In vivo antimalarial activity of extracts of Tanzanian medicinal plants used for the treatment of malaria

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Abstract

Plants used in traditional medicine have been the source of a number of currently used antimalarial medicines and continue to be a promising resource for the discovery of new classes of antimalarial compounds. The aim of this study was to evaluate *in vivo* antimalarial activity of four plants; *Erythrina schliebenii* Harms, *Holarrhena pubescens* Buch-Ham, *Phyllanthus nummulariifolius* Poir, and *Caesalpinia bonducella* (L.) Flem used for treatment of malaria in Tanzania. *In vivo* antimalarial activity was assessed using the 4-day suppressive antimalarial assay. Mice were infected by injection via tail vein with 2×10^7 erythrocytes infected with *Plasmodium berghei* ANKA. Extracts were administered orally, once daily, for a total of four daily doses from the day of infection. Chloroquine (10 mg/kg/day) and solvent (5 mL/kg/day) were used as positive and negative controls, respectively. The extracts of *C. bonducella, E. schliebenii, H. pubescens*, and *P. nummuariifolius* exhibited dose-dependent suppression of parasite growth *in vivo* in mice, with the highest suppression being by *C. bonducella* extract. While each of the plant extracts has potential to yield useful antimalarial compounds, the dichloromethane root extract of *C. bonducella* seems to be the most promising for isolation of active antimalarial compound(s). *In vivo* antimalarial activity presented in this study supports traditional uses of *C. bonducella* roots, *E. schliebenii* stem barks, *H. pubescens* roots, and *P. nummulariifolius* for treatment of malaria.

Key words: *In vivo*, malaria, medicinal plants, *Plasmodium berghei*

INTRODUCTION

Malaria is a preventable and treatable parasitic disease caused by *Plasmodium* parasites that is transmitted to humans by infected mosquito vectors.[1] At present, medicinal plants are the source of effective antimalarial drugs, including artemisinins which are a core component in the currently used antimalarial combination therapies.[2]

Consequently, sustained *in vitro* and *in vivo* screening of medicinal plants for their antimalarial activity will help to provide scientific evidence for their use and contribute to the discovery of antimalarial lead molecules for future drug development.

To identify medicinal plants which would provide potential lead compounds, this study evaluated the extracts of *Erythrina schliebenii* Harms (*Fabaceae*), *Holarrhena pubescens* Buch-Ham (*Apocynaceae*), *Phyllanthus nummulariifolius* Poir (*Euphorbiaceae*), and *Caesalpinia bonducella* (L.) Flem (*Caesalpiniacea*) for *in vivo* antimalarial activity in mice infected with *Plasmodium berghei* ANKA.

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MATERIALS AND METHODS

Chemicals
Diethyl ether (Carlo erba®), ethanol (Carlo erba®), methanol (MeOH) (Carlo erba®), tween 80 (Sigma®), dichloromethane (DCM) (Carlo erba®), dimethylsulfoxide (Carlo erba®), and Giemsa-stain (Sigma) were purchased from Techno-Net Scientific (Dar es Salaam, Tanzania) whereas chloroquine diphosphate was purchased from Sigma (Sigma®, Steinheim, Germany).

Collection of plant materials and extraction
E. schliebenii Harms stem bark (Voucher no. 4661) and *H. pubescens* Buch-Ham roots (Voucher no. 4665) were collected from Lindi region, whereas *P. nummulariifolius* Poir whole plant (Voucher no. RN 30) and *C. bonducella* (L.) Flem roots (Voucher no. RN 93) were collected from Kagera and Dar es Salaam regions, respectively. The plants were identified by a botanist (Mr. Selemani Haji), and the voucher specimens are deposited in the Herbarium at Muhimbili University of Health and Allied Sciences.

Dry powdered plant materials were extracted by maceration using DCM, ethyl acetate (EtoAC), MeOH, or water. Liquid crude extracts were dried by rotary evaporation (at 50°C) to obtain dry crude extracts which were stored at −20°C until they were used.

In vivo antimalarial activity assay
In vivo antimalarial activity of the extracts was determined using the 4-day suppressive test described previously.[3]

Animals
Young adult Theiller's white albino mice, of both sexes, weighing 20–30 g were used. Animals were acclimatized to the laboratory conditions, supplied with food and water *ad libitum* for 5 days before being used for the test. The animals were handled according to the National and International Guidelines for Handling of Laboratory Animals, and the study received ethical clearance from the Institution review board of the Muhimbili University of Health and Allied Sciences.

Malaria parasites
Blood stage *P. berghei* ANKA parasites used in the study were kindly donated by Dr. Lindsay Stewart of the Department of Pathogen Molecular Biology, London School of Hygiene and Tropical Medicine, United Kingdom.

Preparation of infected red blood cells suspension
Donor mice with high parasitemia were anesthetized by diethyl ether; blood was collected by cardiac puncture and diluted with sterile normal saline (0.9% w/v sodium chloride) to make a suspension of 1×10^8 infected red blood cells (iRBCs) per mL, which was used to infect test mice.

Inoculation of parasites and administration of extracts
Each mouse was infected by injection via tail vein with 2×10^7 iRBCs in 0.2 mL suspension of 1×10^8 iRBCs per mL. The extracts were solubilized in 10% Tween 80/6% ethanol, 2% dimethylsulfoxide, or distilled water. Chloroquine was dissolved in normal saline. Three hours post infection, the mice were randomly allocated into groups of 6 mice each: Negative control group received solvent (5 mL/kg/day), positive control group received chloroquine (10 mg/kg/day), and treatment groups received different doses of extracts (200, 400, or 800 mg/kg/day). Dosing was done orally, once daily, starting on the day of infection and continued for a total of four daily doses. Body weight was recorded daily while parasitemia was determined on day 4.

Determination of parasitemia and percentage suppression at day 4
On day 4, tail blood smear was fixed with absolute MeOH and then stained with 10% Giemsa-stain in phosphate buffer (pH 7.2) for 20 min. The parasites were examined under microscope at ×100 oil immersion. Percentage parasitemia was determined by counting the infected erythrocytes in at least 1000 total erythrocytes (infected plus non-infected erythrocytes). The mean percentage suppression of parasitemia for each extract was calculated as follows:

$$\frac{(\text{Mean \% parasitemia in negative control mice} - \text{mean \% parasitemia in treated mice})}{(\text{Mean \% parasitemia in negative control mice})} \times 100.$$

Photographing the parasites
Images were taken with the aid of a light microscope fitted with a camera (Tension®, Axiom) and processed using Tucsen TSView software version 6.2.3.3 (Tucsen, 2013).

Statistical analysis
Percentage parasitemia suppression and survival time in days were presented as mean ± standard deviation for each group. The mean percentage parasitemia on day 4 and the mean survival time were analyzed statistically using the Student's *t*-test to identify the differences between treated groups and negative control group. The difference was considered statistically significant at $P < 0.05$.

RESULTS

The results of *in vivo* antimalarial activity revealed that *C. bonducella*, *E. schliebenii*, *H. pubescens*, and *P. nummulariifolius* exhibited dose-dependent suppression of parasite growth *in vivo* in mice. At a dose of 400 mg/kg/day, MeOH, EtoAC, and DCM extracts of *C. bonducella* roots suppressed the parasites growth by 11.36%, 20.01%, and

37.60%, respectively, while the DCM/MeOH (1:1) extract was found to be inactive [Table 1]. Chloroquine given at 10 mg/kg/day caused 100% suppression of parasitemia. Although all extracts of *C. bonducella* roots reduced parasite growth at this dose, only EtoAC and DCM extracts showed a statistically significant difference (*P* < 0.05) in mean percentage parasitemia as compared to the negative control group.

Among the extracts of *C. bonducella* roots tested at 400 mg/kg/day, the DCM extract had the highest activity with 37.60% parasite suppression [Table 1]. Further evaluation of this extract at three different doses revealed dose-dependent effect. It suppressed parasites growth *in vivo* by 20.70%, 35.3%, and 55.96% at 200, 400, and 800 mg/kg/day, respectively. The mean percentage parasitemia in each of the three groups was significantly different (*P* < 0.05) from the mean in the negative control group [Figure 1 and Table 2]. Similarly, the extracts of *E. schliebenii, H. pubescens*, and *P. nummulariifolius* exhibited dose-dependent suppression of parasitemia. The aqueous extract of *E. schliebenii* stem bark suppressed parasitemia by 24.0% at 400 mg/kg/day and by 28.64% at 800 mg/kg/day. The MeOH extract of *H. pubescens* roots suppressed parasitemia by 32.06% and 43.07% at 400 and 800 mg/kg/day, respectively, while the aqueous extract of *P. nummulariifolius* whole plant suppressed parasitemia by 9.28% and 29.22% at the dose of 400 and 800 mg/kg/day, respectively [Table 3]. With exception of the DCM extract of *C. bonducella* roots at 800 mg/kg/day,

all other extracts did not increase the mean survival time of the mice as compared to the negative control mice.

DISCUSSION

This study reports the *in vivo* antimalarial activity of the extracts of *E. schliebenii, H. pubescens, P. nummulariifolius*, and *C. bonducella* evaluated using the 4-day suppressive test. The results showed that the extracts of these plant species exhibited dose-dependent suppression of parasites growth in mice. The DCM extract of *C. bonducella* roots exhibited the highest *in vivo* antimalarial activity with 55.96% parasite suppression at 800 mg/kg/day compared to all extracts of the other three plant species tested [Table 2 and Figure 1]. The observed efficacy of DCM extract suggests that less to medium polar compounds present in this extract may be responsible for the antimalarial properties of the roots of *C. bonducella*. *C. bonducella* is a medicinal shrub with wide applications common in the tropics and subtropic areas.[4] The leaves of this plant are reported to be used for the treatment of malaria, diabetes, elephantiasis, splenomegaly, leprosy, and convulsions while the roots are used as anthelmintic, astringent, and for fever.[5,6] In previous phytochemical studies, several compounds including caesalpinin 1, caesaldekarin F and G, and demethylcaesaldekarin C have been reported from the roots of *C. bonducella*.[7] This group of cassane-type diterpenoid compounds has been reported to have *in vitro* antimalarial properties. For example, norcaesalpinin E from seeds of *C. crista* was reported to have good antimalarial activity with IC_{50} of 90 nM

Table 1: *In vivo* antimalarial activity of *Caesalpinia bonducella* root extracts at 400 mg/kg/day against *Plasmodium berghei* ANKA

Treatment	Mean percentage parasitemia at day 4±SD (*n*=6)	Mean percentage suppression of parasitemia at day 4	Mean survival time±SD (days)
Group I: Negative control (10% tween 80/6%EtOH)	35.48±4.59		8.0±0.8
Group II: DCM extract	22.14±4.27**	37.60	10.7±2.9
Group III: EtoAC extract	28.38±4.98*	20.01	10.5±3.7
Group IV: MeOH extract	31.45±2.37	11.36	9.3±0.6
Group V: DCM/MeOH (1:1) extract	36.08±5.44	0.00	7.5±1.5
CQ (10 mg/kg/day)	0.00	100	26.0±1.7**

*$P<0.05$, **$P<0.01$ compared with the mean percentage parasitemia in negative control. EtOH: Ethanol, DCM: Dichloromethane, EtoAC: Ethyl acetate, MeOH: Methanol, DCM/MeOH: Dichloromethane/methanol (1:1), SD: Standard deviation, *n*: Number of mice in each group, CQ: Chloroquine

Table 2: *In vivo* antimalarial activity of dichloromethane root extract of *Caesalpinia bonducella* at different doses against *Plasmodium berghei* ANKA

Treatment	Mean percentage parasitemia at day 4±SD (*n*=10)	Mean percentage suppression of parasitemia at day 4	Mean survival time±SD (days)
Group I: Negative control (2% DMSO)	32.95±4.07	0.00	8.1±2.1
Group II: 200 mg/kg/day)	26.13±3.39*	20.70	8.8±3.0
Group III: 400 mg/kg/day)	21.32±3.93**	35.30	11.7±2.7
Group IV: 800 mg/kg/day)	14.51±1.15**	55.96	13.6±3.8*
CQ 10 mg/kg/day	0.00	100	24.0±3.0**

*$P<0.05$, **$P<0.01$ compared with the mean percentage parasitemia in negative control. DMSO: Dimethylsulfoxide, DCM: Dichloromethane, SD: Standard deviation, *n*: Number of mice in each group, CQ: Chloroquine
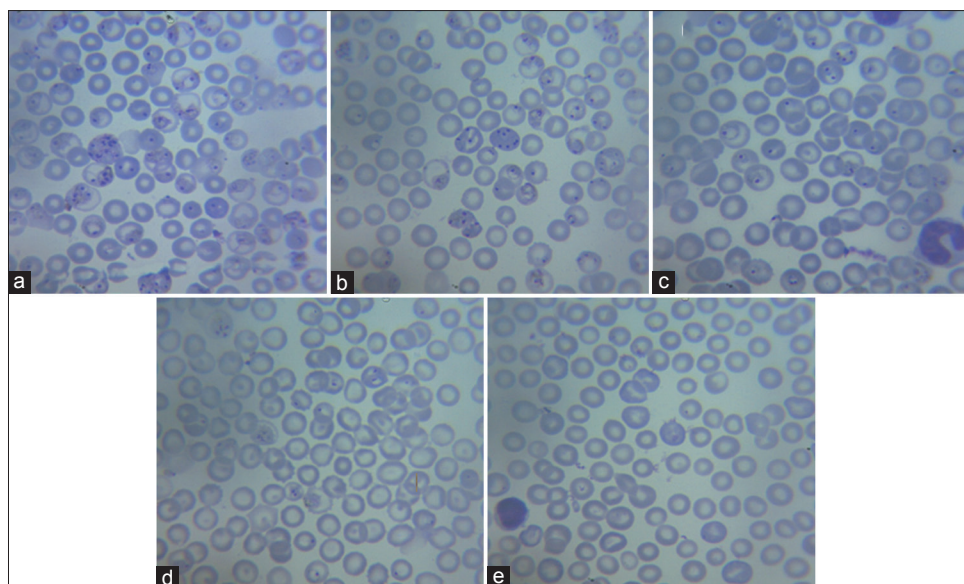
Figure 1: Parasitemia at day 4 in mice treated with different doses of dichloromethane root extract of *Caesalpinia bonducella*. (a) Negative control (2% dimethylsulfoxide); (b) 200 mg/kg/day; (c) 400 mg/kg/day; (d) 800 mg/kg/day; (e) Chloroquine at 10 mg/kg/day

Table 3: *In vivo* antimalarial activity of extracts of *Erythrina schliebenii*, *Holarrhena pubescens*, and *Phyllanthus nummulariifolius* against *Plasmodium berghei* ANKA

Plants	Part (extract)	Mean percentage suppression of parasitemia at day 4 (*n*=6 mice)	
		400 mg/ kg/day	800 mg/ kg/day
Erythrina schliebenii Harms	Stem bark (H$_2$O)	24.0	28.64
Holarrhena pubescens (Buch-Ham)	Root (MeOH)	32.06	43.07
Phyllanthus nummulariifolius Poir	Whole plant (H$_2$O)	9.28	29.22

MeOH: Methanol extract, H$_2$O: Aqueous extract, *n*: Number of mice in each group

against *Plasmodium falciparum* chloroquine resistance – 3/A2.[8] Furthermore, Innocent *et al.*[9] had reported *in vivo* antimalarial activity of 80% ethanolic extract of *C. bonducella* roots. Therefore, the results of *C. bonducella* roots observed in this study give additional scientific evidence on the antimalarial properties of this plant.

This study has further shown that the methanolic root extract of *H. pubescens* inhibited the growth of *P. berghei* ANKA malaria parasites *in vivo* with 43% suppression rate [Table 3]. *H. pubescens* is a tree which is widely studied due to its medicinal properties. The decoction of *H. pubescens* stem bark is used in Asian countries for amoebic dysentery, diarrhea, asthma, bronchospasm, and malaria.[10] Furthermore, the decoction of *H. pubescens* roots was reported as one of the medicinal plant preparations used for treatment of malaria

in Tanzania.[11] The stem bark, seeds, and roots of this plant were reported to be rich in steroidal alkaloid compounds such as conessine, isoconessine, kurchine, conessidine, conkurchicine, and holarrhimine.[12] Conessine is known for its antiamebic properties. However, some studies reported that methanolic extract of *H. pubescens* stem bark exhibited *in vitro* antiplasmodial activity[13] and conessine, the major steroidal alkaloid from the stem bark, was reported to have both antiplasmodial and *in vivo* antimalarial activities.[14] The findings from this study corroborate with the previous reports and therefore support traditional use of *H. pubescens* for the treatment of malaria.

The aqueous extracts of *E. schliebenii* and *P. nummulariifolius* exhibited dose-dependent inhibition of *P. berghei* ANKA parasites *in vivo* [Table 3]. There is no literature which has reported any extract or compound with antimalarial properties from these plants. However, extracts and compounds from other species within the same genera have been reported to have antimalarial properties. For example, the EtoAC extract of *E. sacleuxii* root bark showed good *in vitro* antiplasmodial activity with IC$_{50}$ of 3.0 µg/mL.[15] The aqueous and methanolic extracts of *P. niruri* and aqueous and ethanolic extracts of *P. amarus* whole plant have been reported to possess both *in vitro* and *in vivo* antimalarial activities.[16,17] This suggests that the antimalarial properties reported in the other plant species within the same genera may support the *in vivo* activities of *E. schliebenii* and *P. nummulariifolius* observed in this study.

CONCLUSION

The *in vivo* antimalarial activity presented in this study support traditional uses of *C. bonducella* roots, *E. schliebenii*

stem barks, *H. pubescens* roots, and *P. nummulariifolius* whole plant for treatment of malaria. Although the extracts demonstrated moderate antimalarial activities, their activities may be enhanced by testing their fractions and isolated compounds. Hence, *in vivo* guided isolation and characterization of molecules from these plant species responsible for the observed effect is suggested.

Conflicts of interest

There are no conflicts of interest.

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