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Antimalarial Activity of Some Plants Traditionally used in Meru district of Kenya

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Ten plant extracts commonly used by the Meru community of Kenya were evaluated for the *in vitro* antiplasmodial, *in vivo* antimalarial, cytotoxicity and animal toxicity activities. The water and methanol extracts of *Ludwigia erecta* and the methanol extracts of *Fuerstia africana* and *Schkuhria pinnata* exhibited high antiplasmodial activity ($IC_{50} < 5$ µg/mL) against chloroquine sensitive (D6) and resistant (W2) *Plasmodium falciparum* clones. The cytotoxicity of these highly active extracts on Vero E6 cells were in the range 161.5–4650.0 µg/mL with a selectivity index (SI) of 124.2–3530.7. *In vivo* studies of these extracts showed less activity with chemosuppression of parasitaemia in *Plasmodium berghei* infected mice of 49.64–65.28%. The methanol extract of *Clerodendrum eriophyllum* with a lower *in vitro* activity (IC_{50} 9.51–10.56 µg/mL) exhibited the highest chemosuppression of 90.13%. The methanol and water extracts of *Pittosporum viridiflorum* were toxic to mice but at a lower dose prolonged survival of *P. berghei* infected mice ($p < 0.05$) with no overt signs of toxicity. However, the extracts were cytotoxic (SI, 0.96–2.51) on Vero E6 cells. These results suggest that there is potential to isolate active non-toxic antimalarial principles from these plants. Copyright © 2007 John Wiley & Sons, Ltd.

Keywords: antimalarial; antiplasmodial; plant extracts; toxicity.

INTRODUCTION

The antimalarial properties of *Cinchona* bark (Rubiaceae) has been known for more than 300 years. The isolation of quinine from this plant has played a pivotal role in the chemotherapy of malaria. The subsequent synthesis of chloroquine (CQ) during the Second World War using the structure of quinine as a template was unprecedented in the fight against malaria, with massive worldwide use of the drug in the late 1940s and for a time the use of quinine was neglected until the emergence of *Plasmodium falciparum* strains resistant to chloroquine a decade later (Wellems and Plowe, 2001). In 1978, resistance of *P. falciparum* to CQ was evident in East Africa (WHO, 1984). Although some reports have described the inefficiency of quinine against *P. falciparum* strains in some geographical areas (Wernsdorfer, 1994), it is, however, recognized that this compound is one of the natural products that continues to play an important role in the worldwide fight against malaria. Similarly *Artemisia annua*, an annual herb endemic to the northern parts of Chahar and Suiyuan provinces

of China where it is known as quinghao (green herb) has been used to treat chills and fever for more than 2000 years (Hien and White, 1993). The isolation of artemisinin, a remarkably potent antimalarial agent from the plant, in 1971 has re-affirmed the potential of plant species to provide effective drugs for the treatment of malaria.

The more recent development of artemisinin derivatives, which are more potent than the parent molecule and effective against resistant strains of *P. falciparum*, has rekindled the search for novel antimalarial molecules from plants or as templates for drug development for malaria (WHO, 2000). The need for development of new antimalarials has become increasingly urgent due to the widespread resistance of *P. falciparum* to the previously effective and safe antimalarial drug CQ as well as to other antimalarial drugs (Wellems and Plowe, 2001). In addition, drugs derived from natural products tend to have fewer side effects than synthesized molecules (Bjorkman and Phillips-Howard, 1990). The morbidity and mortality from malaria over the past 20 years has doubled, the main factor for the increase being resistance of malaria parasites to antimalarial drugs (Greenwood and Mutabingwa, 2002).

In malaria endemic countries traditional medicinal plants are frequently used to treat malaria (Gessler *et al.*, 1995). The analysis of traditional medicines that are employed for treatment of malaria represents a potential for discovery of lead molecules for development

into potential antimalarial drugs (Phillipson and Wright, 1991). Many plant preparations are used by the Meru community of Kenya without any available scientific study on efficacy or safety. Meru district is located on the fertile north eastern slopes of Mt Kenya and the latter (3000–5200 m above sea level) has influenced the natural conditions in the district leading to a wide variety of microclimates and agroecological zones. The district is rich in green tropical vegetation cover and its biodiversity nature and uses are claimed to possess medicinal value. It is inhabited by the Ameru, a Bantu tribe who are predominantly agararian. They practice traditional and herbal remedies as an alternative choice of treatment of malaria. It is therefore of interest to screen medicinal plants from this region for an evaluation of *in vitro* antiplasmodial, *in vivo* antimalarial and toxicity tests.

MATERIALS AND METHODS

Plant samples. The plant samples (Table 1) were collected between October and December 2004 from Meru District, Eastern Province, Kenya. The selection of plants was done on the basis of traditional reputation of particular plants for efficacy in the treatment and management of malaria as used by traditional health practitioners (THP) and the local communities. The plants were identified by Mr G. M. Mungai, East Africa Herbarium, National Museums of Kenya, Nairobi and the voucher specimens were deposited in the Herbarium.

Preparation of extracts. The plant materials were air dried under shade at room temperature (25 °C) and then pulverized into fine powders. The powders were packed in air tight polythene bags and stored in the dark until extraction. Each plant sample was separately extracted with methanol and water. For the methanol extracts 50 g of the powdered plant material was macerated with 500 mL of methanol at room temperature for 24 h, filtered through Whatman filter paper No. 1. The marc was re-extracted twice each with 300 mL methanol for the same period and the filtrates pooled and concentrated under vacuum at 40 °C until dry. The concentrate was weighed and transferred to an air tight sample bottle and stored at –20 °C until used.

Another 50 g of the same sample was extracted once with 500 mL of distilled water in a water bath at 60 °C for 1 h, filtered and lyophilized in a freeze dryer. The dry extracts for all the samples similarly treated were weighed into airtight containers and stored at –20 °C until used (Table 1).

Parasites. For antimalarial activities of the aqueous and methanol extracts, the mouse-infective CQ sensitive *P. berghei* strain ANKA (International Livestock Research Institute, ILRI, Nairobi) was used. The CQ susceptible strain (D6 clone) and the CQ resistant *P. falciparum* strain (W2 clone) (Walter Reed Army Institute of Research, Washington D.C.) were used to test the antiplasmodial activities of the plant extracts *in vitro*.

Preparation of test extracts for *in vitro* assay. Stock solutions of aqueous extracts (500 μg/mL) were made in distilled deionized water and filter sterilized using 0.22 μm membrane filters in a laminar flow hood. The methanol extracts (500 μg/mL) were dissolved in DMSO (Sigma Chemical Co., St Louis, MO, USA) followed by subsequent dilution to a lower concentration of DMSO to <1% to avoid carryover (solvent) effect (Dorin *et al.*, 2001). Reference stock drugs of CQ and artemisinin (1 μg/mL) were similarly prepared and all solutions stored at –20 °C until used.

Cultures of *P. falciparum*. *P. falciparum* culture of D6 (CQ sensitive isolate from Sierra Leone) and W2 (CQ resistant isolate from Indochina) were used in the study. The culture media was a variation of that described by Trager and Jensen (1976) and consisted of RPMI 1640 supplemented with 10% serum (Schlichtherle *et al.*, 2000). Uninfected human blood group O⁺ erythrocytes (<28 days old) served as host cells. The cultures were incubated at 37 °C in an atmosphere of 3% CO_2, 5% O_2 and 92% N_2.

***In vitro* determination of antiplasmodial activity.** The *in vitro* semi-automated microdilution assay technique that measures the ability of the extracts to inhibit the incorporation of [G-³H]hypoxanthine (Amersham International, Burkinghamshire, UK) into the malaria parasite was used (Desjardins *et al.*, 1979). For the test, 25 μL aliquots of culture medium were added to all the wells of a 96-well flat-bottom microculture plate (Costar Glass Works, Cambridge, UK). Aliquots (25 μL) of the test solutions were added, in triplicate, to the first wells, and a Titertek motorized hand diluter (Flow Laboratories, Uxbridge, UK) was used to make serial 2-fold

Table 1. Name, voucher specimen number, plant parts collected and % weight of the extracts of evaluated plants

Family/Botanical name/(Voucher specimen no.)	Vernacular (Ameru)	Part collected	% yield of extracts	
			MeOH	H₂O
Capparaceae: *Boscia angustifolia* A. Rich. (CM 076)	Murure	Stem bark	3.02	7.30
Compositae: *Schkuhria pinnata* (Lam) O. Ktze (CM 050)	Gakuinini	Whole plant	2.66	17.06
Compositae: *Sphaeranthus suaveolens* (Forsk.) DC. (CM 052)	Njogu-ya-iria	Whole plant	6.86	4.04
Euphorbiaceae: *Clutia abyssinica* Jaub and Spach (CM 055)	Muthimamburi	Leaves	4.46	10.56
Lauraceae: *Ocotea usambarensis* Engl. (CM 059)	Muura	Stem bark	4.4	10.02
Lamiaceae: *Fuerstia africana* T.C.E. Fries (CM 056)	Muti jwa maigo	Whole plant	2.04	8.86
Onagraceae: *Ludwigia erecta* (L.) Hara (CM 054)	Marimba	Whole plant	7.68	11.04
Pittosporaceae: *Pittosporum viridiflorum* Sims (CM 068)	Munati	Leaves	6.04	13.28
Rubiaceae: *Vangueria acutiloba* Robyns (CM 079)	Mubiru	Stem bark	1.28	6.86
Verbenaceae: *Clerodendrum eriophyllum* Guerke (CM 089)	Muumba	Root bark	3.82	11.66

dilutions of each sample over a 64-fold concentration range. Negative controls treated with the solvent (DMSO) were set up to check for solvent effects (Azas *et al.*, 2001). The susceptibility tests were carried out with initial parasitaemia (expressed as the percentage of erythrocytes infected) of 0.4% by applying 200 µL, 1.5% haematocrit, *P. falciparum* culture to each well. 200 µL of culture media without parasites was added into four wells on the last row of each plate to serve as the background control. Parasitized and non-parasitized erythrocytes were incubated at 37 °C in a gas mixture 3% CO_2, 5% O_2 and 92% N_2. After 48 h, each well was pulsed with 25 µL of culture medium containing 0.5 µCi of [G-^{3}H]hypoxanthine and the plates were incubated for a further 18 h. The contents of each well were then harvested onto glass fiber filter mats, washed thoroughly with distilled water, dried and the radioactivity in counts per minute (cpm) measured by a liquid scintillation and luminescence counter (Wallac Micro Beta Trilux).

Computation of the concentration of drug causing 50% inhibition of [G-^{3}H]hypoxanthine uptake (IC_{50}) was carried out by interpolation after logarithmic transformation of both concentration and cpm values using the formula:

$$IC_{50} = \text{antilog} \left(\log X_1 + \frac{[(\log Y_{50} - \log Y_1)(\log X_2 - \log X_1)]}{(\log Y_2 - \log Y_1)} \right)$$

where, Y_{50} was the cpm value midway between parasitized and non-parasitized control cultures and X_1, Y_1, X_2 and Y_2 were the concentrations and cpm values for the data points above and below the cpm midpoints (Sixsmith *et al.*, 1984).

***In vivo* determination of antimalarial activity.** Chloroquine sensitive *P. berghei* strain ANKA was used to assess the *in vivo* intrinsic antimalarial activity. The assay protocol was based on 4-day suppressive test (Peters *et al.*, 1975). The parasite strain was maintained by serial passage of blood from an infected mouse to a naive mouse. Female Swiss mice (6–7 weeks old; 20–22 g) were randomly infected by intraperitoneal (i.p.) inoculation of 10^7 erythrocytes parasitized with *P. berghei* in a saline suspension of 0.2 mL on day zero (D0) and allocated to several groups of five mice in each cage. They were fed on standard pellets and water *ad libitum*. The animals were housed in the Animal House in KEMRI (Kenya Medical Research Institute) and the Institute's Animal Care and Use Committee gave approval for the study.

Plant extracts were solubilized in 10% v/v Tween 80 (methanol extracts) or in physiological saline (water extracts) and administered to experimental groups of mice 1 h post infection at a dose of 100 mg/kg body weight i.p. in a volume of 0.2 mL, and again after 24, 48, and 72 h (D0 to D3). Two groups (5 mice each) served as negative and positive controls, respectively. The negative group received saline/Tween 80 while the positive group was treated with 5 mg/kg/body weight i.p. with the reference drug CQ diphosphate.

Each day from D1 to D4 thin blood smears were made from the tail of each mouse, Giemsa stained and examined microscopically for assessment of parasitaemia. The mean parasitaemia in each group of mice on day 4 was used to calculate the % chemosuppression of each extract using the formula:

$$(A - B)/A \times 100$$

where *A* was the mean parasitaemia in the negative control and *B* was the parasitaemia in the test group (Tona *et al.*, 2001). Extract activity was determined by % reduction of parasitaemia in treated groups compared with untreated infected mice. The statistical analysis was based on Student's *t*-test where $p < 0.05$ was considered as significant.

Determination of acute toxicity. Healthy Swiss female mice (weight 20–22 g) were divided into groups of five in each cage and had access to tap water and food, except for a short fasting period (12 h) before oral administration of single dose of the extract. The water extracts were dissolved/suspended in distilled water and administered by gavage at a logarithmic dose range of 500–5000 mg/kg body weight to give five dose levels of 500.0, 889.15, 1581.18, 2811.80 and 5000.0 mg/kg body weight. The general behaviour of mice was observed continuously for 1 h after the treatment and then intermittently for 4 h, and thereafter over a period of 24 h (Twaij *et al.*, 1983). The mice were further observed for up to 14 days following treatment for any signs of toxicity, and the latency of death. The LD_{50} value was determined according to a method described by Thompson (1985).

***In vitro* determination of cell cytotoxicity.** The cytotoxic concentration causing 50% cell lysis and death (CC_{50}) was determined for the extracts by a method described by Kurokawa *et al.* (2001). Vero E6 cells (Toyoma Medical and Pharmaceutical University, Toyoma, Japan) were seeded at a concentration of 2.5×10^4 cells/ well in a 24-well plate and grown under 5% CO_2 at 37 °C in Eagle's minimum essential medium (MEM) (Gibco BRL, Scotland) supplemented with 5% fetal bovine serum (FBS) (Gibco BRL, Scotland) for 48 h. The culture media was replaced by fresh media containing extract at various concentrations, and cells further grown for 24 h. The cells were treated with trypsin and the number of viable cells determined by the tryphan blue exclusion method. The concentration of herbal extract reducing cell viability by 50% (CC_{50}) was determined from a curve relating percent cell viability to the concentration of extract.

RESULTS AND DISCUSSION

The *in vitro* activities (IC_{50}) of the extracts against CQ sensitive (D6) *P. falciparum* clone and resistant (W2) clone are shown in Table 2. The activity was categorized as high when IC_{50} was <5 µg/mL, moderate when IC_{50} was 5–20 µg/mL, and weak when IC_{50} was 20–100 µg/mL. Extracts having activity beyond this range were considered inactive. In general the IC_{50s} for W2 were higher than those of D6 whether the plant extracts were extracted with methanol or water. This trend was similar to that of chloroquine and artemisinin, suggestive of a similar mode of action.

The two clones were highly susceptible ($IC_{50} < 5$ µg/ mL) to the methanol and water extracts of *L. erecta*, the water extract of *B. angustifolia* and the methanol extracts of *F. africana* and *S. pinnata* (D6 sensitivity).

Table 2. The mean IC$_{50}$ values (x ± SD) for plant extracts screened against D6 and W2 *P. falciparum* clones

	IC$_{50}$ ± SD (µg/mL)			
	Methanol extracts		Water extracts	
Plant name	D6	W2	D6	W2
L. arrecta	4.10 ± 0.04[f]	11.21 ± 0.65[ae]	0.93 ± 0.05[d]	1.61 ± 0.07[bc]
S. suaveolens	7.93 ± 1.04[f]	56.73 ± 2.31[ae]	9.98 ± 0.17[f]	49.22 ± 7.82[be]
V. acutiloba	13.36 ± 3.4[f]	33.98 ± 1.73[ae]	178.14 ± 9.69[fd]	>250
B. angustifolia	7.43 ± 3.89[f]	35.93 ± 2.16[ae]	1.42 ± 0.076[d]	4.77 ± 0.59[bc]
O. usambarensis	7.69 ± 2.87[f]	29.75 ± 2.23[ae]	41.17 ± 7.49[fd]	76.27 ± 0.57[bce]
P. viridiflorum	18.9 ± 1.70[f]	17.69 ± 1.24[e]	27.61 ± 1.82[f]	224.27 ± 1.7[bce]
C. abyssinica	7.83 ± 1.17[f]	11.35 ± 0.55[ae]	65.23 ± 1.42[fd]	216.81 ± 14.81[bce]
F. africana	0.98 ± 0.05	2.40 ± 0.15[a]	138.26 ± 2.75[fd]	>250
S. pinnata	1.30 ± 0.11	6.83 ± 0.13[ae]	22.51 ± 1.89[fd]	51.82 ± 2.6[bce]
C. eriophyllum	9.51 ± 0.52[f]	10.56 ± 0.13[e]	>250	>250
Chloroquine	8.97 ± 1.18	31.32 ± 2.15	8.97 ± 1.18	31.32 ± 2.15
Artemisinin	0.90 ± 0	3.38 ± 0.49	0.90 ± 0	3.38 ± 0.49

The results are expressed as mean ± SD of three different determinations per experiment. Two reference drugs are included as positive controls and their IC$_{50}$ values are given in ng/mL; Artemisinin 0.90 ± 0 and 3.38 ± 0.49; and chloroquine 8.97 ± 1.18 and 31.32 ± 2.15 for D6 and W2, respectively.

[a] $p < 0.05$ by Welch *t*-test (MeOH W2 vs MeOH D6).
[b] $p < 0.05$ (water W2 vs water D6).
[c] $p < 0.05$ (water W2 vs MeOH W2).
[d] $p < 0.05$ (water D6 vs MeOH D6).
[e] $p < 0.05$ by Dunnett's test (MeOH/water W2 vs CQ).
[f] $p < 0.05$ by Dunnett's test (MeOH/water D6 vs CQ).

The ratio of sensitivity for the two clones was less than that of chloroquine suggesting no cross-resistance with the latter. The results for *L. erecta* are interesting because of the high activity, especially the water extract, which was about five times as active as its methanol extract. Both extracts were not cytotoxic to Vero E6 cells. The water extract, which was most active *in vitro*, exhibited a chemosuppression of parasitaemia in *P. berghei* infected mice of 49.64% while the methanol extract showed 65.28%. The results for *in vivo* antimalarial activity do not necessarily correlate with those for *in vitro* activity probably due to biotransformation of metabolites *in vivo* or poor bioavailability. An extract lacking *in vitro* activity may possess *in vivo* activity and vice versa (Gessler *et al.*, 1995). Cryptolepine an indolisoquinoline, a plant derived antimalarial alkaloid (IC$_{50}$, 0.031 µg/mL KI sensitivity) is biotransformed into inactive metabolite by interaction with adenine–thymine base pair (Kirby *et al.*, 1995) and has no activity *in vivo* (Cimanga *et al.*, 1997). Both extracts prolonged the survival of *P. berghei* infected mice ($p < 0.05$). The entire plant of *L. erecta* is used in East Africa against malaria by boiling the whole plant and taking a bath in the liquid (Kokwaro, 1993). There is no scientific information on this plant and to the best of our knowledge this is the first report on its antimalarial properties.

The *in vivo* antimalarial activities of the selected plants on *P. berghei* infected mice are shown in Table 3. The parasite density for methanol and water extracts was significantly different ($p < 0.05$) when compared with controls (normal saline or 10% Tween 80). There was also a significant difference between all the samples and CQ diphosphate (reference) except the methanol extract of *C. eriophyllum* ($p > 0.05$) whose parasite density was comparable to that of CQ, an indication of the effectiveness of the extract against *P. berghei* infected mice.

The activity of the methanol extract of *C. eriophyllum* was moderate with almost the same range of activity for both clones suggesting no cross-resistance with CQ and a different mode of action. It exhibited the highest chemosuppression of parasitaemia of 90.13%. Although the water extract was inactive, it showed good *in vivo* activity with a chemosuppression of 61.54% indicative of different bioactive constituents from those present in the methanol extract, probably biotransformed into active metabolites *in vivo*. The survival time for mice receiving the extracts was not significantly different ($p > 0.05$) from the negative control. This might be explained by mechanisms of drug action such as having an indirect effect on the immune system, or by other pathways that are not yet understood (Rasoanaivo *et al.*, 1992). On the other hand the suppressed development of *P. berghei* infection in the first days of infection may suggest that the extract affects the blood stages of the parasites. The subsequent development of infection and shorter survival time points to a shorter duration of action of the extract, perhaps limited by rapid metabolism or elimination. Traditionally the root decoction of *C. eriophyllum* is used for intestinal disorders while the extract from the leaves is used for treating malaria (Kokwaro, 1993). There is no scientific information on this plant and to the best of our knowledge this is the first report of its antimalarial activity. Related species have been reported to have antiplasmodial activities in *C. myricoides* (Muregi *et al.*, 2004), as well as alkaloids (Bashwira *et al.*, 1989). Another species *C. glabrum* has also been reported to have antiplasmodial activity (Clarkson *et al.*, 2004).

Deaths for *P. berghei* infected but untreated mice were first observed on day 7 post infection and most of the animals died by day 8. Parasitaemia was evident on the second day and the highest parasitaemia of 30.65% was obtained in the controls on day 4. Mice receiving

Table 3. The mean (x ± SD) parasitaemia, chemosuppression and survival time of *P. berghei* infected mice treated intraperitoneally with methanol and aqueous extracts at 100 mg/kg body weight

| | Plant extracts | | | | | |
| | Methanol | | | Water | | |
Botanical name	Parasite density[a]	% Chemosuppression	Mean survival time (days)	Parasite density[a]	% Chemosuppression	Mean survival time (days)
L. erecta	10.64 ± 1.25[b]	65.28 ± 2.84	15.0 ± 3.0[e]	15.4 ± 4.3[c]	49.64 ± 4.45	12.0 ± 1.0[e,f]
S. suaveolens	16.32 ± 2.12[b]	46.74 ± 4.01	10.67 ± 1.53[f]	16.74 ± 3.02[c]	45.38 ± 3.45	11.0 ± 1.0[f]
V. acutiloba	22.68 ± 2.11[b]	26.01 ± 3.21	10.0 ± 1.0[f]	18.69 ± 2.51[c]	39.02 ± 2.41	10.0 ± 1.0[f]
B. angustifolia	12.22 ± 2.10[b]	60.12 ± 2.10	11.0 ± 1.0[f]	24.35 ± 2.52[c,d]	20.54 ± 6.32	10 ± 1.0[f]
O. usambarensis	17.72 ± 2.84[b]	42.20 ± 5.01	10.0 ± 0.58[f]	20.93 ± 2.15[c]	31.70 ± 1.49	10.33 ± 1.53[f]
*P. viridiflorum**	13.86 ± 1.75[b]	54.77 ± 3.32	15.0 ± 3.0[e]	3.14 ± 0.66[d]	89.76 ± 2.09	14.33 ± 1.53[e]
Clutia abyssinica	18.25 ± 1.02[b]	40.45 ± 3.41	11.0 ± 1.0[f]	8.68 ± 0.43[c,d]	71.69 ± 0.89	11.33 ± 1.53[f]
F. africana	11.69 ± 2.34[b]	61.85 ± 4.61	11.0 ± 1.0[f]	17.42 ± 2.84[c]	43.16 ± 1.69	11.0 ± 1.0[f]
S. pinnata	18.70 ± 4.32[b]	49.90 ± 3.61	10.33 ± 2.08[f]	10.97 ± 1.61[c]	64.22 ± 4.43	10.33 ± 1.53[f]
C. eriophyllum	3.03 ± 1.04	90.13 ± 3.48	11.0 ± 1.0[f]	11.80 ± 0.51[c,d]	61.54 ± 20	11.33 ± 1.53[f]
Saline/10% Tween 80	30.65 ± 1.62	0.0 ± 0	8.0 ± 1.0	30.65 ± 1.62	0.0 ± 0	8.0 ± 1.0
Chloroquine	0.03 ± 0	99.89 ± 0.13	16.33 ± 1.53	0.03 ± 0	99.89 ± 0.13	16.33 ± 1.53

The results are expressed as mean ± SD of 5 determinations per experiment.
[a] Parasite densities (Pd) in percentages, ($p < 0.05$) by Dunnett's test, MeOH and water extracts vs controls.
[b] $p < 0.05$ by Dunnett's test, MeOH extracts vs chloroquine.
[c] $p < 0.05$ water extracts vs chloroquine.
[d] $p < 0.05$ by Welch *t*-test, MeOH vs water extracts.
[e] $p < 0.05$ by Dunnett's test, mean survival time of the extracts vs negative controls.
[f] $p < 0.05$ by Dunnett's *t*-test, mean survival time of the extracts vs chloroquine.
* Concentration of *P. viridiflorum* MeOH and Water extracts was 5 and 20 mg/kg respectively

extracts of *L. erecta* and *P. viridiflorum* had a longer survival time ($p < 0.05$) which was significantly beyond the maximum survival period of the untreated animals. The rest of the extracts showed no significant difference in the survival time when compared with untreated control mice ($p > 0.05$). Mice treated with CQ had low levels of infection over the 4 days of experimental treatment, but started showing signs of infection after withdrawal of CQ and were all dead after 16.33 days.

The safety and or toxicity of the extracts assessed by LD_{50}, CC_{50} or even the survival of mice treated with the extracts in a 4-day suppressive test attest to the safety of most plants evaluated. In the *in vivo* test, it would be considered that death of infected treated animals occurring earlier than those infected and untreated would probably be due to toxicity of the extract. The fact that all the extracts prolonged the survival time of infected mice when compared with infected untreated mice showed that there was some intrinsic antimalarial activity.

P. viridiflorum water extract was toxic (LD_{50} 1000 mg/kg body weight); however, no mortality or signs of toxicity were observed for the rest of the water extracts after oral administration of a single dose at any dose level up to the highest dose tested (5000 mg/kg) which was the no-observed-adverse-effect level (NOAEL) (Alexeeff *et al.*, 2002).

The results for plant extract cytotoxicity (CC_{50}) on Vero E6 cells are shown in Table 4. The extracts of *P. viridiflorum* exhibited the highest cell cytotoxicity on Vero E6 cells with methanol and water extracts presenting CC_{50} of 18.08 and 69.21 µg/mL, respectively, resulting in a selectivity index (SI) of 0.96 and 2.51. Mice treated with the methanol extract of *P. viridiflorum*

Table 4. Cytotoxicity of the plant extracts on Vero E6 cells

| | Plant extracts | | | | | |
| | Methanol extracts | | | Water extracts | | |
Plant species	Vero E6 cells CC_{50} (µg/mL)	*P. falciparum* D6 clone (µg/mL)	Selectivity index (SI)	Vero E6 cells CC_{50} (µg/mL)	*P. falciparum* D6 clone IC_{50} (µg/mL)	Selectivity index (SI)
L. erecta	544.3 ± 11.1	4.10 ± 0.04	132.7	3283.6 ± 31.2	0.93 ± 0.05	3530.7
S. suaveolens	512.2 ± 13.2	7.93 ± 1.04	64.6	2845.3 ± 28.4	9.98 ± 0.17	285.1
V. acutiloba	661.5 ± 23.4	13.36 ± 3.40	49.5	3457 ± 39.2	178.14 ± 9.69	nd
B. angustifolia	1000.0 ± 26.2	7.43 ± 1.89	134.5	6720.0 ± 49.6	1.42 ± 0.07	4732.4
O. usambarensis	461.5 ± 15.3	7.69 ± 2.87	60.0	3973 ± 27.9	41.17 ± 7.49	95.3
P. viridiflorum	18.08 ± 0.4	18.90 ± 1.70	0.96	69.21 ± 1.2	27.61 ± 1.82	2.51
C. abyssinica	331.5 ± 14.2	7.83 ± 1.17	42.3	5574 ± 45.3	65.23 ± 1.42	85.5
F. africana	954.7 ± 21.7	0.98 ± 0.05	974.2	4650.0 ± 34.3	138.26 ± 2.75	nd
S. pinnata	161.5 ± 12.4	1.30 ± 0.11	124.2	3570.0 ± 26.8	22.51 ± 1.89	166.6
C. eriophyllum	233.0 ± 9.5	9.51 ± 0.52	24.5	4571.3 ± 37.8	>250	nd

nd, not determined ($IC_{50} > 100$ µg/mL).

at 100 mg/kg died within 24 h. However, at a much lower dose of 5 mg/kg, there was a significant reduction of parasitaemia with a chemosuppression of 54.77%. Higher doses than this tended to be toxic. The water extract was less toxic than the methanol extract and at a dose of 20 mg/kg there was a significant reduction of parasitaemia with a chemosuppression of 89.76%. In East Africa a decoction or infusion of the stem bark of *P. viridiflorum* is used for malaria and fevers (Gakunju *et al.*, 1995). In South Africa the plant is used as antimalarial and a dichloromethane extract of the whole plant gave an IC_{50} of 3 μg/mL against CQ sensitive *P. falciparum* strain D10 (Clarkson *et al.*, 2004). Gakunju *et al.* (1995) reported an antiplasmodial activity of 30 μg/mL for CQ susceptible *P. falciparum* strain K67. A methanol extract of the leaves from a related species *P. lanatum* was toxic to brine shrimp (LC_{50}, 27.4 μg/mL) and had a comparable antiplasmodial activity (IC_{50}, 24.2 μg/mL) (Wanyoike *et al.*, 2004). In our laboratory, chromatographic analysis on TLC of the methanol and water extracts of the leaves of *P. viridiflorum* revealed the presence of triterpene saponins and this is in conformity with the findings of Seo *et al.* (2002) and Koch *et al.* (2005). It is unknown whether inhibition is due to a specific antiplasmodial action or general cytotoxicity but the *in vivo* test with a lower dose retains the activity without apparent toxicity in mice, which suggests that the toxic compounds could be different from active constituents. Bioactivity guided isolation will allow the isolation of active molecules from the toxic constituents in continuation of the work on this plant.

The water extracts of the rest of the extracts generally had lower cytotoxicities than the methanol extracts. The lowest cytotoxicity on Vero E6 cells was observed for *B. angustifolia* water extract with a CC_{50} of 6720.0 μg/mL and the best selectivity index of 4732.4. In conformity with our results, Cepleanu *et al.* (1994) found out that the stem bark extracts from this plant had neither cytotoxicity nor brine shrimp lethality. These results seem to confirm the validity of their traditional uses as antimalarials (Gessler *et al.*, 1995).

The methanol extract of *B. angustifolia* showed a high chemosuppression of parasitaemia (60.12%), yet the water extract, which was very active *in vitro* exhibited the least chemosuppressive activity, probably due to biotransformation of the constituents into less active metabolites or an indirect immune response. The decoction from the boiled stem bark of *B. angustifolia* is drunk to cure malaria (Kokwaro, 1993). It is reported to have larvicidal activity (Cepleanu *et al.*, 1994).

The sensitivity of the methanol extracts of *S. suaveolens*, *B. angustifolia*, *O. usambarensis* and *C. abyssinica* for D6 sensivity was similar (7.93, 7.43, 7.69 and 7.83 μg/mL, respectively) and these values were higher for W2 sensitivity. The water extracts were less active (9.98, 1.42, 41.17 and 65.23 μg/mL, respectively). The distribution of antiplasmodial constituents was apparently more in methanol extracts than in water extracts and it is noteworthy that all the methanol extracts were active. However, water is mostly the formulation that is used in traditional medicine and apart from the water extracts of *L. erecta* and *B. angustifolia*, the rest of the water extracts had weak activity or were inactive, but exhibited relatively high chemosuppression of parasitaemia in *P. berghei* infected mice. This may explain the consistent reports by traditional healers that these plants are effective in treating malaria in humans.

The *in vitro* activity of methanol extract of *S. pinnata* was higher than that of the water extract; however, the latter presented higher chemosuppression. Both extracts exhibited low cytotoxicity on Vero E6 cells and similarly there was no observed toxicity in mice.

A *Clutia abyssinica* methanol extract had moderate *in vitro* activity and the water extract had weak activity, however, the water extract exhibited high chemosuppression, possibly a prodrug effect. Consistent with our findings Kraft *et al.* (2003) reported antiplasmodial activity of the lipophilic extracts. Boiled leaves of *C. abyssinica* are used to treat malaria while boiled leaves and roots are used for headaches and stomach problems (Kokwaro, 1993).

The *in vitro* activities for methanol and water extracts of *S. suaveolens* were similar for both clones. The two extracts also showed similar chemosuppression of parasitaemia. A decoction of the whole plant of *S. suaveolens* is drunk as a cough remedy. It is also rubbed onto the body of a person suffering from malaria (Kokwaro, 1993). There is no biological information reported and to the best of our knowledge this is the first report of its antimalarial properties.

The *in vitro* activity of the extracts of *O. usambarensis* was between moderate and weak activity (7.69–76.27 μg/mL) and the chemosuppression of water and methanol extracts was partially active. Both extracts exhibited low cytotoxicity and there was no observed toxicity in mice. The infusion from the roots of *O. usambarensis* is taken for backache and malaria (Kokwaro, 1993). Weenem *et al.* (1990) reported weak antiplasmodial activity from this plant.

The water extracts of *F. africana* and *V. acutiloba* were inactive although the methanol extracts had good *in vitro* activities. The chemosuppression of both methanol and water extracts of *V. acutiloba* was low and that of *F. africana* was moderate. The extract from the leaves and young parts of *F. africana* is used as a remedy for malaria, a purgative and anthelmintic particularly for tapeworm (Kokwaro, 1993). Consistent with our findings, Koch *et al.* (2005) reported antiplasmodial activity of the plant and its use as antimalarial remedy among the Masai community. The infusion made from the powdered stem bark of *V. acutiloba* is drunk for malaria (Kokwaro, 1993). There is no biological properties reported and to the best of our knowledge this is the first report of antimalarial activity of this plant. A related taxa *V. infausta* from South Africa has been reported to have antiplasmodial activity (Clarkson *et al.*, 2004).

The selectivity index (SI = ratio of cytotoxicity to biological activity) of the extracts were high (24.5–4732) indicative of selective toxicity to malaria parasites and it is generally considered that biological efficacy is not due to the *in vitro* cytotoxicity when SI ≥ 10 (Vonthron-Senecheau *et al.*, 2003). It has been proposed that the ratio for a good therapeutic remedy be >1000 as found for example with quinine (Likhitwitayawuid *et al.*, 1993). The selectivity index for CQ on L-6 rat skeletal myoblast cells and *P. falciparum* strain KI (CQ resistant) was 942 (Antindehou *et al.*, 2004). Extracts that demonstrated high selectivity (high SI value) should offer the potential for safer therapy.

The study demonstrated the activity of the plant extracts either *in vitro* or *in vivo*. The *in vitro* activity did

not translate directly into *in vivo* activity and vice versa. Mostly the water extracts showed weak or were inactive *in vitro*, but most of them were active *in vivo*, probably through biotransformation of chemical constituents into active metabolites. This may explain the use of these plants in traditional therapy.

The plant extracts tested were less active than reference drugs chloroquine and artemisinin. They were composed of heterogenous mixture of different compounds, and the active principles might show higher activity in their pure form. Most of the plant extracts have IC_{50s} often higher than 5–10 μg/mL (Benoit-Vical *et al.*, 1998) and *Artemisia annua*, for example had an IC_{50} of 3.9 μg/mL in similar conditions (Phillipson and Wright, 1991). Fractionation of *Artemisia annua* yielded the very active molecule artemisinin that is currently the drug of choice in the fight against malaria. *P. viridiflorum* extracts were toxic in mice at the test dose but there is potential for isolation of safe non toxic compound/s since they were active at lower doses with no overt signs of toxicity in mice. Gessler *et al.* (1994) reported different levels of activities in samples of material from the same plant species collected in different regions, which emphasizes the importance of georeference, suggesting that the locality and probably time of plant collection may impact on the amount and composition of active components (Capasso, 1985). It is not known whether such factors have contributed to the composition of phytochemicals in *P. viridiflorum*, which has been reported, used as antimalarial in East and South Africa. It would be of interest to isolate active constituents for identification, antiplasmodial evaluation with further toxicological screening and that care should be taken if this plant is to be promoted as a medicinal plant for treatment of malaria. Consequently phytochemical studies of the most active extracts in order to identify the active principles are underway in our laboratories.

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