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Effect of Methanolic Leaf Extract of *Carica papaya* on *Plasmodium berghei* Infection in Albino Mice

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Authors' contributions

This work was jointly carried out by both authors. Author IYL designed the study and wrote the first draft of the manuscript. Author EAA wrote the protocol, managed the literature search and analyses of the study. Both authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aim: The work was designed to investigate the antmalarial activity of methanolic leaf extract of *Carica papaya* on *Plasmodium berghei* NK65 strain infection *in vivo.*
Place and Duration of Study: Department of Biochemistry and National Institute of Pharmaceutical Research and Development, Abuja, Nigeria, between August and October 2016.
Materials and Method: Twenty five mice were intraperitoneally infected with chloroquine sensitive *P. berghei* strain and shared into 5 equal groups. Group 1 mice were infected and administered only normal saline (negative control). Groups 2, 3 and 4 were treated, after infection, with 100, 200 and 400 mg extract/kg body weight of mouse respectively while group 5 was treated with 5 mg chloroquine /kg body weight. The phytochemical constituents of the plant extract were evaluated.
Result: The extract produced a dose dependent decrease in the level of parasitaemia when compared to the negative control group. Also, at doses of 200 mg/kg and 400 mg/kg, the extract produced increase in body weight and PCV of the infected mice as compared to mice in the negative control group. Phytochemical screening showed that the leaf extract contains alkaloids, anthraquinones, tannins, flavonoids, saponins cardiac glycosides and steroids.
Conclusion: the methanolic leaf extract of *Carica papaya* presented a good effect on *Plasmodium infection* in mice and so could serve as a possible source of antimalarial compounds.

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1. INTRODUCTION

Malaria is still considered a major public health problem in the 106 countries where the risk of contracting the infection with one or more of the *Plasmodium species* exists one of the serious health problems in most of the tropical countries [1]. Malaria is endemic throughout most of the tropics. Ninety-five countries and territories have ongoing transmission. Of the approximately 3.2 billion people living in malarious countries, 1.2 billion are at high risk; the World Health Organization (WHO) states that there were 214 million (range 149 to 303 million) cases of symptomatic malaria in 2015 [2]. It is the leading cause of morbidity and mortality in sub-Saharan Africa, especially in young children and pregnant women [3], with an estimated 429 000 malaria deaths (range 235 000–639 000) worldwide in 2015 [4]. The attack of malaria during pregnancy usually results in severe anemia and impairment of fetal nutrition which contribute to the low birth weight, premature delivery, mental retardation and 60% miscarriages. In 2015, there were an estimated 438 000 malaria deaths worldwide. Most of these deaths occurred in the African Region (90%), followed by the South-East Asia Region (7%) and the Eastern Mediterranean Region (2%) [2].

Malaria is caused by protozoan of genus *Plasmodium* transmitted to the vertebrate by female Anopheles mosquitoes. In the vertebrate host, the sexual blood forms of the parasite are the life cycle stage that is responsible for the morbidity and mortality of plasmodial infections [5]. *P. falciparum* is the most virulent parasite, and is responsible for the majority of malaria related morbidity and mortality [2]. Antimalarial drug resistance has become one of the greatest challenges against malaria control. Resistance to antimalarial drugs has been described for two of the four species of malaria parasites that naturally infect humans, *P. falciparum* and *P. vivax. P. falciparum* has developed resistance to nearly all antimalarial drug in use [3]. With the problems of increasing levels of drug resistance, alternative medicine could be an important and sustainable source of treatment [6]. In Africa, the use of Indigenous medicinal plants with traditional reputation still plays an important role in malaria treatment serving as interesting sources for the detection of novel antiplasmodial compounds [7]. There is so much contribution in the area of plant study in search for potent antimalarial agents [8-11]. In furtherance of these efforts, this research was designed to examine the antimalarial effect of methanolic leaf extract of *Carica papaya* on the strain of *Plasmodium berghei* infection in albino infected mice. *P. berghei* infection of laboratory mouse strains is frequently used in research as a model for human malaria [12].

2. MATERIALS AND METHODS

2.1 Plant Collection, Preparation and Extraction

Fresh leaves of *Carica papaya were* collected from Faringada, Jos, Plateau State, Nigeria. The collection was identified and authenticated.

The fresh leaves were washed with clean-water, air dried and grounded using a grinder mill (GTEK, China). Extraction of plant material was performed by soxhlet apparatus (Sigma-Aldrich, USA) using methanol. The extract was concentrated in a water bath and stored in a refrigerator until required. About 50 g of the extract was put into a 500 ml conical flask and soaked in 70% Methanol. This was then left to stand overnight and mixed thoroughly for 3 hours on a mechanical shaker (Celltron, Switzerland). The content was filtered using a non-absorbent cotton wool on a Buchner funnel-flask using a vacuum pump. The residue was subjected to several parts of rinsing and filtration with fresh solvents to attain some level of exhaustive maceration. The filtrate was concentrated by evaporating to dryness using a rotary evaporator (Sigma-Aldrich, USA).

The dried extract was used for phytochemical analysis and anti-plasmodial assay in experimental albino mice.

2.2 Phytochemical Analysis for the Plant Extracts

The methanolic extract was subjected to phytochemical screening to detect the presence or absence of plant secondary metabolites: saponins, tannins, alkaloids, flavonoids, steroids, anthraquinones, and cardiac glycosides according to the method of Trease and Evans [13].

2.3 Parasite Inoculums

Plasmodium berghei NK65 strain infected erythrocytes were obtained from a donor-infected mouse maintained at animal facility center, NIPRD, Abuja, Nigeria. The inoculum was prepared by determining both the percentage parasitemia and the erythrocytes count of the donor mouse and then diluting with normal saline.

2.4 Experimental Animal and Curative Test

The twenty five (25) Swiss albino mice weighing between 20-25 g used in this study were obtained from animal house of University of Jos Jos, Plateau State Nigeria. They were kept in plastic cages with saw dust bed and given standard laboratory chore and water *ad-libitum.* They were then allowed to acclimatize for two weeks to their new environment before the initiation of the experiments.

In order to evaluate the curative potential of the crude extract, methods described [14,15] in literature were adopted. Each mouse in the treatment group was inoculated intraperitonealy with infected blood suspension (0.2 ml) containing about $1x10^7$ *Plasmodium berghei* parasitized red blood cells on day zero. Groups 2,3 and 4 were dosed once daily for five days with 100, 200 and 400 mg/kg/day of the methanolic leaf extract respectively. Chloroquine diphosphate (5 mg/kg body weight/day) were administered to group 5 mice and 0.2 ml normal saline to group 1 mice (negative control group). All treatments were orally done for five consecutive days from when parasites were first seen in the infected animal blood.

2.5 Parasitemia Count

On each day of treatment, a drop of blood was collected from each infected mouse for parasitemia screening by tail nip. The blood collected was placed on a slide and smeared to make a thick film, fixed with methanol and stained with Giemsa stain. When dried, the film was microscopically viewed by adding a drop of immersion oil and viewing it under x100 magnification of the microscope. The parasitemia density was examined by counting the parasitized red blood cell.

2.6 Determination of Packed Cell Volume

Capillary tubes were filled with blood to about 1 cm or two-third (2/3) of its length and the vacant end of each sealed with plasticin to protect the blood from spilling. The tubes were placed in haematocrit centrifuge with sealed side towards the periphery and then centrifuge for 5-6 minutes. The packed cell volume was read directly from haematocrit reader.

3. RESULTS

3.1 Extract Yield

The percentage yield of the leaf extract *wa*s shown in Table 1. The yield of methanol leaves extract of *C. papaya* was 18%. Phytochemicals Table 2 shows the result of qualitative phytochemical composition of methanol leaf extract of *Carica papaya*. The results revealed the presence of alkaloids, anthraquinones, tannins, flavonoids, saponins cardiac glycosides and steroids.

Table 1. The percentage (%) yield of methanolic leaves extract of *Carica papaya*

Carica papaya	Weight (g)
Leaf powder	100.00
Methanol extract	18.00
Extract yield (%w/w)	18.00

Table 2. Phytochemical composition of methanolic leaf extract of *Carica papaya*

Phytochemicals	Inference
Alkaloids	+++
Cardiac glycosides	++
Anthraquinones	++
Steroids	++
Tannins	++
sapomins	+++
flavonoids	++
Reducing sugars	++
Phlobatannins	-

Key: (-) absent, (+) slightly present, (++) moderately Present, (+++) highly present

3.2 Parasitaemia Count

The average daily parasitaemia level of the *Plasmodium berghei* in infected mice treated with methanolic leaf extract of *Carica papaya* are shown in Fig. 1. The average daily parasitaemia of infected mice treated, respectively, with chloroquine, 400 mg/kg and 200 mg/kg leaf extract of *Carica papaya* significantly ($P<0.05$) reduced when compared with control group. However there is no significant ($p>0.05$) difference in the level of parasitaemia in infected

mice treated with 100 mg/kg leaf extract of *Carica papaya* as compared with the control group.

3.3 Body Weight Changes

Effect of methanolic leaf extract of *Carica papaya* on body weight of *Plasmodium berghei* infected mice was shown in Fig. 2. The body weight of the infected untreated mice and infected treated with 100 mg/kg of *Carica papaya* showed significant decrease in body weight after 4 days post treatment. On the other hand, the infected mice treated with 200 mg/kg, 400 mg/kg of *Carica papaya* as well as those treated with 5 mg/kg chloroquine showed an increase in body weight after 4 days of treatment.

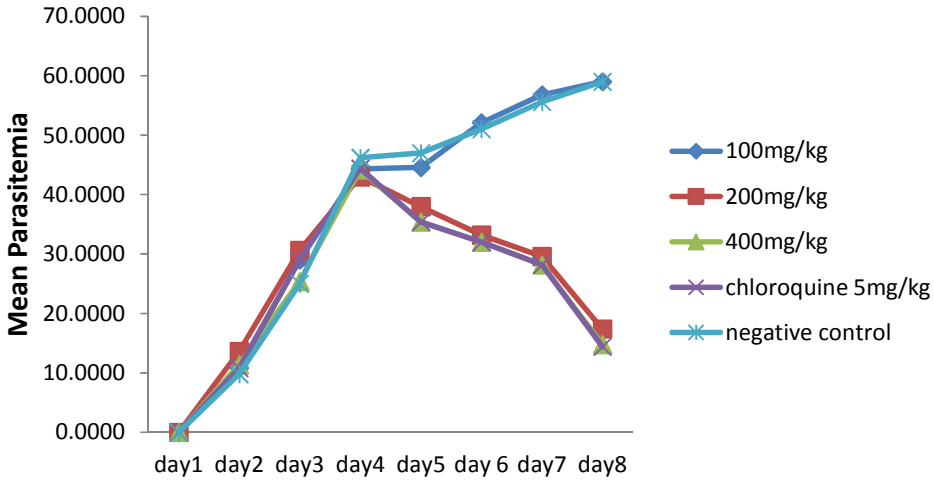

Fig. 1. *In vivo* antiplasmodial activity of methanolic leaf extract of *Carica papaya* against *Plasmodium berghei* in infected mice*:* Each point is a Mean ± SEM where n=5

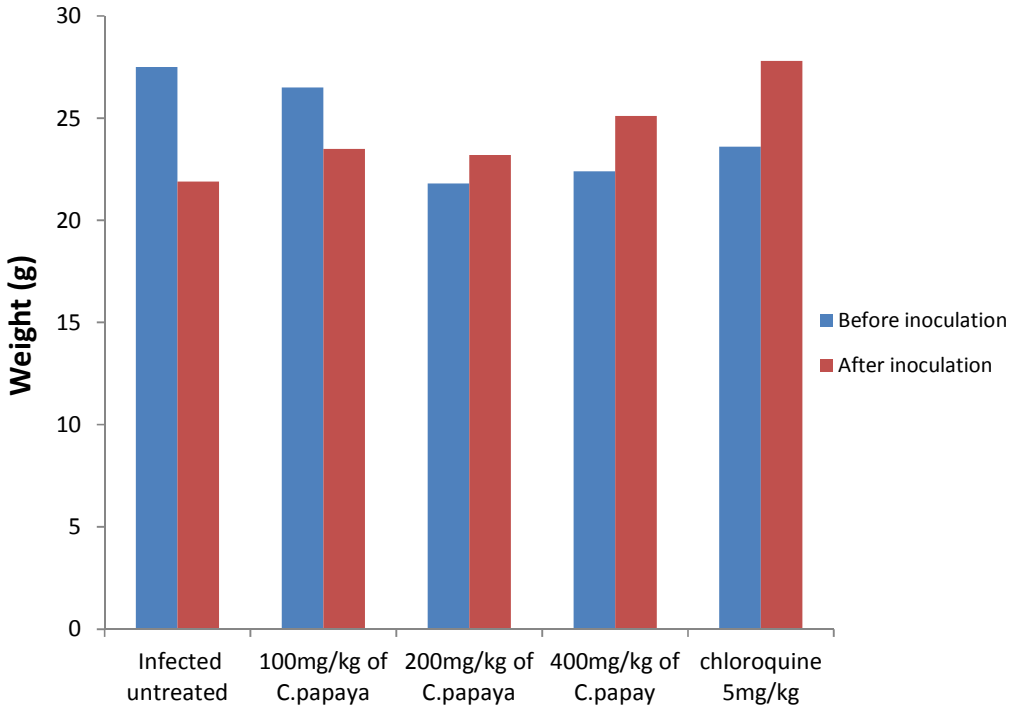

Fig. 2. Effect of methanolic leaf extract of *Carica papaya* on body weight of *P. berghei* infected mice. Values are Mean where n = 5

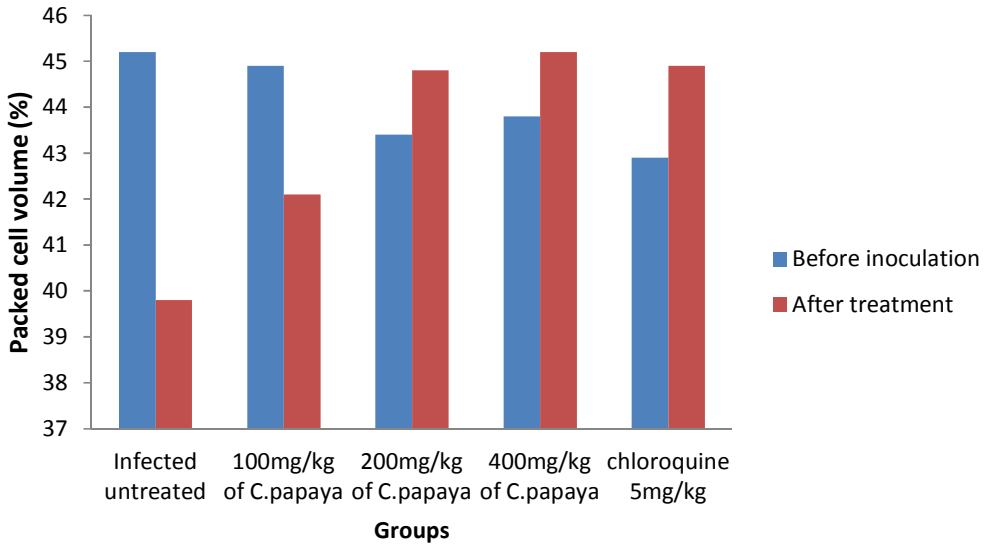

Fig. 3. Effect of methanol leaves extract of *Carica papaya* on PCV of *P. berghei* infected mice

3.4 Packed Cell Volume

Effect of methanolic leaf extract of *Carica papaya* on PCV of *Plasmodium berghei* infected mice is shown in Fig. 3. The PCV of *P. berghei* infected untreated mice and infected treated with 100 mg/kg of *Carica papaya* showed significant decrease in PCV after 4 days of treatment. On the other hand, the infected mice treated with 200 mg/kg, 400 mg/kg of *Carica papaya* leaf extract as well as those treated with 5 mg/kg chloroquine showed significant increase in PCV after 4 days of treatment.

4. DISCUSSION

Plants used in treatment of diseases are said to contain active phytochemicals some of which are responsible for the plants' characteristic adours, pugencies and color while others give virtues as food, medicinal or poisonous [16]. This study revealed the presence of various medicinally important phytochemicals including alkaloids, anthraquinones, tannins, flavonoids, saponins cardiac glycosides and steroids in methanolic leaf extract of *Carica papaya*. This is in line with reports by a number of authors that pharmacologically active phyto compounds like alkaloids, phenolics, flavonoids and also, amino acids s are available in aquous leaf extract[17]; aqueous extract of *Carica papaya* revealed the presence of flavonoids, alkaloids, steroids, monosaccharides, reducing sugars, phlabotannins, free anthraquinones and glycosides while saponins [18]; The qualitative phytochemical analysis of *Carica papaya* leaves showed the presence of alkaloid, flavonoid, Saponin, Tannin and Glycosides [19]. Flavonoids have been reported to have exhibited significant *in vitro* antimalarial activity against *P. falciparum* [20]. This could justify the antimalarial activities exhibited by the plant extract.

The 400 mg/kg and 200 mg/kg methanolic leaf extracts showed a dose dependent and progressive reduction in parasitaemia with time. This finding agrees well with earlier reports of studies using different solvents. Antiplasmodial activity was observed in the ethyl acetate crude extract of C*. papaya* against *P. falciparum* [21]*;* individual administration of aqueous leaf extract of *C. papaya*, *V. amygdalina*, and the combination of both plants significantly ($P < 0.05$) decreased parasite load in mice and enhanced their survival [22]; Methanolic extract of *C. papaya* at 200mg/kg body weight gave significant suppression ($p<0.05$) of parasitemia following five days administration in established infection [23]. This is a very promising feature in the potentials of *Carica papaya* as an antimalarial agent. Good enough, the antilamalarial effect demonstrated by *Carica papaya* leaf extract compared well with chloroquine treatment. Chloroquine has been used as the standard antimalarial drug because of its established activities on *P. berghei* [24]. Anemia, body weight loss and body temperature reduction are the general features of malaria infected mice [25]. So an ideal antimalarial agents obtained from plants are expected to prevent body weight loss in

infected mice [26]. In the present study, extract of *C. papaya* significantly prevented weight loss associated with increase in parasitemia level. The significant increase in level of PCV and body weight in mice treated with *Carrica papaya at* 200 and 400 mg/kg when compared with the negative control group is an indication of ameliorating potentials of the plant extract on the anaemia induced by the malarial infection.

5. CONCLUSION

This indicates that *C. papaya* contains important phytoconstituents that could be implicated in the observed antimalarial effect of the plant. However, the active compound (s) known to give this observed activity need to be identified and nature elucidated.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All experiments have been examined and approved by the University of Jos ethics committee with approval number UJ/EC/2016/015.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Autino B, Noris A, Russo R, Castelli F. Epidemiology of malaria in endemic areas. Mediterr J Hematol Infect Dis. 2012;4(1): e2012060.
   DOI: 10.4084/MJHID.2012.060
2. World Health Organization. Malaria Fact Sheet Report; 2015.
   Available:http://www.who.int/malaria/publications/world_malaria_report_2015/report/en/
   (Accessed on October 29, 2016)
3. World Health Organization. Antimalarial drug resistance; 2014.
   Available:http://www.who.int/malaria/areas/drug_resistance/overview/en/.
   (Accessed on October 29, 2016)
4. World Health Organization. Fact sheet: World Malaria Report; 2016.
   Available:http://www.who.int/malaria/media/world-malaria-report-2016/en/
   (Accessed on June 22, 2017)
5. Ahmed KB. Antibody responses in *Plasmodium faciparum* malaria and their relation to protection against the disease. A thesis from department of immunology, the wenner-green institute stockholm university, stockholm, Sweden; 2004.
6. Wilcox ML, Bodeker G. Traditional herbal medicine for malaria. BMJ. 2004;329: 1156-1159.
7. Hilou A, Nacoulma OG, Guiguemde TR. *In vivo* antimalarial activities of extracts from *Amaranthus spinosus* L. and *Boerhaavia erecta* L. in mice. J. Ethnopharmacol. 2006;103:236-240.
8. Tona L, Mesia K, Musuamba CT, De Bruyne T, Apers S, Hernans N, Van Miert S, Pieters L, Totté J, Vlietinck AJ. *In vitro* antiplasmodial activity of extracts and fractions from seven medicinal plants used in the Democratic Republic of Congo. Journal of Ethnopharmacology. 2004; 93(1):27-32.
9. Iwalokun BA. Enhanced antimalarial effects of chloroquine by aqueous *Vernonia amygdalina* leaf extract in mice infected with chloroquine resistant and sensitive *Plasmodium berghei* strains. African Health Science. 2008;8(1):25-35.
10. Deressa T, Mekonnen Y, Animut A. *In Vivo* anti-malarial activities of *Clerodendrum myricoides, Dodonea angustifolia* and *Aloe debrana* against *Plasmodium berghei.* Ethiopian J. Health Dev. 2010;24(1):25-29.
11. Ajayi EIO, Adeleke MA, Adewumi TY, Adeyemi AA. Antiplas modial activities of ethanol extracts of *Euphorbia hirta* whole plant and *Vernonia amygdalina* leaves in *Plasmodium berghei* -infected mice. Journal of Taibah University for Science.
    Available:https://doi.org/10.1016/j.jtusci.2017.01.008
12. Craig AG, Grau GE, Janse C, Kazura JW, Milner D, Barnwell JW, Turner G, Langhorne J. The role of animal models for research on severe malaria. PLOS Pathogens. 2012;8(2):e1002401.
    DOI: 10.1371/journal.ppat.1002401
13. Trease E, Evans WC. Pharmacognosy. Billiare tindall London. 13th Edition. 1987; 21:61-62.
14. Akuodor GC, Idris UI. Anti-nociceptive, anti-inflammatory and antipyretic effect of the methanolic extract of *Bombax*

buonopozense leaves in rats and mice. Afr. J. Biotechnology. 2011;10:3191-3196.
15. Ryley J, Peters W. The antimalarial activity of some quinoline esters. Ann Trop Med Parasitology. 1995;84(22):209–2.
16. Evans WC, Evans T. Pharmalognosy (15th edition) W.B Saunders company LTD. London. 2002;191-393.
17. Akhila S, Vijayalakshmi NG. Phytochemical Studies on *Carica papaya* leaf juice. IJPSR 2015;6(2):880-883.
18. Biu AA, Buratai LB, Ahmad AA, Hambali IU, Ngulde SI, Zakariah M, Lawal JR. Hytochemistry, toxicity and efficacy of crude aqueous extract of *Carica papaya* leaf against *Trypanosoma brucei*. Bangl. J. Vet. Med. 2016;14(1):99-102.
19. Adachukwu IP, Ogbonna AO, Eze FU. Phytochemical analysis of *Carica papaya* leaves. International Journal Life Science Biotechnology & Pharma Research; 2013. Available:http://new.ijlbpr.com/ijlbpradmin/upload/ ijlbpr_51d451cde89e7.pdf
20. Chanphen R, Thebtaranonth Y, Wanauppathamkul S, Yuthavong Y. Antimalarial principles from *Artemisia indica*. Journal of Natural Products. 1998;61:1146-1147.
21. Melariri P, Campbell W, Etusim P, Smith P. Antiplasmodial properties and bioassay-guided fractionation of ethyl acetate extracts from *Carica papaya* leaves. Journal of Parasitology Research; 2011. Available:http://dx.doi.org/10.1155/2011/104954
22. Okpe O, Habila N, Ikwebe J, Upev VA, Okoduwa SR, Isaac OT. Antimalarial potential of *Carica papaya* and *Vernonia amygdalina* in mice infected with *Plasmodium berghei*. Journal of Tropical Medicine. 2016;6. (Article ID 8738972) Available:https://www.ncbi.nlm.nih.gov/pmc/articles/ PMC5153544/
23. Arise RO, Malomo SO Lawal MM. Comparative Antimalarial and Toxicological effects of Artemisinin with methanolic extract of *Carica papaya* leaves and bark of *Alstonia broonai* in animal models. Advances in Natural and Applied Sciences. 2012;6(2):116-123.
24. Ajaiyeoba E, Falade M, Ogbole O, Okpako L, Akinboye D. *In vivo* antimalarial and cytotoxic properties of *Annona senegalensis* extract. African Journal of Traditional Medicine. 2006;3(1):137-141.
25. Langhorne J, Quin SJ, Sanni LA. Mouse models of blood-stage malaria infections: Immune responses and cytokines involved in protection and pathology. In: Perlmann P, Troye-Blomberg M, editor. Malaria Immunology. Stockholm: Karger Publisher. 2002;204–228.
26. Bantie L, Assefa S, Teklehaimanot T, Engidawork E. *In vivo* antimalarial activity of the crude leaf extract and solvent fractions of *Croton macrostachyus* Hocsht. (Euphorbiaceae) against *Plasmodium berghei* in mice. BMC Complement Altern Med. 2014;14:79. DOI: 10.1186/1472-6882-14-79

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