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In vitro antiplasmodial and cytotoxicity activities of 14 medicinal plants from Kenya

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Abstract

Organic and aqueous extracts obtained from 14 Kenyan medicinal plants were screened for their antimalarial properties on two strains of *Plasmodium falciparum* (K1 chloroquine resistant and NF54 chloroquine sensitive). Dichloromethane extracts had the highest activities with IC_{50} ranging from 1.4 to 35.2 μg/ml. These extracts together with methanol extract of *Turraea robusta* were tested for their cytotoxicity properties *in vitro* on mammalian L6 cell line. The cytotoxicities ranged from >90 to 0.34 μg/ml. Selectivity index (IC_{50} L6 cells/IC_{50} *P. falciparum*) was also determined. *Vernonia lasiopus* had the highest selectivity index (SI) of greater than 10 while *Warbugia ugandensis* had the lowest SI of 0.24. This study suggests that *V. lasiopus* has a high potential for exploitation as a source of antimalarial agents.

Keywords: Antiplasmodial; Medicinal plants; Plasmodium; Cytotoxicity

1. Introduction

In spite of intensive efforts to control malaria, the disease continues to be one of the greatest health problems facing Africa. Although a number of advances have been made towards understanding the disease, relatively few antimalarial drugs have been developed in the last 30 years (Ridley, 2002). Since the treatment and control of malaria depends largely on a limited number of chemoprophylactic and chemotherapeutic agents, there is an urgent need to develop novel, affordable antimalarial treatments. This urgency has further been highlighted by the increasing prevalence of drug resistant strains of malaria parasite *Plasmodium falciparum*, which contribute to the increase of the disease burden. (Clarkson et al., 2004).

Traditional medicines have been used to treat malaria for thousand of years and are a source of two main groups (artemisinin and quinine derivatives) of modern antimalarial drugs (Tagbota and Townson, 2001). Traditional medicine still remains an important and sustainable source of treatment due to difficulties in poor areas in being able to afford and access effective antimalarial drugs. (Willox and Bodeker, 2004; Tagbota and Townson, 2001). It is estimated that in Africa, up to 80% of the population uses traditional medicine for primary health care. Provision of safe and effective traditional medicine therapies could become a critical tool to increase access to health care (WHO, 2003). Use of 'untested' traditional medicines will no doubt continue. Hence, there is an urgent need to distinguish between the efficacious and safe products and the ineffective and/or unsafe products. In our efforts to distinguish between ineffective and/or unsafe products, *in vitro* antiplasmodial and cytotoxicity properties of some medicinal plants from Kenya were investigated.

2. Materials and methods

2.1. Plant collection

Plant materials were collected from Meru and Mombasa regions in Kenya. GM Mungai carried out authentication at the National Museums of Kenya where voucher specimens were deposited (Table 1).

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Table 1
Medicinal plants used in this study

Family/species		Part used	Voucher number
Asclepiadaceae	*Gompocarpus semilunatus* (A. Rich)	whole plant	cm093
Asclepiadaceae	*Gymnema sylvestre* (Retz) Schult	whole plant	cm139
Canellaceae	*Warbugia ugandensis* Sprague	stem bark	cm092
Compositae	*Vernonia auriculifera* Hiern	leaves	cm044
Compositae	*Vernonia lasiopus* O. Hoffm	root bark	cm066
Leguminosae	*Caesalpinia volkensii* Harms	leaves	cm131
Leguminosae	*Strychnos heningsii* Gilg	twigs	cm063
Melianthaceae	*Turraea robusta* Guerke	root bark	cm036
Rubiaceae	*Pentas bussei* Krause	whole plant	cm140
Rubiaceae	*Tarena greveolens* (S.Moore) Brem	stem bark	cm091
Simaroubaceae	*Harrisonia abyssinica* Oliv	stem bark	cm134
Umbelliferae	*Centella asiatica* Urban	whole plant	cm138
Verbenaceae	*Clerodendrum eriophyllum* Guerke	root bark	cm089
Verbenaceae	*Clerodendrum myricoides* (Hochst) Vatke	root bark	cm086

2.2. Preparation of extracts

Air-dried and ground plant materials were extracted successively using Soxhlet with dichloromethane and methanol respectively. Water extracts were extracted for 6 h in a waterbath at 70 °C. Organic extracts were dried using a rotary evaporator while the water extracts were freeze-dried and stored at 4 °C. For bioassays test samples and control were dissolved in 100% DMSO to give stock solutions of 10 mg/ml. They were diluted with media on the day of experiment to desired starting concentration with amount of DMSO not exceeding 1%.

2.3. Parasite cultivation

Continuous *in vitro* cultures of asexual erythrocytic stages of plasmodium strains {NF54-chloroquine sensitive and K1-CQ/pyrimethamine resistant} were maintained following a modified procedure described by Trager and Jensen (1976). Parasite levels in the culture were maintained between 1–10% at a hematocrit of 5% in RPMI 1640 culture medium supplemented with B+ erythrocytes, 0.5% Albumax^R (GIBCO), 50 mg/L hypoxanthine (FLUKA), 2.1g/L sodium bicarbonate (FLUKA) and neomycin (10 ml/L). Incubation was carried out at 37 °C in an atmosphere of 93% N_2, 4% CO_2, 3% O_2.

2.4. Drug assay

This was done using a modification of the semi automated micro dilution technique of Desjardins et al. (1979) which measures the ability of the extracts to inhibit the incorporation of [^{3}H] hypoxanthine into the malaria parasite. Experiments were conducted on 96 well microtiter plates at a parasiteamia of 0.3% and 2.5% hematocrit. Initial drug concentration was 100 μg/ml. Two fold serial dilution was carried out to make seven concentrations the lowest being 1.56 μg/ml. Incubation was carried out at 37 °C in an atmosphere of 93% N_2, 4% CO_2, 3% O_2. Chloroquine was used as the reference drug. After 48 h 50 μL ^{3}H-hypoxanthine (=0.5 μLCi) solution was added to each well of the plate. The plates were incubated for another 24 h after which they were removed and frozen.

2.5. Harvesting

Plates were thawed at room temperature for 1.5 h before harvesting. The plates were then harvested with a Betaplate™ cell harvester (Wallac, Zurich, Switzerland), which transfers the red blood cells onto a glass fiber filter and washes with distilled water. The dried filters were inserted into a plastic foil with 10 ml of scintillation fluid and counted in a Betaplate™ liquid scintillation counter (Wallac, Zurich, Switzerland). The results were recorded as counts per minute (cpm) per well at each drug concentration.

2.6. Data analysis

Data was transferred into a graphic programme (EXCEL) and expressed as a percentage of the untreated controls. The 50% inhibitory concentration (IC_{50}) value was evaluated by logistic regression analysis.

2.7. Cytotoxicity

The assay was done following the method of Page et al. (1993) and Ahmed et al. (1994) where rat skeletal myoblast L6

Table 2
Bioassay results of water, methanol and dichloromethane extracts

Plant/part	K1 strain: IC_{50}'s in μg/ml			NF54 strain: IC_{50}'s in μg/ml		
	Type of extract			Type of extract		
	Water	MeOH	CH_2Cl_2	Water	MeOH	CH_2Cl_2
Caesalpinia volkensii	68.7	51.4	25.6	>100	65.1	11.9
Centella asiatica	ND	19.9	15.4	ND	26	14.9
Clerodendrum eriophyllum	82.7	47	2.7	>100	79	5.3
Clerodendrum myricoides	64	48.2	15.8	94.3	51.5	10.9
Gompocarpus semilunatus	ND	34.4	ND	ND	46.1	ND
Gymnema sylvestre	>100	58.4	ND	>100	40.8	ND
Harrisonia abyssinica	91.1	52.3	4.4	>100	55.4	5.6
Pentas bussei	>100	35.2	ND	>100	40.5	ND
Strychnos heningsii	29.6	14.6	35.2	33.7	17.9	33.3
Tarena greveolens	>100	40.7	ND	>100	52.6	ND
Turraea robusta	91.5	3.5	ND	>100	2.4	ND
Vernonia auriculifera	84.5	53.8	32.7	>100	60.8	27.3
Vernonia lasiopus	52.2	31.2	4.7	>100	50.5	4.9
Warbugia ugandensis	31.8	17.8	1.4	64	24.3	2.2
Chloroquine	0.091	0.05	0.061	0.003	0.003	0.004

NB: values are mean of two independent experiments.
ND: not done.
Score definition: >50 μg/ml=not active; 11–50 μg/ml=moderately active; 5–10 μg/ml=active; <5 μg/ml=highly active.

cell line was used. Cells were maintained in RPMI 1640 media containing 10% fetal calf serum and 1.7 μM L-glutamate. Samples were added to the cultured L6 cells over a concentration range of 90 to 0.12 μg/ml. The plates were incubated for 72 h s at 37 °C/5% CO_2, after which 10 μL of resazurin was added as a viability indicator and incubated for another 2 h. The plates were read with a fluorescence scanner using an excitation wavelength of 536 nm and an emission wavelength of 588 nm (Spectra Max Gemini XS Molecular Devices). Podophyllotoxin (Polysciences Inc. USA) was used as a positive reference.

3. Results

The IC_{50} obtained from two independent experiments with the different extracts on K1 and NF54 strains are summarized in Table 2. None of the extracts tested had as good activity as CQ. The IC_{50} ranged from 1.4 to >100 μg/ml. Dichloromethane extracts had the best activities (IC_{50} <50 μg/ml) except for *Strychnos heningsii* whose methanol extract was the most active. Water extracts were the least active with IC_{50} >100 μg/ml. Cytotoxicity properties of dichloromethane extracts and methanol extract of *Turraea robusta* were tested and the results are summarized in Table 3. For the purposes of this study, a promising antimalarial extract was said to lack toxicity to L6 cells by displaying an IC_{50} value greater than 90 μg/ml. Selectivity index (SI) defined, as the ratio of IC_{50} L6 cells to IC_{50} *P. falciparum* was also determined. The higher the SI the more promising an extract is due to its selectivity for malaria parasites. *Warbugia ugandensis* that had the best antiplasmodial properties with an IC_{50} of 1.4 μg/ml (K1) was also the most toxic to the L6 cells with an IC_{50} of 0.34 μg/ml.

Table 3
Cytotoxicity results and SI of dichloromethane extracts

Plant/part	L6 cells IC_{50}'s (μg/ml)	K1 IC_{50}'s (μg/ml)	SI
Caesalpinia volkensii	82.4	25.6	3.2
Centella asiatica	82.6	15.4	5.4
Clerodendrum eriophyllum	7.9	2.7	2.9
Clerodendrum myricoides	>90	15.8	>5
Gompocarpus semilunatus	ND	ND	ND
Gymnema sylvestre	ND	ND	ND
Harrisonia abyssinica	32.8	4.4	7.5
Pentas bussei	ND	ND	ND
Strychnos heningsii	>90	35.2	>2.6
Tarena greveolens	ND	ND	ND
* *Turraea robusta*	14.3	3.5	4.1
Vernonia auriculifera	84.8	32.7	2.6
Vernonia lasiopus	>90	4.7	>10.7
Warbugia ugandensis	0.34	1.4	0.24
Chloroquine	37	0.061	607
Podophyllotoxin	0.01	ND	ND

Values are mean of two independent experiments.
SI = selectivity index.
* Methanol extract.

4. Discussion and conclusions

We tested 35 extracts from 14 plants for their antiplasmodial properties *in vitro*. Water extracts showed no activity with IC_{50} >50 μg/ml in both strains except for *S. heningsii*, which had moderate activity. Methanol extracts had moderate activity except for *T. robusta* that was highly active with an IC_{50} of 3.5 and 2.4 μg/ml against K1 and NF54 strains respectively. Dichloromethane extracts showed the highest activity with IC_{50} ranging from 35.2 to.4 μg/ml against the two strains except for *S. heningsii* which had dichloromethane extract as the least active. Comparing the IC_{50} values of 55% (5/9) dichloromethane extracts tested with those previously obtained for *Artemisia annua* (3.9 μg/ml) and *Azadirachta indica* (10 μg/ml) it can be said that these extracts have promising results (Maria do Ceu de et al., 2002). This phenomenon of high activity on dichloromethane extracts over water and methanol extracts was also reported by Koch et al. (2005). A probable explanation could be due to lack of tannins, polysaccharides and other water-soluble molecules that have no antiplasmodial properties. It was also noted that sensitivity of the extracts to both CQ-resistant and CQ-sensitive strains did not differ significantly. Literature reveals that some plants tested in this study have also been investigated for their antiplasmodial activity on other strains of *P. falciparum*. Of these are *Vernonia auriculifera*, *Vernonia lasiopus* (Muregi et al., 2003), *Centella asiatica* (Atindehov et al., 2004), *S. heningsii* (Kirira et al., 2006), *Caesalpinia volkensii* (Kuria et al., 2001) and *Harrisonia abyssinica* (Kirira et al., 2006; Clarkson et al., 2004). The results obtained by these authors agree with what was obtained in this study. However, these authors did not determine the cytotoxic levels of these extracts.

The dichloromethane extracts and methanol extract of *T. robusta* were tested for their cytotoxicity properties *in vitro* using L6, rat skeletal myoblast cells. Podophylotoxin was used as the reference drug. Most extracts showed low cytotoxicity as is shown in Table 3 with IC_{50} value of >20 μg/ml (Zirihi et al., 2005). Selectivity index was also calculated with *V. lasiopus* having the highest selectivity of malaria parasite with an SI >10. Though *W. ugandensis* possessed a high antiplasmodial activity (1.4 μg/ml against K1), it also expressed the highest cytotoxicity with an IC_{50} of 0.34 μg/ml and a SI of 0.24. This indicates that the high antiplasmodial activity noticed is probably due to cytotoxicity rather than activity against the parasite themselves.

According to our classification, dichloromethane extract of *V. lasiopus* exhibited promising antimalarial activity with relatively minimal cytotoxicity. This plant is commonly used in traditional medicine in Kenya to manage malaria (Muregi et al., 2003; Bentje, 1994). At least from ethnomedicinal use there is some evidence that the plant may be safe in humans. It is therefore planned to evaluate the activity of *V. lasiopus in vivo* using *Plasmodium berghei* mouse model then followed by isolation of the active compound(s). It is hoped that a lead compound may be identified that could be developed further as antimalarial agent. Meanwhile, the antimalarial activity reported herein probably explains at least part of the therapeutic efficacy claimed for these plants in traditional medicine.

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