1. PROSES REVIEW

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

Re: [AJID] Editor Decision

1 message

Ezrany Tasiam <ezranytasiam@gmail.com> Fri, May 1, 2020 at 6:14 PM
To: "Associate Prof. Gbola OLAYIWOLA" <gbolaolayiwola@gmail.com>
Cc: "wiwied-e@ff.unair.ac.id" <wiwied-e@ff.unair.ac.id>

Dear Prof. Gbola OLAYIWOLA

I have submitted my revisions titled "In vitro Anti-malarial Activity and Toxicity studies of Johar Leaf (Cassia siamea) from Three Different Locations" to African Journal of Infectious Diseases (AJID) via the online revisions submission system on 04/30/2020.

In the revision of reviewer D on RESULTS and DISCUSSION point 5
"What is the maximum range of CC50 values that are generally considered to be safe for a compound / extract to be used in vivo? Incorporate this in your Discussion"
Sorry in my research, it is not related to in vivo, but I have included discussion of the maximum range of CC50 be safe for a compound / extract to be used in vitro.

I would be grateful if you could let me know whether there has been any further progress on my submission.

Sincerely,

Ezrani Tasiam

Min, 5 Apr 2020 pukul 23.22 Associate Prof. Gbola OLAYIWOLA <gbolaolayiwola@gmail.com> menulis:

> Ezrani Tasiam:
>
> We have reached a decision regarding your submission to African Journal of Infectious Diseases (AJID), "In vitro Anti-malarial Activity and Toxicity studies of Johar Leaf (Cassia siamea) from Three Different Locations".
>
> Our decision is: Revisions Required
> Kindly revise your manuscript as per the reviewers' comments below:
>
> Associate Prof. Gbola OLAYIWOLA
> Department of Clinical Pharmacy, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria.E-mail: gbolaolayiwola@yahoo.com
> Phone +2348037115758
> gbolaolayiwola@gmail.com
>
> Department of Clinical Pharmacy, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria.
>
> ---
> Reviewer B:
> Recommendation: See Comments
> Revision required
> ---
> ---
> Reviewer D:
>
> **INTRODUCTION**
>
> Although geographical location is a factor that affects phytochemical constituents of plants, the effect of height above-sea-level (asl) of the location of a plant may be associated with changes in environmental factors. The authors should beef up this Introduction by citing other studies where height asl has been shown to influence plant constituents.

METHOD

The authors stated that *"The materials used in this study were Johar leaf from Pariaman, Surabaya and Palu".*

Geographical or topographical description of these locations should be provided.

Extraction and Fractionation

(i)How was the leaf dried and powdered?

(ii) Provide more details on the liquid fractionation procedure.

(iii) What weight of extract was obtained from the 10g powdered leaf?

Anti-malaria Activity *in vitro* Test

What is the source of *Plasmodium falciparum* strain *3D7* ?.

Anti-malaria Activity *in vitro* Test

These statements "The first microwells in columns 1 and 2 were filled with the first fraction ethanol extract test solution, while the columns 3 and 4 were filled with the second fraction ethanol extract test solution. The third fraction ethanol extract test solution was filled in two columns found in the second microwell" are not clear and should be made more understandable. How were the fractions of ethanol extract test solutions produced? If they are from the liquid fractionation procedure, this should be made more explicit.

All the data should be treated to appropriate statistical analysis

RESULTS

The Calculation of Inhibition Percentage

The given formula for Inhibition Percentage appears incorrect

Safety and Toxicity *in vitro* Test using *MTT ELISA* Method

This subsection should be recast and significantly edited to raise the quality of reportage.

RESULTS and DISCUSSION

1. In Table 2, the Percentage Inhibition and IC50 results should have Standard Deviation values and subjected to statistical analysis. For example, it is not likely that there is a significant difference between the IC50 values of 0.07 ad 0.09.
2. What are the antimalarial activities of fractions from other solvents so as to justify why the authors concentrated on the ethyl-acetate fraction.
3. There should be statistical treatment of the data to determine whether there are significant differences in the CC_{50} values. Eg, 91.08 from Pariaman plant cannot be significantly different from CC_{50} of 193.62 from Palu.
4. At the paragraph after Table 2, the authors stated that "....*the highest toxicity from Surabaya with CC_{50} 135.81 value, then Pariaman with CC_{50} 191.08, and the most dangerous toxicity was from Palu with CC_{50} 193.62.*" (a) How can extract with a CC_{50} value of 193 be more dangerous than that of 135.1?. (b) The authors need to explain what CC50 stands for.
5. What is the maximum range of CC_{50} values that are generally regarded to be safe for a compound/extract to be used in vivo?. Incorporate this in your Discussion
6. In the 1st column in Table 3, are the three samples from Pariaman?. Also, is the 4th Column in Table 3 for IC_{50} or CC_{50}?
7. How was the Selectivity Index calculated?.
8. Is there a correlation between SI and IC50 values in this study?.
9. Discuss the application of selectivity index values in fulfilling pharmacological effect requirements
10. . Discuss how location of plant regarding its height above-sea level can affect environmental factors and invariable the plant growth, development and phytochemical constituents.

Recommendation: Revisions Required

In vitro Anti-malarial Activity and Toxicity studies of Johar Leaf (Cassia siamea) from Three Different Locations.doc

185K

- **NASKAH REVISI**

In vitro Anti-malarial Activity and Toxicity studies of Johar Leaf (*Cassia siamea*) from Three Different Locations

Ezrani Tasiam, Riesta Primaharinastiti, Wiwied Ekasari*

Faculty of Pharmacy, Universitas Airlangga, Surabaya 60115, Indonesia.

*** Coressponding Author's E-mail : wiwied-e@ff.unair.ac.id**

Abstract

Background: Antimalarial activity of *C.siamea* leaves has been proven by the active compound that has been found, i.e Cassiarin A. It is known that the quantity ~~quality~~ of the content of a compound that has the potential as a raw material for medicine can be influenced by various ~~aspects???~~factors including differences in plant origin. This study aims at comparing the antimalarial activity and toxicity of *C.siamea* leaves from three regions with different meters location ~~valuess of meters~~ above sea level (asl), i.e Pariaman (1,000 m asl), Palu (60 m asl), and Surabaya (2 m asl).

Materials and Methods: This study took *C.siamea* leaves from Pariaman, Surabaya, and Palu that were extracted with n-hexane, and ethanol 90% containing 1% tartaric acid. The antimalarial activity test was conducted with *Plasmodium falciparum 3D7*. The toxicity test was conducted with *MTT ELISA* method.

Results: *C.siamea* leaf with the highest antimalarial activity was obtained from Pariaman with an IC_{50} value of 0.006, then the leaves from Palu had ~~obtained~~ 0.037, while ~~and~~ the lowest antimalarial activity was obtained from Surabaya which gave a value of 0.09. During the toxicity test to obtain CC_{50} value, the highest toxicity was obtained from Surabaya with a CC_{50} value of 135.81, then it was followed by the leaves from Pariaman with CC_{50} of 220.82, and the ~~least~~highest??? toxic was obtained from Palu with CC_{50} of 235.52. ~~Please reconcile this statement for clarity~~

Conclusion: *C. siamea* leaf obtained from Pariaman ha~~ds~~ a selectivity index value that satisfies ~~satisfy~~ the requirements of promising~~decent~~ ~~pharmacological~~ antimalarial effect.

Keywords: Anti-malarial, Toxicity, *Cassia siamea*.

List of Abbreviations: *C.roseus* - *Catharanthus roseus, C.siamea – Cassia siamea,* CC_{50} - Cytotoxicity Concentration, DMEM - Dulbecco's modified eagle medium, DMSO – dimethyl sulfoxide, ED_{50} – Effective Dose, ELISA - Enzyme-linked immunosorbent assay, *Huh7it – devirat human hepatocarcinoma*, IC_{50} – ~~Inhibitor ??? Inhibitory~~ Concentration, LD_{50} - Lethal Dose, m asl – meters above sea level, *MTT - 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide, P.falciparum – Plasmodium falciparum*, RBC – red blood cell, SI – Selectivity Index, *3D7 – plasmodium falciparum strain chloroquine sensitive*

Formatted: Font color: Red

Formatted: Font color: Red, Highlight

Formatted: Font color: Red

Formatted: Font color: Red

INTRODUCTION

These days, mMalaria is one of the deadliest and the most dangerous infectious disease that threaten human lives. To cure and prevent malaria disease, therefore, the government through the Department of Health continuously is striving for an alternative treatment. Malaria is one of the life-threatening diseases. It is commonly transmitted through the bite of an infected female *Anopheles* mosquito. This disease commonly constitutes a threat to the lives of s infants, children, and pregnant females. Once the parasite is released into human bloodstream, it threats human immunity.

Numerous attempts have been performedmade by WHO to prevent the infection of mMalaria disease, such as by taking into account the combination treatment ofwith antimalarial drugs (WHO, 2015). The combination treatment of antimalarial drugs is taken when a study on the resistance patterns in certain areas hasve been administereddetermined through resistance survey procedure. When a particular drug for certain disease has been resistant for more than> 25% of the prevalence, then it is not recommended to be used furthermore. The aims of combination threatment areis to improve the efficacy of antimalarial drugs, through antimalarial synergistic activity, and slowing the progression of parasitic resistance to novel antimalarial drugs. through combination drugs that substantiate mutual interaction (WHO, 2016).

Indonesia is commonly known as the richest country offor natural resources. The one natural resource in this archipelago country includes plants that are beneficial for the needs of human life such as for food, industrial needs, and medicine. One plant that has been known empirically to have benefit as a medicinal plant is *Cassia siamea* (Johar leaf) that is considered to be a traditional antimalarial medicine. (Kindly state the family the plant belongs to)

In spite of its traditional usage, *Cassia Siamea* leaf has been examined to identify the antimalarial activity where *Cassia Siamea* leaf extract and fraction wereas assessed through *in vitro* studies on *P.falciparum* and *in vivo* on mouse infected with *P.berghei*. It indicated that the alkaloid fraction ofn the leaf contains a potential activity. Further, Tthe *in vitro* examination of the fraction through *in vitro* on *Plasmodium falciparum* produceds IC_{50} of 0.24 µg/ml and an on isolate Cassiari A prodeuceds IC_{50} of 0.005 µg/ml. The *in vivo* examination through *in vivo* on in mouse infected with *P.berghei* obtainedrevealed an ED_{50} of 0.47 mg/kg (Ekasari *et all*., 2009).

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

An identical study ~~examination~~ with *Cassia siamea*, and *Cassia spetabilis*, ~~was~~ undertaken ~~through *in vivo*~~ to examine the *in vivo* antimalarial activity of~~n~~ the ethanol extract on mice infected with *Plasmodium berghei* ~~and obtained~~gave an ED_{50} of 150 mg/kg (Ekasari, 2018). Furthermore, isolation of active compounds from *Cassia siamea*, alkaloid compound Cassiari A and B, have been reported (Morita *et all*., 2007).

The quality of the composition of an extract ~~compound~~ which has the potential as a drug raw material can be affected by various aspects including differences in plant origin, the plant body parts tested, the condition of the plant area and the type of solvent used (Khan *et al*., 2011). Factors that cause differences in levels of a compound can be divided into two: internal factors (genetic, ontogenic and morphogenetic) and external or environmental factors which are of ~~distinguishes into~~ two types: biotic factors (stress due to bacteria, viruses, fungi and parasites) and abiotic factors (geographic differences in altitude, growth, climate change, soil type and condition, water availability, mineral content, and stress due to temperature, radiation and chemical compounds) (Verma & Shukla, 2015). Other researchers, such as Hendrison (2001) also showed that differences in altitude lead to different secondary metabolites. Furthermore, a research conducted by Laily (2012) explains that altitude serves as one of the factors that influences the growth of a plant. Hence, it is suspected that the altitude difference affects plant growth and components.

On that account, ~~the efficacy of~~ *in vitro* antimalarial activitiesy of leaf extracts of *Cassia siamea* from three areas with different meters above sea level (m asl), i.e. Pariaman (1000 m asl), Palu (60 m asl), dan Surabaya (2 m asl). ~~through *in vitro* is~~ were investigated ~~conducted~~ on *P.falciparum 3D7* culture and their ~~its~~ safety were evaluated ~~through~~ *in vitro* by applying *MTT ELISA* method, ~~by using *Cassia siamea* leaf from three areas with different meters above sea level (m asl), i.e. Pariaman (1000 m asl), Palu (60 m asl), dan Surabaya (2 m asl).~~ From the results of antimalarial activity testing, ~~it was obtained~~ the IC_{50} and ~~it was obtained~~ CC_{50} ~~for~~ values were determined and from which ~~toxicity. Thus, it is able to obtain~~ the ~~value of~~ index selectivity was obtained.

MATERIALS AND METHODS

This study used *Cassia siamea* leaf (known as Johar leaf) from Pariaman, West Sumatera, Indonesia which is located 1000 meters above sea level with a wet tropical climate, an annual rainfinfall average of 368 mm, an average temperature of 26.8^{0}C, and an average humidity of

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font: Italic

Formatted: Font color: Auto, Not Highlight

Formatted: Font color: Auto, Not Highlight

Formatted: Font color: Auto

84.4% (LKIPK Pariaman, 2017). The second place was Palu, Central Sulawesi, Indonesia which is located 60 meters above sea level with a dry climate, an annual rainfall average of 760 mm, an average temperature of 27.7^{0}C, and an average humidity of 76-80% (BPT, 2007) and Surabaya, East Java, Indonesia which is located 2 (two) meters above sea level, an annual rainfall average of 172 mm, an average temperature of 30°C, and an average humidity of 68% -84% (RPJMD Surabaya, 2015). The plant was identified by Anshari Maruzi from Systematic Laboratory, with a voucher specimen number of YK.01.03/2/2861/2019 and deposited in the Herbarium Tawangmanguense at Medicinal Plant and Traditional Medicine Research and Development Centre, Ministry of Health RI.

Extraction and Fractionation

C.siamea leaves were obtained from Pariaman, Palu and dan Surabaya. The leaves were dried at the room temperature. Then, the leaves and were ground into powder, and starch content. Ten grams of *Cassia siamea* leaf powder was macerated consecutively using n-hexane solvent 3 times and ethanol 90% containing 1% tartaric acid three times and evaporated at 40°C using Rotary evaporator to obtain the *y Evaporator* to obtain crude ethanol extract (Pariaman 1.6 g, Palu 1.8 g and Surabaya 1.1 g). The thick ethanol extract was dissolvedwas diluted with distilled water and used for liquid fractionation using 3: 1 ethyl acetate three times fromrom which we obtained crude fraction of ethyl acetate was obtained as follows: Pariaman was 89.5 mg, Palu was 82.5 mg and Surabaya was 89.9 mg. (State the% yield of each of the fractions)

Formatted: Font: Not Italic

Anti-malarial Activity *in vitro* Test

Plasmodium falciparum strain 3D7 (chloroquine-sensitive) was obtained from Institute of Tropical Disease, Universitas Airlangga, Surabaya, Indonesia. The initial parasitemia levels in each well during the experiment of antimalarial activity *in vitro* were 1% parasitemia and 5% hematocrit. In Oone plate, it consisted of 24 wells (Trager and Jensen, 1976). The entire suspensions in Petri (5 mL) were fitted with sterile falcon tubes with 15 ml of caps and then it was centrifuged 1500 rpm for five minutes. A total of 4.5 ml of supernatant was removed hence it was estimated that there were around 5% of red blood cells infected with parasites and approximately 50% of hematocrit with a total amount of 500 µL.

Commented [u2]: This sentence is not clear

The parasite cell suspension was made, thus it obtained 1% of the content of parasitemia and hematocrit reached to 10%, by adding 50% of RBC solution to the tune of 2000 µL into the

test tube, then added the complete solution media counting for 10 ml. In addition, the suspension was mixed cautiously using a micropipette until it was well blended. Before being put into a microwell, a thin blood smear was made, the initial parasitemia level was at 0 hour before being given test substances. The amount of 500 µL of parasitic cell suspensions were introduced ~~inserted~~ into each well containing 500 µL of the test solution. Serial concentrations of the crude fraction of ethyl acetate that were tested for antimalarial activity were 100; 10; 1.0; 0.1; and 0.01 µg/mL. Antimalarial assay was done in a 24 microwell plates with 1% initial and experimental parasitemia (1 mL/well of suspension), then it was placed ~~inserted~~ into the candle jar and incubated in a CO_2 incubator at 37°C for ~~24 and~~ 48 hours. Then, a thin blood smear in a glass slide was made, fixated in methanol, and stained with Giemsa 10% for 10 minutes.

The number of parasitemia was observed under a microscope based on 3000~~'s~~ erythrocytes with 1000 times magnification. ~~Furthermore,~~ Tthe number of parasites at 48 hours incubation were compared. Then, the percentages of parasite growth when ~~by~~ compared with the negative control were calculated. Afterwards, fifty percent inhibitory concentration (IC_{50}) of each extract was determined to identify the antimalarial activity. IC_{50} was defined as the concentration of the compound causing 50% inhibition of parasite growth relative to untreated control.

The Calculation of Inhibition Percentage

$$\text{Inhibition Percentage} = 100\% - \left(\frac{Xu}{Xk}\right) \times 100\%$$

Annotation:
Xu = growth percentage of test solutions
Xk = growth percentage in negative control

IC_{50} Calculation:

IC_{50} is a concentration of the~~ed~~ sample that is able to deter the growth of parasites up to 50%. The calculation of IC_{50} was performed by using Probit analysis (*unit probability*) by making a correlation curve between Probit percent inhibition and logarithm of sample concentration using linear regression of linear equation. Table 1 shows how to categorize the antimalarial activity of plant extract based on theit IC_{50} values.

Table 1. Categorization of plant extract activity to *Plasmodium falciparum* (Kamaraj, 2012)

IC_{50} value (µg/ml)	**Category**
< 10	Promising

Commented [3]: This statement should be made clearer by an English speaking expert.Not communicating

Formatted: Subscript

10- 20	Moderate
20 – 40	Good
40- 70	Marginally Potent
>70	Poor

Toxicity *in vitro* Test using *MTT ELISA* Method

Huh7it tissue culture was grown on the media of DMEM in 96 well microplates with the density of 2,.3 x 10^4 cell/well up to 100 µl/well, the incubation was in~~of~~ CO_2 5%, at 37°C for 24 hours and tissue culture *Huh7it* would accrue and stick to the base of well microplate. The ethyl acetate fraction of *Cassia siamea* leaf ~~starch~~ was diluted with DMSO to achieve a ~~make the~~ concentration of 10 µg/ml. The ~~media of~~ tissue culture *Huh7it* media were removed, and~~then~~ ~~the~~ 100 µl of the leaf extract fraction ~~(Duplo)~~ was added to the ~~tissue culture *Huh7it* to~~ 96 well microplate, and ~~the~~ incubated ~~ion of~~ in CO_2 5% ~~atwas at~~ 37°C for 48 hours. After this~~In~~ ~~the~~ second ~~last~~ incubation, the medium was discarded, and ~~it added~~ 150 µl of *MTT 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide* (10%) solutions was added, followed by ~~the~~ incubation in~~of~~ CO_2 5% ~~was~~ at 37°C for 4 hours. After thi~~s~~e incubation, the media were removed and 100 µl of DMSO was added ~~100 µl of DMSO (as~~ stop solution~~)~~, then mixed~~shaked~~ for one minute. The living cell would interact with *MTT* and changed into purple. The ~~size of~~ absorbance was measured ~~was~~ at wavelength (λ) of 650 nm and 720 nm with *ELISA* reader, using ~~as a negative control~~ cell culture *Huh7*it as negative control ~~was used~~. Then, ~~it calculated~~ the percentage of cell viability was calculated from which ~~at~~ CC_{50} value was determined.

Can you translate? | % cell viability = $\frac{Optical\ Density\ Sel\ Perlakuan}{Optical\ Density\ Sel\ Kontrol}$ x 100%

Data Analysis

IC_{50} values for~~in~~ antimalarial efficacy ~~testing~~ and CC_{50} for ~~in~~ toxicity testing were obtained using Probit Analysis. To determine ~~observe~~ a significant difference in the antimalarial effects ~~testing~~ and the toxicity of *Cassia siamea* leave extracts~~s~~ from Pariaman, Palu and Surabaya, an ANOVA procedure was used.

Formatted: Superscript

Formatted: Highlight

Formatted: Highlight

Commented [u4]: This Equation is not Clear. Which language is it?

Formatted: Highlight

Commented [u5]: The authors should indicate how CC_{30} was calculated. Also how was Selectivity Index calculated?

Formatted: Font: Italic

RESULTS AND DISCUSSION (Please seperate results and discussion in line with the Journal format)

The Activity of Antimalarial Ethyl Acetate Fraction from *Cassia Siamea*

The ethyl acetate fractions of *Cassia Siamea* from Pariaman, Palu, and Surabaya were used in studying their testing of in vitro antimalarial activitiesy. through *in vitro* on *Plasmodium falciparum 3D7* was obtained from ethyl acetate fraction of *Cassia Siamea* from Pariaman, Palu, and Surabaya. The ethyl acetate fraction was used in this study in accordance with the previous research conducted by Morita et al., (2007) and Ekasari (2009) which used ethyl acetate fraction on isolation for extraction of active compounds from *C.siamea*, (i.e. alkaloid Cassiari A and B); and study conducted by Ekasari (2009) which took *C.siamea* leaf isolation fractioned with ethyl acetate. Those studies reported an effective result to inhibition of malarial parasites by the plant extracts.

The data on percentage of inhibition of antimalarial activity test towards *Plasmodium falciparum* as shown in Table 2 were analyzed using the probit analysis. Hence, it obtained Tthe highest IC_{50} value of the *Cassia siamea* Ethyl Acetate Faction was from Pariaman with a value ofwas 0.006, Palu was 0.037, and plants from Surabaya showed the lowest antimalarial activity that was 0.09. According to the Thus, from results of the test of antimalarial activitiesy of from the ethyl acetate fraction of Johar leaf (*C. siamea*) taken from Pariaman, Palu, and Surabaya, it is apparent concludes that different locations resulted in have different IC_{50} values for the plant extractsof antimalarial activities. These differences are further due to differences in the metabolite profile and compound constituents differences (Hendrison, 2001). Therefore, it is suggested suspected that there are differences in the levels of the active compounds in *C.siamea* in responsible for antimalarial activity.

Table 2. The Inhibition Mean and IC_{50} Percentage in each Fraction on *P.falciparum3D7*

Sample	Consentration (µg/mL)	Inhibition Percentage (%)	IC_{50} (µg/mL)
Pariaman	100	98.03	0.006±16.84
	10	88.02	
	1	83.48	
	0.1	78.09	
	0.01	52.99	
Palu	100	88.99	0.037±20.94
	10	80.36	

	1	77.52	
	0.1	64.21	
	0.01	35.38	
Surabaya	100	82.59	0.09±21.37
	10	73.54	
	1	71.29	
	0.1	56.98	
	0.01	27.95	

IC: Inhibitory Concentration

~~The significance value obtained in testing with the ethyl acetate fraction was (p <0.05). It signifies that t~~There is a significant difference ($p < 0.05$) in the IC_{50} values of extracts from the three locations. ~~inhibition percentage among the three samples~~.

Formatted: Subscript

Toxicity of the Ethy~~lia~~l Acetate *Cassia siamea* fraction:

The principle of the *MTT* method is the reduction of *MTT* tetrazolium yellow salt by a reductase system. Tetrazolium succinate, ~~which is included~~ when incorporated in~~to~~ the respiration chain in the mitochondria of living cells becomes reduced to form~~sing~~ purple formazan crystals which~~ and it is~~ are insoluble in water. The addition of the stopper reagent dissolves this colored crystal and then the absorbance is measured using an *ELISA* reader. The intensity of the purple color that is formed is directly proportional to the number of living cells. Hence, if the intensity of the purple color is greater, the number of living cells will increase (Mossman, 1983).

The conversion would only be possible if only the extracts tested do not kill the cells. This ability of the cells is connected to the plant extracts compound that protects the cells on cell-mediated damage. The result from *MTT* assay suggests that the plant extracts have intrinsic biological components that enable them to protect the cells on the damage mediated by *P. falciparum*. Selectivity index (SI) is used to ~~To~~ estimate the potential of molecules or extracts to inhibit parasite growth without toxicity.~~, it is shown by the selectivity index (SI).~~ SI is defined as the ratio of the cytotoxicity on the human cells to the antimalarial activity (Pouplin *et al.*, 2007). Low SI indicates that the antimalaria activity is presumably due to cytotoxic activity of the compound or extract ~~and it is bigger than antimalarial activity~~ towards the parasite themselves. Meanwhile, higher SI value offers the potential of safer therapy. If the CC_{50} > 90 µg / ml, the compound is classified as not cytotoxic, if the CC_{50} is between 2 and 89 µg / ml, the compound

Commented [u6]: Not clear.

Formatted: Font: Italic

is classified as moderately cytotoxic, while If the CC_{50} < 2 µg / ml, the compound is classified as cytotoxic. (Lima, 2015).

In vitro test of toxicity was conducted by utilizing the *MTT-ELISA* method from the ethyl acetate fraction of Johar leaf (*C. siamea*) taken from Pariaman, Palu, and Surabaya. The viability percentage data of *MTT-ELISA* toxicity test as shown in Table 3 was analyzed by using the probit program. The ~~highest~~ ethyl acetate fraction of *Cassia Siamea* ~~was~~ from Surabaya had the highest toxicity with a ~~with~~ CC_{50} value of 135.81, then followed by the leaves from Pariaman with CC_{50} value of 220.82, and the least toxicity was from Palu with CC_{50} value of 235.52.

From ~~According~~ ~~to~~ the results of the toxicity test on *Cassia siamea* ethyl acetate fraction taken from Pariaman, Palu, and Surabaya, it can be asserted~~summarized~~ that the leaves from different location of plants ~~leaves obtained a~~ had different CC_{50}. Therefore, ~~it is further considered that~~ there are other compounds with ~~have~~ toxic effects ~~o~~in the ~~Johar~~ leaves of ~~(~~*C.siamea*~~)~~. Qualitative analysis of *C.siamea* ~~was~~ had been done for various classes of compounds including alkaloids, tannins, saponins, chromone, flavonoids, carbohydrates, proteins, steroids, terpenoids, cardiac glycosides and phlobatannins (Lee, ~~D.S.~~2001., Samia.1978., Usha, V.2011). It is possible that ~~t~~These toxic effects may ~~can~~ be caused by saponins, glycosides, al~~k~~ealoids such as barakol, inter quinones, and tannins (Wiam, 2005). Barakol, a constituent of *C.siamea,* ~~has~~ is the most toxic constituent of ~~effect on~~ *C.siamea*. In ~~Through~~ ~~i~~*In vivo* studies, barakol shows acute and sub-acute hepatotoxic effects with LD_{50} of 2330 mg/kg in Wistar rats (Pumpaiscalai, 2001), and the effects of subchronic toxicity on blood cells in rats fed with a high diet and high cholesterol (Maniratancote 2001). Barakol results in acute toxicity and death in mouse with LD_{50} of 324.09 mg/kg through intraperitoneal injection. Barakol can also interfere with liver function and causes increase in bilirubin in mouse, particularly at a dose of 240 mg/kg. (But you did not isolate barakol, what is the mechanism of the isolate you got fromthe extract?)

Table 3. Percentage of Viability and CC_{50} with The *MTT-ELISA* Method

Sample	Concentration (µg/mL)	Percentage of viability (%)	CC_{50} (µg/mL)
Pariaman	400	9.91	220.82
	200	60.89	
	100	81.82	
	10	96.13	
	1	99.87	

Formatted: Font: Italic

Formatted: Highlight

	0.1	100	
Palu	400	3.35	235.52
	200	55.97	
	100	90.35	
	10	100	
	1	100	
	0.1	100	
Surabaya	400	1.31	135.81
	200	22.57	
	100	78.81	
	10	100	
	1	100	
	0.1	100	

CC: Cytotoxicity Concentration

Significant value obtained during the test with the weight fraction equation was ($p > 0.05$). It further means that there is no significant difference at viability percentage among the three samples.

(How many replicate experiments per concentration did you perform? There should have been SEM or standard deviation!)

Selectivity Index

According to the criteria of antimalarial activity, the extract that ~~obtained~~gave IC_{50}>100 µg/mL was considered inactive. The extract ~~obtaining~~that gave IC_{50}<100 µg/mL can be continued as potential antimalarial. Active extract ~~obtaining~~yielded IC_{50}<10 µg/mL should be selected for the next purification based on bioassay-guided ~~d~~isolation. *In vitro* screening to determine~~observe~~ *P. falciparum* inhibition activity of ~~the~~ plant extracts with IC_{50} value less than 10 µg/mL ~~is was~~ a major phase in the development of new antimalarial drugs. To obtain risk-free antimalarial treatment, ~~it is continued with~~ cytotoxicity assay is carried out to obtain ~~yield~~ SI value. Based on SI value as parameter of pharmacologicaly effect, the extract with SI<4 can be classified as marginally active, SI 4-10 classified as partially active, and active SI>10 classified as antimalarial. Accordingly, as indicated in Table 4, the ~~entire~~ leafve extracts from the three locations tested had satisfactory selectivity index (>10), indicating satisfactory therapeutic potentials. The presence of bioactive compounds indicates a great deal of therapeutic uses possibility (Weniger, 2001).

~~Derived from the IC_{50} value in the antimalarial activity test and the CC_{50} value in the toxicity test,~~ tThe selectivity index value is obtained as follows.

Commented [u7]: The CC50 values should have SD values to enable determination of significant differences between the samples

Formatted: Highlight

Formatted: Font: Italic

$$SI = CC_{50} / IC_{50}$$

Table 4. ~~Index~~ Antimalaria Selectivity Index Values of plant extracts from the three locations.~~Antimalaria Activity and Toxicity~~

Sample	IC_{50}	CC_{50}	SI
Pariaman	0.006	220.82	36803
Palu	0.037	235.52	6365
Surabaya	0.09	135.81	1509

IC: Inhibitory Concentration
CC: Cytotoxicity Concentration
SI: Selectivity Index

~~Index~~ Sselectivity Index value plays a role as a parameter to determine whether or not an extract with antimalarial activity is risk-free~~, that has antimalarial activity toward the use of its toxicity.~~ The value of ~~meaning~~ of ~~the~~ Sselectivity Index~~of this index constitutes~~ of an extract that has antimalarial activity is applied in determining whether the extract can ~~without affecting normal cells and is able to~~ be developed ~~used~~ further as an antimalarial for pharmacological usage.

~~An~~ Aaltitude generate~~d~~s changes in temperature and climate conditions. The study of metabolite profiles of *C. roseus* from diverse regions with different altitudes resulted in differences in the metabolite content of phenolic compounds; resulting in differences of antioxidant activity. This further signifies that geographical conditions affect metabolites (Verma & Shukla, 2015). ~~Other research conducted~~ A study by Figueiredo (2008) reports that the secondary metabolite content in plants is influenced by a great deal of factors, including genetic, environmental (biotic and abiotic) factors, physiological conditions, geographical variations and, evolution.

Differences in location ~~of plant growth~~ based on altitude and geographical conditions are expected to affect plant growth and development. As a result, the process of metabolism in these plants will also be disrupted, hence, ~~accordingly~~ the compounds produced ~~from the process~~ will be different at each altitude.

Metabolites are classified into two: primary and secondary metabolites. Primary metabolites that are formed in limited quantities constitute important factors for the growth and life of organism. Secondary metabolites are not used by plants for growth and are generated

Formatted: Indent: First line: 0 cm

more when plants are in stressful conditions, as stated by Dicosmo (1984). Secondary metabolite compounds are chemical compounds that generally have ~~the ability to~~ bioactivity and can also function as a protectionve to plants from pests and diseases ~~for the plant itself~~ or from the environment.

The production of secondary metabolites is certainly affected by several factors, as reported by Dicosmo (1984) who stateds that light, pH, aeration and microorganisms affect the production of secondary metabolite compounds. Hence, in ~~every~~ different altitudes where the height of the place also affects the ambient temperature, ~~it will affect~~ the biochemical processes taking place ~~contained~~ in the plants are affected .

From the results of the present study,~~According to the results in testing the antimalarial activity and *in vitro* toxicity of ethyl acetate fraction *C.siamea*, it confirm that~~ the differences in ~~of growing areas, with~~ in altitude (above sea level) and geographical locations~~differences, it~~ yielded different ~~results of~~ antimalarial activitiesy and toxicity profiles. ~~This further signifies that location of growth and geographical conditions of *C.siamea* plants affect antimalarial activity and toxicity.~~

The ethyl acetate fraction of *C.siamea* leaf has the potential as an antimalarial drug raw material which obtains a sufficiently appropriate activity value in the *in vitro* testing of *Plasmodium falciparum* and obtains a satisfactory level of safety in the *in vitro* toxicity testing using the *MTT-ELISA* method, where the ethyl acetate fraction leaves from Pariaman acquires selectivity index values fulfilling the pharmacological effect requirements. Antimalarial activity is not in line with toxicity. The ethyl acetate fraction of *C.siamea* from Pariaman acquired a high activity, whereas the ethyl acetate fraction of *C.siamea* from Surabaya yielded the highest toxicity content. This confirms that the active compound of *C.siamea* which plays a role in antimalarial activity has no correlation with the effect of toxicity. The further research is required to determine the degree of active compounds from the ethyl acetate fraction of *C.siamea* from various regions to acquire the highest level as a raw material for antimalarial treatment.

Formatted: Indent: First line: 0 cm

Commented [u8]: This should be re-written for ease of comprehension.

CONCLUSION

This study reports that the Johar leaves (*Cassia siamea*) obtained from Pariaman yielded satisfactory ~~satisfying~~ selectivity index value~~s~~ which meets the requirements for further evalauation for ~~of first-rate~~ pharmacological effects. Accordingly, the results indicate that the leaves can be developed as a raw material for antimalarial treatment. This study further affirms that the location of *Cassia siamea* ~~of plant~~ in terms of its altitude ~~growth~~ affects the antimalarial activity and toxicity of t~~he~~ ~~*Cassia siamea*~~ ethyl acetate extracts of the leaves ~~.fraction.~~

Conflict of Interest The authors hereby declared that we do not acquire a conflict of interest

Acknowledgement +

The authors would like to thank National Institute of Health Research and Development, Ministry of Health (Balitbangkes Kemenkes) of the Republic of Indonesia for providing the financial support.

References **(Please follow the Journal format for the references)**

1. BPOM RI(first letter capital please), (2005). Peraturan Kepala Badan Pengawasan Obat dan Makanan Republik Indonesia Nomor: HK.00.05.41.1384 tentang Kriteriadan Tata Laksana Pendaftaran Obat Tradisional, Obat Herbal Terstandar dan Fitofarmaka, Badan Pengawasan Obat dan Makanan Republik Indonesia, Jakarta.
2. BPT Palu, (2007). Teknologi Pemupukan Spesifik Lokasi dan konservasi Tanah kota Palu, Balai Penelitian Tanah, Palu.
3. Ekasari W, Tutik Sri Wahyuni, Heny Arwaty, Nindya T Putri, (2018). Determination Of Effective Dose Of Antimalarial From *Cassia Spetabilis* Leaf Ethanol Extract In *Plasmodium Berghei*-Infected Mice. African Journal of Infectious Diseases (2018) ; 12(S): 110-115
4. Dicosmo, F, and Tower, G.H.N, (1984). Stress and Seconddary Metabolism inCulture Plant Cell In Phytochemical Adaption to Stress. Plenum Publishing Co. Toronto. Pp 15-50.
5. Ekasari W, Aty W, Noor C, Din S, Toshio H, Hiroshi Morita, (2009). Antimalaria Activity of Cassiarin A from the Leaves Of *Cassia Siamea*. Heterocycles, Vol 78, No7.
6. EMEA, (2016).Guideline on Quality of Herbal Medicinal Products/Traditional Herbal Medicine Products.
7. Figueiredo A. C., Barroso J. G., Pedro, L. G., Scheffer, J. J. C., (2008). Factors Affecting Secondary Metabolite Production in Plants: Volatile Components and essensial Oils. Flavour and Fragrance Journal. 23(4):213-226.
8. Hendrison, M, (2001). The effect of nitrogen fertilization on soil pH is different on yields and levels of alkaloid seeds of kelabet (Trigonella foenum graecum L.) in two locations with different heights and growing seasons. Doctoral Program Dissertation. Bandung: ITB Postgraduate Program.

9. Hiroshi Morita, ShioriOshimi, Yusuke Hirasawa, Koichiro Koyama, Toshio Honda, WiwiedEkasari, GunawanIndrayanto& Noor CholiesZaini, (2007). Cassiarin A and B, Novel Antiplasmodial Alkaloids from *Cassia Siamea*. Vol 9: 3691-3693
10. Kamaraj, N. K. Kaushik, D. Mohanakrishnan et al., (2012). "Antiplasmodial potential of medicinal plant extracts from Malaiyur and Javadhu hills of South India," Journal of Parasitology Research, vol. 111, no. 2, pp. 703–715.
11. Khan S, Singla RK, Abidin MZ, (2011). Assessment of phytochemical diversity in Phyllanthusamarus using HPTLC fingerprints.Indo-global J. Pharm. Sci;1(1):1-12
12. Laily AN, Suranto, Sugiyarto, (2012). Characteristices of Carica pubescens of Dieng Plateau Central Java according to its morphology, antioxidant and protein pattern. Nusantara Bioscience 4 No. 1, halaman 16- 21.
13. Lee, D.S., Lee, S.H & Genistein, (2001). *A Story isoflavone is a potent α-Glucosidase inhibitor*. FEBS Lett : 501:84-86.
14. Lima RBS, Silva LFR, Melo MRS, (2015). In vitro and in vivo antimalarial activity of plants from the Brazilian Amazon. Malaria Journal.;14:508.
15. LKIPK Pariaman, (2017). Laporan Kinerja Instansi Pemerintah Kota Pariaman, Sumatra Barat, Indonesia.
16. Maniratanachote R., Kijsanayotin P., Phivthong-ngam L., Thongsaard W., Niwattisaiwong N., and Lawanprasert S, (2002). Subchronic effects of barakol on blood clinical biochemistry parameters in rats fed with normal and high cholesterol diets. Thai Journal of Pharmacology; 24: 101-111
17. Mossman T, (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assay. Journal of Immunological Methods. 1983;65:55-63.
18. Pouplin JN, Tran H, Tran H, Phan TA, Dolecek C, Farrar J, Tran TH, Caron P, Bodo P, Grellier P, (2007). Antimalarial and cytotoxicity activities of ethnopharmacologically selected medicinal plants from South Vietnam. Journal of Ethnopharmacology.;109:417-427.
19. Pumpaisalchai W.,Kaewvichit S., Siriaunkgul S., Taesothikul T., Niwatananun W., and Sanichwankul K, (2001). Toxicity of barakol: hepatotoxicity and subacute toxicity. International Society for Horticultural Science; 170: 15-18
20. RPJMD Surabaya, (2015). Rencana Pembangunan Jangka Menengah Daerah, Surabaya, Jawa Timur. Indonesia
21. Samia,et al., (1984). New Isoquinone Alkaloids from Leaves of Cassia Siamea. Journal of Natural Product, Vol 47, No. 4 : 708-718
22. Trager,W., Jensen, J.B, (1976). "Human malaria parasites in continuous culture". Science 193, 673–675.
23. Usha, V., & A. K. Bopaiah, (2011). Preliminary phyto-chemical evaluation of the leaf extract of five Cassia Species Chem. Pharm. Res. 3(5):574-583 574
24. Verma, N., Shukla, S., (2015). Impact of Various Factors Responsible for Fluctuations in Plant Secondary Metabolite. J App Res on Med Ar Plants 2(4):105-113
25. World Health Organization, (2015). Guidelines for The Treatment of Malaria. 3rded, Geneva: WHO Press
26. World Health Organization, (2016). World Malaria Report. Geneva: WHO Press
27. Wiam I.M., Jacks T.W., and Zongoma Y.A, (2005). Acute toxicityand phytochemical studies of *Cassia siamea*extracts in rats. Pakistan Journal of Biological Sciences; 8: 586-588.

28. Weniger B, Robledo S, Arango GJ, Deharo E, Aragón R, Muñoz V, Callapa J, Lobstein A, Anton R. (2001) Antiprotozoal activities of Colombian plants. *Journal of Ethnopharmacology*, *78,* 193-20

[AJID] Editor Decision

2 messages

Associate Prof. Gbola OLAYIWOLA <gbolaolayiwola@gmail.com> Thu, May 7, 2020 at 7:20 PM To: Ezrani Tasiam <ezranytasiam@gmail.com>, Riesta Primaharinastiti <r.nastiti@gmail.com>, "Wiwied Ekasari, Dr." <wiwied-e@ff.unair.ac.id>

Ezrani Tasiam, Riesta Primaharinastiti, Wiwied Ekasari, Dr.:

We have reached a decision regarding your submission to African Journal of Infectious Diseases (AJID), "In vitro Anti-malarial Activity and Toxicity studies of Johar Leaf (Cassia siamea) from Three Different Locations".

Our decision is: Revisions Required.

Kindly re-revise your manuscript as follows:

1. Note that the abstract section should contain the following four sub-headings: Background, Materials and Methods, Results, Conclusion. The "abstract" section of your revised manuscript did not contain the sub-heading "Materials and Methods". Please incorporate this sub-heading appropriately, bearing in mind that the entire abstract section should not contain more than 250 words.

2. State clearly the voucher number of the plant studied in the appropriate place of the manuscript. Please note that voucher numbers cannot be obtained from the Ministry of Health.

Thank you.

Associate Prof. Gbola OLAYIWOLA
Department of Clinical Pharmacy, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria.E-mail: gbolaolayiwola@yahoo.com
Phone +2348037115758
gbolaolayiwola@gmail.com

Department of Clinical Pharmacy, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria.

wiwied ekasari <wiwied-e@ff.unair.ac.id> Sat, May 9, 2020 at 10:38 AM
To: "Associate Prof. Gbola OLAYIWOLA" <gbolaolayiwola@gmail.com>

Dear Associate Prof. Gbola OLAYIWOLA
Department of Clinical Pharmacy
Faculty of Pharmacy
Obafemi Awolowo University
Ile-Ife, Nigeria

Thank you for your email. Yes, we will re-revise our manuscript as your suggestion soon.

Best regards,

Dr. Wiwied Ekasari, MSi Apt

[Quoted text hidden]

Re: Revised manuscrip

2 messages

Clement Adewunmi <athmsi2012@gmail.com> Thu, May 7, 2020 at 7:24 PM To: Ezrani Tasiam <ezranytasiam@gmail.com>, Riesta Primaharinastiti <r.nastiti@gmail.com>, "Wiwied Ekasari, Dr." <wiwied-e@ff.unair.ac.id>

Dear Dr. Ekasari,

We have reached a decision regarding your submission to African Journal of Infectious Diseases (AJID), "In vitro Anti-malarial Activity and Toxicity studies of Johar Leaf (Cassia siamea) from Three Different Locations".

Our decision is: Revisions Required.

Kindly re-revise your manuscript as follows:

1. Note that the abstract section should contain the following four sub-headings: Background, Materials and Methods, Results, Conclusion. The "abstract" section of your revised manuscript did not contain the sub-heading "Materials and Methods". Please incorporate this sub-heading appropriately, bearing in mind that the entire abstract section should not contain more than 250 words.

2. State clearly the voucher number of the plant studied in the appropriate place of the manuscript. Please note that voucher numbers cannot be obtained from the Ministry of Health but from Department of Botany and or Plant Taxonomy.

Thank you.

Associate Prof. Gbola OLAYIWOLA

--

COJA House,
7, Road 1, Otunmaiye Square, Ajebamidele,
P. O. Box 2586, Obafemi Awolowo University
Post Office, Ile-Ife, Osun State, Nigeria.
Website: www.athmsi.org
E-mail: athmsi2012@gmail.com, cadewumi@yahoo.com
+2348026522022

wiwied ekasari <wiwied-e@ff.unair.ac.id> Sat, May 9, 2020 at 10:34 AM
To: Clement Adewunmi <athmsi2012@gmail.com>

Dear Mr. Clement Adewunmi
Associate Prof. Gbola OLAYIWOLA

Thank you for your email. Yes, we will re-revise our manuscript as your suggestion soon.

Best regards,

Dr. Wiwied Ekasari

[Quoted text hidden]

Re: Revision

2 messages

Clement Adewunmi <athmsi2012@gmail.com> Tue, May 12, 2020 at 6:52 PM To: Ezrany Tasiam <ezranytasiam@gmail.com>, Wiwied ekasari <wiwiedeka@hotmail.com>, "Wiwied Ekasari, Dr." <wiwied-e@ff.unair.ac.id>

Dear Authors,

We advise you to do a thorough correction of the English text by asking a native English language expert.

Thank you.

On Sat, May 9, 2020 at 5:33 AM Ezrany Tasiam <ezranytasiam@gmail.com> wrote:

> Dear prof. Clement Adewunmi,
>
> I have submitted my revisions titled "In vitro Anti-malarial Activity and Toxicity studies of Johar Leaf (Cassia siamea) from Three Different Locations" to African Journal of Infectious Diseases (AJID) via the online revisions submission system on 05/09/2020.
>
> Sincerely,
>
> Ezrani Tasiam

--

COJA House,
7, Road 1, Otunmaiye Square, Ajebamidele,
P. O. Box 2586, Obafemi Awolowo University
Post Office, Ile-Ife, Osun State, Nigeria.
Website: www.athmsi.org
E-mail: athmsi2012@gmail.com, cadewumi@yahoo.com
+2348026522022

5539In vitro Anti-malarial Activity and Toxicity studies of Johar Leaf (Cassia siamea) from Three Different Locations final revisions-1.doc
186K

wiwied ekasari <wiwied-e@ff.unair.ac.id> Fri, May 15, 2020 at 11:21 AM
To: Clement Adewunmi <athmsi2012@gmail.com>

Dear prof. Clement Adewunmi,

Thank you for your email. Yes, we will immediately ask a native English language expert for correction of the our manuscript English text.

Best regards,

Dr. Wiwied Ekasari, MSi, Apt

[Quoted text hidden]

[AJID] Editor Decision

1 message

Associate Prof. Gbola OLAYIWOLA <gbolaolayiwola@gmail.com> Thu, May 21, 2020 at 4:17 PM To: Ezrani Tasiam <ezranytasiam@gmail.com>, Riesta Primaharinastiti <r.nastiti@gmail.com>, "Wiwied Ekasari, Dr." <wiwied-e@ff.unair.ac.id>

Ezrani Tasiam, Riesta Primaharinastiti, Wiwied Ekasari, Dr.:

We have reached a decision regarding your submission to African Journal of Infectious Diseases (AJID), "In vitro Anti-malarial Activity and Toxicity studies of Johar Leaf (Cassia siamea) from Three Different Locations".

Our decision is: Revisions Required.

We are not convinced that the person who helped you with the writing is a professional English language writer who could give you a certificate for correcting the manuscript. The current revision is not better than the previous.

You did not state a point by point revision of the questions raised by reviewers. Kindly do it carefully answering those questions raised so that anybody reading the work can understand it. The most important aspect is communicating your workto readers and researchers.

Associate Prof. Gbola OLAYIWOLA
Department of Clinical Pharmacy, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria.E-mail: gbolaolayiwola@yahoo.com
Phone +2348037115758
gbolaolayiwola@gmail.com

Department of Clinical Pharmacy, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria.

B-5539-Article Text-18675-1-4-20191115.docx
54K

Re: Revision

1 message

Clement Adewunmi <athmsi2012@gmail.com> Fri, May 22, 2020 at 5:10 PM To: Ezrany Tasiam <ezranytasiam@gmail.com>, "Wiwied Ekasari, Dr." <wiwied-e@ff.unair.ac.id>, "Prof Cyprian O. Onyeji" <cyprian.onyeji@unn.edu.ng>

Dear Authors,

Kindly do your final revision with the attached.

On Thu, May 21, 2020 at 10:19 AM Clement Adewunmi <athmsi2012@gmail.com> wrote:

> Dear Authors,
>
> We have reached a decision regarding your submission to African Journal of Infectious Diseases (AJID), "In vitro Anti-malarial Activity and Toxicity studies of Johar Leaf (Cassia siamea) from Three Different Locations".
>
> Our decision is: Revisions Required.
>
> We are not convinced that the person who helped you with the writing is a professional English language writer who could give you a certificate for correcting the manuscript. The current revision is not better than the previous.
>
> You did not state a point by point revision of the questions raised by reviewers. Kindly do it carefully answering those questions raised so that anybody reading the work can understand it. The most important aspect is communicating your workto readers and researchers.
>
> On Wed, May 20, 2020 at 8:59 AM Ezrany Tasiam <ezranytasiam@gmail.com> wrote:
>
> > Dear prof. Clement Adewunmi,
> >
> > I have submitted my revision titled "In vitro Anti-malarial Activity and Toxicity studies of Johar Leaf (Cassia siamea) from Three Different Locations" to African Journal of Infectious Diseases (AJID).
> >
> > Thank you
>
> --
>
> COJA House,
> 7, Road 1, Otunmaiye Square, Ajebamidele,
> P. O. Box 2586, Obafemi Awolowo University
> Post Office, Ile-Ife, Osun State, Nigeria.
> Website: www.athmsi.org
> E-mail: athmsi2012@gmail.com, cadewumi@yahoo.com
> +2348026522022

--

COJA House,
7, Road 1, Otunmaiye Square, Ajebamidele,
P. O. Box 2586, Obafemi Awolowo University
Post Office, Ile-Ife, Osun State, Nigeria.
Website: www.athmsi.org
E-mail: athmsi2012@gmail.com, cadewumi@yahoo.com
+2348026522022

In vitro Anti-malarial Activity and Toxicity studies of Johar Leaf (Cassia siamea) from Three Different Locationseditorialcorrection.doc
185K

[AJID] Editor Decision

1 message

Associate Prof. Gbola OLAYIWOLA <gbolaolayiwola@gmail.com> Sun, May 31, 2020 at 9:59 PM To: Ezrani Tasiam <ezranytasiam@gmail.com>, Riesta Primaharinastiti <r.nastiti@gmail.com>, "Wiwied Ekasari, Dr." <wiwied-e@ff.unair.ac.id>

Ezrani Tasiam, Riesta Primaharinastiti, Wiwied Ekasari, Dr.:

We have reached a decision regarding your submission to African Journal of Infectious Diseases (AJID), "In vitro Anti-malarial Activity and Toxicity studies of Johar Leaf (Cassia siamea) from Three Different Locations".

Our decision is: Revisions Required

Please address the corrections marked on your manuscript carefully as indicated in the file sent separately.

Aside from these, on page 6, the formula for calculating should be translated to English. This has been highlighted with yellow.

On page 10 of your manuscript you wrote “Significant value obtained during the test with the weight fraction equation was (p> 0.05). It further means that there is no significant difference at viability percentage among the three samples”.

(How many replicate experiments per concentration did you perform? There should have been SEM or standard deviation!).

On page 12, paragraph before the conclusion “The ethyl acetate fraction of *C.siamea* leaf has the potential as an antimalarial drug raw material which obtains a sufficiently appropriate activity value in the *in vitro* testing of *Plasmodium falciparum* and obtains a satisfactory level of safety in the *in vitro* toxicity testing using the *MTT-ELISA* method, where the ethyl acetate fraction leaves from Pariaman acquires selectivity index values fulfilling the pharmacological effect requirements. Antimalarial activity is not in line with toxicity. The ethyl acetate fraction of *C.siamea* from Pariaman acquired a high activity, whereas the ethyl acetate fraction of *C.siamea* from Surabaya yielded the highest toxicity content. This confirms that the active compound of *C.siamea* which plays a role in antimalarial activity has no correlation with the effect of toxicity. The further research is required to determine the degree of active compounds from the ethyl acetate fraction of *C.siamea* from various regions to acquire the highest level as a raw material for antimalarial treatment”.

This paragraph should be rewritten, for ease of comprehension.

It is strongly recommended that an English editing service or help of an English Lecturer in your University should be used for the revision.

Associate Prof. Gbola OLAYIWOLA
Department of Clinical Pharmacy, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria.E-mail: gbolaolayiwola@yahoo.com
Phone +2348037115758
gbolaolayiwola@gmail.com

Department of Clinical Pharmacy, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria.

InvitroAntimalarial-Manuscript

1 message

Clement Adewunmi <athmsi2012@gmail.com> Sun, May 31, 2020 at 3:56 AM To: "Wiwied Ekasari, Dr." <wiwied-e@ff.unair.ac.id>, Ezrani Tasiam <ezranytasiam@gmail.com>, Gbola Olayiwola <gbolaolayiwola@gmail.com>

Dear Authors,

Kindly revise your manuscript as per the attached .
Address the points raised on the manuscript.
You did not allow an English editing service to help you.
Thank you

--

COJA House,
7, Road 1, Otunmaiye Square, Ajebamidele,
P. O. Box 2586, Obafemi Awolowo University
Post Office, Ile-Ife, Osun State, Nigeria.
Website: www.athmsi.org
E-mail: athmsi2012@gmail.com, cadewumi@yahoo.com
+2348026522022

Indonesia 2020AntimalariaB.doc
178K

2. ARTIKEL DITERIMA UNTUK PUBLIKASI

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

[AJID] Editor Decision

1 message

Prof. Anthony O. ONIPEDE <aonipede@oauife.edu.ng> Fri, Jun 19, 2020 at 11:23 PM To: Ezrani Tasiam <ezranytasiam@gmail.com>, Riesta Primaharinastiti <r.nastiti@gmail.com>, "Wiwied Ekasari, Dr." <wiwied-e@ff.unair.ac.id>

Ezrani Tasiam, Riesta Primaharinastiti, Wiwied Ekasari, Dr.:

We have reached a decision regarding your submission to African Journal of Infectious Diseases (AJID), "In vitro Anti-malarial Activity and Toxicity studies of Johar Leaf (Cassia siamea) from Three Different Locations".

Our decision is to: Accept Submission

Prof. Anthony O. ONIPEDE
Department of Medical Microbiology & Parasitology, Faculty of Basic Medical Sciences, College of Health sciences,Obafemi Awolowo University, Ile-ife, Osun-State, Nigeria.E-mail: aonipede@oauife.edu.ng Phone +2348033437850
aonipede@oauife.edu.ng

B-5539-Article Text-18675-1-4-20191115.docx
54K

ArticleProof Reading

2 messages

Clement Adewunmi <athmsi2012@gmail.com> Fri, Jun 26, 2020 at 6:01 PM
To: "Wiwied Ekasari, Dr." <wiwied-e@ff.unair.ac.id>

Dear Dr. Ekasari,

Kindly check your accepted manuscript for errors. Do Not send the whole manuscript back to us but quote the pages, paragraphs and lines where the corrections are needed.
Thank you.

Editor.

--

COJA House,
7, Road 1, Otunmaiye Square, Ajebamidele,
P. O. Box 2586, Obafemi Awolowo University
Post Office, Ile-Ife, Osun State, Nigeria.
Website: www.athmsi.org
E-mail: athmsi2012@gmail.com, cadewumi@yahoo.com
+2348026522022

TasiametalAJID.pdf
137K

wiwied ekasari <wiwied-e@ff.unair.ac.id> Fri, Jun 26, 2020 at 8:47 PM
To: ezrani tasiam <ezrani.tasiam-2017@ff.unair.ac.id>

[Quoted text hidden]

TasiametalAJID.pdf
137K

[AJID] Editor Decision

1 message

Associate Prof. Gbola OLAYIWOLA <gbolaolayiwola@gmail.com> Tue, Jul 7, 2020 at 6:54 PM To: Ezrani Tasiam <ezranytasiam@gmail.com>, Riesta Primaharinastiti <r.nastiti@gmail.com>, "Wiwied Ekasari, Dr." <wiwied-e@ff.unair.ac.id>

Ezrani Tasiam, Riesta Primaharinastiti, Wiwied Ekasari, Dr.:

The editing of your submission, "In vitro Anti-malarial Activity and Toxicity studies of Johar Leaf (Cassia siamea) from Three Different Locations," is complete. We are now sending it to production.

Submission URL: https://athmsi.org/journals/index.php/AJID/authorDashboard/submission/5539

Associate Prof. Gbola OLAYIWOLA
Department of Clinical Pharmacy, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria.E-mail: gbolaolayiwola@yahoo.com
Phone +2348037115758
gbolaolayiwola@gmail.com

Department of Clinical Pharmacy, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria.

ProofReading- Recheck

3 messages

Clement Adewunmi <athmsi2012@gmail.com> Thu, Jul 16, 2020 at 6:44 PM To: "Wiwied Ekasari, Dr." <wiwied-e@ff.unair.ac.id>, Ezrani Tasiam <ezranytasiam@gmail.com>

Dear Dr. Ekasari,

In Table 2: The Column 4 for **IC50**

Correction: The SD values for the IC50 are not realistic as they are much higher than the mean values and should be cross-checked.

Please atend to this urgently.

Thaank you.

--

COJA House,
7, Road 1, Otunmaiye Square, Ajebamidele,
P. O. Box 2586, Obafemi Awolowo University
Post Office, Ile-Ife, Osun State, Nigeria.
Website: www.athmsi.org
E-mail: athmsi2012@gmail.com, cadewumi@yahoo.com
+2348026522022

wiwied ekasari <wiwied-e@ff.unair.ac.id> Fri, Jul 17, 2020 at 2:14 PM
To: Clement Adewunmi <athmsi2012@gmail.com>

Dear Prof. Clement Adewuni

Thank you for your correction. Yes, I'm really sorry for the mistake in calculating the standard deviation (SD).
Following are the revisions to SD values in table 2 and table 3.

Table 2. Mean of inhibition and IC50 percentage on each partition toward *P.falciparum*

Sample	Consentration (µg/ml)	Inhibition Percentage (%)	IC_{50} (µg/ml)
Pariaman	100	98.03	0.006±0.001
	10	88.02	
	1	83.48	
	0.1	78.09	
	0.01	52.99	
Palu	100	88.99	0.037±0.009
	10	80.36	
	1	77.52	
	0.1	64.21	
	0.01	35.38	
Surabaya	100	82.59	0.090±0.063

	10	73.54
	1	71.29
	0.1	56.98
	0.01	27.95

IC: Inhibitory Concentration

Table 3. Percentage of viability and CC50 with the *MTT-ELISA* method

Sample	**Concentration (µg/ml)**	**Percentage of viability (%)**	**CC_{50} (µg/ml)**
Pariaman	400	9.91	220.82±48.32
	200	60.89	
	100	81.82	
	10	96.13	
	1	99.87	
	0.1	100	
Palu	400	3.35	235.52±12.54
	200	55.97	
	100	90.35	
	10	100	
	1	100	
	0.1	100	
Surabaya	400	1.31	135.81±3.75
	200	22.57	
	100	78.81	
	10	100	
	1	100	
	0.1	100	

CC: Cytotoxicity Concentration

Best regard

Dr. Wiwied Ekasari

[Quoted text hidden]

Table 2 & table 3 rev fix.docx
14K

Clement Adewunmi <athmsi2012@gmail.com> Fri, Jul 17, 2020 at 5:11 PM
To: wiwied ekasari <wiwied-e@ff.unair.ac.id>

Noted with thanks.
[Quoted text hidden]