methanol and ethanol extract of *C. spectabilis* leaves have been done before. Therefore, this study used ethyl acetate extract of *C. spectabilis* leaves 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, 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.

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MATERIALS AND METHOD

Plant Materials

Fresh *C. spectabilis* leaves 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 *Cara Pembuatan Simplisia* (Ministry of Health Republic of Indonesia, 1985), and were ground into powder using a pollinating machine.

Determination of Specific Parameter

Identity

Description of the botanical nomenclature, including the scientific name of the plant, part of the plant used, and local name of the plant.

Macroscopic test

Macroscopic test was undertaken by morphological observation of fresh leaves with or without magnifying glass.

Microscopic test

Fresh leaves sections of *C. spectabilis* including transverse section of the costa, transverse section of the mesophyll, longitudinal section of upper epidermis, and longitudinal section of lower epidermis, along with the dried leaves powder were treated with a drop of water in a slide and observed under a microscope. The next observation was continued in a similar way by replacing water with chloral hydrate (heated) and staining with phloroglucinol-HCl reagent.

Organoleptic

Organoleptic test was undertaken by sensory observation including appearance, colour, smell, and taste of dried leaves powder.

Determination of water-soluble essence

Five grams of dried leaves powder were macerated in 100 mL water-chloroform in a plugged flask for 24 h while occasionally shaken for the first 6 h. Let it stand for 18 h then filtered. 20 mL of the filtrate was evaporated to dryness in a heated porcelain cup and the remainder filtered and heated at 105°C until it reached constant weight. Water soluble essence was obtained by calculation of the air-dried materials (Ministry of Health Republic of Indonesia, 2000).

Determination of ethanol-soluble essence

Five grams of dried leaves powder were macerated in 100 mL 95% ethanol in a plugged flask for 24 h while occasionally shaken for the first 6 h. Let it stand for 18 h then filtered. 20 mL of the filtrate was evaporated to dryness in a heated porcelain cup and the remainder filtered and heated at 105°C until it reached constant weight. Ethanol soluble essence was obtained by calculation of the air-dried materials (Ministry of Health Republic of Indonesia, 2000).

Determination of volatile oil

10-50 grams of dried leaves powder were put into a dried flask and added with boiling stones and 450 mL of water. The flask was set in the distillation apparatus and biuret was filled with water. 0.2 mL of xylol was added in the biuret. Flask was heated in the water bath. Therefore, the distillation was streamed slowly yet systematically. Distillation was stopped about 1-6 h. The volume of volatile oil and xylol was achieved in this step. Blank experiment of xylol was undertaken with the same method above without using extract. The aim of this blank experiment is to detect whether there is evaporation of xylol or not. The result can be used as reduction factor (Ministry of Health Republic of Indonesia, 2000).

Determination of total flavonoids level

In this method, rutin was used to make the standard calibration curve. 25 mg of rutin was dissolved in ethanol and then diluted to 125, 250, 500, and 1000 ppm. Stock solution of dried leaves powder were made from one gram of powder were transferred to a flask and added to 25 mL ethanol and centrifuged at 200 rpm for an hour. Samples were then filtered to a 25 mL volumetric flask and the volume was made up with ethanol. Test solution was made from 0.5 mL of dried leaves powder stock solutions and rutin solution were separately added to 1.5 mL ethanol, 0.1 mL aluminium chloride 10%, 0.1 mL potassium acetate 1 M and 2.8 mL distilled water. Each of the solutions was then mixed well and allowed to stand for 30 min at room temperature. Sample blank was prepared in a similar way by replacing aluminium chloride with distilled water. Their absorbance was then measured (Ministry of Health Republic of Indonesia, 2000).

Determination of Non-Specific Parameter

Determination of loss on drying

One gram of dried leaves powder was weighed and put into a weighing bottle heated at 105°C for 30 min. The dried leaves powder was put into the weighing bottle evenly and dried to the constant weight at 105°C.

Determination of water level

A quantity of *C. spectabilis* dried leaves powder expected to give 1-4 mL water was weighed respectively, transferred to a dried flask and then added with a few pieces of boiling stones. 200 mL of water-saturated toluene was added into the flask and the apparatus was assembled. Toluene was filled through the condenser to the receiving tube, followed by heating the flask gently for 15 min. Once the toluene began to boil, the temperature was adjusted to allow the distillation to continue at a rate of 2 drops per second. After the water was completely distilled, the inside of the condenser was rinsed with water-saturated toluene. The distillation was continued for 5 min. The receiving tube was allowed to cool to room temperature. The water and toluene were left to stand a while until completely separated before the volume of water was recorded (Ministry of Health Republic of Indonesia, 2000).

Determination of total ash level

Two grams of dried leaves powder were weighed and put evenly into a preheated porcelain crucible. The porcelain crucibles were heated slowly to 600°C for an hour, then cooled and weighed to the constant weight. Ash level was obtained by calculation of the air-dried material (Ministry of Health Republic of Indonesia, 2000).

Determination of acid-insoluble ash level

Two grams of dried leaves powder and extract were respectively weighed and put evenly into preheated porcelain crucibles. The porcelain crucibles were heated slowly to 600°C for an hour and then cooled. The collected ash was boiled with 25 mL chloride acid for 5 min. The acid-insoluble part was filtered using ash-free filter paper, heated slowly to 600°C for an hour, then cooled and weighed to the constant weight. Ash level was obtained by calculation of the air-dried material (Ministry of Health Republic of Indonesia, 2000).

Determination of pesticide residue, heavy metal and microbial contamination

Residue of pesticide, heavy metal and microbial contamination was undertaken using the standard methodology in Indonesia and the extract common standard parameters of herbal drug (Ministry of Health Republic of Indonesia, 2000).

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

Experimental animal used in this study was BALB/c strain male mice obtained from Faculty of Veterinary Medicine, Universitas Airlangga, with a weight of ±20-30 grams. Mice were acclimatized for two weeks at a temperature of 24±1°C and humidity of 55±5% prior to in vivo test. In all in vivo experiments, the animals were kept in cages with raised, wide-mesh floors to prevent coprophagy. The ethical certificate was obtained from the Ethic Commission of Faculty of Veterinary Medicine of Universitas Airlangga (2.KE.181.10.2018). At the end of the tests, all animals were followed the euthanasia procedure. The mice were sacrificed by cervical dislocation after anaesthesia by intraperitoneal injection of 100 mg/kg ketamine (Carbone et al., 2012). The dead animals were then buried.

***In Vitro* Antimalarial Activity**

The stock sample solution was prepared in dimethyl sulfoxide (DMSO) and diluted to the required concentration with complete media (RPMI 1640, 10% human plasma, 25 mM HEPES, and 25 mM $NaHCO_3$) until the final concentrations of the sample on the well culture plate were: 10; 1; 0.1; 0.01; 0.001 µg/mL. The test was carried out in duplicate. The plates were incubated under CO_2 condition at 37°C in a candle jar for 48 h. After the incubation, the contents of the well were harvested by making a thin smear of blood on clean microscopic slides. The blood smear was then fixed using methanol and stained for 10 min in 10% Giemsa solution (pH 7.3). Each 50% inhibitory concentration (IC_{50}) was calculated based on the inhibition percentage towards *P. falciparum* using probit analysis.

***In Vivo* Antimalarial Activity**

In vivo testing of ethyl acetate extract of *C. spectabilis* leaves was carried out on rodent malaria parasites (*Plasmodium berghei*) using 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, 8 weeks old) were used for each dose. Five other mice were kept as donors who were inoculated but untreated. The two controls (negative and positive) 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 extract of *C. spectabilis* leaves tested were 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 3 h of parasite inoculation (D_0), and then continued at the same time for 3 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 3 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 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 was expressed as mean ± standard error of mean (S.E.M). Statistical significance of means was determined by one-way analysis of variance (ANOVA), followed by Dunnett's (post-hoc test) to compare the measured parameters (parasitemia and level of β-hematin) with negative controls. The analysis was performed with 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 flavonoids 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 observation including fragments of mesophyll, crystal fibers, mesophyll with calcium oxalate crystal, and trichome are shown in Figure 2.

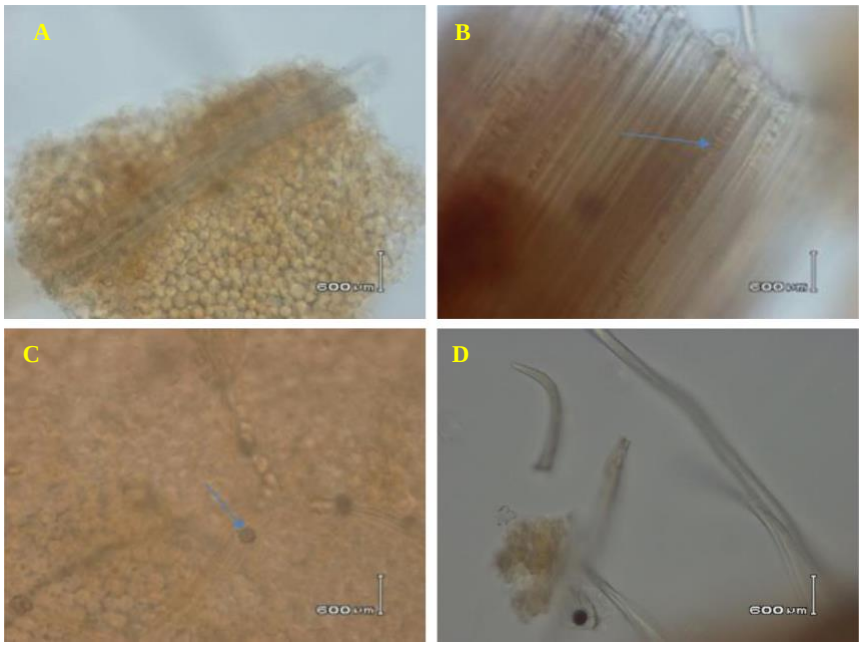

Figure 2. Fragments of (A) mesophyll; (B) crystal fibers (prism shape, blue arrow); (C) mesophyll with calcium oxalate crystal (blue arrow); and (D) trichome

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 requirement to be used as raw material of traditional medicine.

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).

In determination of microbes, the dried leaves powder of *C. spectabilis* already met the requirements regulated by National Agency of Drug and Food Control (2014), as well as the determination of residue of pesticide and heavy metal (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 effect (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 little amounts, but in large amounts it causes the dangerous effect to human.

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

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 percentage of parasitemia and inhibition compared to negative control (p > 0.05). EACS = ethyl acetate extract of *C. spectabilis* leaves. DMSO was used for solubilizing the extracts and as negative control.

Based on the classification according to Gessler et al. (1994), the antimalarial activity of extracts with IC_{50} value less than 10 µg/mL is considered very good; 10 to 50 µg/mL is considered moderate; and more than 50 µg/mL is considered to have low activity. This suggests that in vitro

antimalarial activity from ethyl acetate extract of *C. spectabilis* leaves was moderate because of the IC_{50} value was in the range 10 to 50 µg/mL.

In vivo antimalarial activity test results from ethyl acetate extract of *C. spectabilis* leaves 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.

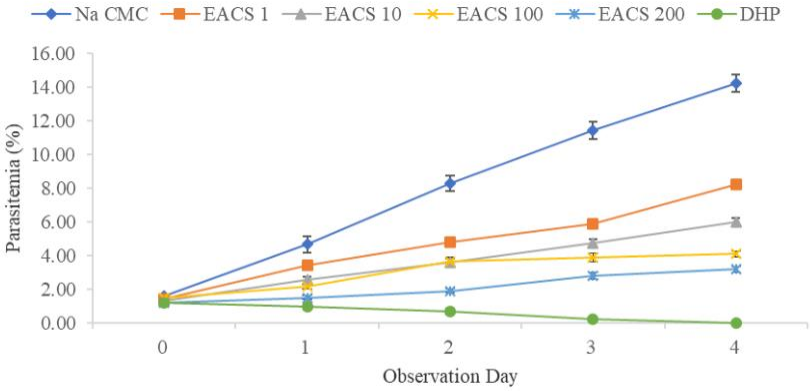

Figure 3. The effect of ethyl acetate extract of *C. spectabilis* leaves on parasitemia percentage of *P. berghei* infected mice on 4-day suppression test; data are mean ± S.E.M. (*n* = 8); Na CMC = sodium carboxymethylcellulose, DHP = dihydroartemisinin + piperaquine, EACS = ethyl acetate extract of *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 extract of *C. spectabilis* leaves 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 inhibit the growth of parasites by 50% of total population. The analysis resulted in an ED_{50} value of 1.74 mg/kg, which revealed the ethyl acetate extract of *C. spectabilis* leaves 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 leaf aqueous extract of *C. siamea* has 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 ED_{50} value of 22.30 mg/kg (Kainama et al., 2019), which was much higher than that of ethyl acetate extract of *C. spectabilis* leaves in this current study.

The heme detoxification inhibitory activity test of ethyl acetate extract of *C. spectabilis* leaves 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 were 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 extract *C. spectabilis* leaves in inhibiting heme detoxification probably by interacting with the alkaloid hydroxyl group in the extract compound which 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 extract of *C. spectabilis* leaves 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.

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

CONCLUSION

Overall, this study was concluded that the dried leaves powder of *C. spectabilis* meet the quality requirements and recommended to be used as raw material of traditional medicine. The results of antiplasmodial activity showed that the ethyl acetate extract of *C. spectabilis* leaves have 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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REFERENCES

Alonso, P., & Noor, A. M. (2017). The global fight against malaria is at crossroads. *The Lancet*, *390*(10112), 2532–2534. https://doi.org/10.1016/S0140-6736(17)33080-5

Baelmans, R., Deharo, E., Muoz, V., Sauvain, M., & Ginsburg, H. (2000). Experimental conditions for testing the inhibitory activity of chloroquine on the formation of β-hematin. *Experimental Parasitology*, *96*(4), 243–248. https://doi.org/10.1006/expr.2000.4558

Basilico, N., Pagani, E., Monti, D., Olliaro, P., & Taramelli, D. (1998). A microtitre-based method for measuring the haem polymerization inhibitory activity (HPIA) of antimalarial drugs. *Journal of Antimicrobial Chemotherapy*, *42*(1), 55–60. https://doi.org/10.1093/jac/42.1.55

Carbone, L., Carbone, E. T., Yi, E. M., Bauer, D. B., Lindstrom, K. A., Parker, J. M., Austin, J. A., Seo, Y., Gandhi, A. D., & Wilkerson, J. D. (2012). Assessing cervical dislocation as a humane euthanasia method in mice. *Journal of the American Association for Laboratory Animal Science*, *51*(3), 352–356.

Ekasari, W., Basuki, D. R., Arwati, H., & Wahyuni, T. S. (2021). Antiplasmodial activity of Ethanolic extract of Cassia spectabilis DC leaf and its inhibition effect in heme detoxification. *BMC Complementary Medicine and Therapies*, *21*(1), 1–12. https://doi.org/10.1186/s12906-021-03239-9

Ekasari, W., Wahyuni, T. S., Arwaty, H., & Putri, N. T. (2018). Determination of effective dose of antimalarial from Cassia spectabilis leaf ethanol extract in Plasmodium berghei-infected mice. *African Journal of Infectious Diseases*, *12*(Special Issue 1), 110–115. https://doi.org/10.2101/Ajid.12v1S.16

Ekasari, W., Widyawaruyanti, A., Zaini, N. C., Syafruddin, D., Honda, T., & Morita, H. (2009). Antimalarial activity of cassiarin A from the leaves of Cassia siamea. *Heterocycles*, *78*(7), 1831–1836. https://doi.org/10.3987/COM-09-11680

El-Nahhal, Y., & Radwan, A. A. (2013). Human health risks: Impact of pesticide application. *Journal of Environment and Earth Science*, *3*(7), 199–210.

Fidock, D. A., Rosenthal, P. J., Croft, S. L., Brun, R., & Nwaka, S. (2004). Antimalarial drug discovery: efficacy models for compound screening. *Nature Reviews. Drug Discovery*, *3*(6), 509–520. https://doi.org/10.1038/nrd1416

Fong, K. Y., & Wright, D. W. (2013). Hemozoin and antimalarial drug discovery. *Future Medicinal Chemistry*, *5*(12), 1437–1450. https://doi.org/10.4155/fmc.13.113

Frölich, S., Schubert, C., Bienzle, U., & Jenett-Siems, K. (2005). In vitro antiplasmodial activity of prenylated chalcone derivatives of hops (Humulus lupulus) and their interaction with haemin. *Journal of Antimicrobial Chemotherapy*, *55*(6), 883–887. https://doi.org/10.1093/jac/dki099

Gessler, M. C., Nkunya, M. H. H., Mwasumbi, L. B., Heinrich, M., & Tanner, M. (1994). Screening Tanzanian medicinal plants for antimalarial activity. *Acta Tropica*, *56*(1), 65–77. https://doi.org/10.1016/0001-706x(94)90041-8

Grace, M. H., Lategan, C., Graziose, R., Smith, P. J., Raskin, I., & Lila, M. A. (2012). Antiplasmodial activity of the ethnobotanical plant Cassia fistula. *Natural Product Communications*, *7*(10), 1263–1266. https://doi.org/10.1177/1934578x1200701002

Kainama, H., Fatmawati, S., Santoso, M., Kakisina, P., & Ersam, T. (2019). In vitro and in vivo antiplasmodial of stem bark extract of Garcinia husor. *HAYATI Journal of Biosciences*, *26*(2), 81–89. https://doi.org/10.4308/hjb.26.2.81

Kaira, B. S., Chawla, S., Gupta, P., & Valecha, N. (2006). Screening of antimalarial drugs: An overview. *Indian Journal of Pharmacology*, *38*(1), 5–12. https://doi.org/10.4103/0253-7613.19846

Kamagaté, M., Koffi, C., Kouamé, N. M., Akoubet, A., Yao, N. A. R., & Die-Kakou, H. M. (2014). Ethnobotany, phytochemistry, pharmacology and toxicology profiles of Cassia siamea Lam. *The Journal of Phytopharmacology*, *3*(1), 57–76.

Ministry of Health Republic of Indonesia. (1985). *How to make simplicia*. Ministry of Health Republic of Indonesia.

Ministry of Health Republic of Indonesia. (2000). *General standard parameters of m medicinal plant extract*. Ministry of Health Republic of Indonesia.

Mosaddegh, M., Irani, M., & Esmaeili, S. (2018). Inhibition test of heme detoxification (ITHD) as an approach for detecting antimalarial agents in medicinal plants. *Research Journal of Pharmacognosy*, *5*(1), 5–11.

Muñoz, V., Sauvain, M., Bourdy, G., Callapa, J., Bergeron, S., Rojas, I., Bravo, J. A., Balderrama, L., Ortiz, B., Gimenez, A., & Deharo, E. (2000). A search for natural bioactive compounds in Bolivia through a multidisciplinary approach. Part I. Evaluation of the antimalarial activity of plants used by the Chacobo Indians. *Journal of Ethnopharmacology*, *69*(2), 127–137. https://doi.org/10.1016/S0378-8741(99)00148-8

National Agency of Drug and Food Control. (2014). *Regulation of Head of National Agency of Drug and Food Control Republic Indonesia Number 12 about Quality Requirements of Traditional Medicine*. National Agency of Drug and Food Control.

Patwekar, S. L., Suryawanshi, A. B., Gaikwad, M. S., Pedewad, S. R., & Potulwar, A. P. (2015). Standardization of herbal drugs: An overview. *The Pharma Innovation Journal*, *4*(9), 100–104.

Purwantiningsih, Purwantini, I., & Santoso, D. (2011). Identification of standard parameters of Kepel leaves [Stelechocarpus Burahol (BL.) Hook. F. & TH.] and the extract as raw material for anti-hyperuricemic medicaments. *Asian Journal of Pharmaceutical and Clinical Research*, *4*(Suppl. 1), 149–153.

Slater, A. F. G., & Cerami, A. (1992). Inhibition by chloroquine of a novel haem polymerase enzyme activity in malaria trophozoites. *Letters to Nature*, *355*(6356), 167–169. https://doi.org/10.1038/355167a0

Talapko, J., Škrlec, I., Alebić, T., Jukić, M., & Včev, A. (2019). Malaria: The past and the present. *Microorganisms*, *7*(6), 179. https://doi.org/10.3390/microorganisms7060179

Turalely, R., Susidarti, R. A., & Wijayanti, M. A. (2011). In vivo antiplasmodial of the most active fraction and its compound of Kapur leaves (Harmsiopanax aculeatus Harms) extract against Plasmodium berghei. *Tropical Medicine Journal*, *1*(2), 131–140. https://doi.org/10.22146/tmj.4575

Veerachari, U., & Bopaiah, A. K. (2011). Preliminary phyto-chemical evaluation of the leaf extract of five Cassia species. *Journal of Chemical and Pharmaceutical Research*, *3*(5), 574–583.

World Health Organization. (2018). *World malaria report 2018*. WHO Press.
Yadav, P., & Prajapati, P. K. (2011). Quality control parameters for medicinal plants, an overview. *Asian Journal of Biomedical and Pharmaceutical Sciences*, *1*(5), 12–16.

- **ANSWER TO REVIEWER(S)**

REVIEWER 1 – 23598-62683-1-RV

1) Abstract section, lines: "The leaves of *C. spectabilis* leaves meet the quality requirement and the ethyl acetate extract from standardized *C. spectabilis* leaves possessed a potential an antimalarial activity which deserves to be further developed."

PERTANYAAN:

Bukankah IC_{50} tinggi, bagaimana bisa disimpulkan potensial dan perlu dikembangkan?

JAWABAN:

Berdasarkan Gessler et al., (1994), nilai IC_{50} dari ekstrak etil asetat daun *C. spectabilis* yang diperoleh dalam pengujian *in vitro* ini tergolong memiliki aktivitas antimalaria yang *moderate*. Sedang pada pengujian secara *in vivo* ekstrak daun *C. spectabilis* terhadap pertumbuhan *P. berghei* pada model mencit terinfeksi (ED_{50} = 1,74 mg/kgBB), berdasarkan Muñoz et al. (2000) tergolong poten karena jika nilai ED_{50} <100 mg/kgBB maka aktivitas antimalaria *in vivo* dari ekstrak dikatakan sangat aktif/poten. Ditambah dengan hasil pengujian ekstrak daun *C. spectabilis* terhadap proses detoksifikasi heme (IC_{50} = 0,33 mg/mL) menunjukkan aktivitas yang kuat karena menurut Baelmans et al. (2000) jika nilai IC_{50} suatu senyawa kurang dari 12 mg/mL maka ekstrak dapat dikategorikan memiliki aktivitas penghambatan detoksifikasi heme.

Sehingga kami menganggap ekstrak ini masih potensial untuk dikembangkan lebih lanjut.

Referensi:

- Gessler, M. C., Nkunya, M. H. H., Mwasumbi, L. B., Heinrich, M., & Tanner, M. (1994). Screening Tanzanian medicinal plants for antimalarial activity. *Acta Tropica*, *56*(1), 65–77. https://doi.org/10.1016/0001-706x(94)90041-8
- Muñoz, V., Sauvain, M., Bourdy, G., Callapa, J., Bergeron, S., Rojas, I., Bravo, J. A., Balderrama, L., Ortiz, B., Gimenez, A., & Deharo, E. (2000). A search for natural bioactive compounds in Bolivia through a multidisciplinary approach. Part I. Evaluation of the antimalarial activity of plants used by the Chacobo Indians. *Journal of Ethnopharmacology*, *69*(2), 127–137. https://doi.org/10.1016/S0378-8741(99)00148-8
- Baelmans, R., Deharo, E., Muoz, V., Sauvain, M., & Ginsburg, H. (2000). Experimental conditions for testing the inhibitory activity of chloroquine on the formation of β-hematin. *Experimental Parasitology*, *96*(4), 243–248. https://doi.org/10.1006/expr.2000.4558

2) Introduction section, paragraph 2: "Drug resistance is a severe global problem and has been reported in all antimalarial drugs although in most of malaria endemic countries, artemisinin-based combination therapy (ACT) remains an effective therapy (Alonso & Noor, 2017). To overcome the problem of resistance, new drugs are needed, where a number of new antimalarial discoveries and developments are ongoing and some of them come from medicinal plants."

PERTANYAAN:

Latar belakang kombinasi tidak relevan. Latar belakang perlu disesuaikan karena tidak terdapat cara kerja terkait resistensi.

JAWABAN:

The sentence has been revised in the manuscript.

"The most critical step in the global eradication of malaria is reducing the number of cases in countries with the highest burden (Talapko et al., 2019). However, this step is still hindered by the existence of parasitic resistance to drugs, resistance to insecticides, and other administrative issues. To overcome the problem of malaria disease, new drugs are needed, where a number of new antimalarial discoveries and developments are ongoing and some of them come from medicinal plants."

Referensi:

- Talapko, J., Škrlec, I., Alebić, T., Jukić, M., & Včev, A. (2019). Malaria: the past and the present. *Microorganisms, 7*(6), 179. https://doi.org/10.3390/microorganisms7060179

3) Introduction section, baris: "Medicinal plants play an important role in promoting health. They are widely spread all over the world, but mostly grow in tropical countries. Until now, it is reported that 25% of modern medicines, directly or indirectly, are derived from plants (Patwekar et al., 2015). The quality of herbal drugs is highly affected by the quality of the raw materials. And the quality of the raw materials can be influenced by factors, including cultivation, harvesting, and production. Quality assurance cannot be achieved unless meeting the specified standard."

PERTANYAAN:

Latar belakang umum. Ada kata "*And*" di awal kalimat "And the quality of the raw materials…"?

JAWABAN:

The sentence has been revised in the manuscript.

"Medicinal plants play an important role in promoting health. They are widely spread all over the world, but mostly grow in tropical countries. Until now, it is reported that 25% of modern medicines, directly or indirectly, are derived from plants (Patwekar et al., 2015). The quality of herbal drugs is highly affected by the quality of the raw materials. The quality of the raw materials can be influenced by factors, including cultivation, harvesting, and production. Quality assurance cannot be achieved unless meeting the specified standard."

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- Patwekar, S. L., Suryawanshi, A. B., Gaikwad, M. S., Pedewad, S. R., & Potulwar, A. P. (2015). Standardization of herbal drugs: an overview. *The Pharma Innovation Journal*, *4*(9), 100–104.

4) Introduction section, baris: "Standardization of raw materials holds the main key in obtaining a qualified drug because raw materials of poor quality can affect the pharmacological activity of the product (Yadav & Prajapati, 2011). Therefore, standardization is an essential step to obtain a safe, effective, and qualified pharmaceutical product (Purwantiningsih et al., 2011). In this research, the specific and non-specific standard parameters of *C. spectabilis* leaves were determined and the results can be

improved as it is one of the Indonesian plants which can be used as the material for traditional medicine, especially for antimalarial medicament."

PERTANYAAN:

Latar belakang ini sangat umum. Terkait dengan tanaman ini, apakah ada latar belakang khusus? Latar belakang khusus perlunya standarisasi *C. spetabilis* apa?

JAWABAN:

Since there are no reports in the literature regarding the standardization of *C. spectabilis* leaves, we tried to gather information on specific and non-specific standard parameters of *C. spectabilis* leaves.

5) Introduction section, paragraph 10, lines: "…both in vitro against *P. falciparum* and in vivo against *P. berghei* infected mice…"

PERTANYAAN:

Tiba-tiba ada *P. berghei* di sini. Di paragraf-paragraf sebelumnya belum pernah dinarasikan.

JAWABAN:

P. berghei has been added/mentioned in paragraph 7 and 8.

Paragraph 7 – "Assessment of antimalarial activity can be done in vitro to *P. falciparum* and in vivo to rodent plasmodia such as *P. berghei* (Fidock et al., 2004)."

Paragraph 8 – "In vivo test includes a four-day primary test for suppression of parasitaemia or inhibition of *P. berghei* growth in mice."

Referensi:

- Fidock, D. A., Rosenthal, P. J., Croft, S. L., Brun, R., & Nwaka, S. (2004). Antimalarial drug discovery: efficacy models for compound screening. *Nature Reviews. Drug Discovery*, *3*(6), 509–520. https://doi.org/10.1038/nrd1416

6) Materials and Methods section, Microscopic test sub-section: "Fresh leaves sections of *C. spectabilis* including transverse section of the costa, transverse section of the mesophyll, longitudinal section of upper epidermis, and longitudinal section of lower epidermis, along with the dried leaves powder were treated with a drop of water in a slide and observed under a microscope. The next observation was continued in a similar way by replacing water with chloral hydrate (heated) and staining with phloroglucinol-HCl reagent."

PERTANYAAN:

Parameter spesifiknya apa?

JAWABAN:

The sentence has been added in the manuscript.

"Plant fragments of genus *Cassia* that can be observed include trichomes; laminae; prismatic calcium oxalate crystal; epidermis; crystal fibers; and vascular bundles (Ministry of Health Republic Indonesia, 1989)."

Referensi:

- Ministry of Health Republic Indonesia. (1989). *Materia medika Indonesia fifth edition*. Jakarta: Ministry of Health Republic Indonesia.

7) Materials and Methods section: "In Vitro Antimalarial Activity" subsection

PERTANYAAN:

Sumber?

JAWABAN:

The reference has been added on the "In Vitro Antimalarial Activity" subsection.

"In vitro testing of ethyl acetate extract of *C. spectabilis* leaves was carried out on continuous cultivation of *P. falciparum* in human erythrocytes (Fidock et al., 2004; Ekasari et al., 2021)."

Referensi:

- Fidock, D. A., Rosenthal, P. J., Croft, S. L., Brun, R., & Nwaka, S. (2004). Antimalarial drug discovery: efficacy models for compound screening. *Nature Reviews. Drug Discovery*, *3*(6), 509–520. https://doi.org/10.1038/nrd1416
- Ekasari, W., Basuki, D. R., Arwati, H., & Wahyuni, T. S. (2021). Antiplasmodial activity of Ethanolic extract of *Cassia spectabilis* DC leaf and its inhibition effect in heme detoxification. *BMC Complementary Medicine and Therapies*, *21*(1), 1–12. https://doi.org/10.1186/s12906-021-03239-9

8) Result and Discussion section, Table 2: "Specific parameters:"

PERTANYAAN:

Mikroskopis?

JAWABAN:

The organoleptic data on the "specific parameters" listed in Table 2 are the results of macroscopic observations (e.g., color, shape, aroma, and taste) on the dried leaf powder of *C. spectabilis*. Meanwhile, the results of microscopic observations (e.g., the constituent fragments) on the dried leaf powder of *C. spectabilis* have been shown in Figure 2.

9) Result and Discussion section, Table 5: "IC_{50} (mg/mL)"

PERTANYAAN:

Umumnya mikrogram per mL.

JAWABAN:

The heme detoxification inhibitory activity of a compound was generally expressed as an IC_{50} value in mM or mg/mL, depending on the concentration of the standard hematin solution used (Basilico et al., 1998; Baelmans et al., 2000; Prakoso et al., 2019; Zakiah et al., 2021).

Referensi:

- Basilico, N., Pagani, E., Monti, D., Olliaro, P., & Taramelli, D. (1998). A microtitre-based method for measuring the haem polymerization inhibitory activity (HPIA) of antimalarial drugs. *Journal of Antimicrobial Chemotherapy*, *42*(1), 55–60. https://doi.org/10.1093/jac/42.1.55

- Baelmans, R., Deharo, E., Muoz, V., Sauvain, M., & Ginsburg, H. (2000). Experimental conditions for testing the inhibitory activity of chloroquine on the formation of β-hematin. *Experimental Parasitology*, *96*(4), 243–248. https://doi.org/10.1006/expr.2000.4558
- Prakoso, N. I., Zakiyah, Z. N., Liyanita, A., Rubiyanto, D., Fitriastuti, D., Ramadani, A. P., Kamari, A., & Mow, S. K. (2019). Antimalarial activity of *Andrographis paniculata* Ness's n-hexane extract and its major compounds. *Open Chemistry*, *17*(1), 788–797. https://doi.org/10.1515/chem-2019-0086
- Zakiah, M., Syarif, R. A., Mustofa, M., Jumina, J., Fatmasari, N., & Sholikhah, E. N. (2021). In vitro antiplasmodial, heme polymerization, and cytotoxicity of hydroxyxanthone derivatives. *Journal of Tropical Medicine*, *2021*, 8866681. https://doi.org/10.1155/2021/8866681

10) Conclusion section, baris: "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."

PERTANYAAN:

Apakah perlu? Jika perlu maka dipertahankan, jika tidak perlu maka dihilangkan.

JAWABAN:

This sentence has been removed/deleted in the manuscript.

11) Acknowledgement section, baris: "…The Ministry of Research, Technology, and Higher Education of…"

PERTANYAAN:

Tahun berapa, nomor kontrak berapa. Terimakasih.

JAWABAN:

The contract/grant number of this research has been added in the acknowledgement section.

"We would like to thank The Ministry of Research, Technology, and Higher Education of the Republic of Indonesia for financial support (Grant number 951/UN3.14/LT/2016)."

REVIEWER 2 – 23598-62738-1-RV

1. Title: "Standardization of *Cassia spectabilis* DC leaves and antimalarial activities of ethyl acetate extract"

   QUESTION: What extract?

   ANSWER:

   Antimalarial activity testing was carried out on the ethyl acetate extract of *Cassia spectabilis* leaves. The title has been changed to "Standardization of *Cassia spectabilis* DC leaves and its antimalarial activities of ethyl acetate extract".

2. Introduction section, paragraph 3, lines: "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."

   QUESTION: Is it the synonym of *Cassia spectabilis*? Or other species?

   ANSWER:

   Cassia siamea is not a synonym for *Cassia spectabilis*. *Cassia siamea*, also known as *Senna siamea*, is the name of another species that also belongs to the genus *Cassia*.

3. Introduction section, paragraph 3, lines: "Apart from that, several other species of *Cassia* with clear antimalarial properties are functioned throughout Africa, such as *C. occidentalis, C. africana, C. floribunda*, and *C. hirsuta*."

   QUESTION: Is it included *Cassia spectabilis*?

   ANSWER:

   In the sentence, it was explained that several other species of genus *Cassia* have been shown to have antimalarial properties (Grace et al., 2012). *Cassia spectabilis* was not included in the four species that have been mentioned. However, based on the similarity of genus between *Cassia spectabilis* and those four species, we suspect that *Cassia spectabilis* also has antimalarial activity.

   Reference:

   - Grace, M. H., Lategan, C., Graziose, R., Smith, P. J., Raskin, I., & Lila, M. A. (2012). Antiplasmodial activity of the ethnobotanical plant *Cassia fistula*. *Natural Product Communications*, *7*(10), 1263–1266. https://doi.org/10.1177/1934578x1200701002

4. Introduction section, paragraph 10, lines: "…both in vitro against *P. falciparum* and in vivo against *P. berghei* infected mice, as well as its heme detoxification inhibitory activity."

   QUESTION: Why did use the species which is different with in vivo test?

   ANSWER:

   Plasmodium species that naturally infect and cause malaria in humans, such as *P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale* and *P. knowlesi*, are essentially unable to infect non-primate animal models (Fidock et al., 2004). Thus, in vivo evaluation of antimalarial

compounds typically begins with the use of rodent malaria parasites, such as *P. berghei*, *P. yoelii*, *P. chabaudi* and *P. vinckei* which have been used extensively in drug discovery and early development (Deharo et al., 2001; Rocha e Silva et al., 2011; Elmi et al., 2021).

Reference:

- Fidock, D. A., Rosenthal, P. J., Croft, S. L., Brun, R., & Nwaka, S. (2004). Antimalarial drug discovery: efficacy models for compound screening. *Nature Reviews. Drug Discovery*, *3*(6), 509–520. https://doi.org/10.1038/nrd1416
- Deharo, E., Bourdy, G., Quenevo, C., Muñoz, V., Ruiz, G., & Sauvain, M. (2001). A search for natural bioactive compounds in Bolivia through a multidisciplinary approach. Part V. Evaluation of the antimalarial activity of plants used by the Tacana Indians. *Journal of Ethnopharmacology*, *77*(1), 91–98. https://doi.org/10.1016/s0378-8741(01)00270-7
- Rocha e Silva, L. F., Silva Pinto, A. C., Pohlit, A. M., Quignard, E. L., Vieira, P. P., Tadei, W. P., Chaves, F. C., Samonek, J. F., Lima, C. A., Costa, M. R., Alecrim, M. d., & Andrade-Neto, V. F. (2011). In vivo and in vitro antimalarial activity of 4-nerolidylcatechol. *Phytotherapy Research: PTR*, *25*(8), 1181–1188. https://doi.org/10.1002/ptr.3424
- Elmi, T., Hajialiani, F., Asadi, M. R., Sadeghi, S., Namazi, M. J., Tabatabaie, F., & Zamani, Z. (2021). Antimalarial effects of the hydroalcoholic extract of *Allium paradoxum* in vitro and in vivo. *Journal of Parasitic Diseases: Official Organ of the Indian Society for Parasitology*, *45*(4), 1055–1064. https://doi.org/10.1007/s12639-021-01359-0

3. ARTIKEL DITERIMA DAN DITERBITKAN

wiwied ekasari <wiwied-e@ff.unair.ac.id>

[Pharmaciana] Editor Decision

1 message

Prof.Dr. apt. Nurkhasanah Mahfudh,M.Si <nurkhasanah@pharm.uad.ac.id> Fri, Jul 1, 2022 at 5:06 AM
Reply-To: "Prof.Dr. Nurkhasanah Mahfudh,M.Si.,Apt" <ibufathan@yahoo.com>
To: "Dr. Wiwied - Ekasari" <wiwied-e@ff.unair.ac.id>

Dr. Wiwied - Ekasari:

We have reached a decision regarding your submission to Pharmaciana, "Standardization of Cassia spectabilis DC leaves and antimalarial activities of ethyl acetate extract".

Our decision is to: GALLEY PROOF

Dear author,
Attached are galley proof and galley revision forms. Would you please design to look at the article, whether by the last edit, the writing of the letters, the address of the author's agency? If there are still things that are not appropriate, please write them in the revision form, we will fix it.
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Prof. Dr.apt. Nurkhasanah, M.Si
Editor in Chief Pharmaciana

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1 message

pharmaciana pharmaciana <pharmaciana@pharm.uad.ac.id> Fri, Jul 1, 2022 at 5:06 AM
To: wiwied-e@ff.unair.ac.id

Dr. Wiwied - Ekasari:

We have reached a decision regarding your submission to Pharmaciana, "Standardization of Cassia spectabilis DC leaves and antimalarial activities of ethyl acetate extract".

Our decision is to: GALLEY PROOF

Dear author,
Attached are galley proof and galley revision forms. Would you please design to look at the article, whether by the last edit, the writing of the letters, the address of the author's agency? If there are still things that are not appropriate, please write them in the revision form, we will fix it. We give a maximum of 2 days from we send this email. If we do not receive a confirmation, we will publish it according to this galley. Thank you

Prof. Dr.apt. Nurkhasanah, M.Si
Editor in Chief Pharmaciana

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