

**Antimalarial activity of methanolic extracts from plants used in Kenyan ethnomedicine and their interactions with chloroquine (CQ) against a CQ-tolerant rodent parasite, in mice.**

Francis W. Muregi<sup>a,c,\*</sup>, Akira Ishih<sup>a</sup>, Toshio Miyase<sup>b</sup>, Tohru Suzuki<sup>a</sup>, Hideto Kino<sup>a</sup>,  
Teruaki Amano<sup>d</sup>, Gerald M. Mkoji<sup>c</sup>, Mamoru Terada<sup>a</sup>

<sup>a</sup>Department of Parasitology, Hamamatsu University School of Medicine, 1-20-1 Handayama,  
Hamamatsu 431-3192, Japan.

<sup>b</sup>Department of Pharmacognosy, School of Pharmaceutical Sciences, University of Shizuoka, Yada  
52-1, Shizuoka 422-8526, Japan.

<sup>c</sup>Centre for Biotechnology Research and Development, Kenya Medical Research Institute (KEMRI),  
P.O. Box 54840-00200, Nairobi, Kenya.

<sup>d</sup>Eastern and Southern Africa Centre for International Parasite Control (ESACIPAC), Kenya Medical  
Research Institute, P.O. Box 54840-00200, Nairobi, Kenya.

\*Corresponding Author. Tel: +81-53-435-2338; Fax: +81-53-435-2337;

*Email address:* [fmuregi@hama-med.ac.jp](mailto:fmuregi@hama-med.ac.jp) (Francis Muregi)

## Abstract

Methanolic extracts from 15 medicinal plants representing 11 families, used traditionally for malaria treatment in Kenya were screened for their *in vivo* antimalarial activity in mice against a chloroquine (CQ)-tolerant *Plasmodium berghei* NK65, either alone or in combination with CQ. The plant parts used ranged from leaves (L), stem bark (SB), root bark (RB), seeds (S) and whole plant (W). When used alone, extracts from 7 plants, *Clerodendrum myricoides* (RB), *Ficus sur* (L/SB/RB), *Maytenus acuminata* (L/RB), *Rhamnus prinoides* (L/RB), *R. staddo* (RB), *Toddalia asiatica* (RB) and *Vernonia lasiopus* (RB) had statistically significant parasitaemia suppressions of 31.7-59.3%. In combination with CQ, methanolic extracts of *Albizia gummifera* (SB), *F. sur* (RB), *R. prinoides* and *R. staddo* (L/RB), *Caesalpinia volkensii* (L), *Maytenus senegalensis* (L/RB), *Withania somnifera* (RB), *Ekebergia capensis* (L/SB), *T. asiatica* (L/RB) and *V. lasiopus* (L/SB/RB) gave statistically significant and improved suppressions which ranged from 45.5-85.1%. The fact that these activities were up to 5-fold higher than that of extract alone may suggest synergistic interactions. Remarkable parasitaemia suppression by the extracts, either alone or in combination with CQ mostly resulted into longer mouse survival relative to the controls, in some cases by a further 2 weeks. Plants, which showed significant antimalarial activity including *V. lasiopus*, *T. asiatica*, *F. sur*, *R. prinoides* and *R. staddo* warrant further evaluation in the search for novel antimalarial agents against drug-resistant malaria.

**Keywords:** Antimalarial activity; Medicinal plants; Drug resistance; *Plasmodium berghei* NK65; Traditional medicine; Synergistic effects

## 1. Introduction

In view of the problems associated with antimalarial drug resistance, new drugs or drug combinations are urgently required today for treatment of malaria. Preferably, the new drugs should have novel modes of action or be chemically different from the drugs in current use (Phillipson and Wright, 1991). Plants have always been considered to be a possible alternative and rich source of new drugs and most of the antimalarial drugs in use today such as quinine and artemisinin were either obtained directly from plants or developed using chemical structures of plant-derived compounds as templates (Basco et al., 1994). Due to limited availability and/or affordability of pharmaceutical medicines in many tropical countries, the majority of the populations depend on traditional medical remedies ((WHO, 2002; Zirihi et al., 2005), mainly from plants. In ethnomedicine, same plants and/or related species are used for the treatment of related ailments within the same region, or across different regions of the world. For instance, whereas *Maytenus senegalensis* is used in many African regions for the treatment of various ailments including chest pains, rheumatism, snakebites and malaria, plants of the genus *Maytenus* are used to prepare decoctions in south America as anti-inflammatory and analgesic remedies (Sosa et al., 2006; Ajaiyeoba et al., 2006). This is however not surprising since malaria manifests itself with symptoms including fever, pains and immunosuppression and some plants may lack direct antiplasmodial activity but may possess antipyretic, analgesic and immune stimulatory effects (Muregi et al., 2003). Among the plants we screened for antimalarial activities, most of them find wide ethnopharmacological use among different Kenyan ethnic groups either for similar or different ailments (Kokwaro, 1976; Beenjte, 1994). While *Vernonia lasiopus* is used against malaria by Kikuyu of central Kenya, the Kamba of eastern Kenya use it against scabies, the Luo of western Kenya against venereal diseases, and pounded leaves are applied to sores by Maasai of southern Kenya (Beentjee, 1994). *Toddalia asiatica* is used traditionally in Kenya by many communities for the treatment of malaria, fever, stomachache, toothache, coughs as well as nasal and bronchial pains, and although all parts of the plant are claimed to have medicinal value, roots are believed to be more potent (Kokwaro, 1976; Beentje, 1994).

In an attempt to impede selection of drug resistance, use of monotherapy is being discouraged for most parasitic diseases (WHO, 2000). In the case of malaria, for instance, not only are novel combinations being tried, but also attempts are being made to enhance the potency and/or even reverse resistance of conventional drugs such as CQ (Winstanley, 2000). Although several synthetic molecules have been shown to restore CQ-sensitivity in resistant *Plasmodium falciparum* strains (Oduola et al., 1998), there are almost no documented data on interactions of herbal remedies with conventional antimalarial drugs such as CQ. In the present study, several medicinal plants used as traditional remedies for malaria in Kenya were evaluated for antimalarial activity against a rodent malaria parasite in mice, alone or in combination with the conventional antimalarial drug, CQ.

## **2. Materials and methods**

### *2.1 Plant materials and extraction*

Based on ethnomedical data, different plant parts (leaf, stem bark, root bark, seed, whole plant) of 15 plant species representing 11 families (Table 1) were collected in January 2004 from central Kenya (Mount Kenya Forest) and southern Rift Valley (Nguruman Escarpment in Magadi). The plants collected were identified by a taxonomic botanist from the East African Herbarium in Nairobi, where voucher specimens were deposited. The plant samples were then catalogued, air-dried at room temperature under shade, and ground into powder using an electric mill. The powder was packaged into one kg-packs and stored in dry and well-ventilated room until use. Organic extraction was done by refluxing 10g of plant material in 500 ml of methanol for one hour. The extracts were then filtered and concentrated to dryness *in vacuo*.

### *2.2 The parasite and infection*

For *in vivo* antimalarial assays of plant extracts, a CQ-tolerant *Plasmodium berghei* (strain NK65), a rodent malaria parasite was used. The blood-stage CQ-tolerant-induced parasite, maintained at the Parasite Bank of the Department of Parasitology, Hamamatsu University School of Medicine was previously a kind gift from Professor Y. Wataya of Okayama University, Japan. A donor mouse to

the experimental mice, having 10-15% parasitaemia was sacrificed and bled by cardiac puncture. 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 intraperitoneally with approximately  $10^5$  parasitized erythrocytes in volumes of 0.2 ml (Ishih et al., 2003). The inoculated mice were then randomized into 5 mice per cage and maintained in an animal care facility on a commercial diet and water *ad libitum*.

### 2.3 *Antimalarial activity of plant extracts either alone or in combination with chloroquine*

In screening of the plant extracts alone, the 4-day suppressive method of Peters et al. (1975) was used. Within 3 hours post-inoculation of mice with the parasite (i.e on 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 (at 8-hour interval) 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 (i.e., on day 4 p.i.), parasitaemia of individual mouse was determined by microscopic examination of Giemsa stained thin blood smears prepared from mouse-tail blood.

In assessing the *in vivo* interactions of CQ and the plant extracts, treatment was started on day 4 p.i. based on the method of Ishih et al. (2004). 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, and an untreated control group, which received water only. For all mice before initial treatment on day 4 p.i., thin blood smears were prepared, after which CQ dose followed by plant extract dose was administered by oral route.

In both studies, *in vivo* antimalarial activity of the test drugs was assessed by monitoring mouse survival and parasitaemia, over a 30-day period. Handling of animals was done in accordance to the *Guide for the Care and Use of Laboratory Animals*, Hamamatsu University School of Medicine.

## 2.4 Data and statistical analysis

Percentage suppression of parasitaemia for the plant extracts was calculated as:  $100 - [(\text{mean parasitaemia treated} / \text{mean parasitaemia control}) \times 100]$  (Gessler et al., 1995). 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.

## 3. Results

### 3.1 Antimalarial activity of plant extracts alone

Table 2 shows a summary of parasitaemia suppression (%) for mice on day 4 p.i. and their corresponding survival on day 9 p.i., when 100% mouse-mortality of the untreated control occurred. Eleven extracts from 7 plant species showed significant parasitaemia suppressions ( $P < 0.05$ ) ranging from 31.7-59.3%. These are *C. myricoides* (RB), *F. sur* (L/SB/RB), *M. acuminata* (L/ RB), *R. prinoides* (L/RB), *R. staddo* (RB), *T. asiatica* (RB) as well as *V. lasiopus* (RB). In contrast, other 14 extracts, *C. volkensis* (S), *M. heterophylla* (RB), *M. senegalensis* (RB), *V. lasiopus* (SB), *A. remota* (W), *E. capensis* (L/SB/RB), *A. indica* (L), *A. gummifera* (L/SB), *F. sur* (L), *R. staddo* (L), and *C. myricoides* (L), had non-significant suppressions ( $P > 0.05$ ), which ranged from 9.8-37.0%. Three extracts from *C. volkensis* (L), *M. heterophylla* (RB) and *V. lasiopus* (L) had no activity at all. Based on day 9 p.i. relative to the untreated controls, 7 extracts gave a 40-60% mouse survival, in some cases up to a further 2 weeks. *T. asiatica* (L) had a 60% and 40% mouse survival on day 9 and 16 p.i. respectively. Although the extract had moderate suppression of 37.0% ( $P > 0.05$ ) on day 4 p.i., it is the only extract that showed sustained effect on day 7 p.i. of 39.8% ( $P < 0.05$ ) relative to the untreated controls. *V. lasiopus* (RB) gave a 60% survival of mice on day 9 p.i. and a 40% survival on day 11 p.i. *C. volkensis* (S) and *A. gummifera* (SB) had 40% survival on day 18 p.i., and 17 p.i. respectively.

### 3.2 Antimalarial activity of plant extracts in combination with CQ

In CQ/plant extract combination studies, the parasitaemia levels on day 4 p.i. (before initial treatment) were not different ( $P>0.05$ ) among all groups and microscopic examination of day 8 p.i. smears detected no parasites. However, the recrudescence parasites reappeared by day 11 p.i., and hence parasitaemia levels at day 11 p.i. were considered the most significant in assessment of chemo-suppression. Table 2 summarizes the parasitaemia suppression (%) for mice on day 11 p.i. and the corresponding survival on day 14 p.i., when all mice of CQ-treated control group died.

Seventeen extracts from *A. gummifera* (SB), *F. sur* (RB), *M. senegalensis* (L/RB), *R. prinoides* (L/RB), *R. staddo* (L/RB), *C. volkensii* (L), *E. capensis* (L/SB), *T. asiatica* (L/RB), *V. lasiopus* (L/SB/RB) as well as *W. somnifera* (RB) in combination with CQ gave parasitaemia suppressions that ranged from 45.5-85.1% ( $P<0.05$ ). The best activities were exhibited by CQ/*A. gummifera* (SB), CQ/*F. sur* (RB), CQ/*R. prinoides* (RB) as well as CQ/*R. staddo* (RB) with activities of 75.2, 79.3, 85.1 and 74.2% respectively. Nine extracts in combination with CQ including *C. volkensii* (S), *Maytenus acuminata* (L), *M. acuminata* (RB) and *M. heterophylla* (RB) showed non-significant ( $P>0.05$ ) activities of up to 45.5%, while 2 extracts, *F. sur* (L/SB) had no suppression at all (Table 2). Relative to day 14 p.i., when 100% mortality of the CQ-alone treated controls occurred, mice in the groups treated with CQ/*R. staddo* (RB) had the highest mouse survival of 80% at day 14 p.i., and 40% survival on day 26 p.i. CQ/*V. lasiopus* (L) had 60 and 40% survival on day 14 and 26 p.i. respectively. The 2 extracts had mice surviving longer by up to a further 2 weeks (day 28 p.i.) relative to the controls. CQ/*A. gummifera* (SB) gave a 60% survival on day 14 p.i., but all the mice died by day 17 p.i. It is remarkable that all the 3 groups had shown significant parasitaemia suppressions of 74.2, 56.1 and 75.2% respectively. Although CQ/*E. capensis* (RB) maintained a 75% survival up to day 23 p.i., and 50% survival on day 28 p.i., it is remarkable that the combination had day 11 p.i. parasitaemia suppression of 37.9% which was not statistically significant ( $P>0.05$ ). Three extracts showed a considerably longer mouse survival than the controls; CQ/*Azadirachta indica* (L), 40% survival on day 25 p.i.; CQ/*R. staddo* (L), 50% survival on day 30 p.i.; CQ/*T. asiatica* (L), 40% mouse survival on day 24 p.i.

#### 4. Discussion and conclusions

When assayed alone against CQ-tolerant *P. berghei* NK65, 38% of the 29 methanolic extracts, representing 53% of all the 15 plant species screened, showed significant parasitaemia suppression ( $P < 0.05$ ) on day 4 p.i. ranging from 31.7-59.3%, which may partially validate the ethnomedical use of the herbs in management of malaria. The plants with considerable *in vivo* chemo-suppression including *V. lasiopus*, *F. sur*, *C. myricoides*, *R. prinoides* and *R. staddo* had shown moderate to significant *in vitro* antiplasmodial activities against both CQ-sensitive and -resistant *P. falciparum* isolates in their water and/or organic fractions in a previous study of some Kenyan medicinal plants (Muregi et al., 2003; 2004). The organic leaf extract of *V. lasiopus* had shown the highest *in vitro* inhibition of parasite growth, with  $IC_{50}$  values as low as 1.0  $\mu\text{g/ml}$ , which is consistent with the findings of the present study, in which the methanolic extract showed a remarkable *in vivo* parasitaemia suppression of 59.3%, albeit in its root bark extract. The presence and/or quantities of bioactive compounds in plants are influenced by several factors including seasons, environment, plant-part used, intra-species variations and plant age (Weenen et al., 1990), and this may explain the discrepancies observed in *in vitro* and *in vivo* activities of plant parts used. Many *Vernonia* species have been investigated chemically and found to contain several metabolites including triterpenes and oxygenated sesquiterpenes, flavones and vernolic acid (Oketch-Rabah, 1996). Oxygenated sesquiterpene lactones are the most abundant secondary metabolites of the genus *Vernonia*, and since artemisinin, isolated from the Chinese herb *Artemisia annua* belongs to the same class of compounds and has been widely used in the synthesis of semi-synthetic antimalarials effective against multi-drug resistant strains of *P. falciparum* (Trigg, 1989; Oketch-Rabah, 1996), it is important to investigate *Vernonia* species further. Two 5-methylcoumarins isolated from the roots of *V. brachycalyx* showed *in vitro* antiplasmodial activity against both CQ-sensitive and -resistant *P. falciparum* isolates (Oketch-Rabah et al., 1997). Methanolic extract of *T. asiatica* (RB) showed remarkable activity (59.3%) in the present study, and was previously reported to possess high *in vitro* antiplasmodial activity, with a mean 50% inhibitory dose of 0.98  $\mu\text{g/ml}$  (Gakunju et al., 1995). Subsequent activity-



guided fractionation and isolation afforded fractions and an alkaloid, nitidine, active against both CQ-sensitive and -resistant *P. falciparum* isolates. Subsequent chemical development of this compound has further improved its potency against CQ-resistant *P. falciparum* isolates *in vitro* (Waigh, 2002). Oketch-Rabah et al. (2000) reported that a coumarin derivative from *T. asiatica* (RB) had a moderate *in vitro* activity of 8.8 µg/ml against *P. falciparum* isolates. Although not much has been reported in literature about biological activity of *F. sur*, it is widely used in Kenyan ethnopharmacology as a cough remedy, against stomachaches and toothaches to relieve pain (Kokwaro, 1976; Beenjte, 1994). The fact that all the *F. sur* parts investigated showed a modest antimalarial activity underscores the need for further investigation of the plant. *R. staddo* and *R. prinoides* are the only 2 Rhamnus species that occur in Africa and the latter is widespread in many parts of eastern and central Africa (Abegaz et al., 1999). In ethnomedicine, the 2 plants, especially their roots are used for treatment of malaria, indigestion, venereal diseases and rheumatism among other ailments (Kokwaro, 1976; Beenjte, 1994). Recent studies have shown that *R. prinoides* can serve as a commercial hopping agent in the brewery industries and 20 compounds including 7 glycosides of emodin anthrone, 5 flavonoids and 3 naphthalenic derivatives were isolated from the plant (Abegaz et al., 1999). Emodin has been reported to possess various pharmacological and biological activities including immunostimulation, antiparasitic, anti-inflammatory and analgesic effects, among others (Izhaki, 2002). In several cases, remarkable suppression of parasitaemia by extracts translated into either a higher and/or a longer mouse survival. However, *V. lasiopus* (RB) gave a 60% survival of mice on day 9 p.i. and a 40% survival on day 11 p.i., which mean that high percentage survival on day 9 p.i. does not translate into a considerably longer mouse survival. This may suggest that the bioactive compound in the plant may have a short half-life, since some antimalarial drugs including artemisinin-based derivatives are known to be fast acting, and to have a short half-life (Van Agtmael et al., 1999). In contrast, *T. asiatica* (L) gave a 60% mouse survival on day 9 p.i., and a relatively longer survival, with 40% mouse survival on day 16 p.i. The extract is the only one which maintained similar suppression levels on day 4 and 7 p.i., suggesting that the bioactive agent (s) in the plant may have a slow onset of action, and/or that it is not fast acting. On the other hand, some extracts with mild parasitaemia

suppression ( $P > 0.05$ ) gave a long mouse survival. *C. volkensis* (SD) and *A. gummifera* (SB), with 40% survival on day 18 p.i., and 17 p.i. respectively are such extracts, implying that other than direct parasiticidal effects, plants may possess other pharmacological benefits to the hosts, such as acting as analgesics, antipyretics or as immune stimulators (Dahanukar et al., 2000).

In combination with CQ, some of the extracts showed up to 5-fold better chemo-suppression as well as longer mouse survival than that of CQ-alone treated controls, suggesting synergistic interactions of the 2 drugs. As expected, high chemo-suppression in most cases led to a high mouse survival at day 14 p.i., and subsequently a longer mouse survival, as in the case of the group treated with CQ/*R. staddo* (RB). Some plant extracts such *V. lasiopus* (L) that lacked activity when used alone, demonstrated both high as well as prolonged mouse survival when used in combination with CQ. As earlier noted, this emphasizes the possibility of other pharmacological effects of plants being involved, besides direct antiparasitic effects. Leaves and seeds of *V. lasiopus* and *V. galamensis* had been reported to possess analgesic effects in rat models (Dahanukar et al., 2000). On the other hand, some extracts including *F. sur* (L/ RB) which had significant activity when used alone showed little or no suppression in combination with CQ. Antagonistic interactions among drugs as well as toxicity cannot be ruled out in such cases, emphasizing the need to avoid simultaneous use of conventional drugs with natural products before the safety and efficacy of such combinations have been authenticated. The fact that about 50% of the 15 plants screened showed moderate to high *in vivo* antimalarial activity when used alone, and that most of the 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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**Table 1.** Plant parts collected based on ethnopharmacological data and percentage yield of dry methanolic extract per 10 g of air-dried plant material used

Plant Family of Botanical name extract	Vernacular name (Kikuyu) <sup>a</sup>	Plant part used (Specimen code)	% Yield plant
Caesalpinaceae			
<i>Caesalpinia volkensii</i> Harms	Mucuthi	L (Cv-L/04)	14
		Sd (Cv-S/04)	18
Celastraceae			
<i>Maytenus acuminata</i> (L.f.) Loes	Rurigi	L (Ma-L/04)	18
		Rb (Ma-RB/04)	20
<i>M. heterophylla</i> (Eckl. & Zeyh.) Robson	Muthuthi	Rb (Mh-RB/04)	6
<i>M. senegalensis</i> (Lam.) Exell	Muthuthi/ Mwenyuke	L (Ma-L/04)	14
		Rb (Ma-RB/04)	14
Compositae			
<i>Vernonia lasiopus</i> O.Hoffm.	Mucatha	L (VI-L/04)	9
		Sb (VI-SB/04)	14
		Rb (VI-RB/04)	22
Labiatae			
<i>Ajuga remota</i> Benth.	Njeri-wa-rurii	Wp (Aj-W/04)	22
Meliaceae			
<i>Ekebergia capensis</i> Sparm.	Mununga/ Murera-hungu	L (Ec-L/04)	9
		Sb (Ec-SB/04)	26
		Rb (Ec-RB/04)	21
<i>Azadirachta indica</i> A. Juss.	Murumbaine	L (Ai-L/04)	10
Mimosaceae			
<i>Albizia gummifera</i> (JF Gmel.) C.A. Sm.	Mukurue	L (Ag-L/04)	5
		Sb (Ag-SB/04)	4
Moraceae			
<i>Ficus sur</i> Forssk.	Mukuyu	L (Fs-L/04)	6
		Sb (Fs-SB/04)	7
		Rb (Fs-RB/04)	9
Rhamnaceae			
<i>Rhamnus prinoides</i> L' Hérit	Mukarakinga	L (Rp-L/04)	24
		Rb (Rp-RB/04)	25
<i>R. staddo</i> A.Rich.	Mubura	L (Rs-L/04)	10
		Rb (Rs-RB/04)	30
Rutaceae			
<i>Toddalia asiatica</i> (L.) Lam.	Mwikunya/ Mururue	L (Ta-L/04)	5
		Rb (Ta-RB/04)	20
Solanaceae			
<i>Withania somnifera</i> (L.) Dunal	Murumbae	Rb (Ws-RB/04)	10
Verbenaceae			
<i>Clerodendrum myricoides</i> (Hochst.) Vatke	Munjuga-iria	L (Cm-L/04)	8
		Rb (Cm-	
RB/04)	25		

L, leaf; SB, stem bark; RB, Root bark; S, seed; W, whole plant

<sup>a</sup> some plant species may share vernacular names while others may have more than one name

**Table 2.** Parasitaemia suppression (%) on day 4 and 11 post infection (p.i.) for mice treated with plants' methanolic extracts alone (from day 0 p.i.) and in combination with CQ (from day 4 p.i), and the corresponding survival (%) on day 9 and 14 p.i. respectively

Drug (%)	Extract alone		CQ +extract	
	Suppression (%)	Survival (%)	Suppression (%)	Survival
	on day 4 p.i.	on day 9 p.i.	on day 11 p.i.	on day 14 p.i.
<i>Caesalpinia volkensii</i> (L)	NS	0	47.0 <sup>b</sup>	20
<i>C. volkensii</i> (S)	33.3	40	33.3	0
<i>Maytenus acuminata</i> (L)	36.6 <sup>b</sup>	0	30.6	40
<i>M. acuminata</i> (RB)	41.5 <sup>b</sup>	0	0.8	20
<i>M. heterophylla</i> (RB)	9.8	0	6.6	0
<i>M. senegalensis</i> (L)	NS	20	55.4 <sup>b</sup>	40
<i>M. senegalensis</i> (RB)	2.4	20	56.2 <sup>b</sup>	20
<i>Vernonia lasiopopus</i> (L)	NS	0	56.1 <sup>b</sup>	60
<i>V. lasiopopus</i> (SB)	14.8	0	63.6 <sup>b</sup>	20
<i>V. lasiopopus</i> (RB)	59.3 <sup>b</sup>	60	62.1 <sup>b</sup>	40
<i>Ajuga remota</i> (W)	26.8	0	7.4	20
<i>Ekebergia capensis</i> (L)	14.8	20	59.1 <sup>b</sup>	20
<i>E. capensis</i> (SB)	33.3	40	45.5 <sup>b</sup>	20
<i>E. capensis</i> (RB)	22.2	20	37.9	75
<i>Azadirachta indica</i> (L)	22.2	20	45.5	40
<i>Albizia gummifera</i> (L)	19.5	0	43.0	40
<i>A. gummifera</i> (SB)	31.7	40	75.2 <sup>b</sup>	60
<i>Ficus sur</i> (L)	43.9 <sup>b</sup>	20	NS	0
<i>F. sur</i> (SB)	34.1 <sup>b</sup>	20	NS	0
<i>F. sur</i> (RB)	48.8 <sup>b</sup>	0	79.3 <sup>b</sup>	20
<i>Rhamnus prinoides</i> (L)	43.9 <sup>b</sup>	0	64.5 <sup>b</sup>	0
<i>R. prinoides</i> (RB)	34.1 <sup>b</sup>	20	85.1 <sup>b</sup>	40
<i>R. staddo</i> (L)	11.1	20	57.6 <sup>b</sup>	50
<i>R. staddo</i> (RB)	48.1 <sup>b</sup>	40	74.2 <sup>b</sup>	80
<i>Toddalia asiatica</i> (L)	37.0	60	57.6 <sup>b</sup>	40
<i>T. asiatica</i> (RB)	59.3 <sup>b</sup>	40	53.0 <sup>b</sup>	20
<i>Withania somnifera</i> (RB)	ND	0	45.5 <sup>b</sup>	50
<i>Clerodendrum myricoides</i> (L)	9.8	0	14.9	0
<i>C. myricoides</i> (RB)	31.7 <sup>b</sup>	0	ND	
Water control	-	0	ND	
CQ control	100	100	-	
0				

L, leaf; SB, stem bark; RB, Root bark; S, seed; W, whole plant

NS, no suppression; ND, not determined

<sup>b</sup>statistically significant (P<0.05)