

In vitro anti-plasmodial and in vivo anti-malarial activity of some plants traditionally used for the treatment of malaria by the Meru community in Kenya

James W. Gathirwa · Geoffrey M. Rukunga · Eliud N. M. Njagi · Sabah A. Omar · Anastasia N. Guantai · Charles N. Muthaura · Peter G. Mwitari · Cecilia W. Kimani · Peter G. Kirira · Festus M. Tolo · Teresia N. Ndunda · Isaiah O. Ndiege

Received: 26 July 2006 / Accepted: 9 January 2007 / Published online: 27 March 2007
© The Japanese Society of Pharmacognosy and Springer 2007

Abstract Extracts of seven medicinal plant species used for treatment of malaria in traditional/cultural health systems of the Ameru people in Kenya were tested in vitro and in vivo against *Plasmodium falciparum* (D6 and W2 strains) and *P. berghei*, respectively. Of the plants tested, 28.57% were highly active ($IC_{50} < 10 \mu\text{g/ml}$) and 42.86% moderately active ($IC_{50} 10\text{--}50 \mu\text{g/ml}$), while 28.57% had weak activity of 50–125 $\mu\text{g/ml}$ in vitro. The water and methanol extracts of *Boscia salicifolia* Oliv. and *Artemisia afra* Jacq. (ex-Willd.) were the most active against both the chloroquine (CQ)-sensitive (D6) and the CQ-resistant (W2) *P. falciparum* strains. *Artemisia afra* and *Rhus natalensis* Bernh. (ex-Krauss) exhibited the highest parasite clearance

and chemo-suppression (>70%) in vivo (in mice). The plants with high in vitro anti-plasmodial (low IC_{50} values) and high anti-malarial activity (high chemo-suppression) in vivo are potential sources of novel anti-malarial drugs.

Keywords Anti-malarial · Anti-plasmodial · Toxicity · *Boscia salicifolia* · *Artemisia afra* · *Rhus natalensis*

Introduction

Malaria is one of the most important infectious diseases in the world, especially the tropics [1]. It is estimated to cause 2.3 and 9% of the disease burden in the world and Africa, respectively [2], thus ranking third after pneumococcal acute respiratory infections (3.5%) and tuberculosis (TB) (2.8%). The incidence of malaria is increasing, with an estimated 300–500 million cases and 3 million deaths annually [3]. Malaria mortality and morbidity are increasing in the highest risk group, which is children under 5 years of age in Africa [4], due to the increasing resistance of the parasites to the available drugs [5]. Drug resistance by *P. falciparum*, the species that causes the greatest number of malaria cases and deaths in Africa [6], has become an issue of utmost concern [7–8] and was implicated in enhanced mortality in hyper- and holoendemic areas and the development of new and expanding foci of *falciparum* malaria in the last 5 decades [9]. It has been identified as a major factor in the economic constraints in malaria control [10]. Consequently, there is an urgent need to discover, develop and use new anti-malarial drugs.

Plant-derived drugs offer another window in chemotherapy [11–12]. Some of the anti-malarial drugs in use today were obtained directly from plants or developed using plant-derived chemical structures as templates. For

J. W. Gathirwa · G. M. Rukunga · C. N. Muthaura · P. G. Mwitari · C. W. Kimani · P. G. Kirira · F. M. Tolo · T. N. Ndunda
Centre for Traditional Medicine and Drug Research,
Kenya Medical Research Institute,
P.O. Box 54840, Nairobi 00200, Kenya

J. W. Gathirwa · E. N. M. Njagi
Department of Biochemistry and Biotechnology,
Kenyatta University,
P.O. Box 43844, Nairobi 00100, Kenya

S. A. Omar
Centre for Biotechnology Research and Development,
Kenya Medical Research Institute,
P.O. Box 54840, Nairobi 00200, Kenya

A. N. Guantai
School of Pharmacy, University of Nairobi,
P.O. Box 30197, Nairobi 00100, Kenya

I. O. Ndiege (✉)
Department of Chemistry, Kenyatta University,
P.O. Box 43844, Nairobi 00100, Kenya
e-mail: indiege@yahoo.com

example, quinine was developed from *Cinchona* sp. [13], and cheap analogues like chloroquine, mefloquine and amodiaquine were developed using the parent molecule as a template. Relatively simple chemical modifications of artemisinin have led to a series of potent anti-malarial drugs (artemether, arteether and artesunate) that are playing an increasingly important role in the treatment of malaria [14]. In Kenya, plant extracts are still widely used in the treatment of malaria and other ailments [15]. A large number of local plants have been studied to establish their potential in malaria treatment [16–20]. Only about 20% of the plants with acclaimed medicinal values have been subjected to rigorous scientific screening and evaluation [21]. In continuation of the efforts to verify the efficacy of traditional/cultural/folkloric anti-malarial preparations, we subjected seven species of medicinal plants to in vitro antiplasmodial and in vivo anti-malarial (rodent) screening. The results are presented in this paper.

Materials and methods

Collection, processing and extraction of plant materials

Following a survey on the ethno-medical use of local plants in the Meru region in the Eastern Province of Kenya through interviews with traditional health practitioners (THPs), seven were selected (Table 1) for the study. The plants were botanically authenticated by a taxonomist and voucher specimens deposited at the East African Herbarium, National Museums of Kenya (NMK). Several plant parts including the leaves, roots, stem or stem-barks depending on the part used by the THPs were collected in August 2004. The plant parts were air-dried under shade

for 14 days, then the root, stems (twigs), stem-bark or root-bark were ground using a laboratory mill (Christy and Norris Ltd., Chelmsford, England) while a kitchen blender was used to grind the leaves. Ground plant material (100 g) was extracted with sterile distilled water. Similarly, 100 g of the plant material was separately extracted in methanol. The water extracts were lyophilized in a freeze-dryer while the methanol extracts were concentrated using a rotary evaporator. The extract was weighed and expressed as a percentage yield (Table 1).

Bioassays

Stock solutions

Stock solutions for in vitro assay of plant extracts were made in deionized autoclaved water and re-sterilized by passing through 0.22- μ m micro-filters in a laminar flow hood. The water-insoluble extracts were dissolved in 100% dimethylsulfoxide (DMSO) (Sigma Chemical Co., St Louis, MO) followed by a subsequent dilution to lower concentration of DMSO to $\leq 1\%$ to avoid solvent carry over (solvent) effect [22]. Stock solutions (1 μ g/ml) of chloroquine diphosphate, artemisinin and mefloquine were similarly prepared for use as reference drugs. All the drug solutions were stored at -20°C until required for bioassay.

Parasite cultures

Test samples were screened against two strains of *P. falciparum*, Sierra Leonean D6 (CQ-sensitive) and Indo-Chinese W2 (CQ-resistant). The strains were obtained from the Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Nairobi. The parasites were

Table 1 Yield (%) of solvent extracts of selected medicinal plants from Meru

Plant	Specimen number	Family	Part	Extract	Yield (%)
<i>Artemisia afra</i> Jacq.	CM035	Asteraceae	Leaves	H ₂ O	8.6
				MeOH	2.0
<i>Boscia salicifolia</i> Oliv.	CM039	Capparidaceae	Stem bark	H ₂ O	14.0
				MeOH	6.4
<i>Catharanthus roseus</i> (L.) Don	CM030	Apocynaceae	Leaves	H ₂ O	13.9
				MeOH	4.0
<i>Clutia robusta</i> Pax, E, FZ.	CM031	Euphorbiaceae	Leaves	H ₂ O	10.3
				MeOH	2.3
<i>Cyathula schimperiana</i> Igifashi	CM043	Amaranthaceae	Root	H ₂ O	8.5
				MeOH	2.6
<i>Rhus natalensis</i> Bernh.	CM037	Anacardiaceae	Stem bark	H ₂ O	10.0
				MeOH	1.9
<i>Ximenia americana</i> L.	CM033	Olacaceae	Root bark	H ₂ O	8.0
				MeOH	2.8

cultivated by a previously described in vitro technique [23]. The culture medium consisted of RPMI 1640 supplemented with 10% human serum [24]. Uninfected human blood group O Rh-positive erythrocytes (<28 days old) served as host cells. The cultures were incubated at 37°C in an atmosphere of 3% CO₂, 5% O₂ and 92% N₂ (BOC, Nairobi).

In vitro anti-plasmodial assay

An in vitro semi-automated micro-dilution assay technique that measures the ability of the extracts to inhibit the incorporation of [G-³H]hypoxanthine (Amersham International, Buckinghamshire, UK) into the malaria parasite was adopted [25]. Briefly, aliquots of the culture medium (25 µl) were added to all the wells of a 96-well flat-bottomed micro-culture plate (Falcon, Becton Dickinson, Franklin Lakes, NJ). Aliquots of the test solutions (25 µl) were added in duplicate to the first wells, and a Titertek motorized hand diluter (Flow laboratories, Uxbridge, UK) was used to make serial twofold dilutions of each sample over a 64-fold concentration range. Negative controls treated with solvent (DMSO) alone were also added to each set of experiments [26] to check for any solvent effect. The susceptibility tests were carried out with initial parasitemia (expressed as the percentage of erythrocytes infected) of 0.4% by applying 200 µl, 1.5% hematocrit, *P. falciparum* culture to each well. Two hundred microliters of culture media without parasites was added into four wells on the last row of each plate to serve as a background. Parasitized and non-parasitized erythrocytes were incubated at 37°C in a gas mixture, 3% CO₂, 5% O₂ and 92% N₂ for 48 h after which 25 µl of culture medium containing 0.5 µCi of [G-³H]hypoxanthine was added to each well. The culture plates were further incubated for 18 h. At the end of the incubation period, the radio-labeled cultures were harvested onto glass-fiber filters using a 96-well cell harvester. [G-³H]hypoxanthine uptake was determined using a micro-beta trilux liquid scintillation and luminescence counter (Wallac MicroBeta TriLux). The drug concentration capable of inhibiting 50% of the *P. falciparum* (IC₅₀) was determined by logarithmic transformation of drug concentration and radioactive counts per minute (cpm) using the formula:

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

where Y_{50} is the cpm value midway between parasitized and non-parasitized control cultures and X_1, Y_1, X_2 and Y_2 are the concentrations and cpm values for the data points above and below the cpm midpoints [27].

In vivo anti-malarial assay

Male Swiss mice (6–8 weeks old, weighing 20 ± 2 g) infected with *P. berghei* ANKA strain were used for anti-malarial assay. The parasite strain was maintained by serial passage of infected blood by interperitoneal (i.p.) injection. The assay protocol was based on the 4-day suppressive test [28]. Briefly, an aliquot of 0.2 ml (2×10^7 parasitized erythrocytes) from an infected donor mouse was transfused into uninfected experimental mice through i.p. injection [29–30]. The infected mice were randomly divided into groups of five individuals each for every test sample. The test extracts were prepared by dissolving the aqueous extracts in sterilized water. Methanol extracts were dissolved in 10% w/v tween 80. CQ diphosphate dissolved in sterilized water was used as a reference anti-malarial drug.

Post-infection (2–4 h on day 0) experimental mice groups were treated with a single dose of test sample at 100 mg/kg at a volume of 0.02 ml by i.p. injection. On days 1 to 3 (24, 48 and 72 h post-infection), the experimental groups of mice were treated again with the same dose and by the same route as on day 0. Two groups (5 mice each) served as negative and positive controls, respectively. The negative group received a placebo (saline) while the positive one was treated with 5 mg/kg day of the reference drug (CQ diphosphate).

Parasitemia was determined daily (24-h interval) by a thin blood film sampled from the tail and stained in 10% Giemsa solution. A thin blood smear was similarly made on day 4 (24 h after the last treatment or 96 h post-infection) from all the animals and stained as described above. Parasitemia was calculated by counting four fields of approximately 200 erythrocytes per field under a microscope. The difference between the mean value of the control group (taken as 100%) and those of the experimental groups was calculated and expressed as percent parasitemia suppression (PS) (activity) according to the following formula [31]:

$$PS = \{(A - B)/A\} \times 100$$

where A is the mean parasitemia in the negative control group on day 4 and B the corresponding parasitemia in the test group. The standard deviations for the mean values were calculated as previously described [32]. For all the groups of experimental mice used, the survival time (in days) was recorded and the mean for each group calculated.

Cytotoxicity evaluation

An actively dividing sub-confluent Vero cell growth-inhibition assay was done [33]. Briefly, Vero cells were grown

in Eagle's minimum essential medium (MEM; GIBCO, Grand Island, NY) supplemented with 5% fetal bovine serum (FBS) in 25-ml cell-culture flasks incubated at 37°C in 5% CO₂. Upon attainment of confluence, the cells were seeded at a concentration of 5×10^4 cells/well in 24-well plates and incubated at 37°C for 2 days. The culture medium was replaced by fresh medium containing test extracts at different concentrations and the cells incubated further for 2 days. Cells in triplicate wells for each sample were detached by trypsinization, and the number of viable cells determined by a trypan blue exclusion test. A hemocytometer was used to aid in counting viable cells. Inhibition data were plotted as dose-response curves, from which the 50% cell growth inhibitory concentration (CC₅₀) was estimated. The selectivity index (SI) was used as the parameter of clinical significance of the test samples by comparing general toxins and the selective inhibitory effect on *P. falciparum*, calculated as: CC₅₀ (vero)/IC₅₀ (*P. falciparum*) [34].

Results

Activity criteria in the in vitro assay were defined as high when IC₅₀ was below 10, moderate when between 10 and 50, and low when between 50 and 100 µg/ml. Samples with IC₅₀ >100 µg/ml were considered as inactive. The anti-plasmodial activity of the seven medicinal plants against CQ sensitive/mefloquine-resistant *P. falciparum* (D6 strain) and CQ resistant/mefloquine-sensitive (W2 strain) are summarized in Table 2.

Extracts of *B. salicifolia* were the most active (IC₅₀ range 1.04 ± 0.03 to 10.09 ± 1.56 µg/ml) against both *P. falciparum* strains followed by that of *A. afra* (IC₅₀ 3.98 ± 0.98 to 11.23 ± 1.98 µg/ml). The methanol extract of *C. roseus* also exhibited high anti-plasmodial activity (4.65 ± 0.08 to 5.34 ± 0.65 µg/ml) against the CQ-sensitive and resistant strains. Apart from *B. salicifolia* and *A. afra*, the water extracts exhibited lower in vitro anti-plasmodial activity than their respective methanol ones in most cases. The water extracts of *X. americana*, *R. natalensis* and *C. schimperiana* were inactive (IC₅₀ > 100 µg/ml), while that of *C. roseus* showed moderate activity (IC₅₀ 10–50 µg/ml).

Anti-malarial effects of extracts from seven selected medicinal plants on *P. berghei* ANKA in mice are summarized in Table 3. The suppression of parasitemia (chemo-suppression) and mean survival rate (time) were used as measures of efficacy. Samples were categorized as highly active when chemo-suppression was above 60% or moderately active between 30 and 60%, but lowly active below 30%.

Methanol extract of *B. salicifolia* and water extract of *R. natalensis* were the most active with chemosuppression of 86.50 ± 4.37 and 83.15 ± 3.61%, respectively. Both water and methanol extracts of *A. afra* also showed high chemosuppression (70.25 ± 5.59 and 77.45 ± 3.06%, respectively). The water extracts of *B. salicifolia*, *C. roseus* and *C. robusta* showed moderate chemo-suppression of parasitemia (43.75 ± 6.90, 42.36 ± 4.87 and 42.35 ± 2.15%, respectively). Similarly, the methanol extracts of *R. natalensis*, *C. schimperiana*, *C. roseus* and *X. americana*

Table 2 In vitro anti-plasmodial activity (IC₅₀) of extracts of selected medicinal plants IC₅₀ ± SD (µg/ml)

Plant	Extract	<i>P. falciparum</i> (D6)	<i>P. falciparum</i> (W2)
<i>Artemisia afra</i>	MeOH	9.04 ± 0.54	3.98 ± 0.98
	H ₂ O	11.23 ± 1.98	4.65 ± 0.64
<i>Boscia salicifolia</i>	MeOH	1.04 ± 0.03	8.86 ± 0.24
	H ₂ O	3.65 ± 0.78	10.09 ± 1.56
<i>Catharanthus roseus</i>	MeOH	4.65 ± 0.88	5.34 ± 0.65
	H ₂ O	32.36 ± 4.31	36.17 ± 7.79
<i>Clutia robusta</i>	MeOH	3.41 ± 0.02	7.58 ± 0.09
	H ₂ O	71.24 ± 1.69	157.81 ± 5.66
<i>Cyathula schimperiana</i>	MeOH	10.40 ± 1.25	17.61 ± 2.61
	H ₂ O	175.92 ± 14.45	>250
<i>Rhus natalensis</i>	MeOH	76.84 ± 8.09	98.32 ± 6.48
	H ₂ O	111.60 ± 22.76	105.25 ± 18.36
<i>Ximenia americana</i>	MeOH	16.74 ± 2.31	68.19 ± 0.12
	H ₂ O	106.66 ± 29.39	129.23 ± 6.55
Artemisinin		0.00164 ± 0.00011	0.00438 ± 0.00049
CQ diphosphate		0.00311 ± 0.00121	0.04057 ± 0.00052
MQ		0.01608 ± 0.00265	0.00255 ± 0.00003

Table 3 Effect of extracts (100 mg/kg day) of selected medicinal plants on *P. berghei* infected mice

Plant	Extract	Parasitemia (%)	Chemo-suppression (%)	Mean survival time (days)
<i>Artemisia afra</i>	MeOH	8.45 ± 0.27	77.45 ± 3.06	19.21 ± 3.57
	H ₂ O	11.15 ± 3.01	70.25 ± 5.59	17.85 ± 2.81
<i>Boscia salicifolia</i>	MeOH	5.06 ± 0.84	86.50 ± 4.37	19.08 ± 4.22
	H ₂ O	21.08 ± 4.52	43.75 ± 6.90	15.71 ± 5.41
<i>Catharanthus roseus</i>	MeOH	24.51 ± 4.32	34.60 ± 1.77	10.85 ± 2.50
	H ₂ O	21.60 ± 2.51	42.36 ± 4.87	12.39 ± 2.95
<i>Clutia robusta</i>	MeOH	28.81 ± 1.40	23.10 ± 6.43	10.45 ± 3.25
	H ₂ O	21.60 ± 2.39	42.35 ± 2.15	12.82 ± 4.70
<i>Cyathula schimperiana</i>	MeOH	20.58 ± 1.69	45.08 ± 6.93	15.38 ± 4.18
	H ₂ O	30.33 ± 2.45	19.06 ± 5.33	7.65 ± 1.89
<i>Rhus natalensis</i>	MeOH	16.40 ± 1.82	56.24 ± 4.85	13.43 ± 5.24
	H ₂ O	6.31 ± 0.97	83.15 ± 3.61	16.52 ± 1.87
<i>Ximenia americana</i>	MeOH	23.80 ± 7.30	36.49 ± 0.28	7.85 ± 1.59
	H ₂ O	29.40 ± 3.89	21.55 ± 6.27	6.72 ± 1.52
CQ diphosphate		0.38 ± 0.43%	99.02 ± 1.26%	21.25 ± 5.28
PBS		37.47 ± 6.62%	0.00	7.14 ± 2.14

PBS phosphate buffered saline (negative control), chloroquine diphosphate (standard drug) administered at 5 mg/kg day; n = 5

showed moderate activity with 56.24 ± 4.85, 45.08 ± 6.93, 34.60 ± 1.77 and 36.49 ± 0.28% chemo-suppression, respectively. *Ximenia americana* and *C. schimperiana* water extracts and the methanol extract of *C. robusta* exhibited the least chemo-suppression of parasitemia (<30%).

Mean survival time of the experimental groups of test animals correlated to treatment. The survival period of mice that received extracts shown to have a high percent of chemo-suppression lived longer compared to those with low activity or no treatment. Mice treated with the methanol extract of *A. afra* had a mean survival time of

19.21 ± 3.57 days while that of the untreated group was 7.14 ± 2.14 days. These differences were statistically significant in some treatments when compared to the control group using the Dunnett multiple comparison procedure [35–36] (PC-PCSM Software, Version 6.0, 1992, Delta-soft, Meylan, France). These included water and methanol extracts of *A. afra* and *B. salicifolia* (*P* < 0.05). Other tested extracts that prolonged the survival time of mice significantly was the water extract of *R. natalensis* (*P* < 0.05). Where survival time of mice was not significantly different from the untreated control group, deaths may be attributed to the effect of high

Table 4 Cytotoxic effect of selected medicinal plants on Vero cells

Plant	Extract	CC ₅₀ (µg/ml)	D6 SI	W2 SI
<i>Artemisia afra</i>	MeOH	594.85	65.8	149.46
	H ₂ O	2,825.21	251.58	607.57
<i>Boscia salicifolia</i>	MeOH	304.92	293.19	34.42
	H ₂ O	1,683.95	461.36	166.89
<i>Catharanthus roseus</i>	MeOH	167.52	36.03	31.37
	H ₂ O	1,285.74	39.73	35.55
<i>Clutia robusta</i>	MeOH	460.29	134.98	60.72
	H ₂ O	4,352.76	61.1	27.5
<i>Cyathula schimperiana</i>	MeOH	241.34	23.21	13.7
	H ₂ O	2,907.12	16.52	ND
<i>Rhus natalensis</i>	MeOH	211.78	2.76	2.15
	H ₂ O	3,958.16	35.47	37.7
<i>Ximenia americana</i>	MeOH	198.11	11.83	2.91
	H ₂ O	1,720.38	16.13	13.31

D6 SI Selectivity index calculated as CC₅₀/IC₅₀ tested against *P. falciparum* D6, W2 SI selectivity index calculated as CC₅₀/IC₅₀ tested against W2, ND not done since IC₅₀ > 250 µg/ml

parasite density or as a result of the toxicity of the extracts.

The highest selective inhibition (ratio of 50% cytotoxic concentration to 50% effective anti-plasmodial concentration) of *P. falciparum* was observed with the water and methanol extracts of *A. afra* and water extract of *B. salicifolia* with selectivity index (SI) ratios of >100 (Table 4). Methanol extract of *C. robusta* also depicted a high selective index. Methanol extract of *R. natalensis* was the most cytotoxic (CC₅₀ 211.78 µg/ml, SI 2.15–2.76) followed by *X. americana* and *C. schimperiana* (SI 2.9–23.21). *Catharanthus roseus* was moderately cytotoxic.

Discussions

Herbal medicine is used by up to 80% of the population in the developing countries [37]. Despite the widespread use, few scientific studies have been undertaken to ascertain the efficacy of traditional remedies. The present investigation demonstrates that water and methanol extracts of selected plants are potential candidates for the discovery of effective anti-malarial drugs or lead compounds. *Catharanthus roseus* exhibited high in vitro anti-plasmodial activity, which may be due to the presence of compounds such as alkaloids, terpenoids [38], flavonoids [39] and sesquiterpenes [40] that were previously isolated from the plant. However, the high in vitro anti-plasmodial activity exhibited was lost when the same was subjected to in vivo assay. *Artemisia afra* has previously been shown to exhibit good in vitro activity against the CQ-sensitive (PoW) and CQ-resistant (Dd2) *P. falciparum* strains [41]. Seven flavonoids have so far been isolated from this plant through bioassay-guided fractionation with acacetin, genkwanin and 7-methoxyacacetin exhibiting good in vitro anti-plasmodial activity (IC₅₀ 4.3–12.6 µg/ml) [41]. Our study confirms similar levels of anti-plasmodial activity and low toxicity of *A. afra* extracts. The in vivo activity of *A. afra* methanol and water extracts was higher than most of the tested plants. *Artemisia afra* is therefore a good candidate with high potential for the discovery of anti-malarial drugs. *Boscia salicifolia* also had high in vitro anti-plasmodial activity (1.04–10.09 µg/ml) against both the CQ-sensitive (D6) and resistant (W2) *P. falciparum* strains. However, the in vivo activity of *B. salicifolia* water extract was moderate while that of the methanol one was high (43.75 ± 6.90 and 86.50 ± 4.37%, respectively). To our knowledge, this is the first report of the anti-plasmodial activity of *B. salicifolia*. The high in vivo anti-malarial activity of *R. natalensis* is also being reported for first time in this study. The methanol extract of *X. americana* stem-bark was previously screened against *P. falciparum* (W2)

and the reported activity (IC₅₀ > 50 (g/ml) agrees with our findings [42] and is supported by earlier findings [43]. Mild brine shrimp lethality (LC₅₀ 11.25 µg/ml) has also been reported for the same extract [44]. This has been confirmed by Vero cell cytotoxicity assay (Table 4). However, the aqueous extract has much lower toxicity. In this study, in vitro activity for several plants was lost in vivo, and yet others with low in vitro activity demonstrated high in vivo activity. Biotransformation or poor bioavailability of the anti-plasmodial compounds may explain the lack of in vivo anti-malarial activity. Cases of high in vivo, but low in vitro anti-plasmodial activity are common, and several reasons have been advanced. Previous studies have shown that, owing to many factors, an extract lacking in vitro activity may still possess in vivo activity [29]. Cytotoxicity results obtained in this study will enable the selection of anti-plasmodial extracts and support further investigation for their potential as sources for lead anti-malarial compounds. Most of the tested plants revealed that selectivity indices were high, thus enabling achievement of therapeutic doses at safe concentrations.

Conclusions

We have demonstrated the anti-plasmodial and anti-malarial effects of seven selected medicinal plants commonly used by the Meru community for the treatment of malaria. The aqueous extracts are generally less toxic than methanol ones and may explain the use of the former in traditional systems of health care delivery. A large number of the tested samples had appreciable anti-plasmodial activity. This demonstrates that the ethno-pharmacological approach used in this study may be useful in the search for new anti-malarial drugs from natural sources. Bioassay-guided fractionation of the anti-plasmodial extracts is underway in order to isolate and identify the active compounds. These will be used as markers for standardization of herbal remedies or may lead to anti-malarial compounds for drug development or templates for synthetic drugs. Cytotoxicity evaluation of a few of the selected plants used in the study revealed low selectivity indices especially for methanol extracts. This is an indication of toxic principles contained in them. We recommend that thorough toxicological studies be done on these plants before they are prescribed for human use.

Acknowledgments The authors acknowledge the World Health Organization (WHO) for funding all aspects of this study under TDR-WHO grant no. A30707. Mr. G. Mungai of the East African Herbarium, National Museums of Kenya (NMK), is acknowledged for assisting in the collection and botanical identification of the medicinal plants. We recognize the Director of KEMRI for allowing the publication of this work.

References

1. Breman JG (2001) The ears of the hippopotamus: manifestations, determinants, and estimates of the malaria burden. *Am J Trop Med Hyg* 64:1–11
2. WHO (2000) Global surveillance on control of leishmaniasis. A report of the scientific group. Technical report series, no. 529. WHO, Geneva
3. Trape JF (2001) The public health impact of chloroquine resistance in Africa. *Am J Trop Med Hyg* 64:12–17
4. Snow RW, Trape JF, Marsh K (2001) The past, present and future of childhood malaria mortality in Africa. *Trends Parasitol* 17:593–597
5. Olliaro PL, Bloland PB (2001) Clinical and public health implications of anti-malarial drug. In: Rosenthal PJ (ed) *Drug discovery*. Humana, Totowa, pp 65–83
6. Ridley RG (1997) *Plasmodium*. Drug discovery and development; an industrial perspective. *Exp Parasitol* 87:293–304
7. Harald N, Chansuda W, Walther H (2003) Malaria drug-sensitivity testing: new assays, new perspectives. *Trends Parasitol* 19:175–178
8. Majori G (2004) Combined anti-malarial therapy using artemisinin. *Parasitologia* 46:85–87
9. Trape JF (2002) Combating malaria in Africa. *Trends Parasitol* 18:224–230
10. Bloland P (2001) Drug resistance in malaria. WHO/CDS/DRS.4. WHO, Geneva
11. Mueller MS, Karhagomba IB, Hirt HM, Wemakor E (2000) The potential of *Artemisia annua* L. as a locally produced remedy for malaria in the tropics: agricultural, chemical and clinical aspects. *J Ethnopharmacol* 73:487–493
12. Tagboto S, Townson S (2001) Anti-parasitic properties of medicinal plants and other naturally occurring products. *Adv Parasitol* 50:199–295
13. Garham PCC (1966) *Malaria parasites and other haemosporidia*. Blackwell, Oxford, pp 470
14. Meshnick SR (2001) Artemisinin and its derivatives. In: Rosenthal PJ (ed) *Anti-malarial chemotherapy: mechanisms of action, resistance, and new directions in drug discovery*. Humana, Totowa, pp 191–201
15. Njoroge GN, Bussmann RW (2006) Diversity and utilization of anti-malarial ethno-phytotherapeutic remedies among the Kikuyus (Central Kenya). *J Ethnobiol Ethnomed* 2:1–8
16. Wanyonyi AW, Chhabra SC, Mkoji G, Eilert U, Njue WM (2002) Bioactive steroidal alkaloid glycosides from *Solanum aculeastrum*. *Phytochemistry* 59:79–84
17. Muregi FW, Chhabra SC, Njagi ENM, Lang'at-Thoruwa CC, Njue W.M, Orago ASS, Omar SA, Ndiege IO (2004) Anti-plasmodial activity of some Kenyan medicinal plant extracts singly and in combination with chloroquine. *Phytother Res* 18:379–384
18. Muregi FW, Chhabra SC, Njagi ENM, Lang'at-Thoruwa CC, Njue WM, Orago ASS, Omar SA, Ndiege IO (2003) In vitro anti-plasmodial activity of some plants used in Kisii, Kenya against malaria and their chloroquine potentiation effects. *J Ethnopharmacol* 84:235–239
19. Wanyoike GN, Chhabra SC, Lang'at-Thoruwa CC, Omar SA (2004) Brine shrimp toxicity and anti-plasmodial activity of five Kenyan medicinal plants. *J Ethnopharmacol* 90:129–133
20. Kirira PG, Rukunga GM, Wanyonyi AW, Muregi FM, Gathirwa JW, Muthaura CN, Omar SA, Tolo F, Mungai GM, Ndiege IO (2006) Anti-plasmodial activity and toxicity of extracts of plants used in traditional malaria therapy in Meru and Kilifi districts of Kenya. *J Ethnopharmacol* (in press)
21. Houghton PJ (2001) Old yet new-pharmaceuticals from plants. *J Chem Ed* 78:175–184
22. Dominique D, Karine LR, Pina S, Pietro A, Daniel P, Patrick P, Laurent M, Christian D (2001) Pfnek-1, a NIMA-related kinase from the human malaria parasite *Plasmodium falciparum*; biochemical properties and possible involvement in MAPK regulation. *Eur J Biochem* 268:2600–2608
23. Trager W, Jensen JB (1976) Human malaria parasites in continuous culture. *Science* 193:673–675
24. Schlichtherle M, Wahlgren M, Perlmann H, Scherf A (2000) *Methods in malaria research*, 3rd edn. MR4/ATCC, Manassas, pp 1–74
25. Desjardins RE, Canfield C, Haynes J, Chulay J (1979) Quantitative assessment of anti-malarial activity in vitro by a semi-automated microdilution technique. *Antimicrob Agents Chemother* 16:710–718
26. Azas N, Laurencin N, Delmas F, Giorgio C, Gasquet M, Laget M, Timon-David P (2001) Synergistic in vitro anti-malarial activity of plant extracts used as traditional herbal remedies in Mali. *Parasitol Res* 88:165–171
27. Sixsmith DG, Watkins WM, Chulay JD, Spencer HC (1984) In vitro anti-malarial activity of tetrahydrofolate dehydrogenase inhibitors. *Am J Trop Med Hyg* 33:772–776
28. Peters W, Portus JH, Robinson BL (1975) The chemotherapy of rodent malaria, XXII. The value of drug-resistant strains of *Plasmodium berghei* in screening for blood schizontocidal activity. *Ann Trop Med Parasitol* 69:155–171
29. Gessler MC, Tanner M, Chollet J, Nkunya MHH, Heinrick M (1995) Tanzanian medicinal plants used traditionally for the treatment of malaria: in vivo anti-malarial and in vitro cytotoxic activities. *Phytother Res* 9:504–508
30. Ajaiyeoba EO, Abalogu UI, Krebs HC, Oduola AMJ (1999) In vivo anti-malarial activities of *Quassia amara* and *Quassia undulata* plant extracts in mice. *J Ethnopharmacol* 67:321–171
31. Tona L, Mesia K (2001) In-vivo antimalarial activity of *Cassia occidentalis*, *Morinda morindoides* and *Phyllanthus niruri*. *Ann Trop Med Parasitol* 95:47–57
32. Armitage P, Berry G (1991) *Statistical methods in medicinal research*. Blackwell, Oxford, pp 90–92
33. Kurokawa M, Nagasaka K, Hirabayashi T, Uyama S, Sato H, Kageyama T, Kadota S, Ohyama H, Hozumi T, Namba T, Shiraki K (1995) Efficacy of traditional herbal medicines in combination with acyclovir against herpes simplex virus type 1 infection in vitro and in vivo. *Antiviral Res* 27:19–37
34. Wright CW, Phillipson JD (1990) Natural products and the development of selective anti-protozoal drugs. *Phytother Res* 4:4–10
35. Dunnett CW (1964) New tables for multiple comparisons with a control. *Biometrics* 1:482–491
36. Benoit F, Robert A, Meunier B (2000) In vitro and in vivo potentiation of artemisinin and synthetic endoperoxide anti-malarial drugs by metalloporphyrins. *Antimicrob Agents Chemother* 44:2836–2841
37. Kingsbury JM (1964) *Poisonous plants of the United States and Canada*. Prentice-Hall, Englewood Cliffs, pp 626
38. Collu G, Unver N, Peltenburg-looman AG, Van der Heijden R, Verpoorte R, Memelink J (2001) Geraniol 10-hydroxylase1, a cytochrome p450 enzyme involved in terpenoid indole alkaloid biosynthesis. *FEBS Lett* 508:215–220
39. Vimala Y, Jain R (2001) A new flavone in mature *Catharanthus roseus* petals. *Indian J Plant Physiol* 6:187–189
40. Hirose F, Ashihara H (1984) Metabolic regulation in plant cell culture-fine control of purine nucleotide biosynthesis in intact cells of *Catharanthus roseus*. *J Plant Physiol* 116:417–423
41. Kraft C, Jenett-Siems K, Siems K, Jakupovic J, Mavi S, Bienzle U, Eich E (2003) In vitro anti-plasmodial evaluation of medicinal plants from Zimbabwe. *Phytother Res* 17:123–128

42. Verotta L, Dell'agli M, Giolito A, Guerrini M, Cabalion P, Bosisio E (2001) In vitro antiplasmodial activity of extracts of *tristanopsis* species and identification of the active constituents: ellagic acid and 3,4,5-trimethoxyphenyl-(6'-*o*-galloyl)-*o*- β -D-glucopyranoside. *J Nat Prod* 64:603–607
43. Benoit F, Valentin A, Pelissier Y, Diafouka F, Marion C, Kone-Bamba D, Kone M, Mallie M, Yapo A, Bastide JM (1996) In vitro anti-malarial activity of vegetal extracts used in West African traditional medicine. *Am J Trop Med Hyg* 54:67–71
44. Mainen JM, Joseph CC, Zakaria H, Mbwambo MK, Nkunya HHM (2004) Testing beyond ethno-medical claims: brine shrimp lethality of some Tanzanian plants. *Pharm Biol* 42:547–551