1. PROSES SUBMIT

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

manuscript submission

5 messages

Editor-in-Chief Tjnpr <editor.tjnpr@gmail.com> **Fri,** Aug 7, 2020 at 1:47 PM To: wiwied-e@ff.unair.ac.id

Dear Dr Wiwied,
Please send your manuscript by email attachment to editor.tjnpr@gmail.com

Best regards

Abiodun

Professor Abiodun Falodun, PhD

Editor-in-Chief:
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Natural Product Research Group, University of Benin
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wiwied ekasari <wiwied-e@ff.unair.ac.id> Sat, Aug 8, 2020 at 8:34 PM
To: Editor-in-Chief Tjnpr <editor.tjnpr@gmail.com>

Dear Professor Abiodun Falodun**,** PhD

Editor-in-Chief:
Tropical Journal of Natural Product Research (TJNPR) Head,

Natural Product Research Group, University of Benin

We would like to submit the enclosed manuscript title **"*In Vitro* Antimalarial and Cytotoxic Activities of *Sauropus androgynus* Leaves Extract"** as a full lenght research article for publication in **Tropical Journal of Natural Product Research** .

All of the authors have read and approved the final submitted manuscript. It can be declared that no portion of the work has been or is currently under consideration for publication elsewhere and also no portion of the manuscript, other than the abstract, has been previously published or posted in the internet.

Thank you for your consideration of this manuscript. We appreciate your time and look forward to your response.

Sincerely yours,

Dr. Wiwied Ekasari, MSi., Apt.
Department of Pharmacognocy and Phytochemistry
Faculty of Pharmacy, Airlangga University, Indonesia
Phone: +62-812-3184-315
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Editor-in-Chief Tjnpr <editor.tjnpr@gmail.com> Sat, Aug 8, 2020 at 8:34 PM
To: wiwied-e@ff.unair.ac.id

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Editor-in-Chief Tjnpr <editor.tjnpr@gmail.com> Sat, Aug 8, 2020 at 10:35 PM
To: wiwied ekasari <wiwied-e@ff.unair.ac.id>

Dear Dr Ekasari,

Thank you for submitting your original article for publication in the Tropical Journal of Natural Product Research (www.tjnpr) SCOPUS Q3.

Kindly send the names, affiliation and email addresses of two potential referees, one from your country and the other from outside your country. The email addresses of the co-authors are also needed.

The peer-review process will commence immediately, and the Editorial Team will get back to you as soon as the review is completed.

Best regards

Abiodun

Professor Abiodun Falodun, PhD

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wiwied ekasari <wiwied-e@ff.unair.ac.id> Mon, Aug 10, 2020 at 8:08 PM
To: Editor-in-Chief Tjnpr <editor.tjnpr@gmail.com>

Dear Professor Abiodun Falodun**,** PhD

Editor-in-Chief:
Tropical Journal of Natural Product Research (TJNPR)
Head, Natural Product Research Group, University of Benin

Thank you for your email. Here I send you the email addresses of the co-author :
Anisah Mahardiani : anisa.mahardiani-2017@ff.unair.ac.id
Suciati : suciati@ff.unair.ac.id

For referees :

1. Dr. Nungruthai Suphrom
   Faculty of Science, Naresuan Universty
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2. Prof. Indah Tantular dr. MKs., PhD
   Faculty of Medicine, Universitas Airlangga
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Best regards
Dr. Wiwied Ekasari., MSi., Apt

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2. PROSES REVIEW DAN ARTIKEL DITERIMA UNTUK PUBLIKASI

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

editorial decison
5 messages

Editor-in-Chief Tjnpr <editor.tjnpr@gmail.com> Sat, Aug 22, 2020 at 4:35 AM
To: wiwied ekasari <wiwied-e@ff.unair.ac.id>

Dear Dr Ekasari,
The manuscript submitted to the Tropical Journal of Natural Product Research (TJNPR) has been carefully reviewed by competent experts.
Find attached the details of the decision.

Please send your response urgently to the editor-in-Chief, to enable us to process your manuscript for the next issue **Vol 4 issue 9 September 2020**.
Kindly acknowledge the receipt of the mail.

Title: In Vitro Antimalarial and Cytotoxic Activities of Sauropus androgynus Leaves Extracts

Authors: Anisah Mahardiani, Suciati, Wiwied Ekasari*
Accepted with some moderate corrections/revisions

Congratulations

Best regards

Abiodun

Professor Abiodun Falodun, PhD

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wiwied ekasari <wiwied-e@ff.unair.ac.id> Mon, Aug 24, 2020 at 9:53 AM
To: Editor-in-Chief Tjnpr <editor.tjnpr@gmail.com>

Dear Professor Abiodun Falodun**,** PhD

Editor-in-Chief:
Tropical Journal of Natural Product Research (TJNPR)
Head, Natural Product Research Group, University of Benin

Thank you for your email,
I am pleased to hear that our manuscript can be published in your journal
Yes, I will pay the ACP as soon as possible.

Best regard
Dr. Wiwied Ekasari., MSi., Apt
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Editor-in-Chief Tjnpr <editor.tjnpr@gmail.com> Mon, Aug 24, 2020 at 9:53 AM
To: wiwied-e@ff.unair.ac.id

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Editor-in-Chief Tjnpr <editor.tjnpr@gmail.com> Tue, Aug 25, 2020 at 4:09 PM
To: wiwied ekasari <wiwied-e@ff.unair.ac.id>

Dear Dr Wiwied,
The review comments will be sent to you in few 48hrs time.

Best regards

Abiodun

Professor Abiodun Falodun, PhD

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Review comments

2 messages

Editor-in-Chief Tjnpr <editor.tjnpr@gmail.com> Wed, Aug 26, 2020 at 4:23 AM
To: wiwied ekasari <wiwied-e@ff.unair.ac.id>

Dear Dr Ekasari

Review comments ***In Vitro* Antimalarial and Cytotoxic Activities of *Sauropus androgynus* Leaves Extracts**

Editorial comments to authors

Give the full name of the author **"Suciati"**

Materials and Methods: include date (Month and Year) and place of plant collection.

- State volume of solvents chloroform and ethanol used for the extraction, also specify if the extraction was successive or each solvent was used on a fresh sample.
- State the source of **Huh7it cells** and the culture conditions.

List of references should follow journal adopted format. See journal website at http://www.tjnpr.org/guideforauthors.aspx# **for details. Abbreviate all Journal names.**

The following references should be cited in the manuscript

1. Okolie NP, EEJ Israel and Falodun A. In-vitro evaluation of antioxidant potential of Rauwolfia vomitoria root extract and its inhibitory effect on lipid peroxidation as indication of aphrodisiac properties. Pharmaceutical Chemistry Journal 2011; 45, 8

2. Abiodun Falodun, Vincent Imieje, Osayemwenre Erharuyi, Joy Ahomafor, Melissa R Jacob, Shabana Khan and Mark T Hamann. Evaluation of three medicinal Plant extracts against Plasmodium falciparum and selected microorganisms. African Journal of Traditional Complementary and Alternative Medicine 2014; 11(4):142-143.

3. Abiodun Falodun, Vincent Imeije, Osayemwenre Erharuyi, Ahomafor Joy, Peter Langer, Melissa Jacobs, Shabanna Khan, Mohammed Abaldry and Mark Hamann. Isolation of antileishmanial, Antimalarial and Antimicrobial metabolites from Jatropha multifida. Asian Pacific Journal of Tropical Biomedicine 2014; 4(5):374 - 378.

Vincent Imieje, Ahmed A. Zaki, Pius Fasinu, Zulfiqar Ali, Ikhlas A. Khan, Babu Tekwani, Shabana I. Khan, Egiebor O. Nosa, Abiodun Falodun. Antiprotozoal and Cytotoxicity Studies of Fractions and Compounds from Enantia chlorantha. Tropical Journal of Natural Product Research 2017; 1(2):89-94.

Osahon K. Ogbeide, Vincent O. Dickson, Randolph D. Jebba, Dennis A. Owhiroro Marvelous O. Olaoluwa, Vincent O.Imieje, Osayemwenre Erharuyi, Bodunde J. Owolabi Pius S. Fasinu, Abiodun Falodun. Antiplasmodial and Acute Toxicity Studies of Fractions and Cassane-Type Diterpenoids from the Stem Bark of Caesalpinia pulcherrima (L.) Sw. Trop J Nat Prod Res 2018; 2(4): 179-184.

O. K. Ogbeide, O. K. Okhomina, I. G. Omoregie, C. A. Unuigbe, A. Ighodaro, I. U. Akhigbe, C. M. Iheanacho, P. C. Akubuiro, A. Solomon, E. E. I. Irabor, B. J. Owolabi, A. Falodun. Antimalarial, ferric reducing Antioxidant Power and Elemental Analysis of Caesalpinia pulcherrima leaf extract. Journal of Chemical Society of Nigeria 2020; 45(4):704 -711.

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The authors should carefully revise and correct the manuscript taking into consideration the comments of all the reviewers. The revised and corrected manuscript should be subjected to plagiarism checker (15% allowed in TJNPR) and English language editing. Evidence of the checks should be attached when submitting the revised/corrected manuscript.

Best regards

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To: Editor-in-Chief Tjnpr <editor.tjnpr@gmail.com>

Dear Professor Abiodun Falodun, PhD

Editor-in-Chief:

Tropical Journal of Natural Product Research (TJNPR) Head,
Natural Product Research Group, University of Benin

Here I send you, my revised manuscript as suggested by reviewers. I also attach the plagiarism check results of my manuscript.

Best regard

Dr. Wiwied Ekasari

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In Vitro Antimalarial and Cytotoxic Activities of Sauropus androgynus Leaves Extracts (2).pdf
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- **NASKAH REVISI**

In Vitro Antimalarial and Cytotoxic Activities of *Sauropus androgynus* Leaves Extracts

ABSTRACT

Malaria is one of the tropical diseases that have ~~still becomes a~~ worldwide implications, ~~problem~~ especially in developing countries. Medical plants have ~~been~~ served as a potential source for the discovery of antimalarial drugs. The leaves of *Sauropus androgynus* is ~~are~~ known for its ~~as~~ antibacterial, analgesic, anti-inflammatory, antihypertensive, and wound healing effects~~er~~. The leaves of *S. androgynus* have been consumed as food for its nutritive values ~~a highly nutrition food~~. However, the antimalarial compound(s) from ~~the~~ its leaves ~~of *S. androgynus*~~ has not been reported before. This research aims ~~of the research are~~ to investigate the *in vitro* antimalarial activities of the n-hexane, chloroform, and 96% ethanol extracts of *S. androgynus* leaves, as well as to study the cytotoxicity activity of the extracts. The leaves of *S. androgynus* were successively extracted with n-hexane, chloroform, and 96% ethanol in order ~~to~~ of increasing ~~e~~ polarity. The antimalarial activity against *Plasmodium falciparum* 3D7 strain (chloroquine-sensitive) was evaluated by using Trager and Jensen method. The cell viability assay was performed to assess the cytotoxic activity of the extracts against Huh7it cells using a tetrazolium based colorimetric method. The chloroform, n-hexane, and 96% ethanol extracts showed antimalarial activity with IC_{50} values of 0.85 µg/mL, 1.23 µg/mL, and 1.88 µg/mL, respectively. On the other hand, the cytotoxic results ~~from~~ of chloroform, n-hexane, and 96% ethanol extracts were 136.00 µg/mL, 766.56 µg/mL, and 896.07 µg/mL, respectively. The findings of the research indicate that the chloroform extract of *S. androgynus* leaves may contain ~~is~~ potential ~~for~~ antimalarial agents.

Keywords: *Sauropus androgynus, Plasmodium falciparum* 3D7, Antimalarial, Cytotoxic

Introduction

Malaria is a tropical disease caused by ~~an infection in red blood cells of~~ protozoan parasites of the genus *Plasmodium*.[1] The parasites infect ~~contaminate~~ the human host through ~~a~~ feeding by female *Anopheles* mosquito.[2] The most dangerous *Plasmodium* species that infect humans is *Plasmodium falciparum*.[3,4] Malaria is an important cause of morbidity and mortality in children and adults in tropical countries.[5,6] Currently, mortality has risen in recent years, probably due to the increasing resistance of the parasite to the various antimalarial medicines.[7] Artemisinin-based combination therapies (ACTs) are now generally considered as the best current treatment for uncomplicated falciparum malaria.[8,9]

The crucial problem in malaria control programmes is the parasite resistance to the most commercially available antimalarial and vector (mosquitoes) resistance to insecticides.[10] As a result, the ~~So that,~~ discovery of new antimalarial drugs from natural product can be a potential source to open new ways in the field of antimalaria therapy.[11,12] In addition, ~~the~~ such compound can be modified to get potential and safe antimalarial drugs. Some of antimalarial compounds from plants are alkaloids, terpenes, flavonoids, and xanthone.[13]

The leaves of *Sauropus androgynus* from Euphorbiaceae family have been consumed as a highly nutritive ~~on~~ food. *S. androgynus* leaves~~f~~ have been known to enhance breast milk production. They are also reported to function as antibacterial, analgesic, anti-inflammatory, antihypertensive, and wound healer.[14] Despite that, the antimalarial compound from the leaves of *S. androgynus* has not been reported before.

This study focuses on the investigation of the *in vitro* antimalarial and cytotoxic activities of n-hexane, chloroform, and 96% ethanol extracts of *S. androgynus* leaves. Furthermore, the various extracts were screened for the presence of plant secondary metabolites.~~this study also performs the phytochemical screening of all extracts.~~

Materials and Methods

Plant materials

Leaves of *S. androgynus* were collected and determined by Materia Medica Batu, East Java. Voucher specimen was deposited at Faculty of Pharmacy, Universitas Airlangga with document number of 074/68/102.7/2018.

Extraction

Dried leaves of *S. androgynus* (960 g) were extracted by macerastion with 4.800 mL n-hexane (3x24 hours) in at room temperature. Subsequently, the marc was successively extracted with chloroform, After macerated with n-hexane during 3x24 hours, the maceration process is continued with chloroform solvent, and 96% ethanol. in the same way. Then, All filtrates from each solvent were evaporated separately with a rotary evaporator to obtain the crude extracts and the yields were calculated. so that a thick extract was obtained.From 960 g of *S. androgynous* dried leaves, 29 grams of n-hexane extract, 39 grams of chloroform extract, and 16.9 grams of 96% ethanol extract were obtained.

Phytochemical screening[15]

Phytochemical screening was carried out by using Thin Layer Chromatography (TLC) method[15]. The samples were applied on to TLC plate, developed with a suitable mobile phase, and then were stained with Dragendorf reagent (alkaloids), anisaldehyde sulfuric acid (terpenes), and NH_4OH concentrated (flavonoids). The spots were identified under UV 254 nm and 366 nm.

In vitro antimalarial assay[16]

The antimalarial activity against *P. falciparum* 3D7 strain (chloroquine-sensitive) was evaluated by using Trager and Jensen method[16]. Cultures were cultivated in human O+ red blood cells with 5% hematocrit in RPMI 1640 medium (Gibco BRL, USA) supplemented with 22.3 mM HEPES (Sigma), hypoxanthine, sodium bicarbonate, and 10% human O+ plasma. Serial concentrations of the extracts (100; 10; 1.0; 0.1; and 0.01 µg/mL) were used. The assay was done in a 24 microwell plate with 1% initial and experimental parasitaemia (1 mL/well of suspension), then inserted into the candlejar, and incubated in a CO_2 incubator at 37°C for 24 and 48 hours. Then, a thin blood smear was made on a glass slide, fixated in methanol, and stained with 10% Giemsa for 10 minutes.

The ~~number~~ level of parasitemia was observed under a microscope on 3000's erythrocytes with 1000 times magnification. Next, the number of parasites in the ring, trophozoite, and schizon stages ~~dium~~ was compared with those at 24 and 48 hours incubation. Then, the percentage of parasites growth was calculated by comparing it with the negative control. Finally, fifty percent inhibitory concentration (IC_{50}) of each extract~~s~~ were determined to express the antimalarial activity. IC_{50} was defined as the concentration of the compound causing 50% inhibition of parasite growth relative to untreated control.[17]

% parasitemia = (number of infected RBC / 3000 RBC's) x 100%

The percentage of the growth inhibition of the parasites was calculated using the formula be~~l~~low[18]:

% growth = % parasitemia - D_o

% inhibition = 100 % - (X_s/X_c x 100%)

Where; X_s = parasitemia in the treated group, X_c = parasitemia in the negative control group

Cytotoxic assay and selectivity index value[19]

Cytotoxic assay was done by using Huh7it cells (hepatoma cancer). Optical density measurements were performed with an ELISA reader on Huh7it cells. Serial concentrations of the extracts (1000; 800; 600; 400; and 100 µg/mL) were used. Huh7it cells in 96 well plates were treated with different concentrations ~~serial dilution~~ of the samples or control. The culture plates were observed using ELISA reader at 560 nm and 750 nm wave lengths. The concentrations at which 50% cytotoxic effect occured (CC_{50}) were then determined by plotting concentrations of extract on x-axis and precentage of cell viability ~~in~~ on y-axis to obtain a ~~with~~ dose response curve[19]. The precentage of viability was measured with the formula as be~~l~~low:

a. Percentage Toxicity = (Absorbance of control-absorbance of sample)/absorbance of control) x 100%
b. Percentage Viability = (Absorbance of sample/absorbance of control) x 100%

Cell viability assay was performed to assess the fifty percent cytotoxic concentration (CC_{50}) of extracts against Huh7it cells. Then, the SI value was calculated from the ratio of cytotoxicity to biological activity[20] ($SI = CC_{50}/IC_{50}$).

Results and Discussion

Antimalarial and study of parasite morphology

The antimalarial activity of the extracts can be shown as a change in the morphology and number of the parasites. The study of parasite morphology in the thin blood smears highlights the fact that extracts from different solvents of the plant have different antimalarial activities. The specific changes in morphology produced by particular extracts may predict different modes of action of the active compound principles in extracts. The parasitemia decreases with increasing concentratios of the extracts, reflecting an inhibitory activity on the parasite replication.[21] Protein synthesis in the parasite begins soon after the merozoite invasion, has reached its peak in ~24 hours, and persists at about this level for another 24 hours, thus spanning the entire parasite erythrocytic life cycle of 48 hours.[22] The result of parasite stages is shown in Table 2. Table 2 explains the different morphology and precentage of parasitemia of *S. androgynus* leaves extracts against *P. falciparum* 3D7 strain. Parasite stages of the specific development were checked at the beginning of incubation (0 hours), 24, and 48 hours. The parasite were classified in three phases: Ring (R), Trophozoites (T), and Schizonts (S).

After 24 hours incubation, the culture that was given the chloroform extract showed that it had lower ring phase than the negative controls. Whereas, the trophozoite and schizon phases were relatively similar. It means the number of merozoites that invades new erythrocytes to be a ring phase from the previous schizon phase is reduced compared to negative controls. Although the parasite morphology was checked every 24 hours until 48 hours of incubation, the IC_{50} values were determined at 48 hours of incubation to account for potential effects on biochemical pathways of the parasite.

Formatt

Cytotoxic assay and selectivity index value

~~Cytotoxic assay used~~ Huh7it cell line was used to investigate the cytotoxicity of the extracts, this is because the metabolism of most drugs and chemical compounds ~~was~~ are carried out in the liver. In addition, malaria can cause damage to liver cells. IC_{50} and CC_{50} values of *S. androgynus* leaves extracts are shown in Table 4 and Table 5. Antimalarial activity of ~~from~~from the chloroform, n-hexane, and 96% ethanol extracts was shown by IC_{50} values of 0.85 µg/mL, 1.23 µg/mL, and 1.88 µg/mL, respectively while the cytotoxic results ~~from~~ of the chloroform, n-hexane, and ethanol 96% extracts were 136.00 µg/mL, 766.56 µg/mL, and 896.07 µg/mL, respectively.

The principle of the MTT method is the reduction of MTT tetrazolium yellow salt by a reductase system. Tetrazolium succinate which belongs to the respiration chain in the mitochondria of living cells forms purple formazan crystals and is insoluble ~~to~~ in water. Addition of stopper reagent ~~will~~ dissolves these colored crystals and then absorbance is measured using an ELISA reader. The intensity of the purple colour formed is directly proportional to the number of living cells. So that if the intensity of the purple colour is greater, it means there are more ~~the number of~~ living cells and thus decrease activity of plant material~~will increase~~.23

The conversion would only be possible if only the extracts tested did not kill the cells. This ability of the cells is linked to the plant extracts compound that protects the cells against cell-mediated damage. The result from MTT assay suggests that the plant extracts have intrinsic biological components that enable them to protect the cells against *P. falciparum* mediated damage. Selectivity index (SI) is defined as the ratio of the cytotoxic on the human cells to the antimalarial activity.[24] It is used to estimate the potential of molecules or extracts to inhibit parasite growth without toxicity. Low SI indicates that the antimalaria activity is probably due to cytotoxic activity that is bigger than antimalarial activity against the parasite themselves. Meanwhile, higher SI value offers the potential of safer therapy.[25]

Based on the criteria of antimalarial activity, extract exhibiting IC_{50}>100 µg/mL is considered inactive. Extract showing IC_{50}<100 µg/mL can be continued as a potential antimalarial agent. Active extract showing IC_{50}<10 µg/mL should be selected for the next purification based on bioassay-guided isolation method. *In vitro* screening to observe *P. falciparum* inhibition activity of the plant extract with IC_{50} value less than 10 µg/mL is a primary step in the development of new antimalarial agent. To ~~get~~ obtain safer antimalarial drug, the development procedure can be monitored with the determination of SI values.~~it can be continued with cytotoxicity assay to get SI value.~~ Based on SI value, extract with SI<4 can be classified as marginally active, SI 4-10 partially active, and active SI>10 can ~~be~~ actively functioned as antimalaria.[26] From the results, all extracts tested had good selectivity indexes (>10), suggesting good therapeutic potentials.

Phytochemical analysis of S. androgynus extracts

The results of phytochemical screening indicated the presence of terpene compounds in all extracts, however, alkaloid and flavonoids were not detected (Table 1). The antimalarial compound from *S. androgynus* leaves can be predicted from terpenes group. Some plants from

the family of Euphorbiaceae are known to have antimalarial activity. Ethanol and n-hexane extracts from the root bark of *Uapaca_nitida* are known to contain terpenoid compounds (betulinic acid and triterpene).[27,28] Meanwhile, the ethanol extract of the leaves of *Croton steenkampianus* contains ~~of~~ diterpenoids and is active as antimalarial.[29] Whereas, based on the Sauropus genus approach, it is reported that a 90% methanol fraction of *S. spatulifolius* leaves has antimalarial activity against *P. falciparum* K1 strains with IC_{50} of 6.10 µg/mL.[30] Terpenes as antimalarial inhibit the growth phase of the plasmodium parasite from ring form to tropozoites. Beside that, terpene inhibits nutrient intake by the parasites by inhibiting the permeation pathway.[31,32]

From the phytochemical screening study, all extracts of *S. androgynus* contain terpenes. Moreover, from the antimalarial activity observed, the chloroform extract has better antimalarial ~~the~~ potential ~~activity~~.

Conclusion

Based on the results of the research, the chloroform extract of *S. androgynus* leaves has good *in vitro* antimalarial activity against *P. falciparum* 3D7 strain (chloroquine-sensitive). The chloroform extract exhibits the highest *in vitro* antimalarial activity with an IC_{50}, CC_{50}, and SI values of 0.85 µg/mL, 136.00 µg/mL, and 160.19µg/mL, respectively. Terpenes were ~~he compound that was~~ present in all extracts and this may be ~~was terpene. Thus, this compound may be~~ responsible for the antimalarial activity.

Conflict of Interest

The authors declare that they have no conflicts of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements

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References

1. Haldar K, Mohandas N. Malaria, erythrocytic infection, and anemia. Hematology Am. Soc. Hematol. Educ. Program. 2009;1:87-93.
2. Toé KH, Jones CM, N'Fale S, Ismail HM, Dabiré RK, Ranson H. Increased pyrethroid resistance in malaria vectors and decreased bed net effectiveness, Burkina Faso. Emerging Infectious Diseases. 2014;20(10):1691–1696.
3. Suh KN, Kain KC, Keystone JS. Malaria. Canadian Medical Association or its licensors. 2004;170:1693–1702.
4. Snow RW, Guerra CA, Noor AM, Myint HY, Hay SI. The global distribution of clinical episodes of *Plasmodium falciparum* malaria. Nature, 2005; 434(7030): 214–217.
5. Geleta G, Ketema T. Severe Malaria Associated with *Plasmodium falciparum* and *P. vivax* among children in Pawe Hospital, Northwest Ethiopia. Malaria Research and Treatment. 2016, Article ID 1240962, 1–7.
6. Komba AN, Makani J, Sadarangani M, Ajala-Agbo T, Berkley JA, Newton CRJC, Marsh K, Williams TN. Malaria as a cause of morbidity and mortality in children with homozygous sickle cell disease on the coast of Kenya. Clin Infect Dis. 2009, 49 (2): 216–222.
7. Cui L, Mharakurwa S, Ndiaye D, Rathod PK, Rosenthal PJ. Antimalarial drug resistance: literature review and activities and findings of the ICEMR Network. The American Society of Tropical Medicine and Hygiene. 2015, 93(3):57–68.
8. Eastman RT, Fidock DA. Artemisinin-based combination therapies: a vital tool in efforts to eliminate malaria. Nat Rev Microbiol. 2009;7:864–874.
9. Pousibet-Puerto J, Salas-Coronas J, Sánchez-Crespo A, Molina-Arrebola MA, Soriano-Pérez MJ, Giménez-López MJ, Vázquez-Villegas J, Cabezas-Fernández MT.

Impact of using artemisinin-based combination therapy (ACT) in the treatment of uncomplicated malaria from *Plasmodium falciparum* in a non-endemic zone. Malaria Journal. 2016,15:339.

10. Matiya DJ, Philbert AB, KidimaW,Matowo JJ. Dynamics and monitoring of insecticide resistance in malaria vectors across mainland Tanzania from 1997 to 2017: a systematic review. Malaria Journal. 2019,18:102.
11. Quinghaosu Antimalarial Coordinating Research Group. Antimalaria studies on qinghaosu. Chinese Medical Journal.1979;92:811-816.
12. Wells TNC, Van Huijsduijnen RH, Van Voorhis WC. Malaria medicines: a glass half full? Nature Reviews Drug Discovery. 2015;14:424-442.
13. Saxena S, Pamt N, Jain DC, Bhakuni RS. Antimalarial agents from plant sources. Current Science. 2003;85:1314-1329.
14. SelviSV,Anusha B. Phytochemical analysis and GC-MS profilling in the leaves of *Sauropus androgynus* (L.) Merr. Int J of Drug Dev& Res.2012;4:162–167.
15. Harborne JB. A guide to modern techniques of plant analysis. (3rd ed.). London, New York: Chapman and Hall; 1998. Phytochemical methods.
16. Trager W,Jensen JB. Human malaria parasites in continuous culture. Science. 1976;193:673–675.
17. Hafid AF, Ariantari NP, Tumewu L, Hidayati AR, Widyawaruyanti A. The active marker compound identification of *Artocarpus champeden* Spreng steambark extract, Morachalchone a as antimalaria. Int J Pharm Pharm Sci. 2012;4:246-249.
18. Hamsidi R, Widyawaruyanti A, Hafid A, Ekasari W, Kasmawati H, Akib NI, Wahyuni, Muhammad HM. *In vitro* antimalarial activity of chloroform, n-butanol, and ethyl acetate

fractions of ethanol extracts of *Carthamus tinctorius* Linn flowers. Asian J Pharm Clin Res. 2018;11:121–123.

19. Widyawaruyanti A, Khasanah U, Tumewu L, Ilmi H, Hafid AF, TantularIS.Antimalarial activity and cytotoxicity study of ethanol extract and fraction from Serratus leaves. International Journal of Pharmacy and Pharmaceutical Sciences.2015;7:250–253.
20. Vonthron-Sénécheau C, Weniger B, Ouattara M, Bi FT, Kamenan A, Lobstein A, Brun R, Anton R. *In vitro* antiplasmodial activity and cytotoxicity of ethnobotanically selected Ivorian plants. Journal of Ethnopharmacology. 2003;87: 221–225.
21. Arnot DE, Gull K. The *Plasmodium* cell-cycle: facts and question. Ann Trop Med Parasitol. 1998;92:361–365.
22. SaxenaS, Pamt N, Jain DC, Bhakuni RS. Antimalarial agents from plant sources. Review Article, Current Science. 2003;85:1314–1329.
23. Mossman T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assay. Journal of Immunological Methods. 1983;65:55–63.
24. Pouplin JN, Tran H, Tran H, Phan TA, Dolecek C, Farrar J, Tran TH, Caron P, Bodo P, Grellier P. Antimalarial and cytotoxicity activities of ethnopharmacologically selected medicinal plants from South Vietnam. Journal of Ethnopharmacology. 2007;109:417–427.
25. Lima RBS, Silva LFR,Melo MRS. *In vitro* and *in vivo* antimalarial activity of plants from the Brazilian Amazon. Malaria Journal. 2015;14:508.
26. Valdes AF, Martinez JM, Lizama RS, Gaiten YG, Rodriguez DA, Payrol JA. *In vitro* antimalarial activity and cytotoxicity of some selected Cuban medicinal plants. *Rev.* Inst. Med. Trop. Sao Paulo. 2010;52:197–201.

27. Kirby, G.C., Khumalo-Ngwenya, N.B., Grawehr, Br. A., Fison, T.W., Warhurst, D.C., Phillipson, J.D., Antimalarial Activity from `Mhekara' (*Uapacanitida*MullArg.), a Tanzanian tree. J. Ethnopharmacol. 1993;40:47–51.

28. Steele, J.C.P., Warhusrst, D.C., Kirby, G.C. Simmonds., *In vitro* and *In vivo* Evaluation of Betulinic Acid as an Antimalarial, Phytotheraphy Research. 1999.13:115-119

29. Adelekan, A.M.,Prozesky, E.A., Hussein, A.A.,Urena, L.D., van Rooyen, P.H., Liles, D.C., Meyer, J.J., Rodriguez, B. Bioactive diterpenes and other constituents of *Croton steenkampianus*. J. Nat. Prod. 2008.71:1919-1922.

30. Zou, Y.S., Kenn, F., Emmy, T., Filip, L., Paul, C., Louis, M., Jan, M.M.Rene de. G., R., Sandra, A., Luc, P. 2012. Antiplasmodial and Cytotoxic Activities of *Striga asiatica*and *Sauropusspatulifolius* Extracts, and Their Isolated Constituents. Phytochemistry Letters 6. 53–58.

31. Gabriel HB, Sussmann RAC, Kimura EA, Rodriguez AAM, Verdaguer IB, Leite GCF, Katzin AM, 2018. Terpenes as Potential Antimalarial Drugs http://dx.doi.org/10.5772/intechopen.75108.

32. Hamilton AC. Medicinal plants, conservation and livelihoods. Biodiversity and Conservation. 2004;13:1477-1517.

Table 1: Phytochemical constituents of extracts

Extracts	Phytochemical constituents		
	Alkaloids	**Terpenes**	**Flavonoids**
n-hexane	-	+	-
Chloroform	-	+	-
96% Ethanol	-	+	-

Key: (+) = present, (-) = absent

Tabel 2. Change of Morphology and Precentage of Parasitemia of *S.androgynus* Leaves Extracts in 24 Hours of Incubation

Sample	**Concentration (µg/mL)**	**% Parasitemia**			**Mean % Parasitemia**	**Mean % Inhibition 24 hours (µg/mL)**
		R	T	S		
D_o		0.60	-	-	0.60	
n-hexana	100.00	0.52	0.03	0.04	0.59	54.58 ±14.33
	10.00	0.56	0.40	0.06	1.02	0
	1.00	0.42	0.59	0.12	1.13	0
	0.10	0.45	0.42	0.14	1.01	0
	0.01	0.43	0.46	0.11	1.00	0
Chloroform	100.00	0.59	0.04	0.04	0.67	44.96±2.97
	10.00	0.73	0.35	0.04	1.12	0
	1.00	0.30	0.41	0.08	0.79	0
	0.10	0.34	0.40	0.09	0.83	0
	0.01	0.56	0.34	0.13	1.03	0
Ethanol 96%	100.00	0.59	0.07	0.04	0.70	53.33±18.86
	10.00	0.49	0.15	0.06	0.70	53.33±18.86
	1.00	0.40	0.58	0.03	1.01	0
	0.10	0.39	0.39	0.03	0.81	0
	0.01	0.43	0.61	0.21	1.25	0

Note : Ring (R), Trophozoites (T), and Schizonts (S)

Tabel 3. Change of Morphology and Precentage of Parasitemia of *S.androgynus* Leaves Extracs in 48 Hours of Incubation

Sample	Concentration (µg/mL)	% Parasitemia			Mean % Parasitemia	Mean % Inhibition 48 hours (µg/mL)
		R	T	S		
D_o		0.60	-	-	0.60	
n-hexane	100.00	0.10	0.73	0.06	0.89	87.12 ± 2.02
	10.00	0.89	0.48	0.29	1.66	52.26±14.32
	1.00	1.16	0.24	0.44	1.84	44.53 ± 7.52
	0.10	1.46	0.38	0.29	2.13	30.45 ± 7.18
	0.01	1.91	0.40	0.23	2.54	13.24 ± 8.78
Chloroform	100.00	0.26	0.45	0.06	0.77	92.22 ± 2.21
	10.00	0.12	0.55	0.48	1.15	74.83 ± 0.97
	1.00	0.89	0.29	0.50	1.68	50.80 ± 2.10
	0.10	1.35	0.20	0.30	1.85	47.87 ± 1.25
	0.01	1.19	1.37	1.37	1.93	36.62 ± 0.72
96% Ethanol	100.00	0.55	0.13	0.04	0.72	93.88± 2.09
	10.00	0.71	0.36	0.57	1.64	48.10±16.68
	1.00	0.93	0.34	0.57	1.84	38.88± 9.28
	0.10	1.29	0.40	0.40	2.09	25.67±21.96
	0.01	1.45	0.20	0.54	2.19	20.72±19.19

Note : Ring (R), Trophozoites (T), and Schizonts (S)

Table 4. Percentage of Viability and CC_{50} Value of *S. androgynus* leaves extracts

Concetration (µg/mL)	% Viability Cell		
	n-hexane	Chloroform	96% Ethanol
1000	4.33	0	36.15
800	45.80	0	64.11
600	67.72	1.84	73.36
400	78.15	7.22	86.75
200	91.01	43.77	95.87
100	96.39	53.87	100.00
CC_{50} (µg/mL)	766.56	136.00	896.07

Table 5. Antimalarial and Cytotoxic Activities of *S. androgynus* leaves extracts

Extracts	IC_{50} in 48 hours (µg/mL)	CC_{50} (µg/mL)	SI
n-hexane	1.23	766.56	625.77
Chloroform	0.85	136.00	160.19
Ethanol 96%	1.88	896.07	476.89

Selectivity Index = CC_{50} / IC_{50}

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