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In Vivo Antimalarial Activity of Aqueous Extracts from Kenyan Medicinal Plants and their Chloroquine (CQ) Potentiation Effects against a Blood-induced CQ-resistant Rodent Parasite in Mice

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Hot water extracts from eight medicinal plants representing five families, used for malaria treatment in Kenya were screened for their *in vivo* antimalarial activity in mice against a chloroquine (CQ) resistant *Plasmodium berghei* NK65, either alone or in combination with CQ. Extracts of three plants, *Toddalia asiatica* (root bark), *Rhamnus prinoides* (leaves and root bark) and *Vernonia lasiopus* (root bark) showed high chemosuppression in the range 51%–75%. *Maytenus acuminata*, *M. heterophylla*, *M. senegalensis* and *Rhamnus staddo* had moderate activities of 33%–49% parasitaemia suppression in the root bark and/or leaf extracts, while *Withania somnifera* (root bark) had a non-significant suppression (21%). In combination with CQ, extracts of *V. lasiopus* (all parts), leaf extracts of *M. senegalensis*, *R. prinoides* and *T. asiatica* as well as root barks of *M. heterophylla*, *R. staddo* and *T. asiatica* had improved parasitaemia suppression in the range 38%–66%, indicating synergistic interactions. Remarkable parasitaemia suppression by the extracts, either alone or in combination with CQ resulted into longer survival of mice relative to the controls, in some cases by more than 2 weeks. Plants, which showed significant antimalarial activity including *V. lasiopus*, *T. asiatica* and *R. prinoides*, should further be evaluated in the search for novel agents against drug-resistant malaria. **Copyright © 2007 John Wiley & Sons, Ltd.**

Keywords: medicinal plants; drug combination; *Plasmodium berghei* NK65; traditional medicine; CQ-resistance; synergistic effects.

INTRODUCTION

Plasmodium falciparum, the most dominant and pathogenic of the four human plasmodia, is responsible for almost all malaria mortality and morbidity in tropical and subtropical countries (Teklehaimanot and Bosman, 1999). In sub-Saharan Africa where over 90% of all the global burden of malaria exists, up to 50% of all outpatient visits in areas with high malaria transmission and 30%–50% of all hospital admissions are attributed to malaria (WHO, 2005). It is estimated that economic losses due to malaria in Africa amount to about $12 billion a year, which by far exceeds the resources needed for malaria control, estimated at about $3 billion (DFID, 2005). Although an effective vaccine is the best long-term control option for malaria, current work on vaccine development largely remains at a preclinical stage, and considering the different phases of vaccine development, it is predicted that a reliable malaria vaccine may be several years away. Thus, the current management strategy mainly depends on chemotherapy. However, multiple antimalarial drug-resistant *P. falciparum* is causing not only the spread of malaria to new areas but also to its reemergence in areas where it had previously been eradicated. Many antimalarial drugs in current usage are chemically related and hence the development of resistance to one drug can facilitate the development of resistance to others. Cross-resistance between chloroquine (CQ) and amodiaquine, both 4-aminoquinolines, has been reported and resistance to mefloquine may also lead to resistance to halofantrine and quinine (Bloland, 2001). The inexorable rise of resistance to the cheap mainstay antimalarial drugs such as CQ and sulfadoxine pyrimethamine by the parasite has prompted a renewed impetus over recent years on research towards the discovery and development of new, safe and affordable antimalarial chemotherapies.

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Today, the commonly used antimalarial drugs, the quinoline blood schizontocides (chloroquine, amodiaquine, mefloquine) and the peroxide antimalarials (artemisinin derivatives) are modeled upon the plant based compounds, quinine and artemisinin, respectively (Ridley, 1997). The discovery of the endoperoxide sesquiterpenes, artemisinin, from *Artemisia annua* has provided the impetus for the investigation of other species of plants for novel antimalarial drugs (Phillipson and Wright, 1991). In developing countries, conventional drugs or formal health systems, unlike traditional medical systems, may not be available or affordable to most of the rural populations. Although up to 80% of the Africa population uses traditional medicine, especially plant remedies, in the management of diseases including malaria, the plants are not yet fully explored (WHO, 2002b). Currently, malaria chemotherapy is targeting the use of drug combinations, providing a possible means of enhancing the antiparasitic activity as well as circumventing or delaying the induction of drug resistance (Rosenthal, 2003). Also, attempts are being made to reverse the parasite's resistance to conventional antimalarial drugs such as CQ, and a range of diverse molecules such as chlorpheniramine, a histamine H1-antagonist have been reported to reverse CQ-resistance (Rosenthal, 2003). There are reports that some local African populations use medicinal plants in association with CQ to enhance its activity (Rasoanaivo *et al.*, 1998), but very few documented data are available on interactions of plant remedies with synthetic drugs. The present study reports the *in vivo* antimalarial activity, alone or in combination with CQ, of aqueous plant extracts used traditionally in the management of malaria in Kenya, against a CQ-resistant rodent malaria parasite, in mice.

MATERIALS AND METHODS

Plant materials and extraction. Based on ethnomedical data, different plant parts (leaves, stem bark, root bark) of eight plant species representing five families (Table 1) were collected in January 2004 from central Kenya (Mount Kenya Forest) and the southern Rift Valley (Nguruman Escarpment in Magadi). The plants were authenticated by Mr Geoffrey M. Mungai, a taxonomic botanist from the East African Herbarium in Nairobi, where voucher specimens were deposited. The specimens were catalogued, dried under shade and ground into powder using a laboratory electric mill. The powder of each plant part was packaged into 1 kg packs and stored in a dry and well-ventilated room until used. Aqueous extraction of each plant part was done by boiling 10 g of the ground material in 500 mL of distilled water followed by filtration and concentration of the extract to 50 mL. The concentrate was then freeze-dried to give a dry sample, and this was stored at 4 °C until used. In the present study, hot water extraction was meant to mimic the procedures used in traditional extractions. The plant parts yielded adequate amounts of dry extracts for *in vivo* antimalarial assays (Table 1).

Table 1. Plants, corresponding parts collected and the yield (g freeze-dried aqueous extract/10 g dry plant material) after hot water extraction

Plant family/Botanical name (specimen code)	Part collected	Yield (g/10 g of plant material)
Rutaceae		
Toddalia asiatica (L.) Lam.		
(Ta-L/04)	Leaves	2.9
(Ta-RB/04)	Root bark	1.9
Celastraceae		
Maytenus acuminata (L.f.) Loes.		
(Ma-RB/04)	Root bark	2.9
M. heterophylla (Eckl. & Zeyh.) Robson		
(Mh-RB/04)	Root bark	0.9
M. senegalensis (Lam.) Exell		
(Ma-L/04)	Leaves	4.9
(Ma-RB/04)	Root bark	1.0
Compositae		
Vernonia lasiopus O.Hoffm.		
(Vl-L/04)	Leaves	1.8
(Vl-SB/04)	Stem bark	3.1
(Vl-RB/04)	Root bark	1.9
Rhamnaceae		
Rhamnus prinoides L' Hérit		
(Rp-L/04)	Leaves	2.2
(Rp-RB/04)	Root bark	1.8
R. staddo A.Rich.		
(Rs-L/04)	Leaves	1.6
(Rs-RB/04)	Root bark	5.3
Solanaceae		
Withania somnifera (L.) Dunal		
(Ws-RB/04)	Root bark	1.6

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The parasite. For *in vivo* antimalarial assays of plant extracts, a blood-induced CQ-resistant *Plasmodium berghei* (strain NK65), a rodent malaria parasite was used. The parasite, maintained at the Parasite Bank of the Department of Parasitology, Hamamatsu University School of Medicine was previously obtained from Professor Y. Wataya of Okayama University, Japan. The frozen parasite specimen (−80 °C) was thawed out and inoculated intraperitoneally (i.p.) into two male outbred ICR mice, which served as the donor mice to the experimental mice. Six days after parasite inoculation, parasitaemia of the donor mice was assessed microscopically by examining Giemsa stained thin blood smears. In addition, the erythrocyte densities were determined using a haemocytometer (F-520, Sysmex Corporation, Japan). Using the parasitaemia and erythrocyte density of the donor mice, the parasitaemia was adjusted downwards using physiological saline and each of the experimental male ICR mice, 7-week-old weighing about 30 g (Japan SLC Inc., Hamamatsu, Japan) was inoculated i.p. with approximately 10^5 parasitized erythrocytes in volumes of 0.2 mL. The inoculated mice were then randomized into groups of five mice and each group put into a cage. The mice were maintained in an animal care facility on a commercial diet (LabDiet, PMI Nutrition International, MO, USA) and water *ad libitum*.

Antimalarial activity of plant extracts either alone or in combination with chloroquine. In screening of the plant extracts alone, the 4-day suppressive method described by Peters *et al.* (1975) was used. Within 3 h post-inoculation of mice with the parasite (day 0), treatment of the experimental groups was initiated by oral administration of the test extract at a dose of 500 mg/kg body weight and treatment was done twice a day for 4 days, up to day 3 post-infection (p.i.). The untreated control group received distilled water only. Twenty-four hours after the last treatment (day 4 p.i.), parasitaemia of the individual mouse was determined microscopically by counting the number of parasites in 5 fields of approximately 100 erythrocytes per field of Giemsa stained thin blood smears.

In assessing the *in vivo* interactions of CQ and the plant extracts, the method of Ishih *et al.* (2004) where treatment starts on day 4 p.i. was used. Infected mice were randomized into CQ/plant extract treated groups (CQ, 20 mg/kg body weight, once a day for 2 days + plant extract, 500 mg/kg body weight, twice a day for 4 days), a CQ-treated positive control group, at a dose of 20 mg/kg body weight, once a day for 2 days, and a water control group. For all mice before initial treatment on day 4 p.i., thin blood smears were prepared followed by administration of drugs by the oral route.

In both studies, the *in vivo* antimalarial activity of the test drugs was assessed by monitoring mouse survival and parasitaemia over a 30-day period. Parasitaemia was assessed by microscopic examination of Giemsa stained thin blood smears prepared from mouse-tail blood. In the present study, the activity of the crude plant extract was described as mild or moderate with a parasitaemia suppression of 30%–50%, while a suppression of 50% or more was considered high or remarkable.

Data and statistical analysis. The percentage suppression of parasitaemia for the plant extracts was calculated as: 100 − [(mean parasitaemia treated/mean parasitaemia control) × 100]. For comparison of average parasitaemia, one-way ANOVA and 2-tailed Student's *t*-test were used (Microsoft® Excel 2004), with $p < 0.05$ being considered significant. Comparison of mouse survival of the treated groups relative to the controls was done by χ^2 (chi-squared) test (Statmate III®), with $p < 0.05$ considered significant.

Ethical considerations. Handling of animals was done in accordance to the *Guide for the Care and Use of Laboratory Animals*, Hamamatsu University School of Medicine.

RESULTS

Antimalarial activity of plant extracts alone

Activities of plants extracts alone were assessed in terms of mean parasitaemia suppression in mice, and survival of mice with respect to untreated controls. Table 2 shows a summary of the percentage parasitaemia suppression for mice on days 4 and 7 post-infection (p.i.). Based on day 4 p.i. smears (i.e. 1 day post-treatment), two plant extracts, *V. lasiopus* (root bark) and *R. prinoides* (root bark), exhibited a remarkable suppression of parasitaemia of 54% and 51% ($p = 0.001$), respectively. Five extracts from four plants showed moderate activities ranging from 33% to 49%, all of which were statistically significant. These were derived from the leaves and root bark of *R. staddo* with suppressions of 33% and 42% ($p = 0.01$), respectively, *M. acuminata* (root bark) and *R. prinoides* (leaves) both with suppressions of 33% ($p = 0.02$, 0.04, respectively), and *M. heterophylla* (root bark) with a suppression of 49% ($p = 0.02$). Seven extracts from the leaves and root barks of both *M. senegalensis* and *T. asiatica*, *V. lasiopus* (leaves and stem bark) and *W. somnifera* (root bark) were inactive, with parasitaemia suppression ranging from 7% to 23%, which were not statistically significant ($p > 0.05$). On day 7 p.i., the percentage parasitaemia suppression for mice treated with extracts of root bark from *M. acuminata* and *M. senegalensis*, leaves of *R. prinoides* and *R. staddo* and *T. asiatica* (leaves and root bark) increased by values ranging from 1.2- to 10.7-fold that of the day 4 p.i. suppressions. The chemosuppressions afforded by the leaf extracts of *R. prinoides* and root-bark extracts of *T. asiatica* were statistically significant, with values of 54% ($p = 0.04$)) and 75% ($p = 0.003$), respectively.

In terms of survival, all mice of untreated controls died between day 7–9 p.i. (Table 2). The group treated with extracts of *T. asiatica* (root bark) had 100% ($p = 0.003$) of mice surviving up to day 15 p.i., 75% ($p = 0.02$) of mice surviving up to day 18 p.i., with the last mouse surviving up to day 20 p.i. *M. heterophylla* (root bark) and *R. prinoides* (leaves and root bark) had 40% of mice surviving beyond day 9 p.i. (Table 2). The group treated with leaves of *R. prinoides* had the longest survival, with the last two mice surviving up to day 22 and 23 p.i. Some groups had a single mouse surviving beyond day 9 p.i., but this survival was not statistically significant ($p > 0.05$).

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Table 2. Days 4 and 7 parasitaemia suppression (%) and % survival on day 9 post-infection, (p.i.) of mice treated from day 0 p.i. with aqueous extracts of plants at a dose of 500 mg/kg, twice a day for 4 days

Drug	Day 4 parasitaemia suppression[a] (%)	Day 7 parasitaemia suppression[a,b] (%)	Day 9 p.i. survival (%)
M. acuminata			
Root bark	33	42	20
M. heterophylla			
Root bark	49	40	40
M. senegalensis			
Leaves	21	20	0
Root bark	19	46	20
R. prinoides			
Leaves	33	54	40
Root bark	51	36	40
R. staddo			
Leaves	33	38	20
Root bark	42	34	20
T. asiatica			
Leaves	21	26	0
Root bark	7	75[d]	100[c]
V. lasiopus			
Leaves	23	12	0
Stem bark	23	16	20
Root bark	54	51	20
W. somnifera			
Root bark	21	11	0
Water control	–	–	0

[a] % parasitaemia suppression on day 4 based on mean parasitaemia of 5 mice per group ($n = 5$), except for *M. senegalensis* (leaves) group where $n = 4$.
[b] % parasitaemia suppression on day 7 based on mean parasitaemia of 5 mice per group ($n = 5$), except for *M. acuminata*, *T. asiatica* (root bark), *V. lasiopus* (leaves and stem bark) and water control groups, where $n = 4$, and *M. senegalensis* (leaves) group, where $n = 3$.
[c] For untreated control, 60% of mice died on day 7 p.i., and the last 2 mice died on day 8 and 9 post infection, respectively. For *T. asiatica* (root bark) group, 100% mice survived to day 15 p.i., 75% to day 18 p.i., and the last mouse died on day 20 p.i.
[d] $p = 0.003$, which is statistically significant ($p < 0.05$ was considered significant).

Antimalarial activity of plant extracts in combination with chloroquine

In the CQ/plant extract combination studies, where treatment of mice started on day 4 p.i., drug activities were assessed in terms of mouse survival as well as parasitaemia reduction, relative to CQ-treated controls. For all the groups, the parasitaemia levels on day 4 p.i. (before initial treatment) were not different ($p = 0.10$) and microscopic examination of day 8 p.i. smears detected no parasites. However, recrudescent parasites reappeared by day 11 p.i., and hence day 11 p.i. parasitaemia levels were considered the most significant in assessment of chemosuppression. Figure 1 shows a characteristic parasitaemia pattern for mice treated with CQ/plant extract combination, between day 4 and 14 p.i. All mice in the CQ control group died between day 13 p.i. (60%) and 14 p.i. (40%), and the survival was therefore monitored with day 14 p.i. as the reference. Table 3 shows a summary of parasitaemia suppression (%) on day 11 p.i. and survival (%) on day 14 p.i. Except for the CQ/*R. staddo* leaf extract, which showed low parasitaemia suppression of 26% that was not significant ($p = 0.12$), all other CQ/extract combinations showed activities ranging from moderate (below 50%) to remarkable (above 50%), all of which were statistically significant ($p < 0.05$). CQ in combination

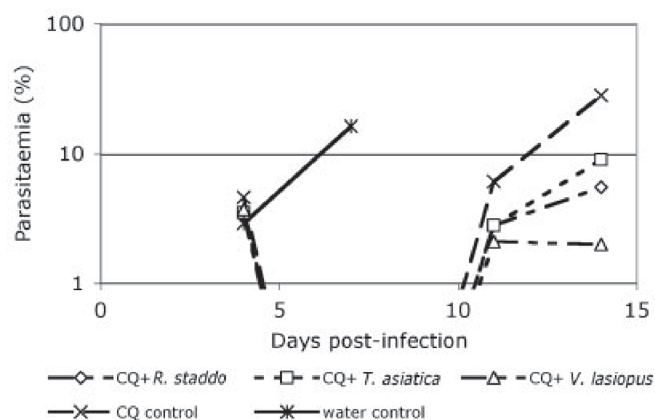

Figure 1. The characteristic parasitaemia patterns of day 4–14 post-infection (p.i.) for mice treated with a combination of CQ/ plant extracts (20 mg/kg body weight, once a day for 2 days + 500 mg/kg body weight, twice a day for 4 days respectively), on a logarithmic scale. Water and CQ controls are included for comparison. For purposes of illustration of the parasitaemia patterns in this figure, the root barks of *R. staddo*, *R. prinoides*, *T. asiatica* and *V. lasiopus* in combination with CQ, which had over 50% suppression on day 11 p.i. and survival of 40% or more on day 14 p.i., were selected. At day 8 p.i., no parasites were observable under the microscope for any group, but this does not mean 0% parasitaemia, since recrudescent parasites reappeared by day 11 p.i., hence the discontinuous lines between days 4 and 11 p.i. The *M. heterophylla* pattern almost overlapped that of *R. staddo*, and hence is omitted.

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Table 3. Day 11 post-infection (p.i.) % suppression of parasitaemia in mice, and the corresponding % survival on day 14 p.i. of mice treated from day 4 p.i. with chloroquine at a dose of 20 mg/kg, once a day for 2 days and plant aqueous extracts at a dose of 500 mg/kg, twice a day for 4 days

Drug	Day 11% parasitaemia suppression[a,b]	Day 14 p.i. survival (%)
CQ + *M. acuminata*		
Root bark	49	20
CQ + *M. heterophylla*		
Root bark	56	40
CQ + *M. senegalensis*		
Leaves	51	20
Root bark	41	40
CQ + *R. prinoides*		
Leaves	38	25
Root bark	34	60
CQ + *R. staddo*		
Leaves	26	40
Root bark	54	40
CQ + *T. asiatica*		
Leaves	51	20
Root bark	54	40
CQ + *V. lasiopus*		
Leaves	48	20
Stem bark	52	20
Root bark	66	60[c]
CQ + *W. somnifera*		
Root bark	59	0
CQ control	–	–

[a] % parasitaemia suppression for day 11 p.i. is based on mean parasitaemia of 5 mice per group ($n = 5$), except for *R. prinoides* (leaves) and *V. lasiopus* (stem bark) groups where $n = 4$.
[b] Values of p for all groups with respect to % parasitaemia suppression were statistically significant ($p < 0.05$), except for *R. staddo* (leaves), $p = 0.12$.
[c] Last 2 mice of *V. lasiopus* (root bark) survived up to day 17 p.i., but it is remarkable that the day 14 mean parasitaemia was at least 2.5–5 times lower in comparison with other groups which had similar survival periods.

with extracts of *M. heterophylla* (root bark), *M. senegalensis* (leaves), *R. staddo* (root bark), *T. asiatica* (leaves and root bark), *V. lasiopus* (stem and root barks) and *W. somnifera* (root bark) had remarkable suppressions of parasitaemia ranging from 51% to 66% (all p values < 0.01). Extracts of *V. lasiopus* (root bark) in combination with CQ showed the highest suppression of 66% ($p = 0.0003$). The extracts of *M. acuminata* and *M. senegalensis* (root bark), *R. prinoides* (leaves and root bark) and *V. lasiopus* (leaves) in combination with CQ showed moderate suppression ranging from 34% to 49% (all p values < 0.05).

With respect to day 14 p.i. when 100% of CQ-control mice died, the survival of mice treated with CQ in combination with some extracts ranged from 40% to 60%. CQ in combination with the root barks of *V. lasiopus* and *R. prinoides* had 60% ($p = 0.04$) survival on day 14 p.i. with the last two mice in the latter group surviving up to day 24 and 30 p.i. CQ in combination with root bark extracts of *M. senegalensis*, as well as leaf extracts of *R. staddo*, which had parasitaemia suppression of 41% and 26%, respectively, showed mice survival of 40% after day 14 p.i. *Rhamnus prinoides* (root bark) in combination with CQ had survival of 60% ($p = 0.04$) and 40% on day 14 and 24 p.i., respectively, with the last mouse surviving up to day 30 p.i. The CQ/*W. somnifera* (root bark) extract had a high chemosuppression (59%). However, all the mice of the group died within the same period as that of the CQ-control group.

DISCUSSION AND CONCLUSIONS

When assayed against CQ-resistant *P. berghei* NK65, 50% of the hot-water extracts of the eight plant species screened showed moderate to remarkable parasitaemia suppression on day 4 p.i. in the range 33%–54%, which partially validates the traditional use of the herbs by local communities in the management of malaria. Of the plants with considerable *in vivo* chemosuppression when used alone were *R. prinoides*, *R. staddo* and *V. lasiopus* in their root bark extracts, with values of 51%, 42% and 54%, respectively. In a previous *in vitro* screening of Kenyan medicinal plants, the three plants had shown moderate to significant antiplasmodial activities against both CQ-sensitive and -resistant *P. falciparum* isolates in their organic fractions, although the water extracts were within the inactive range (Muregi *et al.*, 2003). The organic leaf extracts of *V. lasiopus* had shown the highest *in vitro* inhibition of parasite growth, with IC_{50} values as low as 1.0 µg/mL, which is consistent with the findings of the present study where it showed the best *in vivo* parasitaemia suppression, albeit in its root bark aqueous extract. The presence and/or quantities of bioactive compounds in plants are influenced by several factors including seasons, weather conditions, environment, plant-part used, intra-species variations and plant age, among other factors (Weenen *et al.*, 1990). Therefore, the discrepancies observed between *in vitro* and *in vivo* activities of plant parts used could arise from the fact that the specimens were collected from different locations, at different seasons and possibly different ages since the plants were collected from the wild. *Vernonia* species have been extensively investigated for chemical constituents, and found to contain several metabolites including flavones and vernolic acid, triterpenes and oxygenated sesquiterpenes, with the latter being the most abundant secondary metabolites of the genus *Vernonia*. Two 5-methylcoumarins, 2′-epicycloisobrachycoumarinone epoxide and cycloisobrachycoumarinone epoxide (both isolated from the roots) as well as a germacrane dilactone 16,17-dihydrobrachycalyxolide isolated from the aerial parts of *V. brachycalyx* were reported to possess *in vitro* antiplasmodial activity against *P. falciparum* isolates (Oketch-Rabah *et al.*, 1997, 1998). Two novel elemanolides, epivernodalol and lasiopulide isolated from *V. lasiopus* showed *in vitro* cytotoxicity against human cancer cell lines (Koul *et al.*, 2003). *V. lasiopus* and *V. galamensis* extracts have been reported to possess sedative and analgesic effects in rats (Dahanukar *et al.*, 2000). Parasitaemia suppression by several extracts including *T. asiatica* (root bark) and *R. prinoides* (leaves) increased significantly between day 4 and 7 p.i. *T. asiatica* extract was especially remarkable in that its chemosuppression increased by about 11-fold to 75%, suggesting that the bioactive agent(s) in the plant has a slow onset of action, and/or that it is not fast acting. While some antimalarial drugs such as mefloquine and

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tetracyclines have a slow onset of action and are long acting, other drugs including artemisinin-based derivatives are known to be fast acting, and to have short half-life (Van Agtmael *et al.*, 1999). The slow onset of action for some plant extracts could be attributed to many pharmacological factors such as the active compound in the plant being a pro-drug, which must undergo metabolic changes in the host to an active agent, as in the case with the biguanide antimalarial drug proguanil, which is first metabolized to a slow-acting triazine metabolite, cycloguanil (Fidock *et al.*, 1998). Oketch-Rabah *et al.* (2000) reported high *in vitro* antiplasmodial activity (IC_{50} 1.8 μg/mL) by the organic extracts of *T. asiatica* (root bark) against a CQ-resistant *P. falciparum* isolate VI/S, which correlates with the findings of the present *in vivo* studies. A coumarin derivative, 5,7-dimethoxy-8-(3′-hydroxy-3′methyl-1′-butene)-coumarin isolated from the plant showed moderate *in vitro* antiplasmodial activity against *P. falciparum* isolates (Oketch-Rabah *et al.*, 2000). Previously, nitidine, an alkaloid isolated from the plant, had *in vitro* 50% inhibitory concentrations against both CQ-sensitive and -resistant *P. falciparum* isolates in the range 9–108 ng/mL, and was thought to be the major antimalarial principle of the plant (Gakunju *et al.*, 1995). Previous studies on chemical constituents of the plant yielded 17 coumarins, seven benzo[c]phenanthridine alkaloids, four quinoline alkaloids and a triterpenoid (Ishii *et al.*, 1991). The alkaloids of *T. asiatica* have been reported to possess *in vivo* antiinflammatory and analgesic effects in rats, with no injury to the liver, even after a long-term administration (Hao *et al.*, 2004). For some extracts including *M. heterophylla* (root bark), *M. senegalensis* (leaves), *R. prinoides* and *R. staddo* (root barks), *V. lasiopus* extracts (all parts) and *W. somnifera* (root bark), the suppression on day 7 p.i. in comparison with day 4 p.i. remained more or less the same, or decreased substantially, in some cases giving a 2-fold decrease. In the former situation, it suggests that the active agents in the extracts may be long acting and/or were in higher plasma concentrations, while in the latter, the implication is that the compounds either have short half-life or were not in sufficient concentrations to sustain the desired pharmacological effects. *R. staddo* and *R. prinoides* are the only two *Rhamnus* species that occur in Africa and the latter is widespread in many parts of eastern and central Africa. Twenty compounds including seven glycosides of emodin anthrone, five flavonoids and three naphthalenic derivatives were isolated from *R. prinoides* (Abegaz *et al.*, 1999). Emodin has been reported to possess various pharmacological and biological activities including immuno-stimulation, antiparasitic, antiinflammatory and analgesic effects (Izhaki, 2002). Organic extracts of *M. senegalensis* showed high antiplasmodial activity against *P. falciparum* isolates, and subsequent phytochemical analysis revealed terpenoids and traces of phenolic principles (El Tahil *et al.*, 1999). Maytenoic acid, lupenone and *β*-amyrin isolated from the roots of *M. senegalensis* showed remarkable antiinflammatory activity in mice, comparable to that of the reference drugs indomethacin and hydrocortisone (Sosa *et al.*, 2006). Orabi *et al.* (2001) isolated a new dihydroagarofuran alkaloid from *M. heterophylla*, together with six known compounds including maytenfolic acid. The latter showed moderate antimicrobial activity.

With respect to the survival of mice treated with extracts alone, it is remarkable that longer survival corresponded with significant parasitaemia suppression in mice, either on day 4 or 7 p.i. For instance, whereas all mice of the untreated control died between days 7–9 p.i., 100% and 75% of mice treated with root bark extract of *T. asiatica* survived up to days 15 and 18 p.i., respectively, far beyond the survival of controls. The extract of *R. prinoides* (leaves), which had just a mild chemosuppression of 33%, had 40% of mice surviving up to day 22 p.i. and the last mouse died on day 23 p.i. This suggests that, apart from direct parasiticidal activity, some extracts could have other pharmacological effects that may counter other aspects of malaria illness such as fever, immunosuppression and pain. Such plants may contain principles that may act as immunomodulators, antipyretics or analgesics (Dahanukar *et al.*, 2000).

In combination with CQ, most of the extracts showed better chemosuppression than when used alone, suggesting synergistic interactions with CQ. On comparing the day 7 p.i. parasitaemia suppression of extracts alone with that of day 11 p.i. CQ/extract combinations (since the two time points give equal post-treatment durations), it was noted that extracts of *V. lasiopus* (all parts), leaf extracts of *M. senegalensis*, *R. prinoides* and *T. asiatica* as well as root barks of *M. heterophylla*, *R. staddo* and *T. asiatica* in combination with CQ had 1.2- to 5.4-fold increases in chemosuppression, than when used alone. However, some extracts including *R. prinoides* (leaves and root bark), *R. staddo* (leaves) and root bark of *T. asiatica* and *M. senegalensis* showed no improved activity in terms of parasitaemia reduction when combined with CQ, which may imply additive interactions. With respect to the survival of mice, it is significant that the remarkable suppression of parasitaemia in mice by extracts of *M. heterophylla*, *T. asiatica*, *R. staddo* and *V. lasiopus* (root barks) in combination with CQ translated into more mice with longer survival after day 14 p.i. Interestingly, some extracts which showed low to moderate parasitaemia suppression in combination with CQ, such as the root bark extracts of *M. senegalensis* and *R. prinoides*, as well as the leaf extracts of *R. staddo*, recorded a 40%–60% survival of mice after day 14 p.i., accompanied by long survival. It is remarkable that *R. prinoides* (root bark) in combination with CQ had 60% of mice surviving at this time point, with the last mouse surviving up to day 30 p.i. As observed earlier, this could be attributed to other pharmacological effects of the plant's active principles, rather than direct parasiticidal action. In the present study, CQ/*W. somnifera* (root bark) extract showed a high chemosuppression of 59%, which did not translate to a corresponding long survival of mice. Withanolides are the major chemical constituents of *W. somnifera*, which has been studied extensively for its pharmacological and biological activities. Withanolides are reported to possess wide-ranging activities including adaptogenic, immunostimulatory, antiinflammatory, as well as radioprotective effects. Withaferin A, a steroidal lactone from the plant root is said to have anticancer properties (Dahanukar *et al.*, 2000). Therefore, the plant extracts may have played an immunomodulatory role to the host's immune system during the early days of infection, resulting in a higher parasitaemia suppression of the CQ/extract combination, an effect that was not sustained

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through to the later days of infection. Also, in the case of rodent malaria, there are reported cases in which drug treatment and the subsequent parasite clearance or reduction of parasitaemia may not translate into survival of mice. It has been suggested that interaction of the drugs with the host's system may alter the latter, resulting in exacerbation of malaria illness and the ultimate death of the host, possibly due to adverse effects of the host's own immune factors such as interferon-γ by causing auto-toxicity to the host tissues (Ishih *et al.*, unpublished data). The fact that five out of the eight plants screened showed moderate to high *in vivo* antimalarial activity when used alone, and that almost all extracts enhanced CQ activity forms a basis of further detailed studies of the plants. This includes isolation and characterization of the bioactive compounds with the ultimate objective of finding novel antimalarial compound(s), which can be used in the fight against drug-resistant malaria.

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REFERENCES

Abegaz BM, Ngadjui BT, Bezabih M, Mdee LK. 1999. Novel natural products from marketed plants of eastern and southern Africa. *Pure Appl Chem* **71**: 919–926.

Bloland PB. 2001. *Drug Resistance in Malaria*. Malaria Epidemiology Branch, Centers for Disease Control and Prevention. WHO: Geneva, 1–23.

Dahanukar SA, Kulkarni FA, Rege NN. 2000. Pharmacology of medicinal plants and natural products. *Indian J Pharmacol* **32**: 81–118.

DFID (Department for International Development). 2005. Millennium development goal 6: To combat HIV/AIDS, malaria and other diseases. *Malaria Factsheet*: 1–4.

El Tahil A, Satti GM, Khalid SA. 1999. Antiplasmodial activity of selected Sudanese medicinal plants with emphasis on *Maytenus senegalensis* (Lam.) Exell. *J Ethnopharmacol* **64**: 227–233.

Fidock AD, Nomura T, Wellems ET. 1998. Cycloguanil and its parent compound proguanil demonstrate distinctive activities against *Plasmodium falciparum* malaria parasites transformed with human dihydrofolate reductase. *Mol Pharmacol* **54**: 1140–1147.

Gakunju DMN, Mberu EK, Dossaji SF *et al.* 1995. Potent antimalarial activity from a Kenyan herbal remedy. *Antimicrob Agents Chemother* **39**: 2606–2609.

Hao XY, Peng L, Huang NH, Shen YM. 2004. A study on anti-inflammatory and analgesic effects of alkaloids of *Toddalia asiatica*. *Zhong Xi Yi Jie He Xue Bao* **2**: 450–452.

Ishih A, Suzuki T, Hasegawa T, Kachi S *et al.* 2004. *In vivo* evaluation of combination effects of chloroquine with cepharanthin® or minocycline hydrochloride against blood-induced chloroquine-resistant *Plasmodium berghei* NK65 infections. *Trop Med Health* **32**: 15–19.

Ishii H, Kobayashi J, Ishikawa M *et al.* 1991. [Studies on the chemical constituents of Rutaceae plants. LXVI. The chemical constituents of *Toddalia asiatica* (L.) Lam. (*T. aculeate* Pers.). (1). Chemical constituents of the root bark]. *Yakugaku Zasshi* **111**: 365–375.

Izhaki I. 2002. Emodin – a secondary metabolite with multiple ecological functions in higher plants. *New Phytol* **155**: 205–217.

Koul JL, Koul S, Singh C *et al.* 2003. *In vitro* cytotoxic elemanolides from *Vernonia lasiopus*. *Planta Med* **69**: 164–166.

Muregi FW, Chhabra SC, Njagi ENM *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.

Oketch-Rabah HA, Brogger Christensen S, Frydenvang K *et al.* 1998. Antiprotozoal properties of 16,17-dihydrobrachycalyxolide from *Vernonia brachycalyx*. *Planta Med* **64**: 559–562.

Oketch-Rabah HA, Lemmich E, Dossaji SF *et al.* 1997. Two new antiprotozoal 5-methylcoumarins from *Vernonia brachycalyx*. *J Nat Prod* **60**: 458–461.

Oketch-Rabah HA, Mwangi JW, Lisgarten J, Mberu EK. 2000. A new antiplasmodial coumarin from *Toddalia asiatica* roots. *Fitoterapia* **71**: 636–640.

Orabi KY, Al-Qasoumi SI, El-Olemy MM *et al.* 2001. Dihydroagarofuran alkaloid and triterpenes from *Maytenus heterophylla* and *Maytenus arbutifolia*. *Phytochemistry* **58**: 475–480.

Peters W, Portus JH, Robinson BL. 1975. The four-day suppressive *in vivo* antimalarial test. *Ann Trop Med Parasitol* **69**: 155–171.

Phillipson JD, Wright CW. 1991. Antiprotozoal agents from plant sources. *Planta Med* **57**: 53–59.

Rasoanaivo P, Rafatro H, Ramanitrahasimbola D, Ratsimamanga-Urverg S. 1998. Alkaloids from Madagascan plants as biochemical tools in drug resistant malaria. *Proceedings of the 7th NAPRECA Symposium on Natural Products, (1997), Dar es Salaam Tanzania* **10**: 95–97.

Ridley RG. 1997. Plasmodium: drug discovery and development – An industrial perspective. *Exp Parasitol* **87**: 293–304.

Rosenthal PJ. 2003. Antimalarial drug discovery: Old and new approaches. *J Exp Biol* **206**: 3735–3744.

Sosa S, Morelli CF, Tubaro A *et al.* 2006. Anti-inflammatory activity of *Maytenus senegalensis* root extracts and of maytenoic acid. *Phytomedicine*, doi: 10.1016/j.phymed.2005.11.002.

Teklehaimanot A, Bosman A. 1999. Opportunities, problems and perspectives for malaria control in sub-Saharan Africa. *Parasitologia* **41**: 335–338.

Van Agtmael MA, Eggelte TA, van Boxtel CJ. 1999. Artemisinin drugs in the treatment of malaria: From medicinal herb to registered medication. *Trends Pharmacol Sci* **20**: 1–5.

Weenen H, Nkunya MHH, Bray DH *et al.* 1990. Antimalarial activity of Tanzanian medicinal plants. *Planta Med* **56**: 368–370.

WHO (World Health Organization). 2005. Malaria in Africa. *Roll Back Malaria Infosheet*. WHO: Geneva, 1–3.

WHO (World Health Organization). 2002a. *World Health Organization Fact Sheet* no. 271. WHO: Geneva.

WHO (World Health Organization) Centre for Health Development. 2002b. Traditional Medicine: Planning for cost-effective traditional health services in the new century – a discussion paper. *http:/www.who.or.jp/tm/research/*

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