Malaria Control & Elimination
ISSN: 2470-6965

Somsak et al., Malar Cont Elimination 2015, 4:2
DOI: 10.4172/2470-6965.1000131

Research Article Open Access

Antimalarial Activity of Ethanolic Leaf Extract of *Bauhinia strychnifolia* in Mice Infected with *Plasmodium berghei*

Voravuth Somsak*, Jariya Noilod, Sukanya Chachiyo, and Sawanee Kraithep

Department of Clinical Chemistry, Faculty of Medical Technology, Western University, Kanchanaburi 71170, Thailand

Abstract

The emerging resistance of *Plasmodium* species to currently available antimalarial drugs remains a public health problem, hence the need for new effective, safe and affordable drugs. Plant extracts remain a reliable source of drugs. *Bauhinia strychnifolia* (Yha-nang dang) is widely distributed and has been traditionally used in Thailand to treat fever, alcoholic toxication and allergy. The leaf extract of *B. strychnifolia* had potent antioxidant, anti-cancer and anti-microbial activities. The aim of the study was at investigating antimalarial activities of *B. strychnifolia* in malaria infected rodent models. Acute toxicity of ethanolic crude extract of *B. strychnifolia* leaves was assessed in mice up to a dose of 6,000 mg/kg. ICR mice were inoculated with *Plasmodium berghei* ANKA and treated with the extracts (500, 1500 and 3000 mg/kg) orally by gavage. Four day suppressive and curative effect against established infection and prophylactic models of antimalarial studies were carried out. The extract did not show any toxic effect because doses up to 3,000 mg/kg caused no death or alter the behavior of the tested normal mice. For antimalarial studies, the extract (3,000 mg/kg) exerted significant ($P < 0.01$) effects of prophylactic, suppressive, and curative with percent inhibition of 59%, 84%, and 68%, respectively. However, antimalarial effect of chloroquine at 5 mg/kg was higher than the extract in all test models. These results indicate that ethanolic crude extract of *B. strychnifolia* leaves has excellent *in vivo* antimalarial activities against *P. berghei* ANKA. Hence, this plant extract represents a promising source of new antimalarial agents.

Keywords: Antimalarial; *Bauhinia strychnifolia*; *Plasmodium berghei*

Introduction

Malaria is an endemic infectious disease that is wide spread in tropical and sub-tropical areas of the world and one of the six most important parasitic disease of human [1]. It is a major public health problem in sub-Saharan Africa, where over 85-90% of all global burden of malaria exists with up to 50% of all outpatient visit in areas with high malaria transmission and 30-50% of all hospital admission are attributed to malaria [2]. Each year 1.1 million people die with this disease. Approximately 1 million deaths are in Africa and an estimate 700,000 of them are children. Although an effective vaccine is the best long term control for malaria, current research on vaccine development is still at pre-clinical stage. Therefore, the strategy for malaria mainly focuses on antimalarial drugs capable of reducing or eliminating parasites [3]. However, antimalarial drug resistant *Plasmodium* species and the emergence of insecticide resistant Anopheles mosquitoes cause not only the spread of malaria to new areas but also its re-emergence in areas where it had previously been eradicated [4,5]. Hence, this has prompted research towards the discovery and development of new, safe and affordable antimalarial chemotherapies. In this respect, plant extracts are potential targets for research and development of alternative antimalarial drugs [6].

Bauhinia strychnifolia Craib (Leguminosea-caesalpiniodeae species) is well known in Thai as Yha-nang dang. It is widely distributed and has been traditionally used in Thailand to treat fever, alcoholic toxication, allergy and cancer [7]. Several reports showed that leaf extract of *Bauhinia strychnifolia* (*B. strychnifolia*) had potent antioxidant and free radical scavenging activities. Moreover, anti-cancer and anti-microbial properties of this plant extract have also been described [7,8]. However, antimalarial activity of *B. strychnifolia* leaf extract has not yet been reported. Hence, the aim of the study was to evaluate the antimalarial activity of ethanolic leaf extract of *B. strychnifolia* against *Plasmodium berghei* (*P. berghei*) infection in mice.

Materials and Methods

Plant material and preparation of aqueous crude extract

B. strychnifolia leaves were collected from Kanchanaburi province, Thailand, and authenticated by Dr. Sakaewan Ounjaijean, Faculty of Pharmacy, Payap University, and Chiang Mai, Thailand. The leaves were dried in hot air oven at 50°C, and ground to coarse powder in a mortar. Extraction was carried out by dispensing 100 g of dried powdered plant material in 1 L of 95% ethanol with continuous shaking for 72 h. This was followed with vacuum filtration and powdered crude extract was then obtained using a rotary evaporator. This powder crude extract of *B. strychnifolia* was stored at -20°C. Before use, powdered crude extract was freshly dissolved in tween 80 to obtain appropriate doses [7].

Experimental mice

Healthy ICR mice (female, 4 weeks old and 30-35 g weight) obtained from National Laboratory Animal Center, Mahidol University were used. The mice were conveniently housed under standard environmental condition at 22-25°C. All mice had *ad libitum* access to commercial feed pellets and clean water throughout the study. All animal experiments were approved and ratified by the Animal Ethic Committee, Faculty of Medicine, Chiang Mai University.

***Corresponding author:** Voravuth Somsak, Department of Clinical Chemistry, Faculty of Medical Technology, Western University, Kanchanaburi 71170, Thailand; Tel: +66898009939; E-mail: voravuthsomsak@gmail.com

Received June 21, 2015; **Accepted** July 08, 2015; **Published** July 15, 2015

Citation: Somsak V, Noilod J, Chachiyo S, Kraithep S (2015) Antimalarial Activity of Ethanolic Leaf Extract of *Bauhinia strychnifolia* in Mice Infected with *Plasmodium berghei*. Malar Cont Elimination 4: 131. doi: 10.4172/2470-6965.1000131

Acute toxicity test

Acute toxicity test of *B. strychnifolia* extract was carried out as previously described [9]. Groups of mice (5 mice of each) were given 500, 1500, 3000, and 6000 mg/kg body weight of the extract orally by gavage. The mice were then observed for signs of toxicity which include but not limited to paw licking, salivation, stretching of entire body, weakness, sleeping, respiratory distress, coma and death in first 4 h and subsequently daily for 7 days.

Rodent malaria parasite

P. berghei ANKA strain (PbANKA) was used and maintained in our laboratory by weekly serial passage of $1x10^7$ infected red blood cells (iRBC) in naïve mice. Parasite growth was daily measured by Wright stained thin blood smear under light microscope with 100X lens and reported the percentage of iRBC per 100 red blood cells (% parasitemia) as described below.

$$\% \text{ parasitemia} = \frac{\text{Number of infected erythrocytes}}{\text{Total number of erythrocytes}} \times 100$$

Antimalarial drug

Chloroquine (CQ) was used to study *in vivo* drug susceptibility of PbANKA. The drug was freshly prepared in distilled water and administered orally by gavage. Drug dose, expressed in mg/kg of body weight, was adjusted at the time of administration according to the weight of each mouse. The dose was based on the ED90 (5 mg/kg) of this drug on PbANKA infected mice [10].

Evaluation of prophylactic activity of *B. strychnifolia* (Repository test)

Evaluation of the prophylactic potential of *B. strychnifolia* leaf extract was carried out according to the method described previously [11]. The mice were randomly divided into 5 groups (5 mice of each) and treatment with the extract (500, 1500, and 3000 mg/kg) was initiated orally on day 0 and continued until day 4. Two control groups were used; the positive control group was treated daily with 5 mg/kg of CQ while tween 80 was given to the negative control group. On day 5 of the experiment, mice were injected intraperitoneally with 1×10^7 infected erythrocytes of PbANKA. Seventy two hours later, blood was collected from tail vein and parasitemia was examined by microscopic examination of Giemsa stained thin blood smear. Moreover, average percentage of inhibition was calculated as showed below.

$$\% \text{ inhibition} = ((A\text{-}B) \div A) \times 100$$

Where A is the average % parasitemia in negative control group, and B is the average % parasitemia in the test groups.

Suppressive test of *B. strychnifolia* (4-day suppressive test)

The standard 4-day suppressive test against PbANKA was employed [12]. ICR mice were inoculated by intraperitoneal injection with 1×10^7 infected erythrocytes of PbANKA. The mice were randomly divided into 5 groups (5 mice of each) and treated for 4 consecutive days with 500, 1500, and 3000 mg/kg of the extract orally. Two control groups were used; the positive control was treated daily with 5 mg/kg of CQ while the negative control group was given tween 80. On day 5 of the experiment, blood was collected from tail vein of each mouse and microscopic examination of Giemsa stained thin blood smear was performed to examine parasitemia. Moreover, percentage of inhibition was also calculated as previously described.

Evaluation of curative activity of *B. strychnifolia* (Rane's test)

Evaluation of the curative potential of *B. strychnifolia* leaf extract was carried out according to the method described previously [13]. The mice were injected intraperitoneally with 1×10^7 infected erythrocytes of PbANKA on the first day (day 0). Seventy two hours later, the mice were divided into 5 groups (5 mice of each). The groups were orally treated with the extract (500, 1500, 3000 mg/kg), CQ (5 mg/kg) was given to the positive control and tween 80 was given to the negative control group. The treatment was carried out once daily for 5 days and parasitemia was then examined by microscopy of Giemsa stained thin blood smear. Moreover, percentage of inhibition was also calculated as previously described.

Statistical analysis

Statistical analysis of the data was performed using GraphPad Prism Software (GraphPad Software, Inc., USA). The one way ANOVA test was used to analyze and compare the results at a 95% confidence level. Values of $p< 0.05$ were considered significant. Results were expressed as mean ± standard error of mean (SEM).

Results

Acute toxicity test

Behavioral signs of toxicity observed in mice given 6,000 mg/kg of extract include; paw licking, salivation, stretching and reduce activity However, the mice survived. Moreover, oral administration of the ethanolic extract of *B. strychnifolia* at the doses of 500, 1500, and 3000 mg/kg had no toxic effects throughout the 7-day study period. None of the mice showed any signs of toxicity, such as change of skin, eyes and mucus membranes, behavioral patterns, trembling, diarrhea, falling of the fur, sleep or coma. In addition, no significant changes were observed in their body weight. Hence, 500, 1,500, and 3,000 mg/kg of extract were suitable doses for using in this study.

Blood stage propagation of PbANKA infection in mice

For evaluation of blood stage growth of PbANKA, mice were infected intraperitoneally with $1x10^7$ infected erythrocytes of PbANKA, and parasitemia was daily monitored by microscopy of Giemsa stained thin blood smear. As showed in (Figure 1A), parasitemia was first detectable on day 2 post-infection with a parasitemia less than 1%, and maximum parasitemia was then observed with a parasitemia of 70% on day 14 post-infection. Moreover, infected mice died within 14 days (Figure 1B).

Prophylactic activity of ethanolic leaf extract of *B. strychnifolia*

In order to evaluate protective effect of *B. strychnifolia* leaf extract, prophylactic activity test was done as previously described. The ethanolic leaf extract of *B. strychnifolia* exerted significant ($p<0.01$) reduction in level of parasitemia at 3,000 mg/kg with an inhibition rate of 59% (Figure 2). However, the extract at doses of 500 and 1,500 mg/kg had no activity to reduce in level of parasitemia. Moreover, the standard antimalarial drug, CQ caused reduction of parasitemia significantly ($p<0.01$) with 90% inhibition, which was higher than those of the extract treated groups (Figure 2).

Suppressive activity of ethanolic leaf extract of *B. strychnifolia*

Standard 4-day suppressive test was used in order to evaluate chemosuppressive effect of *B. strychnifolia* leaf extract against PbANKA infected mice. As showed in (Figure 3), the extract exerted

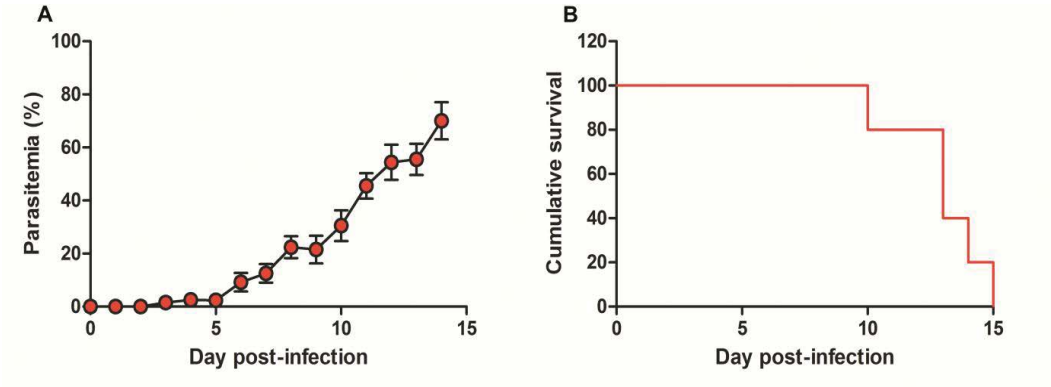

Figure 1: Blood stage propagation of *Plasmodium berghei* ANKA infection in mice. ICR mice (5 mice of each) were infected intraperitoneally with 1×10^7 infected erythrocytes of PbANKA. (a) Parasitemia was daily monitored by microscopy of Giemsa stained thin blood smear and (b) cumulative survival of infected mice was also determined. Results were expressed as mean ± SEM.

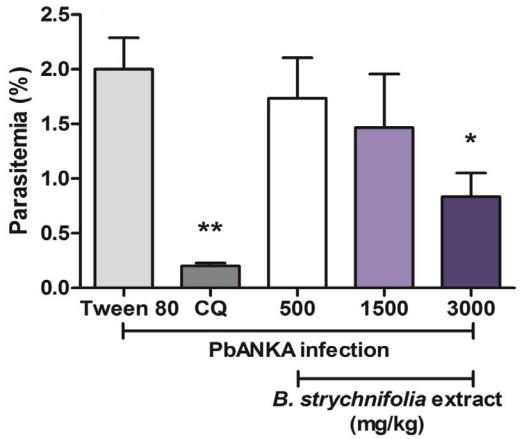

Figure 2: Prophylactic activity of ethanolic crude extract of *Bauhinia strychnifolia* leaves against *Plasmodium berghei* ANKA infected mice. Groups of mice (5 mice of each) were treated with the extracts (500, 1500, and 3000 mg/kg) orally once a day for 4 consecutive days, and subsequently infected intraperitoneally with 1×10^7 infected erythrocytes of PbANKA. CQ (5 mg/kg) was used as positive control, and negative control group was treated with tween 80. Seventy two hours later, parasitemia was measured by microscopy of Giemsa stained thin blood smear. Results were expressed as mean ± SEM. *p<0.05 and **p< 0.01 compared to negative control group.

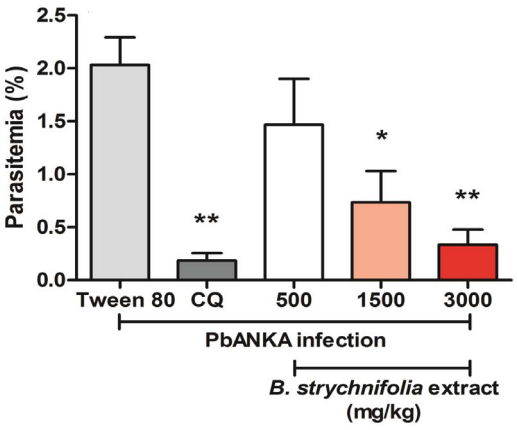

Figure 3: Suppressive test of ethanolic crude extract of *Bauhinia strychnifolia* leaves against *Plasmodium berghei* ANKA infected mice. Groups of mice (5 mice of each) were infected intraperitoneally with 1×10^7 infected erythrocytes of PbANKA, and subsequently treated with 500, 1500, and 3000 mg/kg of the extract orally once a day for 4 consecutive days. Parasitemia was then measured by microscopy of Giemsa stained thin blood smear. Results were expressed as mean ± SEM. *p<0.05 and **p<0.01 compared to negative control group.

dose dependent suppressive effect against PbANKA and significances were also observed with the doses of 1,500 and 3,000 mg/kg (p<0.05 and p<0.01, respectively) when compared to the negative control with inhibition rate of 64% and 84%, respectively. No suppressive effect was found in infected mice treated with 500 mg/kg of the extract. Moreover, 5 mg/kg of CQ showed significant (p<0.01) suppression at 91% inhibition, which was higher than those of the extract treated groups (Figure 3). However, there was an increase in parasitemia in the negative control group.

Curative activity of ethanolic leaf extract of *B. strychnifolia*

In order to evaluate curative activity of *B. strychnifolia* leaf extract, curative activity test *in vivo* against PbANKA was carried out. It was observed that the ethanolic leaf extract of *B. strychnifolia* reduced in parasitemia levels in the extract treated groups. The significant (p<0.01) reduction in parasitemia level was found in infected mice treated with 3,000 mg/kg of the extract with 68% inhibition, compared to the negative control group (Figure 4). However, no curative effect was observed in infected mice treated with 500 and 1,500 mg/kg of the extract. Moreover, standard antimalarial drug, CQ (5 mg/kg) exerted significantly (p<0.01) curative activity against PbANKA infected mice with 89% inhibition, while increasing of parasitemia was found in the negative control group.

Discussion

The in vivo antimalarial activities of the ethanolic crude extract of *B. strychnifolia* leaves were investigated by evaluating the prophylactic, suppressive and curative properties using standard animal model. In vivo models are usually employed in antimalarial studies because they take into account the possible prodrug effect and probable involvement of the immune system in eradication of the pathogen [14]. Plasmodium species that cause human disease are essentially unable to infect

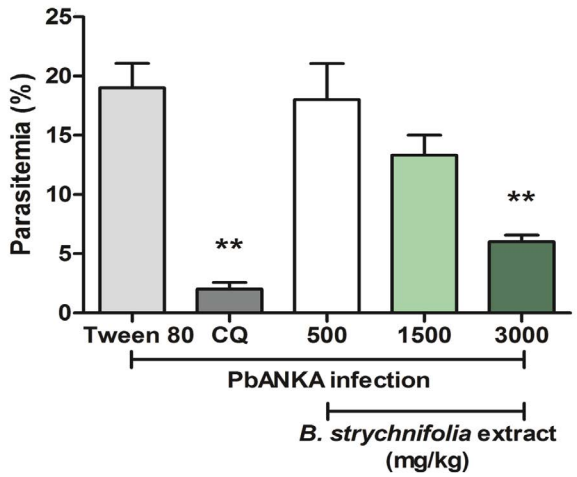

Figure 4: Curative effect of ethanolic crude extract of *Bauhinia strychnifolia* leaves against *Plasmodium berghei* ANKA infected mice. Groups of mice (5 mice of each) were infected intraperitoneally with $1x10^7$ infected erythrocytes of PbANKA. Seventy two hours later, infected mice were treated orally with 500, 1500, and 3000 mg/kg of the extract once a day for 4 consecutive days. Parasitemia was then measured by microscopy of Giemsa stained thin blood smear. Results were expressed as mean ± SEM. $^{**}p<0.01$ compared to negative control group.

non-primate animal models. Therefore, for the in vivo evaluation of antimalarial activities in rodents, the rodent malaria parasite is employed, due to the sensitivity of *P. berghei* ANKA parasite to CQ [15]. The repository test was used to study the prophylactic activity, while Peter's 4-day suppressive test was used to evaluate schizontocidal activity. Rane's test was used to study curative ability during established infection. In all methods, determination of inhibition rate of parasitemia was the most reliable parameter. Plant products are frequently considered to be less toxic and have fewer adverse effects than synthetic ones. Traditional Thai medicine has been used in clinical practice for several centuries. However, the compounds and precise mechanisms of most plant medicine remain to be determined.

In this study, the ethanolic crude extract of *B. strychnifolia* leaves did not show any toxic effects because doses up to 3,000 mg/kg caused no any death or alter the behavior of the tested normal mice. It can be extrapolated that the median lethal dose is thus greater than 3,000 mg/kg making *B. strychnifolia* safety to be between slightly toxic to non-toxic. It was also reported that oral administration is about 100 times less toxic than the intraperitoneal [16,17]. This extract is therefore safe and this could explain the safe use of this plant by the local people who have been using it in traditional management of malaria in Thailand.

It has been found that ethanolic leaf extract of *B. strychnifolia* presented antimalarial activities including prophylactic, suppressive and curative effects against PbANKA infected mice. At the highest dose (3,000 mg/kg), *B. strychnifolia* extract exhibited an *in vivo* suppression of parasitemia of 84%, prophylactic activity of 59%, and curative effect of 68%, suggesting that this extract has very good activity. For solvent extraction of B. strychnifolia, ethanol was found to be an excellent solvent for extraction as indicated by high value of total polyphenolic content and strong antioxidant activity [8]. The ethanolic crude extract of *B. strychnifolia* leaves were reported to contain different classes of metabolites such as polyphenol, flavonoids, diterpenoids, gallic acid, tannins, alkaloids, saponin and kaempferol rhamnoside [7]. Diterpenoids, flavonoids, saponin, alkaloid and kaempferol rhamnoside were known to have antimalarial activities [18-22]. Moreover, polyphenol in this plant extract which has antioxidant effect may also contribute to the antimalarial activity [23-25]. It has been reported that antioxidant activity can inhibit heme polymerization and the unpolymerized heme is very toxic for the *Plasmodium* species [26]. Hence, it might be due to the fact that antimalarial activity against PbANKA infection in mice of *B. strychnifolia* extract might come from these active compounds. Therefore, the efficacy of ethanolic crude extract of *B. strychnifolia* leaves is promising. Moreover, low amounts of these active compounds in 500 and 1,500 mg/kg of the extracts might be a reason for no antimalarial *in vivo*. However, the active compounds are yet to be identified and there is need for the identification. In addition, CQ showed a strong antimalarial effect including prophylactic, suppressive, and curative activities compared to the extract treated groups and negative control.

For all results, it can be concluded that ethanolic crude extract of *B. strychnifolia* leaves presents antimalarial activities against PbANKA infection in mice. However, it can also be suggested for further studies that pure compounds should be studied in order to compare with crude extract. Furthermore, combination treatment with other standard antimalarial drugs such as pyrimethamine, quinine, and artemisinin should be done for developing this extract to be an alternative drug or supplement in malaria treatment in the future.

Acknowledgements

We thank Suthin Audomkasok for excellent assistance with animal experimentation. The authors gratefully acknowledge Dr. Sakaewan Ounjaijean for having provided us with technical advice for preparation of the extract. All animal experiments were performed at Clinical Chemistry Research room, Faculty of Medical Technology, western University in accordance with ethical guidelines.

References

1. Carter KH, Singh P, Mujica OJ, Escalada RP, Ade MP, et al. (2015) Malaria in the Americas: trends from 1959 to 2011. Am J Trop Med Hyg 92: 302-316.
2. World Health Organization. (2014) Malaria in Africa. Roll Back Malaria Infosheet.
3. Lüthi B, Schlagenhauf P (2015) Risk factors associated with malaria deaths in travellers: a literature review. Travel Med Infect Dis 13: 48-60.
4. Ojha PK, Roy K (2015) The current status of antimalarial drug research with special reference to application of QSAR models. Comb Chem High Throughput Screen 18: 91-128.
5. Wang SQ, Wang GZ, Li YC, Meng F, Lin SG, et al. (2015) Sensitivity of Plasmodium falciparum to antimalarial drugs in Hainan Island, China. Korean J Parasitol 53: 35-41.
6. Muregi FW, Chhabra SC, Njagi EN, Lang'at-Thoruwa CC, Njue WM, et al. (2003) In vitro antiplasmodial activity of some plants used in Kisii, Kenya against malaria and their chloroquine potentiation effects. J Ethnopharmacol 84: 235-239.
7. Yuenyongsawad S, Bunluepuech K, Wattanapiromsakul C, Tewtrakul S (2013) Anti-cancer activity of compounds from Bauhinia strychnifolia stem. J Ethnopharmacol 150: 765-769.
8. Kaewpiboon C, Lirdprapamongkol K, Srisomsap C, Winayanuwattikun P, Yongvanich, et al. (2012) Studies of the in vitro cytotoxic, antioxidant, lipase inhibitory and antimicrobial activities of selected Thai medicinal plants. BMC complementary and alternative medicine12: 217.
9. Lorke D (1983) A new approach to practical acute toxicity testing. Arch Toxicol 54: 275-287.
10. Franke-Fayard B, Djokovic D, Dooren MW, Ramesar J, Waters AP, et al. (2008) Simple and sensitive antimalarial drug screening in vitro and in vivo using transgenic luciferase expressing Plasmodium berghei parasites. Int J Parasitol 38:1651-1662.

11. Peters W (1965) Drug resistance in Plasmodium berghei Vincke and Lips, 1948. I. Chloroquine resistance. Exp Parasitol 17: 80-89.

12. Peters W (1975) 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 69: 155-171.

13. Ryley JF, Peters W (1970) The antimalarial activity of some quinolone esters. Ann Trop Med Parasitol 64: 209-222.

14. Waako PJ, Gumede B, Smith P, Folb PI (2005) The in vitro and in vivo antimalarial activity of Cardiospermum halicacabum L. and Momordica foetida Schumch. Et Thonn. J Ethnopharmacol 99: 137-143.

15. Builders MI, Uguru MO, Aguiyi C (2012) Antiplasmodial Potential of the African Mistletoe: Agelanthus dodoneifolius Polh and Wiens. Indian J Pharm Sci 74: 223-229.

16. Wernsdorfer WH, Ismail S, Chan KL, Congpuong K, Wernsdorfer G (2009) Activity of Eurycoma longifolia root extract against Plasmodium falciparum in vitro. Wien Klin Wochenschr 121 Suppl 3: 23-26.

17. Chan KL, Choo CY, Abdullah NR, Ismail Z (2004) Antiplasmodial studies of Eurycoma longifolia Jack using the lactate dehydrogenase assay of Plasmodium falciparum. J Ethnopharmacol 92: 223-227.

18. Walter NS, Bagai U, Kalia S (2013) Antimalarial activity of Bergenia ciliata (Haw.) Sternb. against Plasmodium berghei. Parasitol Res 112: 3123-3128.

19. Adelekan AM, Prozesky EA, Hussein AA, Ureña LD, van Rooyen PH, et al. (2008) Bioactive diterpenes and other constituents of Croton steenkampianus. J Nat Prod 71: 1919-1922.

20. Chen Y, Li S, Sun F, Han H, Zhang X, et al. (2010) In vivo antimalarial activities of glycoalkaloids isolated from Solanaceae plants. Pharm Biol 48: 1018-1024.

21. Gauthier C, Legault J, Lavoie S, Rondeau S, Tremblay S, et al. (2009) Synthesis and cytotoxicity of bidesmosidic betulin and betulinic acid saponins. J Nat Prod 72: 72-81.

22. Barliana MI, Suradji EW, Abdulah R, Diantini A, Hatabu T, et al. (2014) Antiplasmodial properties of kaempferol-3-O-rhamnoside isolated from the leaves of Schima wallichii against chloroquine-resistant Plasmodium falciparum. Biomed Rep 2: 579-583.

23. Addai FK (2010) Natural cocoa as diet-mediated antimalarial prophylaxis. Med Hypotheses 74: 825-830.

24. El Babili F, Bouajila J, Fouraste I, Valentin A, Mauret S, et al.(2010) Chemical study, antimalarial and antioxidant activities, and cytotoxicity to human breast cancer cells (MCF7) of Argania spinosa. Phytomedicine17: 157-160.

25. Sharma SK, Parasuraman P, Kumar G, Surolia N, Surolia A (2007) Green tea catechins potentiate triclosan binding to enoyl-ACP reductase from Plasmodium falciparum (PfENR). J Med Chem 50: 765-775.

26. Vial H (1996) Recent developments and rationale towards new strategies for malarial chemotherapy. Parasite 3: 3-23.