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Traditional herbal antimalarial therapy in Kilifi district, Kenya

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ABSTRACT

Aim of study: To identify plant species used by the traditional health practitioners (THPs) in treatment of malaria, carry out cytotoxicity and efficacy evaluation of the identified plants and to evaluate combination effects.

Materials and methods: Thirteen plants were selected through interviews with traditional healers. *In vitro* antiplasmodial testing was done by measuring ability of the test sample to inhibit the incorporation of radio-labelled hypoxanthine into the malaria parasite. The extracts were tested singly and then in combination using the standard fixed ratio analysis to evaluate synergism. *In vivo* bioassay was done in mice using Peter's 4-days suppressive test and cytotoxicity evaluated *in vitro* using Vero E6 cells.

Results: Of the plants tested *in vitro*, 25% were highly active (IC_{50} < 10 μg/ml), 46% moderately active (IC_{50} 10–50 μg/ml), 16% had weak activity of 50–100 μg/ml while 13% were not active IC_{50} >100 μg/ml. Methanolic extracts of *Azadirachta indica*, *Premna chrysoclada* and *Uvaria acuminata* were the most active (IC_{50} < 10 μg/ml) against both the chloroquine (CQ) sensitive (D6) and the CQ resistant (W2) *Plasmodium falciparum* clones. When tested *in vivo* in a mouse model, *Azadirachta indica*, *Rhus natalensis* and *Grewia plagiophylla* depicted the highest percent parasite clearance and chemo suppression of 89%, 82% and 78%, respectively. Evaluating effect of combining some of these extracts with one another against a multi-drug resistant *Plasmodium falciparum* (W2) clone revealed synergism among some combinations. The highest synergy was between *Uvaria acuminata* and *Premna chrysoclada*. The interaction between *Grewia plagiophylla* and *Combretum illairii* was largely antagonistic. Impressive cytotoxicity results were obtained with most of the plants tested revealing high selectivity indices an indication of enabling achievement of therapeutic doses at safe concentrations. *Uvaria acuminata* was, however, toxic to the cultured cells. Mild cytotoxicity was also observed in *Hoslundia opposita* and *Lannea schweinfurthii* (CC_{50} 37 and 76 μg/ml, respectively).

Conclusions: This study identified plants with low IC_{50} values, high percent chemo suppression and low cytotoxicity thus potential sources for novel antiplasmodial agents. The findings remotely justify use of combined medicinal plants in traditional medicine practices as synergy among some plant species was demonstrated.

1. Introduction

Malaria is one of the most serious and life-threatening vector-borne infection caused by the protozoan Plasmodia parasites. It is widespread in tropical and subtropical regions. Each year, approximately 515 million people are infected resulting in deaths of 1–3 million people, the majority of whom are young children in sub-Saharan Africa (Snow et al., 2005). Malaria is commonly associated with poverty, but is also a cause of poverty and a major hindrance to economic development (Mboera et al., 2007). Mortality has continued to rise in recent years, mainly because of increasing resistance to antimalarial medicines (Talisuna et al., 2007).

Antimalarial therapies, based on the use of the artemisinin derivatives, combined with other drugs; artemesinin combination therapy (ACT) is considered the best current treatment of *Plasmodium falciparum* malaria (Capela et al., 2009; Maude et al., in press). However, efficacy of artemisinin-based combination therapy and artesunate monotherapy has declined in some areas particularly in western Cambodia (Denis et al., 2006a, 2006b; Alker et al., 2007; Noedl et al., 2008; Dondorp et al., 2009). Artemisinin resistance would definitely be disastrous for global malaria control. In addition, ACT treatment regimens face the challenge of high production

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cost thus making the search for cheaper, yet effective alternatives imperative (Fidock et al., 2004).

Due to either limited availability or affordability of pharmaceutical medicines in many tropical countries, about 80% of the rural population in Africa depends on traditional herbal remedies (WHO, 2002; Zirihi et al., 2005). Although there is widespread use of traditional herbal remedies in the management of malaria (Gessler et al., 1995), scientific understanding of the plants is, however, largely unexplored (WHO, 2002) and therefore, there is a need to collect ethnobotanical information on antimalarial plants which is essential for further evaluation of the efficacy and safety of the plants as antimalarial remedies. Plants have played an important role in the treatment of malaria for thousands of years. Potent antimalarial compounds including quinine and artemisinin have been discovered from plants. The medicinal plants are thus a vast reservoir from which potent antimalarial drugs can be developed. Several initiatives in traditional medicine for malaria have been initiated in the last 10 years. These initiatives are designed to develop a strategy for more effective, evidence-based use of traditional medicines that can also inform malaria control policy decisions. Among the many initiatives include Research Initiative on Traditional Antimalarial Methods (RITAM), Natural Products Research in East and Central Africa (NAPRECA), WHO Traditional medicines programme, Global Initiative for Traditional Systems of Health (GIFTS) and many others. Various papers arising from these initiatives reporting on research results in traditional antimalarials have been published (Bodeker and Willcox, 2000; WHO, 2002; Willcox and Bodeker, 2004; Rukunga et al., 2009).

Kilifi district which encompassed our geographical site is rich in ethnomedical knowledge and biodiversity. Populations within this area popularly use traditionally prepared concoctions in management of many ailments including malaria. The medicinal knowledge of the Mijikenda is considered communal; however, there is individually held knowledge by the traditional health practitioners (THP). These are revered and trusted people in the community and play multiple roles as spiritual guides, counselors and healers. In this study, antimalarial plants candidates were identified following the field study. The plant species were collected and evaluated for antimalarial activity, synergism and general cytotoxicology. The findings are herewith discussed.

2. Methods

2.1. Collection of ethnomedical information and plants material preparation

Prior to surveys in the area, a research assistant was identified who had grown up in the area and knew the people and the local language well. We used standardized questionnaires to interview THPs and once plant species were listed, the THPs were instrumental during collection by physically pointing out the plants. Nine THP (mean age: 50 years) were interviewed. Among the areas keenly addressed by the questionnaires included; plant species used in treatment of malaria, methods of disease diagnosis, combination regiment of the traditional preparations, availability of raw material, conservation methods or cultivation practices of the medicinal plants, modes of preparation and route of administration. The questionnaires used in this exercise were a guide for the interviewer to know the kind of questions to ask and was not filled by the THPs; most of them did not have formal education. The plant materials were collected between the months of November and December (low rain season). During raw material collection, sustainable harvesting was practiced in order to protect the habitat. The plants were botanically identified by a botanical taxonomist while in the field prior to collection. Upon transportation to the screening center, they were assigned voucher specimen numbers and voucher specimens deposited at the East African Herbarium, Nairobi. Preparation involved chopping the plant materials into small pieces then air-drying at room temperature. Using a laboratory mill, the dry material was ground and 100 g of powder extracted by soaking in 300 ml of methanol overnight at room temperature. The extracts were then filtered and concentrated using a rotary evaporator. Owing to its ease and convenience in a laboratory situation, methanol was selected as the solvent of choice for this preliminary study. It is agreed most times traditional solvent is water. However, some traditional plant medicines for malaria are prepared by boiling plant materials with meat soup (water plus fat in the meat). In this case, there is introduction of non polar component (organic) of the extraction solvent. In other cases extraction is traditionally done by adding honey and letting it to ferment. Here again the extraction is not only just by water but also bit of ethanol introduced (by fermented honey). So in brief, traditional way of these medicines is not always 100% water as the solvent. Water is very polar solvent. Among the organic solvents methanol is the closest to water in terms of polarity. Therefore in the choice of either water or methanol in doing research on traditional antimalarials there is not much difference because methanol can extract nearly as much as water due to comparable polarity.

2.2. In vitro determination of cell cytotoxicity

Cytotoxic concentration causing 50% cell lysis and death (CC_{50}) was determined by a method described by Kurokawa et al. (1995). Vero E6 cells were seeded at a concentration of 2.5×10^4 cells/well in a 24 well plates and grown under 5% CO_2 at 37 °C in Eagle's Minimum Essential Medium (MEM) supplemented with 5% fetal bovine serum (FBS) for 48 h. The culture media were replaced by fresh media containing extract at various concentrations, and cells further grown for 24 h. The cells were then treated with trypsin and the number of viable cells determined by the tryphan blue exclusion method. The concentration of herbal extract reducing cell viability by 50% (CC_{50}) was determined from a curve relating percent cell viability to the concentration of extract. Selectivity index ($SI = IC_{50}/CC_{50}$) was used as a parameter of clinical significance of the test samples by comparing general toxins and selective inhibitory effect on *Plasmodium falciparum* (Wright and Phillipson, 1990).

2.3. In vitro antiplasmodial assay

Two strains of *Plasmodium falciparum*: the Sierra Leonean (CQ-sensitive) D6; and the Indochinese (CQ-resistant) W2, were used in the study. The parasite cultures were donated by the Malaria Research and Reference Reagent Resource Center (MR4). Parasite cultivation was carried out using previously described procedures (Trager and Jensen, 1976; Schlichtherle et al., 2000). The culture medium consisted of RPMI 1640 (10.4 g/l) powdered medium (without PABA) and lactic acid (LA) dissolved in 960 ml of double-distilled-autoclaved water (DDAW) and supplemented with 10% human serum, 25 mM (5.94 g/l) HEPES and 25 mM $NaHCO_3$. Human O+ red blood cells served as the parasites host cells. Test samples were prepared by dissolving in 100% DMSO and diluting in RPMI to lower the concentration of DMSO to $\leq$1%. Stock solutions (1 μg/ml) of chloroquine and artemisinin were also prepared for use as reference drugs. Semi-automated micro dilution assay technique that measures the ability of the extracts to inhibit the incorporation of [G-^{3}H] hypoxanthine (Amersham International, Burkinghamshire, UK) into the malaria parasite was used in testing antiplasmodial activity (Desjardins et al., 1979). Aliquots (50 μl) of the test solutions were added in the first wells of 96 well flat-bottom microculture plates (Costar Glass Works, Cambridge, UK) in

duplicate and serial diluted 64-fold concentration range down the plate using a Titertek motorized hand diluter (Flow Laboratories, Uxbridge, UK). The last row of wells did not contain the test samples and served as the controls. Two hundred microliters of parasite culture was added into each well. The set 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 each well was pulsed with 25 μl of culture medium containing 0.5 μCi of [G-^{3}H] hypoxanthine. The plates were incubated for a further 18 h. The contents of each well were then harvested onto glass fiber filter mats, washed thoroughly with distilled water, dried and the radioactivity in counts per minute (cpm) measured using a beta-counter (Wallac Micro Beta Trilux). The CPMs obtained were then used to compute the IC_{50} values (Sixsmith et al., 1984).

2.4. In vitro combination experiments

The method described by Canfield et al. (1995) was adapted. Briefly, solutions of initial concentrations 20–50 times the estimated IC_{50} values were combined in different ratios of the various extracts. Thus fixed ratios of pre-determined concentrations needed to inhibit parasite growth by 50% (IC_{50}) was used to determine the interaction of two plant extracts. Single and combined extract solutions were dispensed into the 96-well microtitre plates to give 9 combinations in ratios of 9:1 to 1:9 (extract A:extract B) (Fivelman et al., 1999). Incubation and subsequent procedures were done as described in the previous section and previously by Desjardins et al. (1979) and Le Bras and Deloron (1983). Corresponding IC_{50} values were determined for each sample alone and in combination (Sixsmith et al., 1984). The degree of synergy was evaluated according to Berenbaum (1978). Briefly, sum of fractional inhibition concentration (sum FIC) was calculated using the formula: sum FIC = Ac/Ae + Bc/Be. Where Ac and Bc are the equally effective concentrations (IC_{50}) when used in combination, and Ae and Be are the equally effective concentrations when used alone. In this system: sum FIC < 1 denotes synergism, sum FIC ≥ 1 and <2 denotes additive interaction, sum FIC ≥ 2 denotes antagonism (Gupta et al., 2002).

2.5. In vivo antiplasmodial activity

Male Balb C mice (6–8 weeks old, weighing 20 ± 2 g) were used as the subjects. The mice were bred in standard macrolon type II cages in air-conditioned rooms at 22 °C, 50–70% relative humidity, fed with the standard diet and water *ad libitum*. *Plasmodium berghei* strain ANKA were maintained by serial passage of infected blood through interperitonial injection (ip). The test protocol was based on the 4-day suppressive test (Peters et al., 1975). Briefly, *Plasmodium berghei* infected blood was obtained by heart puncture, mixed with 1% (w/v) heparin in phosphate buffered saline (PBS) (1:1) and the test animals infected by ip injection with 0.2 ml (2×10^7 parasitized erythrocytes). Infected mice were randomly selected into groups of five for one test sample and the experimental groups treated with a single dose of 250 mg/kg of the test sample in 0.2 ml by oral adminstration 2 h post infection (Gessler et al., 1995). Every 1–3 days (24, 48 and 72 h post-infection), the experimental groups were treated again with the same dose through the same route. Two controls groups of five mice each were treated with a placebo (10% Tween 80) and 5 mg/kg.day of the reference drug (CQ) for negative and positive control, respectively. Parasitaemia was determined on day 4 (24 h after the last treatment) by microscopic examination counting parasites in 4 fields of ≈100 erythrocytes per view of thin blood film sampled from the tail of the experimental mouse and stained in 10% giemsa solution. The difference between the mean number of parasites per view in the negative control group (100%) and those of the experimental groups was calculated and expressed as percent parasitaemia suppression (chemo suppression) according to the formula: $PS = [(A - B)/A] \times 100$ (Tona et al., 2001). Where, A is the mean parasitaemia in the negative control on day 4, and B the corresponding parasitaemia in the test group. The standard deviations for the mean values was calculated as described by Armitage and Berry (1991). All *in vivo* experiments were repeated three times. In cases where the standard deviation (SD) was large, the experiment was repeated yet again and in some instances, obvious outliers were not considered while computing the SD.

Percentage parasitaemia was described as number of parasitized erythrocytes per 100 erythrocytes while percentage chemo suppression was taken as inhibition of parasite growth/multiplication relative to control expressed in percentage. Parasites in the negative controls group are assumed to have experienced 0% chemo-suppression. Chemo suppression is thus the potency of the drug to inhibit parasite growth/multiplication.

2.6. In vivo combination experiments

Plant extracts combinations that exhibited strong additive or synergistic interactions *in vitro* were selected for *in vivo* interaction studies. The best performing combinations were mixed from stock solutions and the blends evaluated in mice for parasitaemia suppression (PS) as described above (Section 2.5). Chemo suppression (%) of the blends and individual plant extracts were then compared.

3. Ethical consideration

Permission for sustainable plant harvesting was granted by Kenya Wildlife Service (KWS) in the forest game reserve, local administrators and the local community outside the forest areas. The THPs were interviewed on voluntary basis with each one of them signing a consent form indicating his/her willingness to participate in the exercise. Rules and regulation set by the KEMRI Ethical Committee were adhered to during all the stages of the project. This included conducting the study in accordance with KEMRI guidelines on animal use and care and, the internationally accepted principles for laboratory animal use and care as found in WHO guidelines. A total of 250 male Balb C mice were used in the animal experiment. Needles size (26G-1/2) were used during the interperitoneal infection. The animal experiments were terminated by sacrificing the mice humanely by placing them in an enclosed chamber containing cotton wool soaked in chloroform. All the sacrificed mice as well as those that died in the course of the experiment were put in disposable bags and incinerated.

4. Results

4.1. Plants documentation

Nine THPs, who had many years of experience in the use of traditional medicine, were interviewed on the plants that they used for treatment of malaria. *Azadirachta indica* (Mkilifi) was mentioned by all the informants. *Rhus natalensis*, *Abrus precatorius*, *Allophylus pervillei* and *Lannea schweinfurthii* were mentioned by at least two THPs. The plants mentioned were distributed across several families with no more than three plant species belonging to the same family. The anacardiaceous and combretaceae families had two species mentioned, respectively, for treatment of malaria (Table 1).

The plant parts preferred for medicinal preparations were leaves (48%), roots (22%), stem bark (17%), root bark (9%) and whole plant (4%). In some instances, two to three parts of the same plant are used. A good example is *Hoslundia opposita* where either roots, leaves or the entire aerial part is used in making the antimalarial preparation. The method of preparation was mostly a decoction or a hot water infusion usually prepared just before use. The plant

Table 1
Documentation of plant species collected from the study area based on traditional reputation for their use as antimalarials.

Plant name (voucher number)	Family	Local name	Part used	Other traditional uses
Grewia plagiophylla K. Schum	Tiliaceae	Mkone	Stem bark	Diarrhoea
(*JG701*)			Leaves	Fever
Combretum padoides Engl. & Diels	Combretaceae	Mshinda arume	Roots	Bloody diarrhoea, wounds and conjunctivitis
(*JG702*)			Leaves	
Hoslundia opposita Vahl	Labietaceae	Mtserere/	Roots	Constipation, colds, coughs, sore throat and oral wounds
(*JG703*)		Mgongolo	Leaves	
Rhus natalensis Bernh. ex Krauss	Anacardiaceae	Myahi	Root bark	Coughs, colds, antigiardiac, headache and neck pain
(*JG704*)			Leaves	
Combretum illairii Engl.	Combretaceae	Mshinda arume	Root bark	Coughs, colds
(*JG705*)			Leaves	
Lannea schweinfurthii (Engl.) Engl.	Anacardiaceae	Mnyumbu	Leaves	Gastro-intestinal problems
(*JG706*)			Stem bark	
Premna chrysoclada (Bojer) Gürke	Verbenaceae	Mvuma	Roots	Diarrhoea
(*JG707*)			Leaves	
Allophylus pervillei Blume.	Sapindaceae	Mvundza kondo	Roots Stem bark	Colds
(*JG708*)			Leaves	
Abrus precatorius L. (*JG709*)	Leguminosae/Fabaceae	Mwangala nyuchi	Leaves	Cough, Febrifuge, Fever, Throat
Aganthesanthemum bojeri Klotzsch. (*JG710*)	Rubiaceae	Kahithima	Whole plant	Colds
Uvaria acuminata Oliv.	Annonaceae	Mrori/Mngwene mchetu	Roots	Coughs
(*JG711*)			Leaves	
Azadirachta indica A. Juss (*JG713*)	Meliaceae	Mwarubaine/Mkilifi	Leaves	Many ailments
Flueggea virosa (Willd.) Voigt (*JG712*)	Euphorbiaceae	Mukwamba	Root bark	Chest pains

material was used fresh and in rare cases the dry powdered raw materials were stored for later use, which allowed their utilization throughout the year.

Posology was difficult to quantify but was indicated as drinking boiled but cold decoction half a glass twice daily for adults and half this amount for children which can be approximated to: a half glass ≈125 ml; a pinch: 5 g of powdered plant material in 250 ml (1/2 glass × 2) of water to be taken twice daily; a few leaves: 5 g wet leaves or 10 g dry leaves in 250 ml of water to be taken twice daily; and a handful: 25 g of powdered plant material, or 40 g coarse plant material in 250 ml of water to be taken twice daily. Doses were mainly taken twice a day because people are present at home on the morning and evening. Treatment was supposed to be continued until recovery.

4.2. In vitro assays

Activity criteria in the *in vitro* assay were defined as high when IC_{50} was below 10, moderate when between 10 and 50, and low when between 50 and 100 μg/ml. Samples with IC_{50} > 100 μg/ml were considered as inactive (Table 2).

The leave extracts of *Azadirachta indica*, *Premna chrysoclada* and the root extracts of *Uvaria acuminata* were the most active against both the parasite strains used with IC_{50} values <10 μg/ml. The leave extracts of *Hoslundia opposita* also depicted good activity with IC_{50} values <14 μg/ml in both plasmodium strains. *Grewia plagiophylla*, *Combretum illairii*, *Flueggea virosa* and *Allophylus pervillei* were all moderately active with IC_{50} below 50 μg/ml in both parasite strains. Other plant extracts that were moderately active with IC_{50} values below 50 μg/ml for either of the two parasite strains included; *Rhus natalensis*, *Combretum padoides* and *Lannea schweinfurthii. Aganthesanthemum bojeri* and *Abrus precatorius* had weak activity with IC_{50} values that ranged between 50 and 100 μg/ml. The stem bark extracts of *Grewia plagiophylla* and the leave extracts of *Allophylus pervillei* did not depict antiplasmodial activity at the highest concentration (100 μg/ml) tested.

4.3. Combination results

In vitro drug interaction studies of selected plant extracts with one another against P. falciparum W2 (CQ resistant clone) were undertaken as previously described. The sum FIC values were calculated as previously described (Berenbaum, 1978). The results are summarized in Table 3.

The interaction between extract of *Hoslundia opposita* and that of *Grewia plagiophylla* was additive interaction in most of the combination ratios while the interaction between *Hoslundia opposita* with *Premna chrysoclada* was synergism in all the combination ratios. At combination ratios with high amount of *Uvaria acuminata*, the interaction with *Hoslundia opposita* is additive, however, as the former is increased, the interaction changes to synergism. The net interaction between the extracts of *Hoslundia opposita* and the extracts of *Combretum illairii* was synergism.

The strongest synergism was between the plants extracts combinations was that involving *Uvaria acuminata* and *Premna chrysoclada* at 4:6 ratios where sum FIC value of 0.43 was recorded. The other combination ratios of these two extract all depicted synergistic interaction. *Uvaria acuminata* and *Flueggea virosa* also showed synergism. Combinations involving *Premna chrysoclada* with *Grewia plagiophylla* spanned between additive and synergy. A similar scenario was observed when *Premna chrysoclada* was combined with *Combretum illairii*. Combining extracts of *Grewia plagiophylla* and *Combretum illairii* resulted in an antagonistic interaction. The highest antagonism was at almost equal proportion of the two extracts where sum FIC values >3 were recorded. The net effect of combining *Rhus natalensis* leave extracts with either *Combretum illairii* or *Lannea schweinfurthii* leave extracts was synergism in all the combination ratios.

4.4. Cytotoxicity

All the plant extracts were tested for toxicity using mammalian cells as described in the methodology. Most of the extracts were not cytotoxic at 100 μg/ml; the highest concentration tested. However, *Uvaria acuminata* was found to be cytotoxic with CC_{50} of 2.37 ± 0.76 μg/ml. This recorded the lowest (0.34) selective index (ratio of 50% effective antiplasmodial concentration to 50% cytotoxic concentration) of W2 *Plasmodium falciparum* strain. The root extracts of *Hoslundia opposita* also depicted a low Selectivity index (SI) ratios of 0.58. However, the leave extracts of *Hoslundia opposita* which was among the most active extracts *in vitro* was safe to the mammalian cells with SI values >100. The leave extracts of *Lannea*

Table 2
Antiplasmodial activity ($IC_{50} \pm SD$) of the 13 medicinal plants against CQ sensitive (D6) and CQ resistant (W2) *Plasmodium falciparum* strains.

Plant species	Methanol extract yield (g)	Part used	$IC_{50} \pm SD$ (μg/ml)	
			D6	W2
Grewia plagiophylla	1.81	Leaves	13.28 ± 0.00	34.2 ± 4.7
Grewia plagiophylla	3.78	Stem bark	>100	>100
Hoslundia opposita	3.41	Roots	79.38 ± 5.43	64.21 ± 5.95
Hoslundia opposita	2.99	Aerial part	19.73 ± 4.09	29.41 ± 6.7
Hoslundia opposita	2.12	Leaves	13.22 ± 1.61	12.8 ± 3.49
Rhus natalensis	1.56	Leaves	43.92 ± 2.98	51.2 ± 4.99
Rhus natalensis	2.14	Roots	>100	80.44 ± 3.65
Combretum padoides	3.18	Roots	21.73 ± 0.76	59.43 ± 0.85
Combretum illairii	3.43	Stem bark	55.96 ± 2.07	58.54 ± 3.26
Combretum illairii	1.86	Leaves	24.21 ± 0.65	33.71 ± 5.61
Lannea schweinfurthii	1.80	Leaves	38.87 ± 5.19	54.15 ± 1.42
Premna chrysoclada	2.21	Roots	27.63 ± 4.11	52.35 ± 0.07
Premna chrysoclada	2.49	Leaves	7.75 ± 1.14	9.02 ± 1.16
Allophylus pervillei	3.22	Stem bark	45.62 ± 13.21	48.91 ± 1.67
Allophylus pervillei	2.11	Leaves	>100	>100
Uvaria acuminata	1.89	Leaves	51.13 ± 7.91	>100
Uvaria acuminata	2.66	Roots	8.89 ± 2.12	6.90 ± 0.22
Aganthesanthemum bojeri	0.81	Whole plant	55.3 ± 1.67	55.97 ± 7.78
Abrus precatorius	2.11	Leaves	85.59 ± 4.91	>100
Flueggea virosa	1.93	Roots	27.05 ± 2.02	22.34 ± 2.71
Flueggea virosa	1.24	Aerial part	55.03 ± 0.67	55.92 ± 6.27
Azadirachta indica	2.01	Leaves	6.24 ± 0.03	7.53 ± 0.88
CQ			0.003	0.057
ART			0.0016	0.0019

schweinfurthii was mildly cytotoxic (CC_{50} = 75.80 ± 1.27 μg/ml) and a selectivity index of 1.4.

4.5. In vivo assays

Results of *in vivo* antimalarial assays of the plant extracts using *Plasmodium berghei* in mice are summarized in Table 4. Suppression of parasitaemia (chemo suppression) in mice was used as a measure of efficacy. There was significant parasite density reduction ($p < 0.05$) in animals treated with most of the herbal test samples compared to the ones treated with a placebo (negative control). The reduced peak parasitaemia on day 4 in all treated groups compared to the negative control group was indicative of antimalarial potential of the extracts

The samples were categorized as highly active when chemo suppression was above 60% moderately active between 30% and 60%, but lowly active below 30%. Chemo suppression exhibited by all the extracts was significant ($P < 0.05$) compared to the negative control (PBS). Extracts of *Azadirachta indica*, *Lannea schweinfurthii*, *Rhus natalensis*, *Hoslundia opposita*, *Grewia plagiophylla*, *Flueggea virosa*, *Premna chrysoclada* and *Allophylus pervillei* were the most active with chemo suppression of 89.16%, 83.48%, 82.7%, 79.67%, 77.9%, 68.55%, 65.08% and 62.1%, respectively. *Uvaria acuminata* exhibited the least chemo suppression of 27%. *Aganthesanthemum bojeri* and *Abrus precatorius* which did not exhibit good antiplasmodial activity *in vitro* were not examined in mice.

Combination involving *Rhus natalensis* and *Lannea schweinfurthii* extracts depicted the highest chemossupresion (87.67%). This preparation combined at a ratio of 1:1 inhibited growth of *Plasmodium berghei* allowing only a 3.27% growth of parasites comparing well with standard drug reference CQ whose parasitaemia was 1.27%. Combination of *Rhus natalensis* and *Combretum illairii* extracts also showed good activity with chemo suppression of 74.7%. The enhancement of activity in plant combinations confirms synergistic potential of traditional combinations as reported previously (Gathirwa et al., 2008)

Table 3
Sum FIC values of plant extracts combinations.

Plants combined with *Hoslundia opposita* (leaves)									
Combination ratios	9:1	8:2	7:3	6:4	5:5	4:6	3:7	2:8	1:9
Grewia plagiophylla (leaves)	1.06b	1.24b	1.12b	1.26b	1.2b	1.1b	1.24b	1.1b	0.8c
Premna chrysoclada (leaves)	0.78c	0.98c	0.89c	0.85c	0.85c	0.84c	0.5c	0.9c	0.93c
Uvaria acuminata (roots)	1.42b	1.71b	0.59c	0.79c	0.59c	0.91c	0.81c	0.83c	0.94c
Combretum illairii (leaves)	1.25b	1.33b	0.99c	0.8c	0.96c	0.9c	0.85c	0.74c	0.82c
Plants combined with *Uvaria acuminata* (roots)									
Premna chrysoclada (leaves)	1.61b	0.48c	0.49c	0.56c	0.63c	0.43c	0.63c	0.6c	0.57c
Flueggea virosa (roots)	1.5b	0.66c	0.74c	0.53c	0.42c	0.69c	0.65c	0.61c	0.95c
Plants combined with *Premna chrysoclada* (leaves)									
Grewia plagiophylla (leaves)	0.99c	1.18b	1.08b	1.24b	1.06b	0.69c	0.86c	0.79c	0.69c
Combretum illairii (leaves)	0.74c	0.83c	0.96c	0.87c	0.91c	0.49c	1.29b	1.13b	1.01b
Plant combined with *Grewia plagiophylla* (leaves)									
Combretum illairii (leaves)	1.7b	1.19b	2.77a	2.85a	3.64a	3.93a	2.71a	2.59a	2.81a
Plants combined with *Rhus natalensis* (leaves)									
Combretum illairii (leaves)	0.83c	0.81c	0.82c	0.64c	0.52c	0.46c	0.55c	0.58c	0.58c
Lannea schweinfurthii (leaves)	0.94c	0.99c	0.73c	0.58c	0.44c	0.49c	0.49c	0.61c	0.63c

a—antagonistic, b—additive, c—synergistic.

Table 4
Mean ($x \pm$ SD) parasitaemia, chemo suppression of *Plasmodium berghei* infected mice treated orally with plant extracts (250 mg/kg body weight) or with controls.

Plant extract	Part used	% Parasitaemia	% Chemo suppression
Rhus natalensis	Leaves	4.59 ± 0.32	82.7 ± 0.13
Grewia plagiophylla	Leaves	5.86 ± 0.19	77.9 ± 0.99
Allophylus pervillei	Stem bark	10.05 ± 2.06	62.1 ± 1.60
Uvaria acuminata	Roots	18.98 ± 1.53	27.0 ± 1.01
Combretum illairii	Leaves	17.05 ± 2.11	35.72 ± 1.93
Combretum padoides	Roots	13.12 ± 1.49	50.56 ± 3.45
Premna chrysoclada	Leaves	9.26 ± 0.93	65.08 ± 4.71
Hoslundia opposita	Leaves	5.45 ± 0.02	79.67 ± 4.94
Hoslundia opposita	Aerial part	11.92 ± 2.18	55.05 ± 2.33
Azadirachta indica	Leaves	3.19 ± 0.47	89.16 ± 1.73
Lannea schweinfurthii	Leaves	4.38 ± 0.04	83.48 ± 5.26
Aganthesanthemum bojeri	Whole plant	nd	
Abrus precatorius	Leaves	nd	
Flueggea virosa	Roots	8.36 ± 1.27	68.55 ± 4.73
Rhus natalensis and *Combretum illairii*[a]	4:6[b]	6.71 ± 0.82	74.70 ± 0.60
Rhus natalensis and *Lannea schweinfurthii*[a]	5:5[b]	3.27 ± 1.14	87.67 ± 3.18
10% Tween 80		26.52 ± 1.72	0.00 ± 0.0
Chloroquine (5 mg/kg)		1.27 ± 0.19	95.20 ± 0.29

nd—not done.
[a] Extracts tested in combination.
[b] Best combination ratios determined through the *in vitro* interaction.

5. Discussion

5.1. Ethnobotany

Kilifi District is one of the six districts that encompass the Coast province of Kenya. The district covers an area of 12,464 km^2 inclusive of about 109 km^2 of water. The weather along the coastal strip is moist and warm ranging from a minimum of 21 °C to a maximum of 32 °C. The population of the District is estimated to be 720,000. The district is inhabited by the Mijikenda community 90% of whom are Muslim. Kilifi is a malaria endemic area with the disease being the major causes of infant mortality in the region. Despite the Kenyan government's effort to bring affordable healthcare to its citizens, the practice of traditional medicine is still deep rooted in some rural areas including the entire Kilifi district. The practice continues unabated alongside conventional medicine because of ease of availability, inaccessibility of health centers and also due to social cultural factors. The Mijikenda of Kilifi has much elaborated plant knowledge. The attributes and knowledge on the use of medicinal plants were bequeathed to THPs by their fathers, albeit orally, from generation to generation. The spirit of the departed THP is said to possess the chosen kinsperson who would in turn keep the knowledge to himself and only pass it on, to a lineage in the family a few years before death. This oral transfer of knowledge on medicinal plants without proper documentation is common in many communities and there is therefore the danger of losing this precious cultural heritage. To avoid such loss, ethnobotanical inventories need to be established (Van Wyk et al., 2002). In most parts of Kilifi, the traditional way of life and customary beliefs is quite intact and the acceptability of antimalarial and other medicinal plants as claimed effective remedies is quite high among the population of this area. The Kaya forests were the traditional social–cultural focal point of this coastal community. The fact that these forests were regarded as sacred ceremonial sites and as sources of medicinal plants has largely contributed to their preservation.

The rich ethnobotanical knowledge of the Mijikenda community of Kilifi came in handy in realization of our study objective. Medicinal plants used traditionally against malaria were documented and the data gathered used as a starting point for antimalarial screening. A considerable amount of duplicated information relating to the use of the plants was reported by several THPs which may confirm the antimalarial efficacy of traditional herbal remedies prepared from these species. Some of the plants collected have been reported in the literature, as having been used for malaria or fever confirming validity of the gathered information. The results of this study show that a variety of medicinal plants are traditionally used for treatment of malaria among the Mijikenda community of Kilifi. Thirteen species from 10 families were documented.

There was no definite tendency for the plants mentioned to belong to any particular family. Only two families; combretaceae and anacardiaceae were mentioned more than once. The Combretaceae is a large family with at least 600 species. Combretum are widely used in African traditional medicine. Previous studies had confirmed antimicrobial activity from extracts/isolated compounds of some species belonging to this family (Fyhrquist et al., 2002; Katerere et al., 2003; Martini et al., 2004).

Azadirachta indica (Mwarubaini) also known as neem is a well known folk medicinal plant. This plant was cited by all the traditional healers interviewed. It is a popular belief that Mwarubaini can heal up to 40 different health problems, and it is used all over Africa (Agyepong, 1992; Aikins et al., 1994). Extensive biological studies have been carried out confirming medicinal potential of *Azadirachta indica*. Biswas et al. (2002) have written an elaborated review on some of these findings. *Abrus precatorius* (Mwengala nyuchi) was cited by three THPs. There are species cited in this study that are also known to be used as sources of antimalarial remedies in other parts of Africa. Such includes *Flueggea virosa* (Clarkson et al., 2004).

It was reported that, only selected parts of a specific plant are used for traditional medicinal preparations. It is known that the concentration of the active compounds can vary significantly between different parts of a given plant. Leaves were the most commonly used part of the plant with 48% use in the mentioned plants. This was found to be encouraging as the harvesting of leaves for medicinal purposes is not destructive to the plants. However, the use of root and root bark was also high at 31%. This was found to be destructive where in some cases the whole plant had to be uprooted. Caution should also be advised while obtaining stem bark as improper peeling of stem may result in drying of the plant. It thus calls for conservation and harvesting strategies to facilitate sustainable utilization of these plant resources.

Some of the healers could attribute different modes of action and different targets for improving the well-being of the patient to different plants they used, or to different remedies consisting of different plants. It was also noted that, there is no universal or commonly used treatment procedure. Everything is handled very much

on the individual level. Most often different parts of the same plant are recommended to be used for treating malaria. This could be due to certain uniform healing properties shown in taste, odour or medicinal reactions. In some instances, some parts are considered to be stronger than others and the selection of the part to be used depends on the condition of a patient. While some plants are considered powerful enough to cure malaria on their own, and are used singly, other plants are used only in combination with other plants. It is believed that the plants will have different functions leading to 'whole' healing. These functions are attributed to reducing joint pains, lowering the temperature, giving strength, etc.

When it comes to collection, use and storage of plant materials, it was interesting to note the healers do not consider this a vital issue. They do their collection any time as demand dictates. The fact that every healer relies on 2–3 plants for treatment of malaria, buffers them during seasons when a certain species is not available. In such instances, they go for the second or even the third option. The healers in the study area usually do not grow medicinal plants in their gardens or fields; they collect most of their raw materials from wild plants found in different places. Sometimes these places are far from their dwellings. The Kaya forests in this region are to a large extent intact. It serves as a major collecting point of the medicinal plants. In cases where the collecting places are very far away or difficult to get to, the THPs normally do not go for collections so often. They collect a big stock and dry the material to store it for future use. Much of the plant material (especially root and stem bark) is air-dried and stored over long periods. Glass bottles, plastic containers and gunny sacks are among the most popular storage containers. It was noted that certain rituals are adhered to when collecting the plant materials. A healer would at time ask for a white chicken. It was not exactly clear why such a demand is necessary. It can be assumed that, this probably acts as part of healer's payment for the service offered. However, to some healers they emphasized that failure to appease the gods with such tokens make the medicinal preparations not to work.

In terms of preparation, application and dosage of the remedies, the traditional remedies are very often freshly prepared (to make a personal medicine) specifically for one patient. The remedies for malaria are most often prepared as a decoction or less frequently as pure leaf juice. Decoctions are prepared by placing the plant material in cold water, bringing it to boil and simmering it for about 15 min. Every healer has his own methods to determine the dosage of the traditional remedies. Very often kitchen utensils like cups, glasses, or spoons are used. The dosage of a remedy depends mainly upon the drug to be administered, the sex and the age. For instance, pregnant women and children more often are given lower dosages compared to other adults. The duration of the treatment varies depending on the remedy and the severity of the health condition, normally between 3 and 7 days.

5.2. Bioassays

These results confirm the high activity of *Rhus natalensis*. Gathirwa et al. (2007) reported a chemo suppression 83.15% for *Rhus natalensis* collected in Meru district of Kenya. The current study confirms this plant's potential albeit having been collected from a different region. In the same study, the plant was also shown not to be cytotoxic to mammalian cells with a CC_{50} of 211.78 and a selectivity index of 2.78.

In a previous study, the methanol extract of *Flueggea virosa* was shown to have high antiplasmodial activity with IC_{50} values that were <4 μg/ml against both D6 and W2 *Plasmodium falciparum* strains (Muthaura et al., 2007), while CC_{50} for the same was >600 μg/ml with a selectivity index of 299. The methanol extract of *Flueggea virosa* examined in our study confirms that earlier report of the plant species collected in another region of the country. This plant was tested for safety in mice with no apparent signs of toxicity observed at the highest dosage (LD_{50} > 5000 mg/kg) tested. A chemo suppression of 70.91% in mice had been reported earlier (Muthaura et al., 2007). There are other studies on *Flueggea virosa* that reported good activity consistent with our results (Clarkson et al., 2004). *Flueggea virosa* is traditionally used for malaria in Tanzania and South Africa (Hedberg et al., 1983).

Abrus precatorius had previously been investigated for antiplasmodial activity and the pentane extract found to have moderate activity with IC_{50} value <20 μg/ml (Mĩenan et al., 2006). In China, this plant is used in folklore medicine for various inflammatory diseases (Chang, 1981). Limmatvapirat et al. (2004) reported the presence of antimalarial and antitubercular isoflavanquinone in its aerial parts. *Uvaria afzelii* closely related to *Uvaria acuminata* is used widely in Côte d'Ivoire by traditional healers as antimalarial. *In vitro* evaluation has shown that it has good antimalarial activity with IC_{50} ranging between 5 and 10 μg/ml (Mĩenan et al., 2006). *Uvaria afzelii* has also been shown to have antiplasmodial activity (Nkunya et al., 1991). Subsequently, indole alkaloid-(DL) schefflone has been isolated (Nkunya et al., 1990). Root decoction is traditionally used for malaria (Kokwaro, 1993; Beentje, 1994). Caution is, however, advised on the use of *Uvaria acuminata* as the results indicated some toxicity. Actually, several studies have confirmed potential toxicity in *Uvaria* species with some studies having isolated the cytotoxic compounds (Ichimaru et al., 2004). However, the interest in the plant's metabolites has remained high due to their antiparasitic activities and potential for exploitation as anticancer agents (Zafra-Polo et al., 1996). Toxicity involving this plant was not reported by any of the THP. However, it emerged that it is never administered alone. The fact that it is always given in combination with others such as *Hoslundia opposita* or *Flueggea virosa*, probably masks the toxicogical components.

The stem bark of *Lannea schweinfurthii* is boiled in water and drunk for treatment of syphilis, cellulitis, abscesses and oral candidiasis (Maregesi et al., 2007). Bark decoction is used for gingivitis while the root decoction is used for nasal ulcers and asthma (Neuwinger, 2000). Ground fresh leaves of *Hoslundia opposita* are soaked in water and the extract douched to treat vaginitis and drunk for treatment of hypertension. The roots are boiled and taken orally to cure children fever and convulsions (Maregesi et al., 2007). Leaves are used for skin diseases and herpes zoster while the whole plant is used for liver cancer (Azuine, 1998). The plant is also used for gonnorrhoea, blenorrhoea cystitis, liver disease, chest pain, cough, fever, hookworm, stomach disorders, wounds and mental disturbances (Watt and Breyer-Brandwijk, 1962). The roots are used to treat malaria, epilepsy, convulsions and measles like swellings on the skin (Hedberg et al., 1983)

Antimalarial compounds from *Azadirachta indica* one of the most commonly used medicinal plant have been studied in the past. Gedunin isolated from the leaves and barks of *Azadirachta indica* was found to be responsible for the high activity (Bray et al., 1990; MacKinnon et al., 1997). *Azadirachta indica* is the third most commonly used herbal medicine to treat malaria in Kenya after *Ajuga remota* and *Caesalpinia volkensii* (Kuria et al., 2001). As Sofowora (1982) noted, many people in several African countries take a decoction of *Azadirachta indica* (neem tree) for malaria fever. Their reasons for doing so include reaction to chloroquine, a dislike for synthetic drugs, and the expense and unavailability of synthetic antimalarials.

Some of the plants tested did not exhibit high antiplasmodial activity. This is despite having been reported by the THPs as being good antimalarials. These plants could be effectively more active on *Plasmodium falciparum* in man, as it is the case for plants containing prodrugs non-active by themselves but which are metabolized to active forms as has been demonstrated for *Azadirachta indica* extracts (Parida et al., 2002). This underlies the limit of the *in vitro*

tests. The potency of the extract may also be affected by solvent of extraction, georeference, time and season of harvesting or other environmental factors (Prance, 1994). Plants that are frequently reported as used as antimalarials in various countries do not necessarily show high activity in the *in vitro* test. These findings can probably partly be explained because many of the plants used in the treatment of malaria could have other therapeutic activities rather than antiparasitic effect, such as antipyretic or immunomodulatory.

Most of the plants were collected from the community land, which is facing great pressure due to over-utilization of indigenous trees and medicinal plants may disappear before their uses are documented and their efficacy scientifically proven. The majority of the population in Kilifi district is in the low social–economic bracket and very often the use of medicinal plant is the only affordable treatment option. Medicinal plant use therefore, will remain an integral part of the health care system to the community for a long time to come. Consequently, ethnobotanical exploration should not only be a cost-effective means of locating new and useful tropical plant compounds but also be linked to the urgent need for sustainable conservation strategies for medicinal plants.

6. Conclusions

The traditional healers in our study treat malaria with herbal remedies, each made from 1 to 3 plants. The different plant species which can be used belong to different plant families. Multiple citations of the same plants were rare. The fact that traditional healers use a large number of plants for the treatment of malaria may suggest that the acquisition of knowledge of medicinal plants is a dynamic process. The choice of these medicinal plants by traditional healers was shown to be legitimate as confirmed by the antiplasmodial screening carried out. Some of the plants mentioned when tested for antimalarial activity against malarial parasites *in vitro* and *in vivo* showed promising results. This indicates that some of these traditional remedies probably owe their effectiveness at least in part to the presence of a component that can kill the malaria parasite. When looking for new leads for drug development the selection of ethnomedically used plants seems to be a better approach than random screening (Cordell, 1995; Unander et al., 1995). There is a need to send back useful findings to information providers at the level of their understanding and practices in order to reduce health hazards that might be an outcome of the treatment offered by them in especially where toxicological potential is demonstrated in bioassays.

Extracts with a high selectivity for the parasites offer the potential for safe antimalarial therapy. The 13 plants studied revealed antiplasmodial activities of variable intensity. Many plant species reported in this study have been investigated for their phytoconstituents and pharmacological activities, the latter are in agreement with ethnomedical uses reported in this study. In Kilifi, treatments based on medicinal plants are still an important part of social life and culture and the acceptability of these plants as claimed effective remedies is quite high among the population of this area. The claimed therapeutic value of the reported species and the positive preliminary findings calls for further examination geared towards isolation of pure compounds as biomarkers or templates for antimalarial drug development. Other efforts should be on encouragement of preservation and documentation of this flora which may otherwise be lost due to erosion of age old traditional methods of biodiversity conservation and medicinal knowledge as in the current practice. These results remotely validate the traditional use of the selected medicinal plants in management of malaria.

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