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Research Article

Antimalarial activities and toxicity levels of selected medicinal plants used in Kenya

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Background: Resistance development to antimalarial drugs necessitates the look at traditional medicinal plants as sources of novel compounds that could have the otential to be developed into new antimalarial therapies. Four medicinal plants used in Kenya to treat malaria were investigated.

Objective: To determine the *in vitro* and *in vivo* antimalarial activity and safety of four medicinal plants used in Kenya to treat malaria.

Materials and Methods: *Ximenia americana, Sericocomopsis hilderbrandtii, Pentas lanceolata and Fuerstia africana* were collected from their habitat, dried, and extracted with methanol and aqueous solvents. *In vitro* antiplasmodial activity carried out using *Plasmodium falciparum, In vivo* antimalarial activity using *Plasmodium berghei* ANKA strain in Swiss albino mice. Cytotoxicity was carried out using MTT assay on VeroE99 cell lines, acute toxicity was investigated in Swiss albino mice.

Results: All extracts had good *in vitro* activity against D6 strain of *Plasmodium falciparum* with IC_{50}<20µg/ml. Aerial parts of *Fuerstia africana* methanol extract had the highest *in vitro* activity. Seven extracts showed good *in vivo* activity with chemosuppresion >30% while three demonstrated low activity. *Fuerstia africana* was moderately cytotoxic. Except for *Ximenia americana* water extract, all the extracts were safe with LD_{50} > 5000mg/Kg.

Conclusion: Results of this study support medicinal use of these plants and indicate that useful compounds can be isolated for further exploitation, formulation and use.

Keywords: Medicinal plants, antiplasmodial activity, cytotoxicity, acute toxicity

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1. Introduction

Malaria is a life threatening vector borne infection, caused by the protozoan *Plasmodium* parasite. Globally, an estimated 3.4 billion people are at risk of malaria (WHO 2014), with Africa bearing over 90% of the global malaria burden (Snow et al, 2005). Malaria accounts for 30% of outpatient cases and 19% of admissions (WHO 2008; Kigondu et al, 2011).

Symptoms of malaria include fever, headache, loss of appetite, diarrhea, joint pains and chills. Severe malaria presents frequently as severe anemia and or cerebral malaria. Malaria is also responsible for complications in course of pregnancy (Malaney, et al, 2004). And can lead to chronic debilitation with delays in cognitive and physical development (Breman, et al, 2004) all of which have a negative impact on the social and economic development of affected countries (Sachs, et al, 2002).

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Currently artemisinine combination treatments are the recommended first line therapy for *falciparum* malaria (Gathirwa et al, 2011). However *P. falciparum* parasites with reduced *in vivo* susceptibility to artemisinine derivatives have emerged in western Cambodia (Noedl, 2008, Dondorp, 2009). Using artemisinine or its derivatives as monotherapy leads to resistance development (Dondorp, et al, 2010; Gathirwa et al, 2008), while addition of artemisinine with a drug that has failed locally leads to a failed combination (Mutabingwa, et al, 2005). Furthermore production of ACTs to meet global demand is costly as it is limited by global shortage of materials (Whitty, et al, 2004; Kigondu et al, 2011). The threat of parasite resistance to artemisinine and its derivatives (Noedl, 2010), having lost other drugs to resistance such as chloroquine (Spencer, 1985), quinine (Pickard and Wermsdofer, 2002), sulphadoxine pyrimidimine (White, et al, 2010), and mefloxine (Wongsrichanalai et al, 2001) is worrying. There is therefore need for development of new antimalarial drugs that are affordable to combat increasing parasite resistance.

Plants have been considered a rich source of new drugs (Kiria et al, 2006). Some antimalarial drugs in use today such as quinine and artemisinine originated from plants (Ravikumar, et al, 2010; Muthaura et al, 2007). Quinine for example was extracted from the bark of *Cinchona* plant originally grown in Peru (Greenwood, 1992; Rukunga et al, 2009), while artemisinine was extracted from a Chinese herb, *qinghao* or *Artemisia annua* (Klayman, 1985; Muthaura et al, 2007). Medicinal plants provide cheap alternative therapeutic agents than conventional drugs (Muregi et al, 2007; Gathirwa et al, 2011). Additionally drugs derived from natural products tend to have fewer side effects (Breman et al, 2004). Infact, traditional medicinal plants have been used for a long time in malaria endemic regions to treat malaria (Gessler et al, 1995; Kokwaro, 1993; Koch, 2005; Gathathi, 2009) without the exact knowledge on efficacy and safety.

In the effort to determine the efficacy and safety of traditionally used medicinal plants, four medicinal plants, used to treat malaria in Kenya were targeted. These included *Sericocomopsis hildebrandtii* Schinz, *Pentas lanceolata* (Forssk.) Defleurs, *Fuerstia africana* T.C.E. Friers, and *Ximenia americana* L. The antimalarial and toxicity activities of these four medicinal plants were investigated.

2. Methodology

2.1 Plant materials

The plant samples used in this study were collected in Kajiado, Embu and Baringo Counties based on ethnopharmacological information, and were identified by a taxonomist at the East Africa Herbarium, National Museums of Kenya, Nairobi where voucher specimens were deposited. They were dried indoors and powdered using an electric mill.

2.2 Preparation of the plant materials

For each plant part, 50 g of powder was extracted by soaking in methanol and distilled water, separately. The extracts were decanted and filtered through Whatman filter paper and re-extracted again for 24 hr. The extraction process was repeated until a clear extract was obtained. The filtered extracts were combined and methanol removed under reduced pressure at 40 °C (Harborne, 1998) while aqueous extracts were freeze dried with a freeze drier (Edwards freeze dryer Modulyo) dry samples were stored in a freezer at −4 °C. **Table 1** presents the plant species and yields of the water and methanol extracts.

2.3 Parasites

Chloroquine sensitive *P. berghei* strain ANKA donated by International Livestock Research Institute, ILRI, Kenya was used in the *in vivo* assay. While two strains were used for *in vitro* sensitivity test ; Chloroquine sensitive (D6) strain from Sierra Leone and Chloroquine resistant (W2) strain from Indochina, donated by the Division of Experimental Therapeutics, Walter Reed Army Institute of Research, Washington DC.

2.4 Preparation of test extracts

Stock solutions of aqueous extracts (10 mg/ml) were made in distilled deionized water and filter sterilized using 0.22 µm membrane filters in a laminar flow hood. Methanol extracts were dissolved in DMSO (Sigma Chemical Co., USA) followed by subsequent dilution to lower concentration of DMSO, to <1% to avoid carry over (solvent) effect (Dorin et al, 2001). Reference drugs, CQ diphosphate and Artemisinine at a concentration of 1 µg/ml each, were similarly prepared and all solutions stored at −20 °C until used.

2.5 Cultures of *Plasmodium falciparum*

P. falciparum culture of D6 and W2 strains were used in the study. The culture media was a variation of that described by Trager and Jensen (1976) and consisted of RPMI 1640 supplemented with 10% serum (Schlichtherle et al, 2000). Uninfected human blood group O+ erythrocytes (<28 days old) served as host cells. The cultures were incubated at 37 °C in an atmosphere of 3% CO_2, 5% O_2, and 92% N_2 (BOC, Nairobi).

2.6 *In vitro* antiplasmodial assay

The assay protocol that measures the ability of the extracts to inhibit the incorporation of [G-^{3}H] hypoxanthine (Amersham International, Burkinghamshire, UK) into the malaria parasite (Desjardins et al, 1979) was used. Aliquots of culture medium (25 µl) were added to all the wells of a 96 well flat-bottom microculture plate (Costar Glass Works, Cambridge, UK). Aliquots (25 µl) of the test solutions were added, in triplicate, 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. The sensitivity tests were carried out with attainment of ring stage parasite growth and initial parasitaemia of 0.4% by applying 200 µl, 1.5% haematocrit of *P. falciparum* culture to each well. As control, 200 µl of culture media without parasites was added into four wells on the last row of each plate. Parasitized and non-parasitized erythrocytes were incubated at 37 °C in a gas mixture of 3% CO_2, 5%

O_2 and 92% N_2. After 48 hr 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 hr. were then harvested onto glass fiber filter mats using a 96 well harvester, washed with distilled water and dried. [G-^{3}H] hypoxanthine uptake was determined using a micro beta trilux liquid scintillation counter (Wallac Micro Beta Trilux). Computation of the concentration of drug causing 50% inhibition of [G-^{3}H] hypoxanthine uptake (IC_{50}) was carried out by interpolation after logarithmic transformation of both concentration and cpm values using the formula described by Sixsmith et al*,* (1984).

$$IC_{50} = \frac{\text{antilog } (\log X_1 + [(\log Y_{50} - \log Y_1)\ (\log X_2 - \log X_1)])}{(\text{Log } Y_2 - \log Y_1)}$$

Where Y_{50} was the cpm value midway between parasitisized and non-parasitisized control cultures and X_1, Y_1, X_2, and Y_2 were the concentrations and cpm values for the data points above and below the cpm midpoints.

2.7 *In vitro* determination of cell cytotoxicity

Cytotoxic concentration causing 50% cell lysis and death (CC_{50}) was determined by a method described by Mosmann (1983). Briefly, Vero E99 cells were seeded at a concentration of 2.5×10^4 cells/well in a 24 well plate 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 hr. The culture media were replaced by fresh media containing extract at various concentrations, and cells further grown for 24 hr. The cells were then treated with trypsin and the number of viable cells determined by the tryphan blue exclusion method. 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 *P. falciparum* (Wright and Phillipson, 1990).

2.8 *In vivo* antiplasmodial activity

Swiss albino mice (20±2 g) were used to evaluate antimalarial activity using *P. berghei* strain ANKA, maintained by serial passage. The assay protocol was based on the 4-day suppressive test described by Peters et al*,* (1975). Briefly, the mice were infected interperitonially 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 500 mg/kg of the test sample in 0.2 ml orally 2 hr post infection (i.e. on day 0) (Gessler et al*,* 1995). This was repeated after every 24 hr (Day 1, Day 2, and Day 3). Two control groups of five mice each were treated with a placebo (10% Tween 80) and 5 mg/kg/day of Chloroquine for negative and positive controls, respectively. Parasitaemia was determined on day 4 (24 hr after the last treatment) by microscopic examination by counting parasites in 4 fields of approximately 100 erythrocytes per view of thin blood smear 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 (Tona et al*,* 2001).

$$PS = [(A-B)/A] \times 100$$

Where, A is the mean parasitaemia in the negative control on day 4, and B the corresponding parasitaemia in the test group. Standard deviation for the mean values was calculated as described by Armitage and Berry (1991).

2.9 Acute toxicity

Female Swiss albino mice (20±2 g), grouped in to five per cage were starved overnight (12 hr) before oral administration of a single dose of the extract and provided with tap water and food. The extracts were dissolved in distilled water and administered by gavage at logarithmic dose ranging between 500 and 5000 mg/kg body weight giving five dose levels of 500, 889.15, 1581.18, 2811.80 mg/kg body and 5000 mg/kg body weight. Behavior observed for 1 hr after the treatment, intermittently for 4 hr, thereafter over a period of 24 hr (Twaij et al*,* 1983). Were further observed for up to 14 days following treatment for any signs of toxicity, and the latency of death. LD_{50} value was determined according to a method described by Thompson, (1985).

3. Results

3.1 *In vitro* antiplasmodial assay

Methanol extracts exhibited higher antiplasmodial activity than water extracts for both strains of *P. falciparum*. All the methanol extracts posted IC_{50} values <5µg/ml, (**Tables 2**) while aqueous extracts exhibited a range of good to moderate activity with IC_{50} values <20µg/ml.

IC_{50} value against W2 strain was higher than that of D6 strain of *P. falciparum* (**Table 3**). Methanol extract of *F. africana* (aerial part) showed good activity while the others exhibited moderate antiplasmodial activities against W2 strain of *P. falciparum*. Water extracts of of *F. africana* (aerial parts) and *P. lanceolata* (aerial parts) had moderate antiplasmodial activities where as the others had low antiplasmodial activities (IC_{50} >50 µg/ml) against W2 strain of *P. falciparum.*

3.2 *In vivo* assay.

For extracts subjected to *in vivo* assay, 7 had chemosuppression >30% while three were in active (**Table 4**). Methanol extract of *P. lanceolata* and water extract of *X. americana* were the most active and had the highest mean survival time. All the water extracts were active against *P. berghei* in mice. Only methanol extracts of *P. lanceolata* and *X. americana* were active *in vivo*.

Table 1: Plant species and yield of water and methanol extracts

Botanical name	Family	Part used	Dry weight of extract %	
			Water extract	Methanol Extract
F. africana T.C.E. Friers	Lamiaceae	Aerial part	9.4	3.73
S. hilderbrandtii Schinz	Amaranthacea	Aerial part	7.04	2.08
		Roots	10.58	2.32
P. lanceolata (Forssk.) Defleurs	Rubiaceae	Aerial part	7.06	2.53
X. americana L	Olacaceae	Stem bark	16.38	11.62

Table 2: *In vitro* antiplasmodial activity (IC_{50}) of extracts of selected medicinal plants against D6 strain of P. falciparum

Plant	Part Used	Aqueous extract IC_{50} ± SD (µg/mL)	Methanol extract IC_{50} ± SD (µg/mL)
S. hilderbrandtii	AP	18.004 ±3.62	3.153 ±1.24
	R	4.096 ±1.69	2.12 ±0.56
F. africana	AP	17.035 ±4.31	1.841 ±0.82
P. lanceolata	AP	7.127 ±1.73	3.744 ±0.42
X. americana	SB	9.621 ±2.45	2.108 ±0.27
Chloroquine	-	7.649 ±1.82	
Artemisinin	-	0.962 ±0.33	

The IC_{50} values are expressed as mean ± S.D. of three different determinations per experiment;
Key: AP = aerial part, R = root SB = stem bark

Table 3: *In vitro* antiplasmodial activity (IC_{50}) of extracts of selected medicinal plants against W2 strain of *P. falciparum*

Plant	Part used	Aqueous extract IC_{50} ± SD (µg/ml)	Methanol extract IC_{50} ± SD (µg/ml)
S. hilderbrandtii	AP	78.695 ±0.70	12.688 ±4.69
	R	54.166 ±4.71	14.851 ±3.37
F. africana	AP	37.981 ±2.43	6.227 ±0.12
P. lanceolata	AP	43.151 ±6.32	14.903 ±2.14
X. americana	SB	83.489 ±7.21	36.791 ±4.51
Chloroquine	-	56.117 ±0.22	
Artemisinin	-	3.56 ±0.17	

The IC_{50} values are expressed as mean ± S.D. of three different determinations per experiment;
Key: AP = aerial part, R = root SB = stem bark

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3.3 Cytotoxicity Assay

Table 5 summarizes cytotoxicity (CC_{50}) against the mammalian Vero E cells of the plant extracts. Methanol extracts of *F. africana* were moderately cytotoxic with CC_{50} of 63.45 µg/ml. The rest of the extracts were not cytotoxic at 100 µg/ml.

Stem bark of *X. americana*, aqueous extract demonstrated 2 deaths within 40 min. The mice treated with the rest of plant extracts continued to gain weight at a similar rate to that seen in the untreated controls with no external toxic effects observed within 14 days. *X. americana* methanol extract was not tested since it could not dissolve at 5000 mg/kg.

3.4 Acute toxicity

Table 4: *In vivo* anti-malarial activity of selected medicinal plant extracts on *Plasmodium berghei* in mice

Extract/Drug	Part Used	Solvent	Route	Dose (Mg/kg/Day)	Parasite Density	% chemo-Suppression	Mean survival time
F. africana	AP	MeOH	Oral	500	25.14 ± 3.65	27.88 ± 3.47	10.6 ±1.51
F. africana	AP	H_2O	Oral	500	20.79 ± 2.02	30.80 ± 2.80	10 ± 2.94
S. hilderbrandtii	AP	MeOH	Oral	500	28.74 ± 0.72	17.53 ± 2.07	10 ± 2.91
S. hilderbrandtii	AP	H_2O	Oral	500	23.04 ± 1.88	33.89 ± 3.41	8.8 ± 1.30
P. lanceolata	AP	MeOH	Oral	500	12.23 ± 0.04	64.92 ± 0.14	11.4 ±3.28
P. lanceolata	AP	H_2O	Oral	500	20.55 ± 1.42	41.04 ± 2.02	10 ± 2.91
X. americana	SB	MeOH	Oral	500	17.15 ± 3.58	50.81 ± 0.28	11.6 ±3.28
X. americana	SB	H_2O	Oral	500	15.73 ±0.036	54.87 ± 0.38	9.3 ± 1.25
S. hilderbrandtii	R	MeOH	Oral	500	30.45 ± 2.18	12.63 ± 1.26	9.8 ± 1.64
S. hilderbrandtii	R	H_2O	Oral	500	24.30 ± 4.29	30.29 ± 2.31	9.2 ± 1.30
Positive control Chloroquine	-	-	Oral	10	1.11 ± 0.46	96.80 ± 1.37	15 ± 1.34
Negative control Tween 80	-	-	Oral	0.2 ml/day	34.85 ± 2.87	-	8.8 ± 1.28

Results are expressed as means ± SD of 5 determinations per experiment

Table 5: Cytotoxicity (CC_{50}) of plant extracts to Vero E cells.

Plant	Plant part used	Extract	CC_{50} µg/ml	IC_{50} µg/ml	Selectivity index (SI)
F. africana	AP	Methanol	63.45	1.841	34.465
		Water	≥100	17.035	NC
S. hilderbrandtii	AP	Methanol	≥100	3.15	NC
		Water	≥100	18.004	NC
P. lanceolata	AP	Methanol	≥100	3.744	NC
		Water	≥100	7.127	NC
X. americana	SB	Methanol	≥100	2.108	NC
		Water	≥100	9.621	NC
S. hilderbrandtii	R	Methanol	≥100	3.948	NC
		Water	≥100	4.046	NC

SI: selectivity index, is defined as the ratio of the CC_{50} value determined on the mammalian cell line on the IC_{50} value determined on P. falciparum (D6)
NC: Not cytotoxic at the highest concentration tested

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4. Discussion

Yields of water extracts were higher than those of methanol extracts. Water being more polar than methanol is able to extract more compounds, evident in other studies (Muthaura et al, 2007 ; Kigondu et al, 2011). W2 being a resistant *P. falciparum* strain, exhibited lower activity than D6. Methanol extracts had, higher activity against both strains of *P. falciparum* than water extracts. This could be due to active constituents responsible for activity which extracts more in methanol as already observed by Kirira *et al* (2006)

Generally the plants tested were not as active as the reference drug artemisinine (IC_{50} 0.962 µg/ml for D6 strain and 3.56 µg/ml for W2 strain of *P. falciparum*). Since the extracts were heterogeneous mixture of different compounds. Isolating pure compounds from the extracts may give similar IC_{50} values as artemisinine, considering that plant extracts with IC_{50} values often <10 µg/ml (Nkunya et al, 1991) are potential source of antimalarial compounds. For instance *Artemisia annua* (IC_{50}, 3.9 µg/ml) (Phillipson and Wright 1991) resulted in lead antimalarial compound artemisinine. The fact that all methanol extracts were more active than CQ confirms and validates the medicinal plants as already used in traditional setting.

Methanol extract of *F. africana* (aerial parts) recorded the highest activity *in vitro* against both strains of *P. falciparum*. This is in agreement with a previous study where the dichloromethane extract of the leaves of *F. africana* were reported to exhibit high *in vitro* antiplasmodial activity (IC_{50} 3.8 µg/ml, Kigondu et al, 2011). Aerial parts consist of leaves and flowers. Flowers may contain more active compounds against *P. falciparum* than the leaves alone as used in literature. Methanol is a more polar solvent than dichloromethane, consequently, it may have resulted in extraction of more antimalarial compounds, hence the higher activity in this study. However the methanol extracts of *F. africana* (aerial parts) were inactive *in vivo.* This differs with literature where earlier investigations by Muthaura, (2007), revealed *F. africana* to have a chemosuppression of 61.85±4.61% and 43.16±1.69% for methanol and water extracts respectively. This disparities could be due to difference in seasons of harvesting, geo-reference, plant-part used intra-species variations and plant age that affect the presence and quantities of bioactive compounds in plants (Weenen et al, 1990).

Notably apart from methanol extracts of *F. africana* (aerial parts), *S. hilderbrandtii* (aerial parts and roots) also exhibited inactivity in the mouse model despite high *in vitro* activity. As described by Gassler et al, (1995), *in vitro* activity may not necessarily translate to *in vivo* activity. This can be attributed to poor bioavailability, low absorption, poor transport (Muthaura, 2007) and or alteration of structures that enhance activity by metabolic processes in mice (Muregi, 2007).

However, the water extracts of *F. africana* (aerial part) and *S. hilderbrandtii* (roots and aerial parts) showed *in vivo* antimalarial activity, despite moderate *in vitro* activity against D6 strain of *P. falciparum*. It is likely that some of the compounds in the extracts are converted to active metabolites resulting in bioactivity *in vivo* (Muregi et al, 2007). In addition, the results presented here are supported by Gachathi, 1989 and Kokwaro, 1993 who have documented the ethnomedicinal use of water extracts of *S. hilderbrandtii* and *F. africana* and others in traditional medicine.

The methanol extract of the aerial parts of *P. lanceolata,* had high activity *in vitro*, (IC_{50} 3.744± 0.42 µg/ml) previous investigation has shown petroleum ether root extract of *P. lanceolata* to have an IC_{50} of 58.5±4.7, and 33.91±1.06 µg/ml against D6 strain,and W2 strain of *P. falciparum* respectively (Kigondu, 2011). This difference can be attributed to the solvents and the plant parts used. Methanol appears to be a better solvent for extraction of active antimalarial compounds than petroleum ether, hence the observed higher activity in this study. Secondly aerial parts (leaves and flowers) used in this study possess a higher number of antiplasmodial compounds. This is mainly because the aerial parts manufacture more secondary metabolites than the roots. This finding is encouraging as this prevents the plant from over exploitation since aerial parts can be harvested leaving behind the rest of the plant to continue growing hence environmental conservation.

Aerial parts of *P. lanceolata* consistently exhibited good *in vivo* and *in vitro* activity, lack of cytotoxicity on Vero E cells and the lack of toxicity in mice, confirming that *P. lanceolata* is an effective and safe antimalarial herb. This validates its use as an antimalarial as used in traditional medicine. The knowledge that the methanol extract is more active *in vivo* than water extract is useful since water is mainly used in preparation of herbal remedies.

The methanol extracts of the roots and aerial parts of *S. hilderbrandtii* had high *in vitro* activity in this study. This agrees with literature where the roots of *S. hilderbrandtii* ($CHCl_3$ extract) have been reported to have IC_{50} of 3.8 µg/ml against D6 strain of *P. falciparum* (Koch et al, 2005). This affirms more the potential of this plant as a future antimalarial.

The roots of *S. hilderbrandtii* (methanol extracts) had higher antiplasmodial activity than the aerial parts. This trend was similar for water extracts, where the roots had higher antiplasmodial activity than the aerial parts. This could be due to the fact that roots act as storage organs in most plants (Babitha et al, 2002) and hence store phytochemical compounds in high concentration manufactured in the leaves and transported to the roots for storage. This is encouraging since extract yields from the roots were found to be higher than from the aerial parts hence more availability for use.

Both methanol and water extracts of *X. americana* had good *in vitro* and *in vivo* activity against D6 strain of *P. falciparum* and *P. berghei* respectively. Notably the water extract had higher activity than the methanol extract *in vivo.* This is probably due to biotransformation of constituents into more active metabolites in mice (Muregi, et al, 2007). This knowledge is encouraging since water is mainly the formulation used in the traditional preparation of herbal remedies (Kokwaro 1993, Gachathi, 1989; Bentjee, 1994). Although not much has been reported about antimalarial activity of *X. americana,* its

traditional use as an antimalarial has been reported by Gronhaug et al, (2008) and Diallo et al, (2002). Therefore this study confirms the high potential of this plant as a future antimalarial. And partially validates its use as an antimalarial as used in the traditional medicine.

Stem bark of *X. americana* and aerial parts of *P. lanceolata* methanol extracts had the highest chemosuppression with higher mean survival time (11.60 and 11.40 days respectively), higher than untreated mice (8.60 days). The prolonged survival time is probably due to curative action of the extracts. Interestingly methanol extract of *F. africana* was inactive with a mean survival time of 10.6 days. This implies as proposed by Dahanukar et al, (2000), that this plant extracts may possess other pharmacological benefits to the hosts, other than direct parasiticidal effects, such as acting as analgesics, antipyretics or as immune stimulators. On the contrary water extract of *X. americana* had a good activity with shorter mean survival time of 9.3 days. This indicates that the extracts act on the blood stages of the parasites, suggesting that the active compounds in these plant extract may have a short half-life. Some antimalarial drugs including artemisinine based derivatives are known to be fast acting, and to have a short half life (Muregi et al, 2007).

In acute toxicity assay no deaths were observed for eight of the ten extracts at the highest concentration tested which was 5000 mg/kg body weight, indicating the safety of the plant extracts. Another pointer to the safety of most of the samples tested is that all the animals that received the extracts stayed alive for the entire period of the 4 day suppressive test. If the test mice die before day 5, then the cause of death may be attributed to the effect of the test drugs rather than the parasites (Jutamaad et al, 1998) suggesting that the therapeutic index is too low. Moreover the doses of the extracts orally administered up to 5000mg/kg body weight were much higher than those taken as infusion by people. *X. americana* water extract displayed mortality though less than 50% of population. Since its antimalarial activity was encouraging and was not cytotoxic against Vero E99 cell lines the plant can still be used at safe doses as an antimalarial. Furthermore safe antimalarial compounds can still be isolated from the plant.

The methanol extract of *F. africana* had good antimalarial activity with no signs of acute toxicity in mice. However it exhibited moderate cytotoxicity (CC_{50} of 63.45 µg/ml) with selectivity index of 34.465 on Vero E99 cells, meaning its antiplasmodial activity was not due to cytotoxicity as described by Vonthron-Senecheau et al, (2003). For this reason, safe antimalarial compounds can be pursued from this plant (Kokwaro 1993; Baraza et al, 2008). The findings here demonstrate the potential of this plant as a safe antimalarial. Most extracts were not cytotoxic on Vero E 99 cell lines (CC_{50} values ≥100 µg/ml), suggesting the safety of the medicinal plants that have been used over many years..

5. Conclusion

Antiplasmodial potential of the four selected medicinal plants used in Kenya has been demonstrated in this study. The observed high *in vitro* activity indicates their high potential and should therefore be pursued further for development of new antimalarial drugs. This study supports the use of *P. lanceolata* and *X. americana* as traditional antimalarials. Lack of cytotoxicity of most of the plant extracts demonstrates the safety of these medicinal plants that have been used for a long time. Methanol extract of *F. africana* (aerial parts) though moderately cytotoxic can still be administered at safe doses. The fact that most of the plant extracts were not toxic to mice at 5000mg/kg body weight, validates their use in high doses as used by traditional medicine. Since *X. americana* water extract showed mortality of less than 50% of the population, it can still be used as antimalarial at safe lower doses in traditional medicine.

Conflict of Interest declaration

The authors declare no conflict of interest.

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