Journal of Ethnopharmacology 170 (2015) 148–157

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jep

Antiplasmodial potential of traditional antimalarial phytotherapy remedies used by the Kwale community of the Kenyan Coast

C.N. Muthaura [a,*], J.M. Keriko [b], C. Mutai [a], A. Yenesew [d], J.W. Gathirwa [a], B.N. Irungu [a], R. Nyangacha [a], G.M. Mungai [c], S. Derese [d]

[a] Kenya Medical Research Institute, P.O. Box 54840, 00200 Nairobi, Kenya
[b] Department of Chemistry, Jomo Kenyatta University of Agriculture and Technology, P.O. Box 62000, Nairobi, Kenya
[c] East Africa Herbarium, National Museums of Kenya, P.O. Box 40658, 00100 Nairobi, Kenya
[d] Department of Chemistry, University of Nairobi, P.O. Box 30197, Nairobi, Kenya

ARTICLE INFO

Article history:
Received 19 February 2015
Received in revised form
22 April 2015
Accepted 12 May 2015
Available online 20 May 2015

Keywords:
Traditional remedies
Antimalarial plants
Kwale County

ABSTRACT

Ethnopharmacological relevance: In Kenya, 22 million people are at risk of malaria, 70% of them are in rural areas and most of these people use traditional plant based medicines to treat malaria. The aim of the study was to escalate documentation, from an earlier study of medicinal plants, traditionally used to treat malaria by the Digo community of Kwale County, taking cognizance of their pharmacological information by evaluating their antiplasmodial efficacies.

Materials and methods: The study was carried out in Kwale County at Shimba Hills Game Reserve and adjoining part of Kinango. Traditional health practitioners (THP) were interviewed with a standard questionnaire to obtain information on medicinal plants traditionally used for management of malaria. Group interviews were also held among THPs and members of the community. The plant samples collected were tested for antiplasmodial activity against chloroquine sensitive (D6) and resistant (W2) *Plasmodium falciparum* using the ability of extracts, prepared from the plant species, to inhibit the incorporation of [G-3H] hypoxanthine into the malaria parasites.

Results: Fifty seven (57) species in forty eight (48) genera and thirty (30) families were documented and evaluated for in vitro antiplasmodial activity. Apocynaceae, Euphorbiaceae, and Rubiaceae families had each about 12% of the plant species reported as antimalarial remedy and represented the species that are most commonly used. Twelve species (21.1%) showed antiplasmodial efficacy of $IC_{50} < 5$ μg/ml and these were *Boscia salicifolia*, *Cissampelos mucronata*, *Clerodendrum myricoides*, *Commiphora schimperi*, *Flueggea virosa*, *Maytenus undata*, *Maytenus senegalensis*, *Maytenus putterlickioides*, *Vernonia amygdalina*, *Warburgia stuhlmannii*, *Zanthoxylum chalybeum* and *Tabernaemontana pachysiphon.*

Conclusions: These results seem to indicate that ethnopharmacological inquiry used in search for new herbal remedies as predictive and could form the basis of an ethnopharmacopoeia and search for new active principles. This is the first report on traditional use of *T. pachysiphon* for malaria and its antiplasmodial activity.

1. Introduction

Malaria is a major tropical parasitic disease responsible for significant morbidity and mortality and in the absence of practical preventive measures; the current options are chemoprophylaxis and chemotherapy (Hardie and Dürrheim, 2013). The chemotherapy of malaria is one of the medicinal fields that are known to use pharmaceuticals originating from natural product research; examples are quinine from *Cinchona succirubra* and artemisinin from *Artemisia annua* (Kayser and Atta-ur-Rahman, 2002; Renslo, 2013). The use of natural product-derived drugs and drugs from other sources in combating malaria has however been faced with several challenges, including the emergence of drug resistance parasites. This has made many of the first line drugs such as chloroquine (CQ) not effective. The need for new drugs, preferably with new mode of action is therefore urgently needed (Jansen et al., 2012).

In Kenya, 22 million people are at risk of malaria, 70% of them are in rural areas. About 34,000 Kenyan children die every year from malaria compared to a total estimate of 42,000 people dead

* Corresponding author. Tel.: +254 722430222; fax: +254 20 2720030.
E-mail address: cmuthaura@yahoo.com (C.N. Muthaura).

http://dx.doi.org/10.1016/j.jep.2015.05.024
0378-8741/

(DMS, 2006). 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, 2002b; 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, 2002a) 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. To meet the criteria of efficacy, safety and quality control like synthetic drug products (Wagner, 1997); the pharmacological, toxicological and phytochemical profiles of the extracts have to be scientifically evaluated.

Malaria is endemic in Kwale and prevalent in many other communities in Kenya. The situation has become worse with increasing drug resistance by the malaria parasite, *Plasmodium falciparum* (WHO, 2001). Western style healthcare provided by the government has been expanded in the last decades, but is often not readily available and many regions remain completely underserved. Subsequently, most communities still use herbal remedies as readily and cheaply available alternative. Many tribes in Africa have much elaborated plant knowledge (Barrow, 1996). Most knowledge on medicinal plants is transferred orally in many communities (Fratkin, 1996) and there is therefore the danger of losing this precious cultural heritage. In view of the rapid loss of natural habitats, traditional community life, cultural diversity and knowledge of medicinal plants, an increasing number of ethnobotanical inventories need to be established (Van Wyk et al., 2002). In most parts of Kwale, the traditional way of life and customary beliefs are however, 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 the Digo community in Kwale County, one of the nine deeply traditional ethnic groups that form the Mijikenda community of the coast province. More than half of the Kenyan's rare plants grow in the coastal region; most have been identified within the Kaya forest patches, which comprise about 10% of the Kenya's coastal forest. The traditional medicinal knowledge from the resources of these forests, in possession of a few traditional healers, requires documentation for the benefit of current and future scientific research on the plants to determine their efficacy, safety and phytochemical properties. As a follow up of an earlier ethnobotanical study, twenty-five (25) species in twenty one (21) genera and sixteen (16) families (Muthaura et al., 2007a) were encountered; in the present study this escalated to fifty seven (57) species in forty eight (48) genera and thirty (30) families were documented. In addition, the in vitro antiplasmodial efficacy of the plant species was carried out as a step in proposing appropriate scientific measures through ethnopharmacological evaluation of the plant extracts for further pharmacological and phytochemical studies.

2. Materials and methods

2.1. Study area

In Kwale County, the study area centered around at 4°12' S and 39°25' E in the Shimba Hills Forest Game Reserve and in Kinango division (Fig. 1). The area is hot and humid all year round with annual mean temperatures ranging between 23 °C and 34 °C and the average relative humidity ranging between 60% and 80%. The Coastal uplands commonly known as Shimba Hills rise steeply from the Coastal belt to 462 m above sea level. The soils are made of sandstone and grit and are fairly fertile for cultivation. The type of climate is monsoon, hot and dry from January to April while June to August is the coolest period. Rainfall comes in two seasons with long rains from March/April to July and short rains from October to December. The total precipitation varies from 900 mm to 1500 mm per annum along the Coastal belt to 500–600 mm in the hinterland, which comprises 92% of the land whose agricultural potential is low (Muthaura et al., 2007a).

The population of Kwale County is 649,931 (2009 National Population Census Report) people, inhabited mainly by the Digo and Duruma who belong to the Mijikenda ethnic group of Coastal Kenya. The Digo are the major Bantu tribe, 90% of who are Muslim and are concentrated on the Southern Coastal strip of Kenya between Mombasa and the border with Tanzania. The community is rural and depends on agriculture as its major source of livelihood. In most parts of Kwale, the traditional way of life and customary beliefs are quite intact (Muthaura et al., 2007a). Traditional practices (such as animism and ancestor worship) have more influence on the Digo community than does Islam. The Kaya forests are the traditional social–cultural focal point of the Digo community. The medicinal knowledge of the Digo is considered communal. However, the Kaya elders are reputed as historical repository holders of cultural knowledge, with a comprehensive understanding of the Digo plant knowledge. These are revered and trusted people in the community and play multiple roles as spiritual guides, counselors and healers. These attributes and the knowledge on the use of medicinal plants were bequeathed to them from their fathers, albeit orally, from generation to generation (Muthaura et al., 2007a). The spirit of the departed THP was supposed to possess the chosen THP who would in turn keep the knowledge to himself and only transmit it to a lineage in the family a few years before death (Muthaura et al., 2007a).

The community plant knowledge is inseparable from the day-to-day life of the people, and the acceptability of antimalarial and other medicinal plants as claimed effective remedies is quite high among the population. Malaria is common in the study area and is associated with significant morbidity and mortality, especially children aged 5 years of age and below and pregnant women (DMS, 2006). The prevalence of *P. falciparum* malaria is reported to exceed 50% and the area is classified as a malaria endemic zone (DMS, 2006). Most parts of the County are remote and health facilities far apart. The inhabitants are generally poor and cannot afford conventional antimalarial drugs (Nguta et al., 2010) with consequent widespread use of traditional medicine. The traditional medicinal knowledge from the resources of the forests, in possession of a few traditional healers requires documentation and evaluation of their efficacy for the benefit of current and future generations.

2.2. Data collection

Fieldwork to collect plant material was carried in the month of October and December, 2008. Permission for a sustainable plant harvesting was granted by KWS at the Shimba Hills Forest Game Reserve, and the local community outside the forest areas. To obtain information on medicinal plants traditionally used for the management of malaria, local people facilitated access to THPs who were interviewed with a standard questionnaire. Prior to surveys in each area, a research assistant was identified who had grown up in the area and knew the people and the local language well. Several contacts were made with THPs before the actual interview to win their trust. A taxonomist who was conversant with the flora of the area was part of the collection team. Nine THPs (2 women and 7 men; mean age: 55 years) were interviewed.

2.3. Collection of plant samples

Traditionally the disease is treated in function of symptomatology and those plant parts claimed to treat malaria, fevers and joint

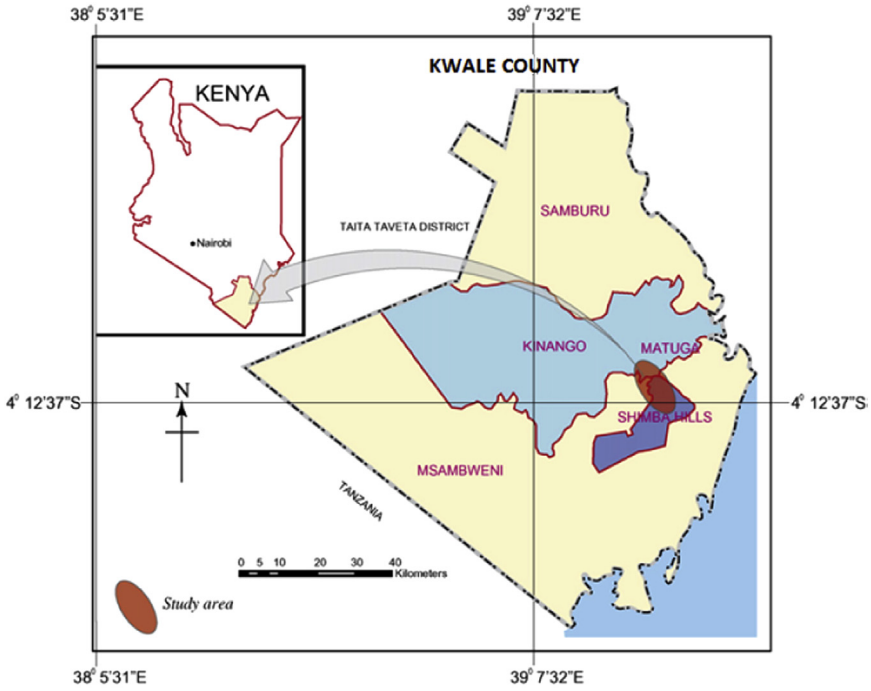

Fig. 1. Map of Kenya showing Kwale County with study areas Shimba Hills Game Reserve and Kinango.

pains as identified by THP were collected. The plants were identified by the taxonomist and voucher specimens deposited at the East Africa Herbarium, National Museums of Kenya.

2.4. The extracts for antiplasmodial efficacy

All the plant species collected were extracted separately in water (which is the traditional formulation) and also in methanol (MeOH) at a concentration of 10% dry weight per volume. The aqueous extracts were left to extract in 1 L conical flasks over a warm water bath at 60 °C for 1 h, while MeOH extracts were prepared by maceration of the plant material with MeOH at room temperature for 24 h (25 °C). The water extracts were lyophilized after filtration and the organic extracts were concentrated under vacuo until dry. The choice of methanol was due to its polarity which is close to that of water and the fact that some traditional medicine is prepared in alcohol (ethanol) from beverages like palm wine, maize, fermented honey, and other edible seed foods as well as edible oils. And again for further work on phytochemical analysis of the active constituents from the extracts, the results of methanol extracts would be an appropriate reference point. The yields of the water extracts were higher than the corresponding methanol extracts and ranged between 4.5 and 12.8%, while those of the methanol extracts were between 1.6 and 8.4%. All the extracts were stored in airtight containers at −20 °C until used.

2.5. Preparation of test extracts

Stock solutions of aqueous extracts (500 μg/ml) were made in distilled deionized water and filter sterilized using 0.22 μm membrane filters in a laminar flow hood. The methanol extracts were dissolved in DMSO (Sigma chemical CO., St. Louis, MO, USA) followed by subsequent dilution to lower concentration of DMSO, to < 1% to avoid carry over (solvent) effect (Dorin et al., 2001). Reference drugs, chloroquine diphosphate and artemisinin at a concentration of 1 μg/ml each, were similarly prepared and all solutions stored at −20 °C until used.

2.6. In vitro antiplasmodial assay

An in vitro semi-automated micro-dilution assay technique was used that measures the ability of the extracts to inhibit the incorporation of [G-3H] hypoxanthine into the malaria parasite (Desjardins et al., 1979; O'neill et al., 1985), *P. falciparum* culture of D6 (CQ sensitive) and W2 (CQ resistant) was used in the study. Aliquots of the culture medium (25 μl) were added to all the wells of a 96-well flat-bottomed micro-culture plate (Costar Glass Works, Cambridge, UK). The test solutions (25 μl) were added in duplicate to the first wells and a Titertek motorized hand diluter (Flow Laboratories, Uxbridge, UK) was used to make two-fold serial dilutions of each sample over a 64-fold concentration range. The susceptibility tests were carried out with an initial 200 μl of parasite culture (0.4% parasitaemia, 1.5% hematocrit) in each well. The stock solutions of the plant extracts were diluted in the plate to give a 100 μg/ml concentration (as the highest concentration) and then diluted two-fold until reaching a concentration of 1.56 μg/ml. A suspension (200 μl) of parasitized erythrocytes (0.4% parasitaemia) in the culture medium was added to all the test wells. Non-parasitised erythrocytes were used in control experiments. The plates were incubated at 37 °C in an atmosphere of 3% CO_2, 5% O_2 and 92% N_2. After 48 h each well was pulsed with 25 μl of culture medium containing 0.5 μCi of [G-3H]-hypoxanthine and the plates were incubated for a further 18 h. The contents of each plate were harvested onto glass fiber filters, washed thoroughly with distilled water, dried and the radioactivity measured using a BetaplateTM liquid scintillation counter (Wallac, Zurich, Switzerland). The CPMs obtained were then used to compute the IC_{50} values (Sixsmith et al., 1984).

3. Results and discussion

3.1. Ethnopharmacological study

Table 1 shows a documentation of plant species collected from the study area (Fig. 1) based on traditional reputation for their use

Table 1
Antiplasmodial efficacy of plant species collected from Kwale County based on traditional reputation for their use as antimalarials.

Species/family/voucher no.	Plant part	Extract	*P. falciparum* (strain)/antiplasmodial activity IC_{50}, μg/ml	
			D6	W2
Albizia antihelmintica Brongn. (Mimosaceae) (cm123)	Rb	H_2O	> 100	> 100
		MeOH	> 100	> 100
Agathisanthemum bojeri Klotzsch (Rubiaceae) (cm 57)	Wp	MeOH	49.8	55.9
Acacia nilotica (L.) Del. (Mimosaceae) (cm 132)	Sb	H_2O	> 100	> 100
		MeOH	70.33	73.6
Acacia seyal Delile Willd. (Mimosaceae) (cm56)	Sb	H_2O		> 100
		MeOH		89.3
Acalypta fruticosa Forssk (Euphorbiaceae) (cm54)	L	MeOH	13.8	
Azadirachta indica A. Juss.(Meliaceae) (cm120)	L	H_2O	49.5	> 100
		MeOH	39.5	> 100
	Sb		50.1	89.3
Adansonia digitata L. (Bombacaceae) (cm50)	Sb	MeoH	78.9	67.3
Boscia salicifolia Oliv. (Rubiaceae) (cm45)	Sb	H_2O	3.6	10.1
		MeOH	1.1	8.8
Bridelia micrantha (Hochst) Baill. (Euphorbiaceae) (67)	Sb	MeOH	19.4	14.2
Cassia abbreviata Oliv. (Fabaceae) (cm122)	Rb	H_2O	> 100	> 100
		MeOH	> 100	> 100
Cassia occidentalis L.(Fabaceae) (cm46)	Rb	MeOH	18.8	
Centella asiatica (L.) Urban (Umbelliferae) (cm138)	Wp	H_2O	58.6	
		MeOH		15.5
Cissampelos mucronata A. Rich. (Menispermaceae) (cm137)	Rb	MeOH	8.8	9.2
	L	MeOH	4.4	
Carissa edulis (Forsk.) Vahl (Apocynaceae) (045)	Rb	H_2O	> 100	> 100
		MeOH	25.5	
Clerodendrum myricoides (Hochst.) Vatke (Verbenaceae) (cm125)	Rb	MeOH	4.7	8.3
		DCM	10.2	4.3
	L	MeOH	18.8	
Commiphora schimperi (O. Berg) Engl (Burseraceae) (cm57)	Sb	MeOH	3.9	5.2
Combretum padoides Engl. and Diels (Combretaceae) (cm42)	Rb	MeOH	21.7	59.3
Flueggea virosa (Willd.) Voigt (Euphorbiaceae) (cm118)	L	H_2O	25.5	37.8
		MeOH	2.2	3.6
	Rb		31.5	
Flacourtia indica (Burm.f.) Merr. (Flacourtiaceae) (cm39)	L	H_2O	89.7	
		MeOH	33.7	
Gymnema sylvestre (Retz) Shultes (Asclepiadaceae) (cm61)	Climber	MeOH		69.3
Grewia plagiophylla K. Schum (Tiliaceae) (cm44)	L	MeOH	13.2	34.2
Harrisonia abyssinica Oliv. (Simaroubaceae) (cm134)	Rb	H_2O		
		MeOH	7.8	11.4
Harungana madagascariensis Poir. (Guttiferae) (cm128)	L	H_2O	> 100	> 100
		MeOH	39.1	43.7
Hoslundia opposita Vahl. (Labiatae) (cm37)	L	MeOH	15.2	25.6
Hugonia castaneifolia Engl. (Linaceae) (cm33)	Twigs	H_2O		152.1
		MeOH	23.4	
Maytenus undata (Thunb.) Blakelock (Celastraceae) (cm133)	L	H_2O	0.95	1.9
		MeOH	7.4	9.8
	Rb	H_2O	5.5	7.9
		MeOH	5.1	4.9

Table 1 (*continued*)

Species/family/voucher no.	Plant part	Extract	*P. falciparum* (strain)/antiplasmodial activity IC_{50}, μg/ml	
Maytenus putterlickioides (Loes.)	Rb	H_2O	> 100	> 100
Excell and Mendonca (Celastraceae) (cm116)		MeOH	4.4	10.2
	L	MeOH	5.6	8.2
Maytenus senegalensis (Lam. Excell) (Celastraceae) (cm129)	Rb	H_2O	> 100	> 100
		MeOH	4.7	9.8
Mangifera indica L.(Anacardiaceae) (cm29)	Sb	MeOH	< 25	
Moringa oleifera Lam. (Moringaceae) (cm36)	L	MeOH	9.8	
Ocimum basilcum L.(Lamiaceae) (38)	L	MeOH	16.4	
Ocimum gratissimum L. (O.suave) (Lamiaceae) (cm41)	L	MeOH	5.9	
Pentas bussei Krause (Rubiaceae) (cm139)	Rb	MeOH	23.3	27.9
Pentas longiflora Oliv. (Rubiaceae) (cm126)	Rb	MeOH	13.3	
Premna chrysoclada (Bojer) Gürke (Verbenaceae) (cm35)	L	MeOH	11.1	
Ricinus communis L. (Euphorbiaceae) (cm32)	L	MeOH	< 25	
	F		> 25	
Rauvolfia mombasiana Stapf (Apocynaceae) (cm130)	Rb	H_2O		> 100
		MeOH		9.1
Schizozygia coffaeoides Baill. (Apocynaceae) (cm26)	L	MeOH		10.5
Scolopia zeyheri (Nees) Harv. (Flacourtiaceae) (48)	L	MeOH		24.9
Solanum incanum L. (Solanaceae) (cm23)	Rb	H_2O	> 100	> 100
		MeOH	> 100	> 100
Suregada zanzibarensis Baill. (Euphorbiaceae) (cm141)	L	MeOH	6.7	5.8
Tabernaemontana pachysiphon Stapf (Apocynaceae) (cm33)	F	H_2O	4.8	4.4
		MeOH	3.9	53.7
	L	H_2O	25.3	70.8
		MeOH	14.7	25.4
Terminalia spinosa Engl. (Combretaceae) (cm081)	F	H_2O	62.9	
	Sb	MeOH	7.9	
Turraea floribunda Hochst. (Meliaceae) (cm21)	Sb	MeOH		5.5
Tridax procumbens L. (Compositae) (cm115)	Wp	H_2O		> 100
		MeOH		15.4
Toddalia asiatica (L.) Lam. (Rutaceae) (cm136)	Rb	MeOH	6.82	13.9
Tamarindus indica L. (Fabaceae) (cm24)	Rb	MeOH		35.2
Uvaria lucida Benth. (Annonaceae) (cm47)	L	H_2O	> 100	> 100
		MeOH	5.9	10.3
Uvaria scheffleri Diels. (Annonaceae) (cm088)	L	H_2O		97.2
		MeOH		6.8
	Rb	H_2O		58.9
		MeOH		8.9
Uvaria acuminata Oliv. (Annonaceae) (cm49)	L	MeOH	51.1	> 100
	Rb		8.9	6.9
Vernonia amygdalina Del. (Compositae) (cm27)	L	H_2O		3.8
		MeOH	4.9	7.2
Vitex strickeri Vatke and Hildebr. (Verbenaceae) (cm29)	L	H_2O	> 100	> 100
		MeOH	26.7	24.8
Warburgia stuhlmannii Engl. (Canellaceae) (cm119)	Sb	H_2O	> 100	> 100
		MeOH	1.8	2.3
Zehneria scabra (Cucurbitaceae) (30)	Wp	MeOH		9.8
Ziziphus mucronata Willd. (Rhamnaceae) (cm19)	L	H_2O	> 100	

Table 1 (*continued*)

Species/family/voucher no.	Plant part	Extract	*P. falciparum* (strain)/antiplasmodial activity IC_{50}, μg/ml	
		MeOH	21.9	
Zanthoxylum chalybeum Engl. (Rutaceae) (cm127)	Rb	H_2O	5.3	3.1
		MeOH	3.7	2.9
Chloroquine phosphate (ng/ml)			8.97	31.32
Artemisinin (ng/ml)			0.9	3.38

Rb=root bark, Sb=stem bark, L=leaf, Wp=whole plant, C=climber, T=twigs.

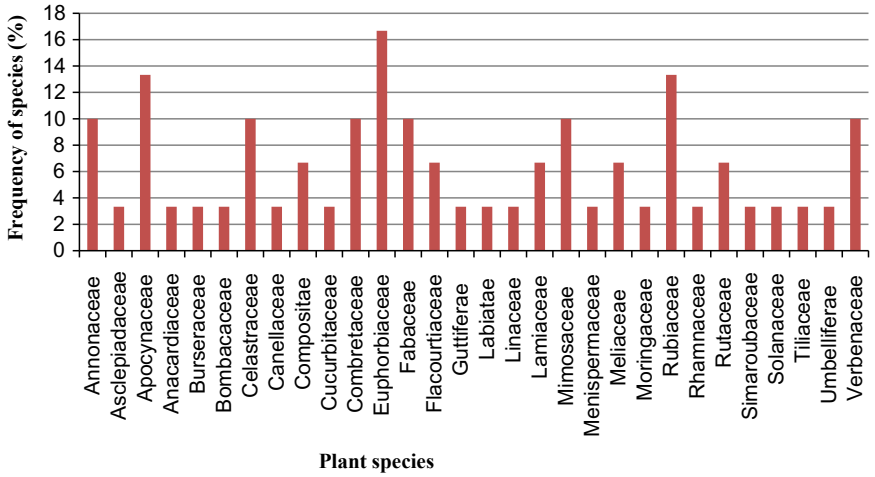

Fig. 2. Frequency of the species in the families

as antimalarials. The plant species are listed in alphabetical order, with their respective families and voucher specimen number in parenthesis in the first column. Subsequent columns indicate the plant part used for herbal preparation, the type of extract prepared for the test, and the antiplasmodial efficacy with different strains of *P. falciparum*. The ethnopharmacological survey documented plants used traditionally against malaria taking cognizance of their pharmacological information by evaluating their antiplasmodial efficacies. The results of the pharmacological efficacy seemed to support the basic ethnobotanical criterion that is the frequency of citation, or how widely a remedy is used as the basis of biological efficacy. It shows that a large number of medicinal plants are traditionally used in treatment of malaria in study area. A considerable amount of duplications of information relating to the use of the plants by several healers, and the fact that some of the plants collected have been reported in the literature, as having been used for treatment of malaria or fever; indicates that the healers could be trusted for the information they imparted about the plants they used.

Medicinal plants play a major role in many communities over the world in the treatment and prevention of disease and the promotion of general health. Previous studies have shown that more than 1200 medicinal plants from 160 families are used worldwide to treat malaria or fever (Willcox and Bodeker, 2004) and still many antimalarial plant species remain to be discovered. A study in Zimbabwe documented 28 plants from 16 plant families that were used in the prevention and treatment of malaria by traditional healers in four villages in the Chipinge district (Ngarivhume et al., 2015). The present study documented fifty seven (57) species in forty eight (48) genera and thirty (30) families that were used in the treatment of malaria in the study area. The plant species were mostly woody plants (shrubs and trees) and the plant parts reported were the root bark, stem bark, leaf, whole plant, twigs and climber in that order of frequency. Respondents reported that the appropriate plant parts were collected as and when they were needed. The plant material was used fresh or dried and most plants to be used as a remedy were stored for later use in the dry state, which allowed their utilization throughout the year. The method of preparation was mostly a hot water infusion, a decoction or a concoction usually prepared just before use and filtered through a piece of cloth or just decanted.

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 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: 10 g wet leaves or 5 g of dry leaves in 250 ml of water to be taken twice daily; and a handful: 10 g of powdered plant material, or 15 g coarse plant material in 250 ml of water to be taken twice daily. Treatment was supposed to be continued until recovery and it was indicated that the herbal remedy was effective with no side effects if the correct dose was taken.

Nine plant families had at least 3 species mentioned in the treatment of malaria (Fig. 2). The root bark was the most frequently mentioned part of the plant used in preparation of the herbal remedies. Apocynaceae, Euphorbiaceae, and Rubiaceae families had the highest number of species mentioned in treatment of malaria. This may confirm the effectiveness of traditional herbal remedies prepared from species represented in the above families from the study area. Studies from other regions of Africa indicate Rubiacea to have many species used in the management of malaria in different countries (Iwu, 1994). This is in agreement with the results of this study; but it also revealed other families with similar frequency on the number of species cited as sources of antimalarial remedies. The family Euphorbiaceae had a higher number of species while Apocynaceae had the same number of species (5 and 4 species, respectively) cited as sources of antimalarial remedies compared to Rubiaceae (4 species) (Fig. 2)

which would indicate the importance of these families as possible sources of antimalarial plants.

The information on the frequently utilized antimalarial plant species (Fig. 2) is also an important lead to the species that can be targeted for antiplasmodial tests and phytochemical analysis. The root bark and stem bark were commonly used parts of the plant and it has been shown that these plant parts are the major sources of traditional medicine preparations (Githae, 1995; Bronner, 1990). The bark is rich in secondary metabolites hence its popularity as a source of traditional medicine (Watt and Breyer-Brandwijk, 1962; Cunningham, 2001).

Phytochemical studies carried out on some *Strychnos* species showed that the root or stem bark had twice the amount of alkaloidal constituents as the leaves (Quetin-Leclercq et al., 1990). It is possible that local people have through continued use learnt that the roots and the stem bark are more potent in treating malaria. However, there is an increasing use of the leaf part of the plant due to conservation concern. The harvesting of the roots was found to be destructive where in some cases the whole plant had to be uprooted. This calls for conservation and harvesting strategies to facilitate sustainable utilization of these plant resources (Cunningham, 2001).

There are species, which were commonly cited in this study that are known for use as sources of antimalarial remedies in other parts of Africa. They are also reported to contain antiplasmodial activity against *P. falciparum*. Those from South Africa included plants screened against *P. falciparum* on CQ sensitive strain D10 such as *Carissa edulis* stems (IC_{50}, 33 μg/ml) and *Maytenus undata* leaves (IC_{50}, 21 μg/ml). Others were *Centella asiatica* leaves (IC_{50}, 8.3 μg/ml), *Tridax procumbens* whole plant (IC_{50}, 17 μg/ml), *Maytenus senegalensis* roots (IC_{50}, 15.5 μg/ml), *Flueggea virosa* leaves (IC_{50}, 19 μg/ml) and *Acacia nilotica* twigs (IC_{50}, 13 μg/ml) (Clarkson et al., 2004). The convergence in use of the same species in different cultures over a long period suggests strongly that these species may be effective in the treatment of malaria (Wyk and Wink, 2004). It is however, important to validate all claims of therapeutic efficacy and safety by undertaking pharmacological, toxicological, and controlled clinical studies.

Within a context of growing antimalarial resistance and the difficulties for households to afford and access effective antimalarials, the development and promotion of phytomedicines may be the only sustainable solution to malaria treatment. This focus is justified because herbal medicines are widely accepted as safe and efficacious remedies by the study community in Kwale. Indeed many drugs used in malaria treatment have been derived from higher plants using leads from traditional knowledge (Wyk and Wink, 2004). These include the quinoline based antimalarials as well as artemisinin and its derivatives (Waako et al., 2005).

Most of the plants under study were collected within the forest areas of Shimba Hills Game Reserve in Kwale, the latter is facing threats of enchroachment and over-utilization of indigenous trees and medicinal plants may disappear before their uses are documented. Kenya's strategy for conservation of forests involves intensification of timber and other non-wood products outside forest areas (Njuguna et al., 2000). Domestication of medicinal plants is a suitable option for optimizing resource utilization, as well as decreasing overdependence on wild habitats. It is also important that the entire ethnoflora of the study area be documented as a measure of conservation strategies for target species that could support the health and economy of the communities concerned.

Many traditional health practitioners are old men, and since in many cases the information on traditional use of herbal medicine has not been recorded and has been passed orally from one generation to another there is a danger that this cultural heritage and basis for future research may be lost forever unless documented. As a result, this study has indentified, documented and assessed the efficacy of a large number of plant species that have been used in Kenyan folk medicine for treatment of malaria and which could form the basis of an ethnopharmacopoeia.

3.2. Antiplasmodial efficacy of the plant species

All the species plant parts collected were assessed for antiplasmodial efficacy and the results obtained seemed to justify the traditional use of the plants in treatment of malaria. The high activity range ($IC_{50} < 5$ μg/ml) was emphasized with the reasoning that inhibition of parasite growth at low concentrations indicates selective activity as opposed to higher concentrations where nonspecific toxicity is often observed. The IC_{50} of the reference drug CQ diphosphate, for CQ resistant (W2) and sensitive (D6) *P. falciparum* strains were 31.3 (60.7 nM), and 8.97 (17.4 nM) ng/ml, respectively and this defines the range of CQ resistance to sensitivity. The threshold for in vitro CQ resistance has been defined as $IC_{50} > 100$ nM (Ringwald et al., 1996). The mean in vitro efficacies (IC_{50}) for the extracts against CQ sensitive (D6) and resistant (W2) *P. falciparum* strains is presented in Table 1. The activity (Fig. 3) was categorized as active when $IC_{50} \leq 5$ μg/ml; moderate when $5 < IC_{50} \leq 50$ μg/ml and inactive when $IC_{50} >$ 50 μg/ml (Gessler et al., 1994, Sha'a et al., 2011).

One hundred and seven (107) extracts from fifty seven (57) species in forty eight (48) genera and thirty (30) families were tested for antiplasmodial efficacy. Twelve species (21.1%) showed an efficacy of $IC_{50} \leq 5$ μg/ml; 62.3% (36 species) exhibited $IC_{50} > 5 \leq 50$ μg/ml and 15.8% (9 species) showed an efficacy of $IC_{50} > 50$ μg/ml (Fig. 3). Twenty one percent (21.1%), of the extracts showed $IC_{50} < 5$ μg/ml, a value at which plant extracts were considered to be active with potential for isolation of active constituents (Clarkson et al., 2004). Plant species of which at least one extract had an $IC_{50} < 5$ μg/ml against any *P. falciparum* strain were chosen to represent that particular species irrespective of other different extracts of the same species showing different activity (Fig. 4).

The plant extracts tested were less active than reference drugs CQ and artemisinin. They were composed of heterogeneous mixture of different compounds and the active principles might show higher activity in their pure form. Most of the reported plant extracts have IC_{50} often higher than 5–10 μg/ml (Phillipson and Wright, 1991; Benoit-Vical et al., 1998). *Artemisia annua* and *Azadirachta indica* (from where artemisinin and gedunin were isolated with IC_{50} in nanomolar concentrations), for example, had IC_{50} of 3.9 and 10 μg/ml, respectively (Phillipson and Wright, 1991). Nevertheless, plants that are frequently reported 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

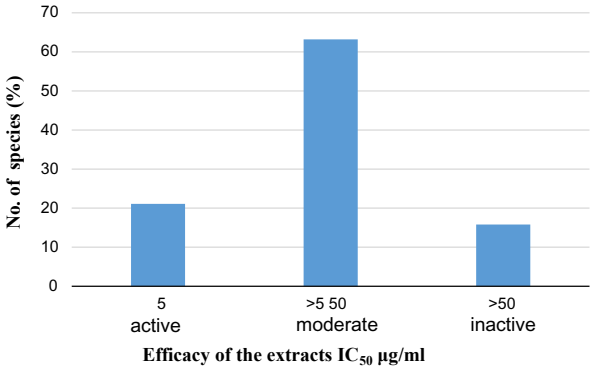

Fig. 3. Efficacy (IC_{50}) of the plant species collected from Kwale.

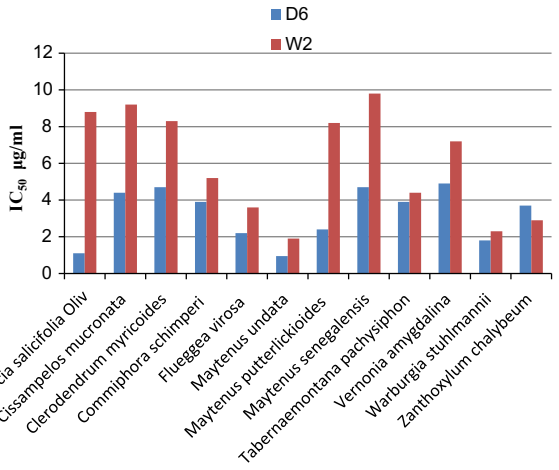

Fig. 4. Efficacy (IC_{50}) of the plant species whose activity was < 5 µg/ml in any extract.

malaria could have therapeutic activities other than antiparasitic effect such as antipyretic, antioxidant, anti-inflammatory, analgesic, immunomodulatory and cytotoxic properties (Parida et al., 2002; Mbatchi et al., 2006). This suggests that in vitro assay cannot be solely relied on to determine the antimalarial property of a plant.

Several classes of secondary plant metabolites are responsible for antiplasmodial activity; the most important and diverse biopotency has been observed in alkaloids, quassinoids, sesquiterpene lactones, courmarins, triterpenoids, limonoids and saponins (Schwikkard and van Heerden, 2002; Kaur et al., 2009). Twelve species (21.1%) showed an efficacy of $IC_{50} < 5$ µg/ml a value that was considered highly active with potential for isolation of antiplasmodial compounds (Clarkson et al., 2004). Previous studies on antiplasmodial activities support these findings with *B. salicifolia* (IC_{50}, 1.03 µg/ml, on D6 strain) (Gathirwa et al., 2008); *Cissampelos mucronata* (IC_{50}, 0.38 µg/ml, on KI strain) (Gessler et al., 1994); *Clerodendrum myricoides* (IC_{50}, 4.0 µg/ml, on V1/S strain) (Muregi et al., 2004); *Commiphora schimperi* (IC_{50}, 4.6 µg/ml, on D6 strain) (Koch et al., 2005); *Flueggea virosa* (IC_{50}, 2.0 µg/ml, on THP1 cells) (Kaou et al., 2008); *Maytenus undata* (IC_{50}, 21 µg/ml, on D10 strain) (Clarkson et al., 2004); *Maytenus senegalensis* (IC_{50}, 3.9 µg/ml, on 3D7 strain) (El Tahir et al., 1999); *Vernonia amygdalina* (IC_{50}, < 3 µg/ml) (Tona et al., 2004); *Zanthoxylum chalybeum* (IC_{50}, 8.1 µg/ml) (Bbosa et al., 2014) and *Warburgia ugandensis* (IC_{50}, 8.10 µg/ml) (Wube et al., 2010).

Warbugia stuhlmannii is found on the Kenyan Coast and a dichloromethane stem bark extract from its close variety found inland, *W. ugandensis*, had a similar antiplasmodial activity (IC_{50s} 1.4 and 2.2 µg/ml for CQ sensitive (NF54) and resistant (KI) *P. falciparum* strains, respectively (Irungu et al., 2007). Kigondu et al. (2009) reported nonantiplasmodial activity for *Maytenus putterlickioides* in agreement with aqueous antiplasmodial activity reported by Muthaura et al. (2007b), however the MeOH extract was active ($IC_{50} < 5$ µg/ml). The fruit of *Tabernaemontana pachysiphon* has been used traditionally to prevent miscarriages, treatment of sores and ulcers in Nigeria (Thomas, 1910; Green, 1994). Chukwujekwu et al. (2005) and Onasanwo and Elegbe (2006) reported its use as anti-inflammatory, antibacterial and antinociceptive. The plant is reported to have antimicrobial activity and the phytochemical screening of leaf extract showed the presence of active principles such as alkaloids, saponins, resins, flavonoids, polyphenols and carbohydrates (Duru and Mbata, 2010). Alkaloidal compounds have been reported from some species of the genus Tabernaemontana namely *T. dichotoma* Roxb. (Perera et al., 1984) and *T. pandacaqui* Poir. (Abe et al., 1993). It is probable that *T. pachysiphon* may contain similar alkaloids. In East Africa the latex of *T. pachysiphon* is applied to sore eyes and a decoction of the root bark is used against stomach-ache, constipation, flatulence, headache and as a hypnotic. Headache is also treated with a leaf infusion. The bark is used as medicine for hypertension. In Kenya grated roots and crushed leaves are also used to treat scabies (Kokwaro, 1993). This is the first report of its traditional use for malaria and antiplasmodial activity.

Methanol extracts were generally more active in vitro than water extracts probably due to active lipophilic constituents, which do not extract into the water extract. This is in agreement with Tona et al. (1999) who observed that the distribution of biologically active constituents was more in methanolic extracts than in aqueous extracts. This data supports the proposition that scientific validation of herbal medicine will lead to more widespread use of traditional medicines as is the case in cheaper health care systems in India and China, provided that safety and efficacy through pre-clinical and controlled clinical studies is carried out. African traditional knowledge and medicine thus have the potential to play a larger role in primary healthcare, particularly in poor and isolated rural areas (Ngarivhume et al., 2015).

4. Conclusion

Among the fifty seven species tested, 21.1% were identified as having good antimalarial effects ($IC_{50} < 5$ µg/ml), 63.2% with moderate effects (5 µg/ml $< IC_{50} \leq 50$ µg/ml), and 15.8% as inactive ($IC_{50} > 50$ µg/ml). The documented antimalarial plants could be developed into a database for compilation of material medica or herbal pharmacopeia that would contribute to value addition of traditional medicine, conservation and search for new active principles. The traditional use of *T. pachysiphon* for treatment of malaria and its antiplasmodial activity of leaf and fruit extract are reported for the first time.

The main problem facing the use of traditional medicines is the proof requirement that the active components contained in medicinal plants are useful, safe, and effective. This is required to assure the medical field and the public regarding the use of medicinal plants as drug alternatives. The proofs of pharmacological activity that are available at present are mostly based on empirical experience. The scientific proof then becomes the most important thing, in order to eliminate the concern of using medicinal plants as drugs for alternative treatment, and hence the requirement for pre-clinical studies and controlled clinical trials of herbal medicines. Documentation of medicinal plants traditionally used in treatment of malaria will lead to their recognition and conservation for sustainable utilization. It is also important that the entire ethnoflora of the study areas be documented as a measure of conservation strategies for target species that could support the health and economy of the communities concerned. The local community of Kwale County, Kenya is the owners of ethnotraditional knowledge presented in this paper. Consequently, any intellectual property rights that may accrue following the use of this knowledge must be shared with them.

Acknowledgments

This work received financial support from UNICEF/UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR). We thank the Director, KEMRI for allowing publication of this study.

References

Abe, F., Tatsuo, Y., Guevara, B.Q., 1993. Indole alkaloids from Tabernaemontana pandacaqui in the Philippines. Biochem. Syst. Ecol. 21, 847–848.

Barrow, E.G.C., 1996. The Dry Lands of Africa: Local Participation in Tree Management. Initiative Publishers, Nairobi.

Bbosa, G.S., Mwebaza, N., Lubega, A., Musisi, N., Kyegombe, D.B., Ntale, M., 2014. Antiplasmodial activity of leaf extracts of *Zanthoxylum chalybeum Engl*. Br. J. Pharm. Res. 4 (6), 705–713.

Benoit-Vical, F., Valentin, A., Cournac, V., Pélissier, Y., Mallié, M., Bastide, J.M., 1998. In vitro antiplasmodial activity of stem and root extracts of *Nauclea latifolia* S.M. (Rubiaceae). J. Ethnopharmacol. 61, 173–178.

Bronner, G., 1990. Vegetation and land use in Matthews Range area, Samburu District, Kenya. Crame, Berlin.

Chukwujekwu, J.U., Staden, J.V., Smith, P., 2005. Antibacterial, anti-inflammatory and antimalarial activities of some Nigeria medicinal plants. S. Afr. J. Bot. 71, 316–325.

Clarkson, C., Maharaj, V.J., Crouch, N.R., Grace, O.M., Pillay, P., Matsabisa, M.G., Bhagwandin, N., Smith, P.J., Folb, P.I., 2004. In vitro antiplasmodial activity of medicinal plants native to or naturalised in South Africa. J. Ethnopharmacol. 92, 177–191.

Cunningham, A.B., 2001. Applied Ethnobotany; People, Wild Plant Use and Conservation. Earthscan Publishers Limited, London.

Desjardins, R.E., Canfield, C.J., Haynes, J.D., Chulay, J.D., 1979. Quantitative assessment of antimalarial activity in vitro by a semi-automated microdilution technique. Antimicrob. Agents Chemother. 16, 710–718.

Dorin, D., Le Roch, K., Sallicandro, P., Alano, P., Parzy, D., Poullet, P., Meijer, L., Doerig, C., 2001. Pfnek-1, a NIMA-related kinase from the human malaria parasite *Plasmodium falciparum*; biochemical properties and possible involvement in MAPK regulation. Eur. J. Biochem. 268, 2600–2608.

DMS., 2006. Director of Medical Services. Malaria Fact Sheet. Ministry of Health, Republic of Kenya.

Duru, C.M., Mbata, T.I., 2010. The antimicrobial activities and phytochemical screening of ethanolic leaf extracts of *Hedranthera barteri* Hook and *Tabernaemontana pachysiphon Stapf*. J. Dev. Biol. Tissue Eng. 2 (1), 1–4.

El Tahir, A., Satti, G.M.H., Khalid, S.A., 1999. Antiplasmodial activity of selected Sudanese medicinal plants with emphasis on *Maytenus senegalensis* (Lam.) Exell. J. Ethnopharmacol. 64, 227–233.

Fratkin, E.M., 1996. Traditional medicine and concepts of healing among Samburu pastoralists of Kenya. J. Ethnobiol. 16, 63–97.

Gathirwa, J.W., Rukunga, G.M., Njagi, E.N.M., Omar, S.A., Mwitari, P.G., Guantai, A.N., Tolo, F.M., Kimani, C.W., Muthaura, C.N., Kirira, P.G., Ndunda, T.N., Amalemba, G., Mungai, G.M., Ndiege, I.O., 2008. The in vitro antiplasmodial and in vivo antimalarial efficacy of combinations of some medicinal plants used traditionally for treatment of malaria by the Meru community in Kenya. J. Ethnopharmacol. 115, 223–231.

Githae, J., 1995. Ethnomedicine practice in Kenya: the case of Karati rural service. In: Sindiga, I., Nyaigotti-Chacha, C., Kanuna, M. (Eds.), Traditional Medicine in Africa. Nairobi: East African Educational Publishers, Ltd., pp. 55–60.

Green, B.O., 1994. Taxonomic and Ethanobotanical Studies on the Family Apocynaceae in Nigeria Ph.D Thesis. University of Port Harcourt, Nigeria.

Gessler, M.C., Nkunya, M.H., Mwasumbi, L.B., Heinrich, M., Tanner, M., 1994. Screening Tanzanian medicinal plants for antimalarial activity. Acta Trop. 56, 65–77.

Gessler, M.C., Msuya, D.E., Nkunya, M.H.H., Mwasumbi, L.B., Schär, A., Heinrich, M., Tanner, M., 1995. Traditional healers in Tanzania: the treatment of malaria with plant remedies. J. Ethnopharmacol. 48, 131–144.

Hardie, K., Dürrheim, D.N., 2013. Tropical fevers: important human parasitic fevers of the tropics. In: Goldsmid, J.M., Leggat, P.A. (Eds.), Primer of Tropical Medicine. Revised Edition. The Australasian College of Tropical Medicine, Brisbane, Ch. 5B.

Irungu, B.N., Rukunga, G.M., Mungai, G.M., Muthaura, C.N., 2007. In vitro antiplasmodial and cytotoxicity activities of 14 medicinal plants from Kenya. S. Afr. J. Bot. 73, 204–207.

Iwu, M.M., 1994. African medicinal plants in the search for new drugs based on ethnobotanical leads. In: Chadwick, D.J., Marsh, J. (Eds.), Ethnobotany and the Search for New Drugs, 185. John Wiley & Sons, Ltd., Chichester, pp. 116–129, Ciba Foundation Symposium.

Jansen, O., Tits, M., Angenot, L., Nicolas, J.P., De Mol, P., Nikiema, J.B., Frédérich, M., 2012. Antiplasmodial activity of *Dicoma tomentosa* (Asteraceae) and identification of urospermal A-15-O-acetate as the main active compound. Malar. J. 11, 289.

Kaou, A.M., Mahiou-Leddet, V., Hutter, S., Aïnouddine, S., Hassani, S., Yahaya, I., Azas, N., Ollivier, E., 2008. Antimalarial activity of crude extracts from nine African medicinal plants. J. Ethnopharmacol. 116 (1), 74–83.

Kaur, K., Jain, M., Kaur, T., Jain, R., 2009. Antimalarials from nature. . Bioorg. Med. Chem. 17, 3229–3256.

Kayser, O.A.F.K., Atta-ur-Rahman, E., 2002. Natural Products as Potential Anti-Parasitic Drugs. Elsevier Science Publishers, Amsterdam B.V, Chapter 16.

Kigondu, E.V.M., Rukunga, G.M., Keriko, J.M., Tonui, W.K., Gathirwa, J.W., Kirira, P.G., Irungu, B., Ingonga, J.M., Ndiege, I.O., 2009. Anti-parasitic activity and cytotoxicity of selected medicinal plants from Kenya. J. Ethnopharmacol. 123, 504–509.

Koch, A., Tamez, P., Pezzuto, J., Soejarto, D., 2005. Evaluation of plants used for antimalarial treatment by the Masai of Kenya. J. Ethnopharmacol. 101, 95–99.

Kokwaro, J.O., 1993. Medicinal plants of East Africa. East African Literature Bureau, Nairobi.

Mbatchi, S.F., Mbatchi, B., Banzouzi, J.T., Bansimba, T., Nsonde Ntandou, G.F., Ouamba, J.M., Berry, A., Benoit-Vical, F., 2006. In vitro antiplasmodial activity of 18 plants used in Congo Brazzaville traditional medicine. J. Ethnopharmacol. 104, 168–174.

Muregi, F.W., Chhabra, S.C., Njagi, E.N.M., Lang'at-Thoruwa, C.C., Njue, W.M., Orago, A.S., Omar, S.A., Ndiege, I.O., 2004. Antiplasmodial activity of some Kenyan medicinal plant extracts singly and in combination with chloroquine. Phytother. Res. 18, 379–384.

Muthaura, C.N., Rukunga, G.M., Chhabra, S.C., Mungai, G.M., Njagi, E.N.M., 2007a. Traditional antimalarial phytotherapy remedies used by the Kwale community of the Kenyan Coast. J. Ethnopharmacol. 114, 377–386.

Muthaura, C.N., Rukunga, G.M., Chhabra, S.C., Omar, S.A., Guantai, A.N., Gathirwa, J.W., Tolo, F.M., Mwitari, P.G., Keter, L.K., Kirira, P.G., Kimani, C.W., Mungai, G.M., Njagi, E. N.M., 2007b. Antimalarial activity of some plants traditionally used in treatment of malaria in Kwale district of Kenya. J. Ethnopharmacol. 112, 545–551.

Ngarivhume, T., van't Klooster, C.I.E.A., de Jong, J.T.V.M., Van der Westhuizen, J.H., 2015. Medicinal plants used by traditional healers for the treatment of malaria in the Chipinge district in Zimbabwe. J. Ethnopharmacol. 159, 224–237.

Nguta, J.M., Mbaria, J.M., Gakuya, D.W., Gathumbi, P.K., Kiama, S.G., 2010. Traditional antimalarial phytotherapy remedies used by the South Coast community, Kenya. J. Ethnopharmacol. 131, 256–267.

Njuguna, P.M., Holding, C., Munyasya, C., 2000. On-farm Woody Biomass Surveys (1993 and 1998): A Case Workshop held in Arusha. In: Temu, A.B., Lund, G., Malimbwi, R.E., Kowero, G.S., Klein, K., Malande, Y., Kone, I. (Eds.), The African Academy of Sciences, pp. 54–77.

O'neill, M.J., Bray, D.H., Boardman, P., Phillipson, J.D., Warhurst, D.C., 1985. Plants as sources of anti-malarial drugs part. 1. In vitro test method for the evaluation of crude extracts from plants. Planta Med. 51, 394–398.

Onasanwo, S.A., Elegbe, R.A., 2006. Anti-nociceptive and anti-inflammatory properties of the leaf extracts of *Hedranthera barteri* in rats and mice. Afr. J. Biomed. Res. 9, 109–117.

Parida, M.M., Upadhyay, C., Pandya, G., Jana, A.M., 2002. Inhibitory potential of neem (Azadirachta indica Juss) leaves on dengue virus type-2 replication. J. Ethnopharmacol. 79, 273–278.

Perera, P., van Beek, T.A., Verpoorte, R., 1984. 16(S)-Hydroxy-16,22-dihydroapparicine, a new alkaloid from the leaves of *Tabernaemontana dichotoma*. J. Nat. Prod. 47 (5), 835–838.

Phillipson, J.D., Wright, C.W., 1991. Can ethnopharmacology contribute to the development of antimalarial agents? J. Ethnopharmacol. 32, 155–165.

Quetin-Leclercq, J., Angent, L., Bisset, N.G., 1990. South American strychnos species; ethnobotany (except curare) and alkaloid screening. J. Ethnopharmacol. 28, 1–52.

Renslo, A.R., 2013. Antimalarial drug discovery: from quinine to the dream of eradication. ACS Med. Chem. Lett. 4 (12), 1126–1128.

Ringwald, P., Bickii, J., Basco, L.K., 1996. In vitro activity of antimalarials against clinical isolates of *Plasmodium falciparum* in Yaounde, Cameroon. Am. J. Trop. Med. Hyg. 55, 254–258.

Sha'a, K.K., Oguche, S., Watila, I.M., Ikpa, T.F., 2011. In vitro antimalarial activity of the extracts of *Vernonia amygdalina* commonly used in traditional medicine in Nigeria. Sci. World J. 6 (2).

Schwikkard, S., van Heerden, F.R., 2002. Antimalarial activity of plant metabolites. Nat. Prod. Rep. 19, 675–692.

Sixsmith, D.G., Watkins, W.M., Chulay, J.D., Spencer, H.C., 1984. In vitro antimalarial activity of tetrahydrofolate dehydrogenase inhibitors. Am. J. Trop. Med. Hyg. 33, 772–776.

Thomas, N.W., 1910. Report on Edo speaking people of Nigeria London.

Tona, L., Ngimbi, N.P., Tsakala, M., Mesia, K., Cimanga, K., Apers, S., De Bruyne, T., Pieters, L., Totté, J., Vlietinck, A.J., 1999. Antimalarial activity of 20 crude extracts from nine African medicinal plants used in Kinshasa, Congo. J. Ethnopharmacol. 68, 193–203.

Tona, L., Cimanga, R.K., Mesia, K., Musuamba, C.T., De Bruyne, T., Apers, S., Hernans, N., Van Miert, S., Pieters, L., Totté, J., Vlietinck, A.J., 2004. In vitro antiplasmodial activity of extracts and fractions from seven medicinal plants used in the Democratic Republic of Congo. J. Ethnopharmacol. 93 (1), 27–32.

Van Wyk, B.E., Van Oudtshoorn, B., Gericke, N., 2002. Medicinal Plants of South Africa. Briza Publications, Pretoria, South Africa.

Waako, P.J., Gumede, B., Smith, P., Folb, P.I., 2005. The in vitro and in vivo antimalarial activity of *Cardiospermum halicacabum* L. and *Momordica foetida* Schumch. Et Thonn. J. Ethnopharmacol. 99, 137–143.

Wagner, H., 1997. Guidelines for quality control of phytomedicines. In: World Congress on Medicinal and Aromatic Plants for Human Welfare. Vol. 2, Abstracts, pp. L–4.

Watt, J.M., Breyer-Brandwijk, M.G., 1962. The Medicinal and Poisonous Plants of Southern and Eastern Africa, 2nd ed. E.&S. Livingstone Ltd., London.

WHO, 2002a. WHO Traditional Medicine Strategy 2002–2005. World Health Organisation, Geneva.

WHO, 2002b. Traditional Medicine: Planning for Cost-effective Traditional Health Services in the New Century-A Discussion Paper. Centre for Health Development.

WHO, 2001. Promoting the Role of Traditional Medicine in Health Systems: A Strategy for the African Region. WHO Regional Office for Africa, Harare, pp. 17.

Willcox, M.L., Bodeker, G., 2004. Traditional herbal medicines for malaria. Br. Med. J. 329, 1156–1159.

Wube, A.A.1, Bucar, F., Gibbons, S., Asres, K., Rattray, L., Croft, S.L., 2010. Antiprotozoal activity of drimane and coloratane sesquiterpenes towards

Trypanosoma brucei rhodesiense and *Plasmodium falciparum* in vitro. Phytother. Res. 24 (10), 1468–1472.
Wyk, van, B.E., Wink, M., 2004. Medicinal Plants of the World, 1st ed. Timber Press, Portland.
Zirihi, G.N., Mambu, L., Guede-Guina, F., Bodo, B., Grellier, P., 2005. In vitro antiplasmodial activity and cytotoxicity of 33 West African plants used for treatment of malaria. J. Ethnopharmacol. 98, 281–285.