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Anti-plasmodial Activity of Some Kenyan Medicinal Plant Extracts Singly and in Combination with Chloroquine

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Sixty organic and aqueous extracts of eleven plants used for the control of malaria by local communities in Kisii District, Kenya were screened for *in vitro* anti-plasmodial activity. The plants selection was based on existing ethnobotanical information and interviews with local communities. The extracts were tested against chloroquine sensitive and resistant laboratory adapted strains of *Plasmodium falciparum*. The study revealed that 63.6% of the plants were active ($IC_{50} \leq 100$ μg/mL). Extracts of four plants, *Ekebergia capensis*, *Stephania abyssinica*, *Ajuga remota* and *Clerodendrum myricoides* gave IC_{50} values below 30 μg/mL against both chloroquine sensitive and resistant *P. falciparum* strains. Combination of extracts of *E. capensis* and *C. myricoides* with chloroquine against the multi-drug resistant *P. falciparum* isolate (V1/S) revealed synergistic effect. The plants which showed activity may be useful as sources for novel anti-plasmodial compounds. Copyright © 2004 John Wiley & Sons, Ltd.

Keywords: plant extracts; anti-plasmodial; ethno-botany; synergism; *Plasmodium falciparum*; *Acacia hockii*; *Ajuga remota*; *Clerodendrum myricoides*; *Cucumis figarei*; *Ekebergia capensis*; *Leonotis mollissima*; *Leucas calostachys*; *Melia azedarach*; *Parinari curatellifolia*; *Senna didymobotrya*; *Stephania abyssinica*.

INTRODUCTION

Each year, 300–500 million clinical cases and 1.5–2.7 million deaths associated with malaria are reported globally (Nchinda, 1998). In Africa, malaria accounts for about one million deaths per year in children under 5 years of age, and it is estimated that both direct and indirect global costs of malaria exceeded US$ 2 billion in 1997 (Teklehaimanot and Bosman, 1999). More than 90% of malaria morbidity and mortality worldwide is found in Africa, and in Kenya, 26 000 children under 5 years of age die annually from associated complications (Teklehaimanot and Bosman, 1999). The resurgence of malaria is partly attributed to the development of drug resistance by the most common malaria parasite (*Plamodium falciparum*) to the commonly used anti-malarial drugs such as chloroquine and sulfadoxine-pyrimethamine (Basco *et al*., 1994). Therefore, new drugs are urgently required for the treatment of malaria. These should preferably have different modes of action from those of the current conventional drugs, in order to circumvent or delay the development of drug resistance.

Plants have always been considered to be an alternative source of new drugs, and some of the anti-malarial drugs in use today such as quinine and artemisinin were obtained directly from plants or developed using chemical structures of plant-derived compounds as templates (Phillipson and Wright, 1991). In tropical countries, modern medicines are not affordable to most of the rural populations and WHO estimates that 80% of the world's population use botanical medicines for primary health care (Farnsworth *et al*., 1985). In addition, research on malaria chemotherapy is targeting use of combinations of two or more drugs as a way of delaying or overcoming development of drug resistance (WHO, 2000). Although combinations of conventional drugs have been applied in the management of drug-resistant malaria (Oduola *et al*., 1998), little is known about the outcome of combination with herbal drugs (Rasoanaivo *et al*., 1998). We hereby report the *in vitro* anti-plasmodial studies on extracts from eleven plants used by local communities in traditional malaria therapy in Kisii District, Kenya and results of their combination with chloroquine.

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MATERIALS AND METHODS

Plant materials. The plant materials were collected from Kisii District, Nyanza Province, Kenya between October 1999 and May 2000 based on the ethnomedical data and interviews with local communities (Table 1). These were authenticated by Mr Simon Mathenge, Department of Botany, University of Nairobi, Nairobi and the voucher specimens were deposited in the Herbarium. The plant samples were air-dried under shade and ground using a laboratory mill (for rootbark, stembark) and a kitchen blender (for leaves).

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Table 1. Plants and the parts used as tradtitional anti-malarials with % yield of extracts

Family/ Botanical name	Vernacular (Ekegusii)	Part collected	% Yield of extracts				
			C_6H_{12}	$CHCl_3$	EtOAc	MeOH	H_2O
Caesalpinaceae							
Senna didymobotrya (Fresen.) Irwin & Barneby (MP-SM/16/99)	Omobeno	Leaves	6.7	6.0	7.6	7.5	13.9
Chrysobalanaceae							
Parinari curatellifolia Benth. (MP-SM/74/99)	Omoraa	Aerial parts	5.6	5.7	5.5	5.1	10.2
Cucurbitaceae							
Cucumis figarei Naud. (MP-SM/33/99)	Egwagwa	Whole plant	5.1	5.3	5.3	6.1	7.0
Labiatae							
Ajuga remota Benth. (MP-SM/39/99)	Omonyantira	Leaves	6.8	5.0	7.6	4.5	10.7
Leonotis mollissima Gürke (MP-SM/44/99)	Risibi	Leaves	5.2	5.9	4.8	6.1	8.5
Leucas calostachys Oliv. (MP-SM/45/99)	Ekemwa	Leaves	5.8	5.1	4.5	6.5	15.2
Meliaceae							
Ekebergia capensis Sparrm. (MP-SM/57/99)	Omonyamari	Stembark	4.9	4.7	4.7	4.5	10.6
Melia azedarach L. (MP-SM/58/99)	Omwarubaine	Leaves	6.6	6.3	6.3	7.0	15.8
Menispermaceae							
Stephania abyssinica (Dillon & A. Rich.) Walp. (MP-SM/64/99)	Omotabararia	Roots	4.1	4.6	4.5	4.2	5.6
Mimosaceae							
Acacia hockii De Wild. (MP-SM/54/99)	Omokonge	Rootbark	4.5	4.6	4.5	4.6	8.6
Verbenaceae							
Clerodendrum myricoides (Hochst.) Vatke (MP-SM/84/99)	Omonyasese	Leaves/Rootbark	2.4 1.2	1.7 1.1	0.8 0.6	7.5 3.6	11.8 7.2

Extraction. The chaff (25 g) of each plant part was boiled for 5 min in 250 mL of water followed by thorough mixing on a shaker and soaking for 12 h. The extracts were filtered and freeze-dried to give between 1–3 g of dry solid. Sequential cold organic extraction was also performed by soaking 25 g of the plant chaff for 48 h in solvents of increasing polarity (hexane, chloroform, ethyl acetate, methanol). The solvent was removed under reduced pressure at 40 °C and dried under vacuum over anhydrous silica gel to give a dry solid or paste (Table 1). The extracts were preserved at –20 °C until used.

Preparations of drugs. In micro-titre plates, stock solutions of 250 and 1 μg/mL of crude extracts and chloroquine, respectively, were made with double-distilled autoclaved water and successively filtered through 0.45 and 0.22 μm filters, under sterile conditions (laminar flow hood). The extracts that were not soluble in water were first dissolved in dimethylsulphoxide (DMSO) (solvent concentration < 0.02%) (Elueze *et al*., 1996). All drugs were stored at –20 °C and retrieved only during use.

Cultures of *Plasmodium falciparum* and bioassays. Two laboratory adapted local *P. falciparum* isolates (K 39 chloroquine-sensitive and ENT 30 chloroquine-resistant) and two reference isolates (NF 54 chloroquine-sensitive and V1/S chloroquine/multidrug resistant) were used for this study. The strains have been cultured and maintained at the laboratories of Kenya Medical Research Institute, Nairobi. The culture medium was a variatrion of that described by Trager and Jensen (1976) and consisted of RPMI 1640 supplemented with 10% human serum, 25 mM N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid (HEPES) and 25 mM $NaHCO_3$. Human type O^+ red blood cells (<28 days old) served as host cells and the cultures were incubated at 37 °C in an atmosphere of 3% CO_2, 5% O_2 and 92% N_2.

The *in vitro* semi-automated micro-dilution assay technique that measured the ability of the extracts to inhibit the incorporation of [G-^{3}H]hypoxanthine into the malaria parasite was used (Desjardins *et al*., 1979; Omulokoli *et al*., 1997). Aliquots of the culture medium (25 μL) were added to all the wells of a 96-well flat-botommed microtitre plate (Nunc, Roskilde, Denmark). 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) used to make two fold serial dilutions of each sample over a 64-fold concentration range. The susceptibility tests were carried out with initial parasitaemia of 0.4% by applying 200 µL of 1.5% haematocrit *P. falciparum* culture to each well. Parasitized and non-parasitisized erythrocytes were incorporated into all tests. The plates were incubated at 37 °C in a gas mixture, 3% CO_2, 5% O_2 and 92% N_2 for 48 h after which 25 µL of culture medium containing 0.5 µCi of [G-^{3}H] hypoxanthine was pulsed and incubated for a further 18 h. Each well was harvested onto a glass fibre filter, dried, and radioactivity in counts per min (cpm) measured by liquid scintillation.

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,

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

where Y_{50} is the cpm value midway between parasitisized and non-parasitisized 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 (Sixsmith *et al*., 1984).

Drug interaction experiments. The method described by Canfield *et al*. (1995) was used with initial concentrations 20–50 times the estimated IC_{50} values combined in various ratios of anti-plasmodial drugs. Single and combined drug solutions were dispensed into the 96-well micro-titre plate to give duplicate rows of chloroquine alone, the test drug (plant extract), and nine different combinations (90:10 to 10:90 chloroquine:extract) (Fivelman *et al*., 1999). Incubation and subsequent procedures were followed as previously described. The degree of synergy was evaluated according to the method of Berenbaum (1978) using the formula

$$\frac{A_c}{A_e} + \frac{B_c}{B_e} = K$$

where A_c and B_c are the equally effective concentrations (IC_{50}) when used in combination, and A_e and B_e are the equally effective concentrations when used alone. The value of K less than 1 indicates synergism, equal to 1 additive effect and greater than 1 antagonism.

RESULTS AND DISCUSSION

All the 60 extracts were initially screened against K 39 and those that afforded high activity ($IC_{50} < 20$ µg/mL) were tested further against NF 54, ENT 30 and V1/S. Table 2 lists the mean IC_{50} values of all the plant extracts against K 39 while Table 3 lists the same for the selected plant extracts against NF 54, ENT 30, and V1/S, respectively. *E. capensis* hexane extract was found to exhibit no anti-plasmodial activity *in vitro* against *P. falciparum*. However, the chloroform, ethyl acetate, methanol and water extracts gave good IC_{50} values (<5 µg/mL) suggesting that the plant extracts have a high *in vitro* anti-plasmodial activity. The *C. myricoides* leaves showed good anti-plasmodial activity of the methanolic extract. The ethyl acetate extract was within the mild activity range. Although the rootbark lacked activity in most of the extracts, the methanolic one gave high activity (IC_{50} = 8.55, 5.81, 6.71, 3.96 µg/mL for K 39, NF 54, ENT 30 and V1/S, respectively), which is twice as active as that of the leaves (IC_{50} = 16.78, 12.81, 13.46, 15.96 µg/mL). This is an indication of a better *in vitro* anti-plasmodial activity of the rootbark than that of the leaves. There are cases in which the chloroquine resistant isolates seemed to be more responsive to the plant extracts than the susceptible ones. For example, V1/S is more than two times susceptible than K 39 to *C. myricoides* rootbark methanolic extracts (IC_{50} = 3.96 and 8.55 µg/mL, respectively). This suggests lack of cross-resistance with chloroquine probably due to differences in the mode of action of compounds present in the extracts. This indicates that the extracts from these plants have the potential of solving the problem of multi-drug resistance. These plants warrant further detailed biological and chemical studies. The bioactive principles could be isolated from these plants and used to develop cheap anti-malarial drugs. The *in vitro*

Table 2. The mean IC_{50} values (x ± SD) for plants extracts against K 39

Plant species	IC_{50} ± SD (µg/mL)				
	C_6H_{12}	$CHCl_3$	EtOAc	MeOH	H_2O
A. hockii	>100	>100	>100	>100	91.5 ± 4.78
A. remota	>100	>100	>100	21.6 ± 1.36	>100
C. figarei	>100	>100	>100	>100	>100
C. myricoides					
Leaves	>100	>100	48.6 ± 1.43	16.8 ± 2.65	>100
Rootbark	>100	>100	>100	8.6 ± 3.51	>100
E. capensis	>100	3.9 ± 0.11	4.7 ± 0.14	4.6 ± 0.28	3.8 ± 0.07
L. calostachys	66.5 ± 2.10	36.2 ± 3.98	>100	87.7 ± 1.77	>100
L. mollissima	50.8 ± 5.66	35.7 ± 2.34	>100	80.7 ± 4.07	>100
M. azedarach	>100	>100	>100	>100	>100
P. curatellifolia	>100	>100	>100	>100	>100
S. abyssinica	>100	67.1 ± 2.94	>100	63.8 ± 3.44	22.9 ± 1.03
S. didymobotrya	>100	>100	>100	>100	>100

Chloroquine IC_{50} value = 0.019 µg/mL (positive control).

Table 3. The mean IC_{50} values (x ± SD) for selected plant extracts against various *P. falciparum* strains

Extracts	IC_{50} ± SD (μg/mL)			
	K 39	NF 54	ENT 30	V1/S
E. capensis				
Stembark				
Chloroform	3.9 ± 0.11	8.7 ± 1.32	8.3 ± 1.13	13.4 ± 2.09
Ethyl acetate	4.7 ± 0.14	15.4 ± 2.71	4.9 ± 1.04	23.1 ± 1.87
Methanol	4.6 ± 0.28	8.9 ± 2.66	6.1 ± 1.38	8.3 ± 2.14
Water	3.9 ± 0.07	22.9 ± 1.94	17.6 ± 2.34	26.0 ± 2.83
C. myricoides				
Leaves				
Methanol	16.8 ± 1.06	12.8 ± 1.25	13.5 ± 4.39	16.0 ± 2.06
Rootbark				
Methanol	8.5 ± 0.15	5.8 ± 2.03	6.7 ± 3.61	4.0 ± 2.51
* Chloroquine	0.019	0.016	0.071	0.078

* positive control.

activities of the Labiatae plant species *A. remota*, *L. mollissina* and *L. calostachys* were within the mild activity range (20–100 μg/mL). *A. remota* had only its methanol extract showing good activity (IC_{50} = 21.60 μg/mL). Apart from the hexane and ethyl acetate extracts of *S. abyssinica* that showed no activity, the others were within the mild activity range. The aqueous extracts also exhibited good activity (IC_{50} = 22.90 μg/mL). For *A. hockii*, only the aqueous extract showed a mild activity of IC_{50} = 91.48 μg/mL whereas the rest were inactive. *Melia azedarach* showed no activity against K 39 for all the extracts (IC_{50} > 100 μg/mL) although it is in the same family (Meliaceae) as *E. capensis*. *C. figarei*, *S. didymobotrya* and *P. curatellifolia* extracts did not show any reasonable *in vitro* activity.

The *E. capensis* (chloroform, ethyl acetate, methanol, aqueous) and C. *myricoides* rootbark (methanol) extracts with IC_{50} < 10 μg/mL were combined with chloroquine and tested against the multi-drug resistant *P. falciparum* isolate, V1/S. The results of the drug combination studies are presented in Table 4. Except for a few combinations of *E. capensis* ethyl acetate and methanol extracts with chloroquine, the rest exhibited strong synergistic effect *in vitro*. *C. myricoides* methanol extract in combination with chloroquine also showed good synergy as indicated by low sum FIC values.

The study reveals that 63.6% of the plants used as traditional anti-malarials in Kisii are active (IC_{50} ≤ 100 μg/mL) against K 39, a chloroquine sensitive *P. falciparum* isolate. Four plants, *Ekebergia capensis*, *Stephania abyssinica*, *Ajuga remota* and *Clerodendrum myricoides* gave at least one extract with IC_{50} value below 30 μg/mL against chloroquine sensitive *P. falciparum* (K 39) strain.

E. capensis showed the best anti-plasmodial activity (IC_{50} = 3.92, 4.66 and 4.61 μg/mL for $CHCl_3$, EtOAc and MeOH extracts, respectively). The aqueous extract had good anti-plasmodial activity (IC_{50} = 3.87 μg/mL), which compares well with the activity of the organic extracts. The bark of this plant is used traditionally as an emetic for heartburn and respiratory complaints; to treat abscesses and boils; the hot water infusions for treatment of pimples; heart ailments and infertility in South Africa (Mulholland *et al*., 1998). Despite several phytochemical investigations of *E. capensis* and *E. benguelensis* (Taylor, 1981; Nashiyama *et al*., 1996; Nkunya and Jonker, 1997; Mulholland and Iourine, 1998; Muholland *et al*., 1998), no anti-plasmodial compound has been isolated.

Methanolic extracts of leaves and rootbark of *C. myricoides* showed good activity against all the four isolates used in this study. *C. myricoides* has been also reported to be useful in the management of other parasitic diseases such as theileriosis (Baerts and Lehman, 1991). Phytochemical investigations in the genus *Clerodendrum* has revealed macrocyclic alkaloids

Table 4. Interactions of chloroquine with *E. capensis* and *C. myricoides* extracts against V1/S

Combination ratios	SUM FIC				
	E. capensis (Stembark)				*C. myricoides* (rootbark)
	$CHCl_3$	EtOAC	MeOH	H_2O	MeOH
90:10	0.32	1.76	2.97	0.56	0.18
80:20	0.25	1.69	3.50	0.54	0.20
70:30	0.23	1.44	1.69	0.32	0.15
60:40	0.19	1.27	0.74	0.18	0.18
50:50	0.21	0.87	0.78	0.28	0.18
40:60	0.23	0.63	0.78	0.45	0.08
30:70	0.33	0.54	0.82	0.51	0.09
20:80	0.31	0.45	0.94	0.78	0.07
10:90	0.34	0.35	0.96	0.66	0.13

(Lumbu and Hootele, 1993), triterpenoid saponins (Toyota *et al*., 1990) and iridoid glycosides (Calis *et al*., 1994), with no indications of their anti-plasmodial activity.

Stephania abyssinica water extract showed good activity (IC_{50} = 22.90 μg/mL) against K 39. Previous phytochemical investigations on *S. abyssinica* revealed the presence of hasubanan alkaloids (Kupchan *et al*., 1973; Southon and Buckingham, 1989; Dagne *et al*., 1993) in the ethanol extract of the roots.

Ajuga remota methanolic extract also exhibited reasonable anti-plasmodial activity (IC_{50} = 21.60 μg/mL). Previous bioassay-guided phytochemical investigations showed cardiotonic (Kuria and Muriuki, 1984), anti-mycobacterial (Cantrell *et al*., 1999) and anti-plasmodial (Kuria *et al*., 2001) activities. Recently, the anti-plasmodial activity has been shown to be due to ergosterol-5, 8-peroxide (Kuria *et al*., 2002).

It is therefore necessary to carry out detailed phytochemical studies to identify the active constituents in these plants. There are cases where the individual isolated compounds may not exhibit activity unlike their combinations in the crude extracts. This may be explained by synergestic effects of the constituents of the crude extracts or presence of prodrugs. It has also been found that some extracts may show *in vitro* activity and no *in vivo* activity or vice versa (Gessler *et al*., 1995). This situation therefore calls for detailed *in vitro* and *in vivo* investigations of the crude extracts and the isolated compounds.

Four plants (*M. azedarach*, *P. curatellifolia*, *C. figarei* and *S. didymobotrya*) were inactive (IC_{50} > 100 μg/mL). Plant extracts may not have direct effect to the parasite *in vitro* but may deal with malaria symptoms such as fever *in vivo*. For instance, they may act as febrifuges, immuno-modulators or prodrugs. Gedunin isolated (Mackinnon *et al*., 1997) from the bark of *Melia azedarach* and *Azadirachta indica* (Meliaceae) has been found to be an anti-plasmodial compound (Khalid *et al*., 1989). Many communities around the world use these plants as traditional anti-malarials. However, activity depends on many factors such as the season in which the plant is collected, the age of the plants, intraspecies variation, part collected and the environmental conditions among others. Therefore, lack of *in vitro* anti-plasmodial activity in this case does not disqualify the use of these plants as traditional anti-malarials.

In the drug interactions studies, ethyl acetate and methanol extracts of *E. capensis* are more antagonistic or mildly synergistic to chloroquine. However, chloroform and water extracts showed good synergistic effects. *C. myricoides* (rootbark) methanol extract gave the strongest synergistic effects with chloroquine. Most extracts exhibited good synergy in combination with chloroquine *in vitro*. If this can be reproduced *in vivo*, then the crude preparations and/or isolated compounds could be used in combination with chloroquine against resistant strains of *P. falciparum*.

Although several compounds have been shown to restore chloroquine sensitivity in resistant *P. falciparum* strains (Oduola *et al*., 1998), little is known about the mechanism of the reversal of resistance by herbal remedies. Rasoanaivo *et al*. (1998) have investigated several medicinal plants used in association with chloroquine by local populations in Madagascar. They have shown that not only do the crude alkaloids significantly enhance the *in vitro* and *in vivo* chloroquine action but the isolated compounds also reverse resistance significantly. The mechanism(s) of reversal of chloroquine resistance through combination with herbal remedies is not well understood currently. Further studies on these extracts are important since they can probably serve as biochemical tools for the understanding of the chloroquine resistance and the mechanism of reversal in *P. falciparum*.

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REFERENCES

Baerts M, Lehman J. 1991. Plantes medicinales vetarinaives de la region des Cretes Zaire-Ni au Burundi. *Annales Economischa Weternschappen Koninkilijk Museum voor Middeen Africa* **21**: 133.

Basco LK, Mitaku S, Skaltsounis AL, *et al.* 1994. *In vitro* activities of furoquinoline and acridone alkaloids against *Plasmodium falciparum. Antimicrob Agents Chemother* **38**: 1169–1171.

Berenbaum MC. 1978. A method for testing for synergy with any number of agents. *J Infect Dis* **137**: 122–130.

Calis I, Hosny M, Yuruker A, *et al.* 1994. Inerminosides A and B, two novel complex iridoid glycosides from *Clerodendrum inerme*. *J Nat Prod* **57**: 494–500.

Canfield CJ, Pudney M, Gutteridge WE. 1995. Interactions of atovaquone with other antimalarial drugs against *Plasmodium falciparum in vitro*. *Exp Parasitol* **80**: 373–381.

Cantrell CL, Rajab MS, Franzbau SG, *et al.* 1999. Anti-microbial ergosterol-5,8-endoperoxide from *Ajuga remota*. *Planta Med* **65**: 732–734.

Dagne E, Ganatilaka AAL, Kingston DGI, Alemu M. 1993. 4′-O-Methylstiphavanine from *Stephania abyssinica*. *J Nat Prod* **56**: 2022–2025.

Desjardins RE, Canfield CJ, Haynes JD, Chulay JD. 1979. Quantitative assessment of antimalarial activity *in vitro* by a semi-automated microdilution technique. *Antimicrob Agents Chemother* **16**: 710–718.

Elueze EI, Croft SL, Warhust DC. 1996. Activity of pyronaridine and mepacrine against twelve strains of *Plasmodium falciparum in-vitro*. *J Antimicrob Chemother* **37**: 511–518.

Farnsworth NR, Akerere O, Bilngel AS, *et al.* 1985. Medicinal plants in therapy. *Bull WHO* **63**: 965–981.

Fivelman QL, Walden JC, Smith PJ, *et al.* 1999. The effect of artesunate combined with standard antimalarials against chloroquine sensitive- and chloroquine resistant strains of *Plasmodium falciparum in vitro*. *Trans R Soc Trop Med Hyg* **93**: 429–432.

Gessler MC, Tanner M, Chollet J, *et al.* 1995. Tanzanian medicinal plants used traditionally for the treatment of malaria*: In vivo* antimalarial and *in vitro* cytotoxic activities. *Phytother Res* **9**: 504–508.

Khalid SA, Duddeck H, Gonzalez-Siena M. 1989. Isolation and characterization of the antimalarial agents of the neem tree, *Azadaracta indica*. *J Nat Prod* **52**: 922–927.

Kupchan SM, Leipa AJ, Fujita J. 1973. New phenolic husubanan alkaloids from *Stephania abyssinica*. *J Org Chem* **38**: 151–153.

Kuria KAM, Chepkwony H, Govaerts C, *et al.* 2002. The antiplasmodial activity of isolates from *Ajuga remota*. *J Nat Prod* **65**: 789–793.

Kuria KAM, De Coster S, Muriuki G, *et al.* 2001. Anti-malarial activity of *Ajuga remota* Benth. (Labiatae) and *Caesalpinia volkensii* (Caesalpiniaceae) *in vitro* confirmation of ethnopharmacological use. *J Ethnopharm* **74**: 141–148.

Kuria KAM, Muriuki G. 1984. A new cardiotonic agent from *Ajuga remota* Benth. (Labiatae). *East Afr Med J* **61**: 533–537.

Lumbu S, Hootele C. 1993. Buchnerine and N-(Z)-p-methoxycinnamoylbucherine, two new macrocyclic alkaloids from *Clerodendrum buchneri* roots. *J Nat Prod* **56**: 957–960.

Mackinnon S, Durst T, Arnason JT, *et al.* 1997. Antimalarial activity of tropical Meliaceae extracts and gedunine derivatives. *J Nat Prod* **60**: 336–341.

Mulholland DA, Coombes PH, Iourine SE, *et al.* 1998. The Chemistry of South African *Ekebergia species*. Proceedings of the 7th NAPRECA Symposium on Natural Products, (1997). *Dar es Salaam, Tanzania* **10**: 41–48.

Mulholland DA, Iourine SE. 1998. Limonoids from *Ekebergia capensis*. *Phytochemistry* **47**: 1357–1361.

Nashiyama Y, Moriyasu M, Ichimaru M, *et al.* 1996. Acyclic triterpenoids from *Ekebergia capensis*. *Phytochemisty* **42**: 803–807.

Nchinda TC. 1998. Malaria: A re-emerging disease in Africa. *Emerg Infect Dis* **4**: 399–403.

Nkunya MHH, Jonker SA. 1997. Antiprotozoan and other natural products from Tanzanian lianas, shrubs and small trees. Proceedings of the 7th NAPRECA Symposium on Natural Products, (1997), *Dar es Salaam, Tanzania* **10**: 49–59.

Oduola AM, Sowumi A, Milhow WK, *et al.* 1998. The effect of combining promethazine with chloroquine against *Plasmodium falciparum in vitro*. *Am J Trop Med Hyg* **58**: 625–629

Omulokoli E, Khan B, Chhabra SC. 1997. Antiplasmodial activity of four Kenyan medicinal plants. *J Ethnopharm* **56**: 133–137.

Phillipson JD, Wright CW. 1991. Antiprotozoal agents from plant sources. *Planta Med* **57** (S*uppl* 1): 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.

Sixsmith DG, Watkins WM, Chulay JD, Spencer HC. 1984. *In vitro* antimalarial activity of tetrahydrofolate dehydrogenase inhibitors. *Am J Trop Med Hyg* **33**: 772–776.

Southon IW, Buckingham J. 1989. *Dictionary of Alkaloids*. Chapman and Hall: London; 1001.

Taylor DAH. 1981. Ekebergin, a limonoid extractive from *Ekebergia capensis*. *Phytochemistry* **20**: 2263–2265.

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

Toyota M, Msonthi JD, Hostettman K. 1990. A molluscidal and antifungal triterpenoid saponin from roots of *Clerodendrum wildii*. *Phytochemistry* **29**: 2849–2851.

Trager W, Jensen JB. 1976. Human malaria parasites in continuous culture. *Science* **193**: 673–675.

World Health Organization. 2000. Severe falciparum malaria. *Trans R Soc Trop Med Hyg* **94** (*Suppl* 1): 1–71.

