Pharmaceutical Biology

ISSN: 1388-0209 (Print) 1744-5116 (Online) Journal homepage: informahealthcare.com/journals/iphb20

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To cite this article: H.A Oketch-Rabah, S.F. Dossaji & E.K. Mberu (1999) Antimalarial Activity of Some Kenyan Medicinal Plants, Pharmaceutical Biology, 37:5, 329-334, DOI: 10.1076/phbi.37.5.329.6053

To link to this article: https://doi.org/10.1076/phbi.37.5.329.6053

Published online: 29 Sep 2008.

Pharmaceutical Biology
1999, Vol. 37, No. 5, pp. 329–334

1388-0209/99/3705-0329$15.00

ANTIMALARIAL ACTIVITY OF SOME KENYAN MEDICINAL PLANTS

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ABSTRACT

This paper describes the in vitro *antimalarial activity of eight species of plants popularly used traditionally to treat malaria in Kenya. Organic and aqueous extracts from different parts of the plants were tested. Generally, a stronger antimalarial activity was observed in the organic extracts. The most active extracts were of* Vernonia brachycalyx *O. Hoffm. Schreber. (Compositae) leaves which showed an* IC_{50} *of 6.6* μ*g/ml for methylene chloride: ethyl acetate (1:1) extracts, while the aqueous and more polar methanolic extracts gave* IC_{50} *values of 29.6 and 30* μ*g/ml, respectively. The findings of this study support the use of this plant as a traditional remedy for malaria. The rest of the plants tested gave* IC_{50} *values between 30–100* μ*g/ml.*

INTRODUCTION

Malaria is currently one of the world's most serious health problems, especially in Africa, Asia and Latin America, with 300–500 million people susceptible (WHO, 1997). Estimates of annual mortality due to malaria range from 0.5–2.5 million (WHO, 1990; Watkins & Marsh, 1997). Of the four species of *Plasmodium* that infect man, *Plasmodium falciparum* causes the most severe form of infection and is becoming increasingly difficult to control due to the development of drug resistance (Olliaro et al., 1996).

Keywords: Antimalarial, traditional medicine, antiplasmodial, ethnopharmacology, plants, extracts, *in vitro* antimalarial, *Plasmodium falciparum*, folklore, phytomedicines.

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Most of the available drugs have been developed through synthesis and screening: an approach which has proved inefficient and very costly. Out of 250,000 compounds synthesised since the 1970s, only mefloquin and halofantrine have been developed into antimalarial drugs at a cost of over US $150 million (Salako, 1985). Since the development of a vaccine is not obvious in the near future, chemotherapy and chemoprophylaxis remain the major methods of controlling this disease. However, with the increase in cases of drug resistance and failure, there is an urgent need for new drugs with novel modes of action.

Herbal medicines are considered a potential source of new drugs or a source of templates for developing new drugs. Some of the most important antimalarial drugs known today were originally obtained from plants. Quinine is obtained from *Cinchona* species (Phillipson & Wright, 1991; Phillipson, 1994) while the most recent drug to the Western Pharmacopoeia, artemisinin, comes from *Artemisia annua,* a Chinese traditional febrifuge that has been in use for 2000 years (Klayman et al., 1984, 1985; Trigg, 1985). Recent studies have also revealed a diverse range of plant secondary metabolites with varied levels of antimalarial activity (Nkunya, 1992). Some of these compounds are currently being investigated as lead compounds (Chen et al., 1994).

The present study was intended to contribute toward this search for natural antimalarial compounds, from traditional herbal remedies, which have the potential for development into new antimalarial drugs. Although the target was antimalarial compounds, plants which are used to treat fever and swollen spleen have been included since malaria is usually accompanied with fever and, in cases of repeated infections, splenomegaly may occur.

Traditional herbal remedies are mostly prepared and administered in aqueous medium and therefore the

aqueous extracts that were evaluated were prepared in a similar fashion as done traditionally. In addition, organic extracts of different polarities were also prepared and tested, in order to evaluate the antimalarial activity of other secondary metabolites in the plant that are insoluble or only sparingly soluble in water but soluble in organic solvents and which may also have significant antimalarial activity.

MATERIALS AND METHODS

Plant Materials

Eight plants were selected for study based on ethnopharmacological information in the literature as well as interviews with local inhabitants in the collection areas.

Plants were collected during the dry season (in August, 1995, corresponding to the time when herbalists collect the herbs and they also claim that the plants collected in the dry season are more potent than those collected in the rainy season) from Kithembe hill in Machakos District, Kenya. Voucher specimens have been deposited at the East African Herbarium at the National Museums of Kenya where identification of the plants were confirmed by a taxonomist, Mr. Onesmus Mwangangi.

Preparation of Extracts

Except for *Aloe secundiflora*, for which the leaf exudates tested were prepared from fresh material that was obtained from plants growing in the National Museums of Kenya Botanic garden, fresh plant material was transported to the laboratory where it was spread out to dry at room temperature (22–26°C). The material was considered dry when upon crushing with the hand it broke to finer pieces. This was ground to powder using a grinding mill. The pulverised material (500 g) was then divided into two portions; one of 100 g was used to prepare the aqueous extract and, the rest was used for organic extraction.

Organic extracts were prepared by soaking in solvent at room temperature for 48 h at the ratio of 200 ml of solvent: 100 g plant material. The material was successively extracted with CH_2Cl_2: EtOAc (1:1) and MeOH. The extracts obtained were clarified by filtration using natural cotton wool first then Whatman No. 1 filter paper. The filtrate obtained was concentrated under reduced pressure and lyophilised to powder.

The aqueous extract was prepared by boiling the powdered plant material in water for 2 h. The extract was allowed to cool, filtered, using natural cotton wool first, then Whatman No. 1 filter paper, and the filtrate lysophilsed to powder. All extracts were stored at 4°C before use.

The extracts were denoted a, b and c, repectively, for [CH_2Cl_2: EtOAc], MeOH and aqueous extracts.

Preparation of Test Solutions

Stock solution of 1 mg/50 μl in dimethyl sulfoxide (DMSO) or 70% EtOH (depending on solubility) was diluted with complete medium with serum (CMS) to achieve final well concentrations of 111.1, 55.6, 27.8, 13.9, 6.9, 3.45, and 1.74 μg/ml for the crude extracts. The final concentration of DMSO/EtOH in the test solution (below 0.5%) was shown to have no effect on parasite growth by including solvent controls in the tests. Each concentration of test material was tested in duplicate and each test was repeated a minimum of three times using different cultures of the parasite test strains.

In Vitro Testing for Antiplasmodial Activity

P. falciparum laboratory reference isolates were used. A chloroquine-sensitive strain of *P. falciparum* (K39) originally obtained from Kisumu District, Kenya and a multi drug-resistant strain (V1/S) originally from Vietnam were used in the tests. These parasites were maintained in O positive RBC, in standard RPMI medium supplemented with 10% normal human serum (NHS) as previously described (Trager & Jensen, 1976).

In vitro tests

For tests of *in vitro* antimalarial activity, the method of Desjardins et al. (1979) modified as described by Watkins et al. (1987) was used. A 96-well flat bottomed microtitre plate (Sterilin UK) was first prepared with 25 μl CMS added to each well. Extract solutions (25 μl) were added in duplicate columns across the second row (row B) and diluted 2-fold from one row to the next, with a 'Tritertek' motorised hand diluter (Flow Lab. UK), down to the eighth row (row H). The concentration of test compound in row B was thus 64× of the concentration in row H. Aliquots of 200 μl of culture, diluted with fresh 50% red blood cells (RBCs) and CMS to a parasitaemia of 0.4% and haematocrit of 1.5%, were added to each well (parasites were used only after attaining a growth rate of ≥3-fold per 48 h cycle). In the top 12 wells on each plate were drug-free controls. The first 8 wells received parasitised RBC and the last 4 non parasitised RBC. The plates were placed in a gas-tight box saturated with water vapour, flushed

with 5% O_2, 3% CO_2, 93% N_2 gas mixture for 2 min and incubated at 37°C. After 48 h, the plates were removed and 25 μl of 1 μCi/ml ^{3}H-hypoxanthine solution (made up with distilled H_2O and CMS) was added to each well and re-incubated for a further 18 h before harvesting.

Harvesting the parasites

After the second incubation period, the parasites were harvested using a Mash II harvester on mini Mash glass filters (Wittaker M A Bioproducts), filtering the contents of each well onto the glass fibre filters and flushing out the wells with distilled water. The filter papers were dried at 60°C for 30 min and the individual discs representing parasite DNA from parasites in each well were placed in a scintillation vial. To each vial was added 2 ml of scintillation cocktail and the ^{3}H-hypoxanthine incorporation measured by liquid scintillation counting on a 1211 Minibeta Counter LKB (Wallac). The readings were obtained as counts per min (cpm).

Data analysis

The drug concentration causing 50% inhibition of radioisotope uptake (IC_{50}) was calculated by interpolation after logarithmic transformation of both drug concentration and label uptake as previously described (Sixmith et al., 1984).

RESULTS

The results are presented in Table 1 as the concentration of crude extract that inhibited 50% (IC_{50}) uptake of hypoxanthine by treated parasites as compared to the control non-treated parasite's IC_{50} values.

DISCUSSION

In general, the organic extracts showed a higher antimalarial activity compared to the aqueous extracts tested, except in the case of *Ethulia scheffleri* where a higher activity was noted in the aqueous extracts when tested for activity against a chloroquine sensitive K39. Of the seven plants tested, the most active was *Vernonia brachycalyx.* The leaves showed a relatively high activity with IC_{50} values of 5–9 μg/ml. This was an encouraging finding given that the leaves of this plant are used to treat malaria (Kokwaro, 1993). Further phytochemical work, reported elsewhere, led to the isolation of a sesquiterpene lactone (SQL), 16,17-dihydrobrachycalyxolid, as 0.02% of the crude material (Oketch-Rabah et al., 1998). This compound elicited a slightly higher level of antimalarial activity than the crude extract (IC_{50} 3–4 μg/ml). Thus, being the major compound in the extract, the activity elicited by the extract is probably mainly due to this compound.

Previous reports on toxicity of sesquiterpene lactones indicate that these compounds are highly toxic to various human cell lines (Kupchan et al., 1968; 1969a,b). The toxicity of 16,17-dihydrobrachycalyxolid was assessed by its ability to inhibit the proliferation of phytohaemmaglutinin (PHA) treated human lymphocytes (Oketch-Rabah et al., 1998). An IC_{50} of 7.8 ± 4.2 μg/ml was obtained, which was comparable to that obtained in the antiplasmodial tests. In order to assess whether the observed antiplasmodial activity was due to a specific or a general toxicity effect, the experimental selectivity index (SI) was calculated (Angerhofer et al., 1992) for this sesquiterpene lactone. The SI was calculated as the ratio of lymphocyte cytotoxicty (IC_{50}) to *P. falciparum* cytotoxicity (IC_{50}) and was calculated as [IC_{50} of drug in PHA response tests/IC_{50} of drug in antiplasmdial tests]. A larger ratio indicates a more selective compound, while a smaller value (less than one) indicates lack of selectivity. The value obtained for this compound was approximately one, indicating that the compounds acts nonselectively. Its activity may be attributed to general toxicity. The compound may be targeting a general metabolic pathway that is essential not only to the *Plasmodium* parasite but also to the lymphocytes. Such general toxicity may be attributed to activities that have been previously demonstrated in SQLs having the α-methylene-γ-lactone or an α,β-unsaturated ester functionalities that intefere with DNA and RNA synthesis and consequently inhibit cell division (Picman, 1986). Since 16,17-dihydrobrachycalyxolid possesses an α,β-methylene ester, this functionality may be presumed responsible for the observed biological activities.

V. brachycalyx organic roots extract (VBR-b) was also subjected to bioactivity-guided fractionation and yielded two isomers of a 5-methylcoumarin, both of which showed moderate antimalarial activity (Oketch-Rabah et al., 1997).

Many members of the genus *Vernonia* are also known to contain sesquiterpene lactones (Bohlman & Jakupovic, 1990), a group of terpenoids which have been shown to have strong cytotoxic effects (Kupchan et al., 1968; 1969a,b). Since a number of other species of this genus are used traditionally for the treatment of malaria, further research is necessary to determine the

Table 1. IC_{50} values for crude extracts tested against *Plasmodium falciparum* parasite strains K39 and V1/S. Chloroquine was used as the positive control.

Plant name and code	Part used and extract		IC_{50} μg/ml ±S.D (number of replicates)	
			K39	VI/S
Aloe secundiflora Eng. (Aloaceae, formerly Liliaceae) ASL	leaf exudate	ASL-c	66.20 ± 5.83 (3)	–
Clerodendrum myricoides (Hochst.)Vatke (Verbenaceae)	root	CMR-a	47.1 ± 14.6 (3)	24.1 ± 9.3 (3)
CMR		CMR-b	84.7 ± 12.9 (3)	75.6 ± 8.0 (3)
		CMR-c	63.6 ± 17.5 (3)	81.2 ± 15.4 (3)
Ethulia schefleri S. Moore (Compositae)	leaves	ESL-a	49.8 ± 3.7 (3)	32.3 ± 10.4 (3)
ESL		ESL-b	NA	NA
		ESL-c	20.6 ± 8.3 (3)	62.9 ± 0.7 (3)
Kigelia africana (Lamk.) Benth. (Bignoniaceae)	bark	KAB-a	53.2 ± 9.8 (3)	42.2 ± 12.2 (3)
KAB		KAB-b	59.9 ± 4.1 (3)	83.8 ± 33.0 (2)
		KAB-c	NA	NA
Kigelia africana (Lamk.) Benth. (Bignoniaceae)	fruit	KAF-a	165.9 ± 3.7 (3)	NA
KAF		KAF-b	NA	NA
		KAF-c	NA	NA
Lippia javanica (Burm.f.) Spreng (Verbenaceae)	root	LJR-a	16.7 ± 6.3 (6)	19.2 ± 11.1 (3)
LJR		LJR-b	40.6 ± 24.1 (5)	40.1 ± 29.6 (3)
		LJR-c	NA	NA
Ormocarpum trachycarpum (Taub.) Harms (Papilionaceae)	stem bark	OTS-a	19.6 ± 5.5 (3)	17.5 ± 0.5 (3)
OTS		OTS-b	41.7 ± 19.2 (3)	32.8 ± 2.8 (3)
		OTS-c	79.4 ± 37.78 (3)	NA
Plectranthus sylvestris G rke (Labiatae)	leaves	HPS-a	41.1 ± 12.2 (50)	NA
HPS		HPS-b	56.2 ± 28.1 (5)	61.0 ± 23.2 (3)
		HPS-c	NA	NA
Vernonia brachycalyx O. (Hoffm.) Schreber (Compositae)	leaves	VBL-a	6.6 ± 1.6 (3)	8.4 ± 1.2 (3)
VBL		VBL*	36.2 ± 1.6 (5	–
		VBL-b	29.6 ± 0.1 (3)	16.6 ± 1.4 (3)
		VBL-c	31.2 ± 4.8 (5)	30.2 ± 1.8 (3
VBR	root	VBR-a	18.8 ± 3.8 (3)	11.6 ± 4.30 (3)
		VBR-b	29.6 ± 0.1 (3)	18.6 ± 6.8 (3)
		VBR-c	–	NA
Control Chloroquine phosphate (CQ)			0.0026 ± 0.0080 (20)	0.0520 ± 0.0229 (25)

The numbers in parenthesis after the IC_{50} values represents the replicates for each experiment. The figure after each code name refers to type of extracts; (a) is for the CH_2Cl_2: EtOAc (1:1), (b) for MeOH, and (c) is for the aqueous extract. NA indicates extracts for which the IC_{50} value obtained was $>$ 111.1 μg/ml, therefore considered not active; – indicate extracts not tested. All units are in μg/ml. VBL** indicates VBL-a extracts tested after storing for 48 h on the bench.

toxicity of these plant drugs and the possible implications of their use in malaria therapy.

Boiled leaves of E*thulia schefleri* (Compositae) are rubbed over the entire body to cure malaria (Kokwaro, 1993). Unlike most of the other plants tested, the leaf aqueous extracts showed a higher antimalarial activity against chloroquine-sensitive and resistant-strain of *P. falciparum* compared to the organic extracts (Table 1). This may be an indication that the antiplasmodial activity is due to compounds, probably glycosides, which are better extracted with polar solvents. Further work is in progress to identify the active principles.

Kigelia africana, the African sausage tree, has many uses. The bark is used to treat headaches, and the leaves are used to treat malaria and as an abortifacient. The fruits are used to treat haemorrhoids wounds and gynaecological disorders. It is also believed to be used for witchcraft. Slices from from the dried fruits are used as an additive in beer brewing (Kokwaro, 1993). In this study neither the leaves nor the fruit extracts showed significant antimalarial activity.

Lipia javanica (Verbenaceae) is known to contain volatile oils. An infusion of the leaves and stem or a decoction made from the leaves is used to treat malaria (Kokwaro, 1993). Treatment is also effected by rubbing the body with pounded leaves which suggests that compounds with curative properties can be absorbed through the skin. Such compounds are likely to be lipophilic and this might explain the higher antiplasmodial activity observed in the nonpolar CH_2Cl_2: EtOAc (1:1) extracts. In a previous study on another species of *Lippia* from Mali, *L. Chevalieri* (both *in vitro* and *in vivo*) a weak antiplasmodial activity was reported (Valentin et al., 1995).

Plectranthus sylvestris (Labiatae) is also known to contain volatile oils. In East Africa, a decoction made from a mixture of roots of this plant together with those of *Cassia didymobotrya* and *Clerodendrum johnstinii*,

is used as a remedy for malaria and headache (Kokwaro, 1993). In this study, none of the extracts tested showed significant antiplasmodial activity. This could be attributed to the fact that the roots are used as a concoction together with materials from other plant species and, thus, the antimalarial property could be due to either synergistic activity of several compounds in the extracts or potentiation of the activity of *Plectranthus* compounds by other compounds from the other plants.

Ormocarpum trachycarpum leaves and stem bark are used to treat fever (Kokwaro, 1993). In this study, only the stem bark was analysed and the results obtained indicate that the less polar CH_2Cl_2: EtOAc (1:1) extracts have a higher antiplamodial activity, compared to the more polar methanol and aqueous extracts. Bioactivity-guided fractionation was attempted but after the first fractionation, with methylene chloride containing increasing amounts of ethyl acetate, the fractions obtained had very low antiplasmodial activity of $IC_{50} \geqslant 100$ μg/ml, therefore no further fractionation was done.

CONCLUSION

Although among the eight plants studied, only *V. brachycalyx* leaves elicited significant antiplasmodial activity, the rest are not necessarily without therapeutic value. In the traditional medicine practice, plants prescribed may have antipyretic or immunomodulatory effects rather than direct antiparasitic activity. Such plants may contain antipyretics which may have been missed in this study. Furthermore, antiprotozoal efficacy of some natural products may be potentiated *in vivo* by other compounds which occur in the extracts, and yet other compounds may need to be metabolised *in vivo* to produce the active compounds. Such compounds may not be detected by assays using *in vitro* methods.

The positive antimalarial activity shown by *V. brachycalyx* extracts and the subsequent isolation of a strongly antimalarial sesquiterpene lactone derivative supports the traditional use of this plant for malaria therapy. However, users should be cautioned about its potential toxicity given that the major active principle in the extracts is a cytotoxic sesquiterpene lactone.

ACKNOWLEDGEMENT

H.A.O. is grateful to DANIDA for a Ph.D fellowship during which this work was done.

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Accepted: April 21, 1999