Trop J Nat Prod Res, March 2019; 3(3):80-84

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Antimalarial Activity of the Methanol Aerial Extract of *Alysicarpus glumaceus* (Vahl) DC. in *Plasmodium berghei* Infected Mice

Amina B. Olorukooba*, Ferhat Khan, Charles Madu

Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria.

ARTICLE INFO

Article history:
Received 25 February 2019
Revised 17 March 2019
Accepted 22 March 2019
Published online 04 April 2019

ABSTRACT

Medicinal plants are a potential source of affordable and effective drugs used in the treatment of many diseases. The plant *Alysicarpus glumaceus* has been used in traditional medicine for the treatment of many ailments including; thrush, sore, asthma, burn, and fever. The present study was designed to investigate the antimalarial activity of the methanol aerial extract of *Alysicarpus glumaceus* in *Plasmodium berghei* infected mice using suppressive, curative and prophylactic models. Phytochemical screening using standard procedures and acute toxicity studies via the oral route in mice were also conducted. Results from the phytochemical screening revealed the presence of glycosides, saponins, tannins, flavonoids, triterpenes, and alkaloids. The oral mean lethal dose (LD_{50}) was estimated to be greater than 5,000 mg/kg in mice. The methanol aerial extract of *Alysicarpus glumaceus* at doses of 250, 500 and 1,000 mg/kg produced a significant ($p<0.05$) dose-dependentchemosuppression in the suppressive, curative and prophylactic tests respectively. There was a statistically significantly ($p<0.05$) prolongation of mean survival time in the extract treated mice in the curative study. The results of this investigation suggest that the methanol aerial extract of *Alysicarpus glumaceus* contains bioactive constituents with antimalarial activity.

Keywords: *Alysicarpus glumaceus,* Antimalarial, Acute toxicity, Chemosuppression, *Plasmodium berghei*

Introduction

The impact of malaria on human life still remains a global problem as the disease is responsible for the death of more than 435,000 people each year, largely in the African region.[1] Though substantial progress had been previously made in the fight against malaria, recent reports show increases in malaria cases with a decrease in funding of malaria control programs.[1] Malaria is preventable and curable; however, it still remains a major cause of mortality and morbidity especially in the developing countries.[2] Five species of *Plasmodium* parasite have been identified to cause the mosquito-borne disease in humans including *P. falciparum*, *P. ovale*, *P. malariae*, *P. vivax* and *P. knowlesi*.[3]*Plasmodium falciparum* is the deadliest parasite responsible for the majority of malaria-related morbidity and mortality with a significant social and economic impact in developing countries including Nigeria.[1]

Various strategies including the use of long-lasting insecticidal nets (LLINs), indoor residual spraying (IRS), intermittent preventive treatment in pregnancy (IPTp) and the management of infection with available antimalarials, have been employed in the fight against malaria.[4] Though these strategies have resulted in a reduction in the burden of malaria disease, they are however still insufficient to achieve elimination in many countries, especially in the developing world.[5] Lack of an effective antimalarial vaccine, side effects associated with the use of some currently used antimalarials, the emergence of new antimalarial drug resistance cases and insecticide resistant *Anopheles* mosquitoes is seriously threatening malaria control.[6] This scenario has thus prompted the research towards the discovery and development of new, safe and affordable antimalarial drugs.

Medicinal plants have been the main source of treatment of malaria in many developing countries.[7] These plants have served as sources for new drugs including some of the most successful antimalarial agents, such as quinine and artemisinin.[8]*Alysicarpus glumaceus* (Family-Fabaceae) is a shrubby annual plantwhich can grow up to 1 m high. The plant is found in the grassy savanna of Upper Volta, Northern Ghana and Northern Nigeria, and is also widely dispersed over the rest of tropical Africa.[9] Traditionally various parts of the plant have been used for the treatment of many ailments including ; nasopharyngeal infections, pulmonary troubles, fever, stomach disorders, diarrhea, dysentery, dropsy, swellings, edema, and gout.[9] However, there is no scientific data to validate the antimalarial properties of this plant. This study was designed to evaluate the *in vivo* antimalarial effect of aerial parts of *Alysicarpus glumaceus*.

Materials and Methods

Plant Collection and Extract Preparation

The aerial parts, leaves, and fruits of *Alysicarpus glumaceus* were collected in Igabi Local Government Area of Kaduna state. The plant parts were taken to the herbarium section of the Department of Botany, Ahmadu Bello University, Zaria where it was identified and authenticated by comparing with existing voucher specimen (446). The aerial parts were air-dried under shade till a constant weight was obtained and subsequently sized reduced using mortar and pestle. The powder (2 kg) was extracted with 10 L of methanol (70% v/v) by maceration technique for three days with occasional shaking. The extract was freed of solvent by evaporation to drynessresulting in a dark green sticky mass(315.63g) subsequently referred to as methanol

*Corresponding author. E mail: aolorukooba@gmail.com
Tel: +2348057665703

Citation: Olorukooba AB, Khan F, Madu C. Antimalarial Activity of the Methanol Aerial Extract of *Alysicarpus glumaceus* (Vahl) DC. in *Plasmodium berghei* Infected Mice. Trop J Nat Prod Res. 2019; 3(3):80-84. doi.org/10.26538/tjnpr/v3i3.3

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

aerial extract of *Alysicarpus glumaceus* (MEAG). The extract was stored in airtight container placed in a desiccator until needed for work. An aqueous solution was freshly prepared for each study using distilled water.

Phytochemical Screening
The methanol aerial extract of *Alysicarpus glumaceus* was subjected to phytochemical screening using standard methods as described by Trease and Evans.[10]

Experimental Animals
Swiss albino mice of both sexes weighing 18–22 g and 6–8 weeks old, were used for the study. The animals were kept at the Animal House of the Department of Pharmacology and Therapeutics and housed in plastic cages with softwood shavings and chips as beddings. They were provided with free access to standard pellet food (Vital feeds(R), Jos) and clean drinking water. All experiments performed on the laboratory animals were in accordance with the criteria outlined in the Guide for the Care and Use of Laboratory Animals by the National Institute of Health (Publication No. 80-23, 2011). Ethical approval was sought from the ABU Committee on Animal Use and Care(ABUCAUC/2017/122).

Acute Toxicity Study
Acute toxicity studies using Lorke's method[11] was carried out to determine thesafety of the methanol aerial extract of *Alysicarpus glumaceus* after oral administration. The study was carried out in two phases; in the first phase, three groups consisting of three mice each were administered graded doses of the extract (10 mg/kg, 100 mg/kg and 1,000 mg/kg) via theoral route. The animals were then observed for signs of physical toxicity (writhing, decreased respiration, decreased body tone, decreased motor activity and death) for the first four hours and then intermittently for 24 hours.
A second phase was then initiated where four animals were administered the extract at doses of 1,200, 1,600, 2,900, and 5,000 mg/kg respectively. The animals were then observed for signs of physical toxicity for the first four hours and then intermittently for 24 hours. The oral median lethal dose (LD_{50}) was estimated as the geometric mean of the minimum dose producing mortality and maximum dose producing no mortality as shown in the formula below.
$LD_{50} = \sqrt{}$ (Lowest lethal dose x Highest non-lethal dose)

Rodent Malaria Parasite
Plasmodium berghei NK65 chloroquine sensitive strain was obtained from the Department of Microbiology, National Medical Research Institute (NIMR), Lagos. The parasite was maintained by weekly serial passage of $1x10^7$ infected red blood cells (RBC) in naïve mice.

Determination of Percentage Parasitemia
The method by Kalra *et al*.,[12] was used to determine the percentage parasitemia. Blood was obtained from the tail of an infected mice and thin smears were prepared on slides. The slides were allowed to dry and then fixed with methanol. The slides after fixing were dried and then stained with 10% Giemsa in methanol for 30 min. Slides were rinsed with water, allowed to dry and carefully observed under amicroscope using a×100 objective lens with immersion oil in 4 different fields on each slide. The percentage parasitemia was calculated using the formula:

$$\% \text{ parasitemia} = \frac{no\ of\ parasitized\ RBC}{total\ no\ of\ RBC} \times 100$$

Infection of Mice
Donor mice with *P. berghei* parasitemia level of about 20-25% were used for the study. Through cardiac puncture, blood from a donor mouse was collected into a Petri dish treated with 0.5% trisodium citrate. The blood was then diluted with physiological saline (0.9%) such that 1 mL of blood contained 5×10^7 infected erythrocytes. Each mouse was administered intraperitoneally with 0.2 mL of this diluted blood containing approximately 1×10^7*P. berghei*-infected red blood cells.

In vivo Antimalarial Tests
Animal Grouping and Dosing
Thirty mice of both sexes were used for each model employed in this study. For the suppressive and curative studies, the negative (Group I) and positive (Group V) controls were orally administered with distilled water (1mL/kg) and artesunate (5 mg/kg), respectively. In the prophylactic study, the positive control was administered pyrimethamine (1.2 mg/kg). Groups III, IV and V were orally administered with the extract at doses of 250, 500 and 1,000 mg/kg respectively.

Four-day Suppressive Test
Evaluation of the suppressive activity of the extract against chloroquine sensitive *P. berghei* infection in mice was based on the method as described by Peters *et al*.[13] Thirty (30) mice were weighed and randomly divided into five groups of six mice per group. Each mouse was administered with 0.2 mL standard inoculum containing approximately 1 x 10^7*P. berghei*infected red blood cells via theintraperitoneal route.Three hours later, the extract and standard drug were administered to the mice. Groups I and V received distilled water (10 mL/kg) and Artesunate (5 mg/kg) respectively, while groups II, III and IV were administered with the extract at doses of 250, 500 and 1,000 mg/kg, respectively. All drug and extract administration was through the oral route. Treatments continued for four consecutive days (Day 0–3). On the fifth day (day 4), drops of blood were collected from the tail of each mouse. Thin blood smears were made on a microscopic slide, air-dried and fixed with absolute methanol. The slide was then stained with 10% Giemsa solution for 30 min. Parasitemia level was determined by counting the number of parasitized erythrocytes in 4 random fields using the x100 objective lens under a light microscope. The percentage parasite suppression relative to the negative control was calculated using the formula below:

$$\% \quad \text{Suppression} \quad = \frac{\text{Average parasitemia in control} - \text{Average parasitemia in each treated group}}{\text{Average parasitemia in control}} \times 100$$

Curative Test
The curative test was carried out using the method as described by Ryley and Peters.[14] Thirty (30) mice were injected intraperitoneally with 0.2 mL of standard inoculum containing $1x10^7$ *P. berghei* infected erythrocytes on the first day (Day 0) and left for 72 h. Seventy-two hours (72 h) after inoculation, mice were randomly divided into five groups containing six mice each. Groups I and V received distilled water (10 mL/kg) and Artesunate (5 mg/kg) while groups II, III and IV were administered with the extract at doses of 250, 500 and 1,000 mg/ kg, respectively. All drug and extract administration was through the oral route and treatment continued daily for four days. On the fifth day, Giemsa's stained blood smears were prepared from the tail blood sample of each mouse to determine parasitemia level. Percentage chemosuppression was assessed using the formula as stated above. The animals were then kept, fed *ad libitum* and observed for 28 days. Any death that occurred during this period was recorded to determine the mean survival time (MST) using the formula as shown below.

$$\text{MST} = \frac{\text{Sum of survival time of all mice in a group (days)}}{\text{Total number of mice in that group}}$$

Prophylactic Test
The prophylactic activity of the extract was evaluated using the method described by Peters.[15] On the first day (Day 0), mice were randomized into five groups of six mice per group. Groups I and V were used as negative (10 mL/kg distilled water) and positive (1.2 mg/kg pyrimethamine) controls respectively. Groups II, III and IV received graded doses of the extract (250, 500 and 1,000 mg/kg), respectively for three consecutive days. All drug and extract administration was through the oral route. On the fourth day, mice were injected with 0.2 mL standard inoculum containing approximately 1×10^7*P. berghei* infected erythrocytes intraperitoneally. Seventy-two (72) hours later blood was collected from the tail of each mouse and was prepared as described above to determine the level of

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

parasitemia. Percentage chemosuppression was also determined using the previously stated formula.

Statistical Analysis

Results were expressed as Mean ± Standard Error of Mean. Data were compared using one-way analysis of variance (ANOVA) followed by Dunnett's post-hoc test. Results were considered as statistically significant at $p<0.05$.

Results and Discussion

The huge economic burden due to malaria in developing countries still remains a serious cause for concern.[1] This burden in addition to the development of parasite resistant strains and side effects of some antimalarials presently in use has necessitated the need to search for cheaper, affordable and more effective antimalarial drugs.[3] Many pharmaceuticals in use today were developed from medicinal plants.[16] In this context, this study was carried out to investigate the *in vivo* antimalarial activities of the methanol extract of the aerial parts of *Alysicarpusglumaceus* by evaluating the suppressive, curative and prophylactic activities using a rodent malaria model (*Plasmodium berghei* infected mice). In antimalarial studies, the evaluation of the percentage inhibition of parasitemia is usually taken as the most reliable parameter. This is because reduced parasitemia levels have been found to be necessary for the recovery of an organism from symptomatic malaria.[17] According to Mzena *et al*.[18] plant extracts with the ability to decrease parasitemia levels have been shown to produce high chemosuppression activity. The present study was thus carried out to investigate the ability of the methanol aerial extract *Alysicarpus glumaceus* to decrease parasitemia levels in *P. berghei* infected mice.

The methanol aerial extract of *Alysicarpus glumaceus* produced a significant dose-dependent reduction ($p<0.05$) in parasitemia levels compared to the control (distilled water treated group). The highest percentage chemosuppression(68.3%) was observed at the 1,000 mg/kg dose, the standard drug (Artesunate, 5 mg/kg) however produced 84.9% chemosuppression (Figure 1).Peter's 4-day suppressive test is used to evaluate schizontocidal activity of anextract orcompound. It is the most widely used preliminary test in antimalarial studies whereby the antimalarial activity of a test compound is evaluated by comparing blood parasitemia levels between treated and untreated mice.[12]The parasite suppressive effect (percentage chemosuppression of 41.1%, 56.4% and 68.3%) produced at the 250, 500 and 1,000 mg/kg doses respectively,thus suggests that the extract possesses antimalarial activity.

The curative or Rane's test evaluates the efficacy of a chemical substance against established malaria infection by using the level of parasitemia suppression and survival time as important parameters.[19] A significant ($p<0.05$) dose-dependent reduction in parasitemia level was observed on the administration of the methanol aerial extract of *Alysicarpus glumaceus* to the parasitized mice compared to the negative control (Figure 2). The extract was also able to prolong the mean survival time of *P. berghei* infected mice compared to the distilled water treated group (Table 1).The mean survival time of the mice treated with the highest extract dose (1,000 mg/kg) was 28 days, and this was comparable to that of the artesunate treated group who also survived for 28 days.Test compounds that can prolong the survival time of *P. berghei* infected experimental animals compared to the negative controls are considered as active agents against malaria.[20] The ability of the methanol extract of *A. glumaceus*to produce a significant curative effect in established *P. berghei* infection and increase in mean survival time suggests that the extract was able to suppress the proliferation of *P. berghei* and also reduce the overall pathologic effect of the parasite in the infected mice. In the untreated mice group, death occurred much faster than in the extract treated group. However, neither the extract nor the standard drug (artesunate) cured the infection. This could be due to recrudescence

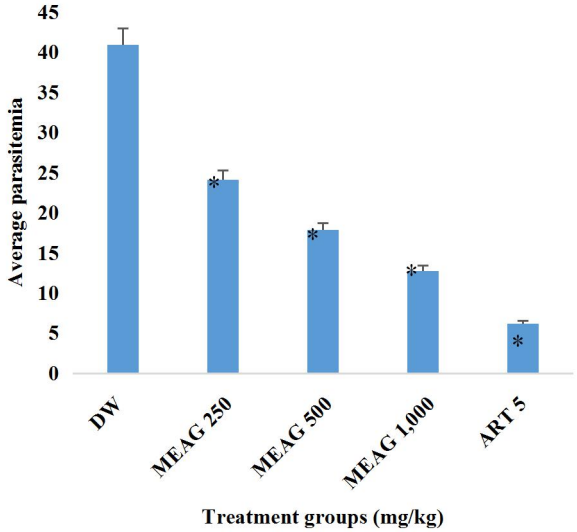

Figure 1: Effect of Methanol Aerial Extract of *Alysicarpus glumaceus* on 4-day Suppressive Antiplasmodial Test in *P. berghei* infected Mice.

Values are presented as mean SEM; Data analyzed by one-way ANOVA followed by Dunnett's post-hoc test; n=6; *= $p<0.5$ versus control; DW = Distilled water; MEAG= Methanol Aerial Extract of *Alysicarpusglumaceus*; ART= Artesunate; Route of administration = oral.

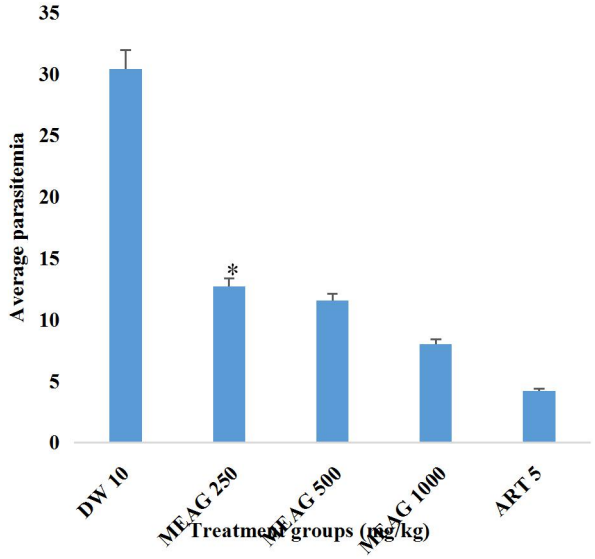

Figure 2: Effect of Methanol Aerial Extract of *Alysicarpus glumaceus* on Curative Antipasmodial Test in *P. berghei* Infected Mice.

Values are presented as mean SEM; Data analyzed by one-way ANOVA followed by Dunnett's post-hoc test; n = 6; * = $p<0.5$ versus control; DW = Distilled water; MEAG= Methanol Aerial Extract of *Alysicarpus glumaceus*; ART = Artesunate; Route of administration =oral.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

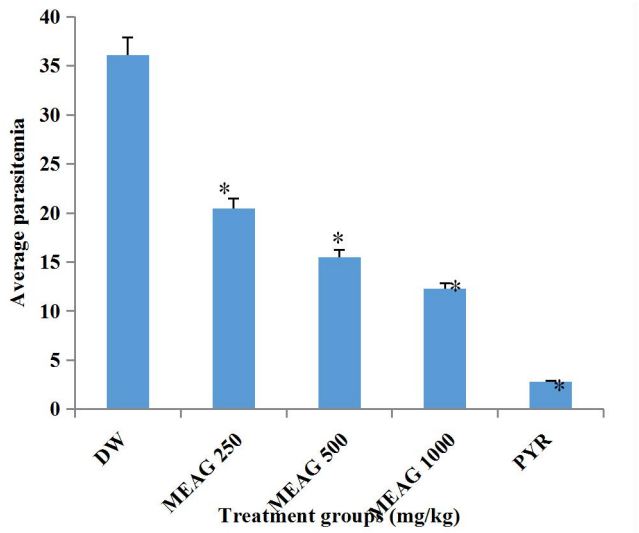

Figure 3: Effect of Methanol Aerial Extract of *Alysicarpus glumaceus*on Prophylactic Antiplasmodial Test in *P. berghei* Infected Mice.
Values are presented as mean SEM; Data analyzed by one-way ANOVA followed by Dunnett's post-hoc test; n = 6; * = p<0.5 versus control; DW= Distilled water; MEAG= Methanol Aerial Extract of *Alysicarpus glumaceus*; PYR = Pyrimethamine; Route of administration = oral.

Table 1: Effect of Methanol Aerial Extract of *Alysicarpus glumaceus* on Mean Survival Time in *P.berghei* Infected Mice.

Treatment group	Dose (mg/kg)	MST (days)
DW	10 mL/kg	6.83 ± 1.38
MEAG	250	23.00 ± 0.84*
MEAG	500	26.17 ± 0.49*
MEAG	1,000	28.00 ± 0.00*
ART	5	28.00 ± 0.00*

Values are presented as Mean ± SEM; Data analyzed by one-way ANOVA followed by Dunnett's Post-hoc test; n = 6; * = p<0.05 versus control; DW = Distilled Water; MEAG = Methanol Aerial Extract of *Alysicarpus glumaceus*; ART = Artesunate; MST = Mean Survival Time; Route of administration = oral.

Table 2: Phytochemical Constituents Present in the Methanol Aerial Extract of *Alysicarpus glumaceus.*

Phytochemical constituents	Inference
Anthraquinones	-
Saponins	+
Cardiac glycosides	+
Tannins	+
Flavonoids	+
Steroids	+
Triterpenes	+
Alkaloids	+

Key: + = present, - = absent

of *P. berghei* parasites after apparent cure.[21]According to Mbah *et al*.,[22] the curative property of potential antimalarial agents of plant-based origin should be detectable during screening for antimalarial activity. Thus, the ability of the extract to decrease parasitemia levels and prolong survival time suggests that it has antimalarial activity.
The repository test was used to study the chemoprophylactic activity of the extract. The dose-dependent reduction in parasitemia levels produced by the methanol aerial extract of *Alysicarpus glumaceus* (average parasitemia 20.46 ± 1.31, 15.48 ± 1.37 and 12.26 ± 0.79 at 250, 500, and 1,000 mg/kg, respectively), is an indication of the extracts ability to inhibit the proliferation of *Plasmodium*parasite in mice (Figure 3). The current finding agreed with other reports on medicinal plants used for malaria, such as *Indigofera spicata*,[8] *Strycnos mitis*[21] and *Trema orientalis*.[23]
The oral median lethal dose was estimated to be greater than 5,000 mg/kg as there was no mortality recorded after the first and second phases of the acute toxicity study. This suggests a good safety profile of *Alysicarpus glumaceus* in mice.[11]From the study, the extract was reported to possess different phytochemical constituents including glycosides, tannins, flavonoids, alkaloids, saponins, triterpenes and steroids. Anthraquinones were however absent (Table 2). Flavonoids, saponins, alkaloids, diterpenoids and kaempferol rhamnoside found in various medicinal plants have demonstrated antimalarial activities.[24-27] Thus the antimalarial activity of *Alysicarpus glumaceus* methanol extract in *P. berghei* infected mice might be due to the presence of one or a combination of these phytochemical constituents acting singly or in combination.

Conclusion

The results obtained in the present study revealed that the methanol aerial extract *Alysicarpus glumaceus* possesses significant antimalarial activity against *Plasmodium berghei* which may be due to the presence of the phytochemical constituents present in the plant.

Conflict of interest

The authors declare no conflict 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.

Acknowledgments

The authors wish to acknowledge Mr. Balarabe Abubakar of the Animal House, Department of Pharmacology and Therapeutics, ABU Zaria for his technical assistance.

References

1. World Malaria Report 2018. Geneva: World Health Organization; 2018 Published 2018. https://www.who.int/malaria/publications/world-malaria-report-2018/en/. Accessed December 22, 2018.
2. Mekonnen LB. In vivo antimalarial activity of the crude root and fruit extracts of *Croton macrostachyus* (Euphorbiaceae) against *Plasmodium berghei* infected mice, J Trad Complement Med. 2015; 5(2015):168-173.
3. Obey JK, Ngeiywa MM, Kiprono P, Omar S, von Wright A, Kauhanen J, Tikkanen-Kaukanen C. Antimalarial activity of *Croton macrostachyus* stem bark extracts. J Pathog. 2018; 1:1-6.

4. World Malaria Report 2016. Geneva: World Health Organization; 2016 http://www.who.int/malaria/publications/world-malaria-report-2016/report/en/. Published in 2016. Accessed December 22, 2018.
5. Olasehinde GI, Ayanda OI, Egwari LO, Ajayi AA, Awofeso T. In vivo antiplasmodial activity of crude ethanolic and n-hexane extracts of *Moringa oleifera* leaves. Int J Agric Biol. 2016; 18(5):906–910.
6. Alout H, Roche B, Dabiré RK, Cohuet A. Consequences of insecticide resistance on malaria transmission. PLoS Pathog. 2017; 13(9):3-7.
7. Ntie-Kang F, Onguéné PA, Lifongo LL, Ndom JC, Sippl W, Mbaze LM. The potential of anti-malarial compounds derived from African medicinal plants. Part II: a pharmacological evaluation of non-alkaloids and non-terpenoids. Malar J. 2014; 13(81):1-9.
8. Birru EM, Geta M, Gurmu AE. Antiplasmodial activity of *Indigofera spicata* root extract against *Plasmodium berghei* infection in mice. Malar J. 2017; 16(1):198-206.
9. Burkhill HM. The Useful Plants of West Tropical Africa. Volume 3, Royal Botanic Gardens, Kew, UK. 1985. 233 p.
10. Trease GE and Evans WC. Phytochemistry, In: Textbook of Pharmacognosy 13th Edition, Balliere, Tindall andCansell Ltd. London. 2002. 224-243 p.
11. Lorke D. A new approach to practical acute toxicity testing, Arch Toxicol. 1983; 54(4):275–287.
12. Kalra BS, Chawla S, Gupta P, Valecha N. Screening of antimalarial drugs: an overview," Indian J Pharmacol. 2006; 38(1):5–12.
13. Peters W, Portus JH, Robinson BL. The chemotherapy of rodent malaria, XXII. The value of drug-resistant strains of *P. berghei* in screening for blood schizontocidal activity. Ann Trop Med Parasitol. 1975; 69(2):155-71.
14. Ryley JF and Peters W. The antimalarial activity of some quinolone esters. Ann Trop Med Parasitol. 1970; 64(2):209-222.
15. Peters W and Robinson BL. The chemotherapy of rodent malaria, XXII. XLVII. Studies on pyronaridine and other Mannich base antimalarials. Ann Trop Med Parasitol. 1992; 46(5):455-465.
16. Cragg GM and Newman DJ. Natural products: A continuing source of novel drug leads. Bio et Biophy Acta. 2013; 1830(6):3670–3695.
17. Mengistie B, Makonnen E, Urga K. In vivo antimalarial activity of *Dodonaea_angustifolia* seed extracts against *Plasmodium berghei* in mice model. *Momona* Ethiop J Sci. 2012; 5 (4) 47–63.
18. Mzena T, Swai H, Chacha M. Antimalarial activity of *Cucumis metuliferus* and *Lippia kituiensis* against *Plasmodium berghei* infection in mice. Res Rep Trop Med. 2018; 9(2018):81-88.
19. Trigg PI and Kondrachine AV. Global overview of malaria. Indian J Med Res. 1997; 106:39-52.
20. Peter IT and Anatoli VK. The current global malaria situation. Malaria parasite biology, pathogenesis, and protection, ASM Press, Washington, DC, USA. 1998. 11-22 p.
21. Fentahun S, Makonnen E, Awas T, Giday M. In vivo antimalarial activity of crude extracts and solvent fractions of leaves of *Strychnos mitis* in *Plasmodium berghei* infected mice, BMC Complement Altern Med. 2017; 17(1):1-12.
22. Mbah CC, Akuodor GC, Anyalewechi NA, Iwuanyanwu TC, Osunkwo UA, *In vivo*antiplasmodial activities of aqueous extract of *Bridelia ferruginea* stem bark against *Plasmodium berghei* in mice, Pharma Biol. 2012; 50(2):188-194.
23. Olanlokun JO, David OM, Afolayan AJ. In vitro antiplasmodial activity and prophylactic potentials of extract and fractions of *Trema orientalis* (Linn.) stem bark. BMC Compl Altern Med. 2017; 17(1):407-419.
24. Adelekan AM, Prozesky EA, Hussein AA, Ureña LD, van Rooyen PH. Bioactive diterpenes and other constituents of *Croton steenkampianus*. J Nat Prod. 2008; 71(11):1919-1922.
25. Chen Y, Li S, Sun F, Han H, Zhang X. In vivo antimalarial activities of glycoalkaloids isolated from Solanaceae plants. Pharm Biol. 2010; 48(9):1018-1024.
26. Walter NS, Bagai U, Kalia S. Antimalarial activity of *Bergenia ciliata* (Haw.) Sternb. against *Plasmodium berghei*, Parasitol Res. 2013; 112(9):3123-3128.
27. Barliana MI, Suradji EW, Abdulah R, Diantini A, Hatabu T. Antiplasmodial properties of kaempferol-3-O-rhamnoside isolated from the leaves of *Schima wallichii* against chloroquine-resistant *Plasmodium falciparum*. Biomed Rep. 2014; 2(4):579-583.