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Evaluation of the antiplasmodial activity of extracts of plants used in traditional medicine in Kenya

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Malaria is still a major public health problem because of resistance to therapeutic drugs. Among strategies for the development of new antimalarial, a study of plants traditionally used in Africa particularly that of Kenya against malaria has been pursued. The present study is to evaluate In vitro antiplasmodial activity of extracts from Kenyan plants commonly used by traditional healers for the treatment of malaria and other diseases. For each species, *n*-hexane, dichloromethane, and methanol extracts were evaluated on a chloroquine (CQ) -resistant (FcB1-Colombia) and on a CQ-sensitive (F32-Tanzania) strain of *Plasmodium*. The extracts were tested at 50, 10, 5, 1, 0.5, 0.1 and 0.05 µg/ml. Cytotoxicity on a fibroblast cell line (VERO) was also evaluated for the potentiality active extracts. Ten extracts from six plants with a good selectivity index (SI) whose, *Alangium chinense* (Lour.) Harms (Alangiaceae) (IC₅₀= 2.81 µg/ml; SI= 12.3 and 6.15 µg/ml with SI= 14.4 on FcB1 and F32, respectively), *Cadaba farinosa* Forssk. (Capparaceae) (IC₅₀ = 3.05 µg/ml with SI> >32 on FcB1), *Schizogygia coffaeoides* Baill. (Apocynaceae) (IC₅₀ = 4.80 µg/ml; SI=14.4 on FcB1) were found to have a promising antiplasmodial activity and should be pursued to characterize the constituents responsible for antiplasmodial activity.

Key words: Malaria, antiplasmodial, *falciparum*, *scolopia zeyheri*, apocynacea.

INTRODUCTION

There were an estimated 216 million episodes of malaria in 2010, of which approximately 81% (about 174 million cases), were in the African Region. On the same year, malaria killed more than 600,000 people (91% were in Africa) and is second only to tuberculosis for its impact on world health (WHO, 2011). Malaria is one of the main causes of childhood death and is still a major threat to child health. Moreover, malaria contributes to the loss of in-

come in the endemic countries (Malaney et al., 2004).

Despite clear progress in the fight against malaria, treatments are less and less effective, mainly due to drug resistance developed by most of the field isolates of *Plasmodium falciparum*. One of the reasons for resistance spread might be the unlimited misuse of chloroquine or other antimalarial drugs in all kinds of fever-inducing diseases without confirmed diagnosis of malaria (Kuria et al., 2001). Due to the resistance and the cost of efficient drugs, people living in endemic countries often use traditional remedies either to cure themselves or as an alternative or complementary treatment (Wilcox et al., 2004). The study of traditional medicine has led to the

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discovery of numerous new pharmacology active molecules such as artemisinin or even of active extracts (Benoit-Vical et al., 2003). It is generally admitted that more than 50% of new available drugs in the world are still derived from natural products (Newman and Cragg, 2007). An important role in the challenge is to discover new anti-malarial drugs (Jansen et al., 2012). In the present work, the *in vitro* antiplasmodial activity of plant selected after an ethnobotanical survey are presented. Various extracts obtained from 19 plants collected in Kenya were tested for antiplasmodial activity. The selected plants, *Acokanthera oppositifolia* (Lam) (Apocynaceae) is a species encountered in Eastern Africa where it's used as an arrow poison. The whole tree, except possibly the ripe fruits, is known to be poisonous (Kokwaro, 1993). Powdered dried leaves and roots are traditionally employed to treat headaches and snake bites (Kokwaro, 1993; Dharani, 2002); *A. chinense* (Lour.) Harms (Alangiaceae) is used in Kenya by traditional healers in magic ceremonies (Kokwaro, 1993). Though there had been no previous evaluation of the antiplasmodial and cytotoxic activities of *A. chinense*, five other *Alangium* species already showed cytotoxic and/or antiproliferative activities (Hayat et al., 1977; Rao and Venkatachalam, 1999; Sakurai et al., 2006); *Cadaba farinosa* Forssk. (Capparaceae) is a bush that grows in arid and semi-arid zones to grassy desert savannah. This plant is widely used in traditional medicine in Western and Eastern Africa. It was reported to relieve pain, dysentery, cough or fever (Gohar, 2002); *Schizozygia coffaeoides* Baill. (Apocynaceae) is a monotypic shrub used in Kenyan traditional medicine for the treatment of malaria (local ethnopharmacological investigations) and various skin diseases (Omino and Kokwaro, 1993); *Scolopia zeyheri* Warb (Flacourtiaceae) is a medium size tree from tropical East Africa which is traditionally used to treat malaria and general pain; *Toddalia asiatica* Lam. (Rutaceae) root is a widely known climbing shrub or liana used by traditional healers to cure various symptoms. *In vitro* antiplasmodial activity of coumarins from *T. asiatica* root extracts was already reported (Oketch-Rabah et al., 2000). Most Rutaceae extracts also contains benzo[c]phenanthridines such as nitidine and dihydronitine which were involved in their cytotoxic (Iwasaki et al., 2006) and antiplasmodial (Gakunju et al., 1995; Jullian et al., 2006; Bouquet J et al., 2012) properties. *In vivo* antimalarial activity was also previously reported by Muregi et al. (2007). All these plants were species mostly used in traditional medicine against fevers (one of the main symptoms of malaria) and against various infectious diseases.

MATERIAL AND METHODS

Material

Twenty-seven parts of plants collected from nineteen

plants species belonging to ten families were selected on the basis of ethnopharmacological data. Collection of the different plants was conducted between September and November 2009 in Kenya by PBCM (author). The plants and their parts that were tested are listed in Table 1. These plants are used by traditional healers in Kenya to treat malaria or other infectious diseases of which fever is a common symptom. The plants were identified by PBCM (author) in the University of Nairobi and a voucher specimen of each of them was deposited at the herbarium, Botany Department, University of Nairobi Nairobi, Kenya. Samples were air-dried, powdered and extracted before being tested for antiplasmodial activity. Some of the more active plants were harvested twice (independently, since collection was conducted in 2009) in order to confirm the results obtained on new samples (Table 1).

Extract Preparation

The air-dried parts of plants were powdered and an aliquot (3 to 5 g) of each selected part was successively extracted with 2 x 10 ml of *n*-hexane, dichloromethane and methanol for 10 min each time. Extractions were carried out using a Dionex® accelerated solvent extraction system (ASE 100®) pressurized cell (100 bars) of 10 ml at 40°C.

The 81 crude extracts were air-dried, weighed and then dissolved in dimethylsulphoxide (DMSO) to give stock solutions (10 mg/ml) which were stored at -20°C before use. The stock solution of each extract was then serially diluted with culture medium just before being tested for antiplasmodial activity or cytotoxicity.

Antiplasmodial Activity Evaluation

Antiplasmodial activity of the extracts was evaluated *in vitro* on *P. falciparum* as described elsewhere (Benoit-Vical et al., 1996). Two strains of *P. falciparum* were used, F32-Tanzania (chloroquine (CQ)-sensitive strain) and FcB1 Columbia (CQ-resistant strain). *In vitro* culture of *P. falciparum* was carried out according (Trager & Jensen, 1976) with modifications described elsewhere (Cachet et al., 2009). *P. falciparum* infected erythrocytes were plated at 0.5-1% parasitaemia (2% PCV) in the presence of growing dilutions of the extracts for 24 h. [³H]-hypoxanthine (18.5 kBq, Perkin-Elmer, Courtaboeuf, France) was then added and the plates incubated for 24h more. The [³H]-hypoxanthine incorporation was determined by scintillation counting with a β-counter (Perkin Elmer, Courtaboeuf, France). Each experiment was repeated three times. The incorporation of [³H]-hypoxanthine by the parasites in the presence of the extract was compared with control parasite cultures without extract (referred to as 100%). For this radioactive

Table 1. List of the plants selected for evaluation of antiplasmodial activity.

Plants species	Family	Parts investigated	Vouchers Numbers
<i>Alangium chinense</i> (Lour.) Harms	Alangiaceae	Aerial parts	2005/26
<i>Acokanthera oppositifolia</i> (Lam.) Codd	Apocynaceae	Stems , Leaves	2005/26
<i>Acokanthera schimperi</i> Benth. and Hook.	Apocynaceae	Stems, Leaves	2005/25
<i>Schizogygia coffaeoides</i> Baill.	Apocynaceae	Stems, Roots, Leaves	2005/35
<i>Cryptolepis apiculata</i> K. Schum.	Asclepiadaceae	Whole part	2007/70
<i>Cryptolepis hypoglaucua</i> K. Schum.	Asclepiadaceae	Whole part	2007/74
<i>Cynanchum altiscandens</i> K. Schum.	Asclepiadaceae	Roots	2007/80
<i>Mondia whiteii</i> Skeels	Asclepiadaceae	Aerial parts	2007/60
<i>Maerua angolensis</i> DC.	Capparaceae	Stems bark	2005/28
<i>Cadaba farinosa</i> Forssk.	Capparaceae	Aerial parts	2005/27
<i>Microglossa pyrifolia</i> (Lam.) Kuntze	Asteraceae	Stems, Leaves	2005/30
<i>Rhamnus staddo</i> A. Rich.	Rhamnaceae	Leaves	2005/21
<i>Dovyalis abyssinica</i> Warb.	Flacourtiaceae	Whole part	2005/32
<i>Scolopia zeyheri</i> Warb.	Flacourtiaceae	Aerial parts	2007/42
<i>Ekebergia capensis</i> Sparrrm	Meliaceae	Stems, Leaves,	2007/48
<i>Melia volkensii</i> Gürke	Meliaceae	Fruits, Stems, internal fruits	2005/29
<i>Trichillia emetica</i> Vahl	Meliaceae	Leaves	2007/41
<i>Toddalia asiatica</i> Lam.	Rutaceae	Roots	2007/43
<i>Vitex strickeri</i> Vatke and Hildebrandt	Lamiaceae	Roots	2005/40

method, IC₅₀ values were determined graphically in terms of concentration versus inhibition percentage (Roumy et al., 2007). A control corresponding to the highest amount of DMSO (0.1%) was added and as previously described, no effect was observed on parasite growth (Ménan et al., 2006). Chloroquine was used as control drug.

Cytotoxicity assay

The effect of extracts on eukaryotic cells was evaluated by an XTT assay (Portet et al; 2009). Briefly, VERO cells (fibroblast cell line from African green monkey kidney) at confluence were trypsinized (Trypsin EDTA, Biowittaker, Belgium). The cells were counted and seeded at 5000 cells/well in 96-well plates and incubated (37°C, 5% CO₂) in order to adhere. The cells were washed with PBS (pH 7.4, Biowittaker) and growing dilutions of the extracts were added in the wells in 100 µl EMEM (Eagle's minimum essential medium). After 48 h of incubation at 37°C in a humidified incubator, an XTT sodium salt solution (sodium 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide) was added to each well. The cells were incubated at 37°C for further 3 h more and the colorimetric reaction stopped by the addition of 100 µl of SDS 10% (Sodium Dodecyl Sulfate, Sigma) to each well. The spectrometric absorbance of

each well was measured using a POLAR star (Galaxy BMG) at 450 nm. Doxorubicin served as positive control. IC₅₀ values were also determined graphically.

Selectivity Index (SI)

The SI value allows the evaluation of the selective activity of the extracts against the parasite compared to its toxicity for VERO cells. The SI value is calculated as the ratio between cytotoxic IC₅₀ values and FcB1 or F32 parasitic IC₅₀ values.

RESULTS

The extraction was performed with three solvents with gradient of polarity and 81 extracts were tested for antiplasmodial activity on two *P. falciparum* strains. For maximum readability, only the plants of which at least one extract had an IC₅₀ lower than 20µg/ml are listed.

The mean values of at least three experiments (± SD) are presented in Table 2. For most of the extracts with an IC₅₀ lower than 20 µg/ml, the cytotoxicity on VERO cells was also evaluated. The cytotoxicity results are presented in Table 2 which also includes the results of the selectivity index calculation. On *P. falciparum* culture,

Table 2. In vitro antiplasmodial and cytotoxic activities of plant extracts and selectivity index (a).

Plants species	Extracts	FcB1(µg/ml)	F32 (µg/ml)	VERO(µg/ml)	SI ^a /FcB1	SI ^a /F32
<i>A. oppositifolia</i> Stem	H ^b	31.5 ± 12	> 50	–	–	–
	D ^c	9.8 ± 2.2	8.95 ± 1.0	4.60	<1	<1
	M ^d	> 50	> 50	–	–	–
<i>A. oppositifolia</i> Leaves	H	> 50	> 50	–	–	–
	D	19.5 ± 5.2	47.5 ± 12.6	85.5	4.4	1.8
	M	> 50	> 50	–	–	–
<i>A. chinense</i> Aerial parts	H	> 50	> 50	–	–	–
	D	6.15 ± 4.0	38.5 ± 6.4	88.5	14.4	2.3
	M	2.8 ± 0.5	6.5 ± 0.7	34.5	12.3	5.3
<i>C. farinosa</i> Aerial parts	H	> 50	> 50	–	–	–
	D	3.0 ± 1.9	6.2 ± 10.6	> 100	>32	>16
	M	> 50	> 50	–	–	–
<i>S. coffaeoides</i> Stem	H	19.75 ± 4.3	8.0 ± 1.4	38.0	2.1	4.75
	D	9.70 ± 4.9	7.50 ± 3.5	60.0	6.2	8
	M	> 50	> 50	–	–	–
<i>S. coffaeoides</i> Roots	H	20.7 ± 5.6	6.5 ± 1.5	7.0	<1	1.1
	D	4.8 ± 0.9	6.15 ± 1.7	69.0	14.4	11.2
	M	> 50	34 ± 5.6	–	–	–
<i>S. zeyheri</i> Aerial parts	H	24.5 ± 2.12	>50	>100	>4.1	–
	D	29.3 ± 6.7	7.5 ± 2.1	>100	>3.4	>13.3
	M	> 50	> 50	–	–	–
<i>T. asiatica</i> Roots	H	31.75 ± 9.4	10.0 ± 1.4	63.0	2.0	6.3
	D	5.0 ± 2.2	5.75 ± 1.0	59.0	11.8	10.3
	M	32.0 ± 12	39.0 ± 4	–	–	–
CQ ^e		0.140 µM	0.045 µM	–		
DOX ^f		–	–	0.899 µM		

IC₅₀ of the extracts ranged from 2.81 to more than 50 µg/ml. Among all the extracts tested those from, *Alangium chinense* (Lour.) Harms (Alangiaceae), *C. farinosa* Forssk. (Capparaceae), *S. coffaeoides* Baill. (Apocynaceae), *Toddalia asiatica* Lam. (Rutaceae), *S. zeyheri* Warb (Flacourtiaceae) and *Acokanthera oppositifolia* (Lam.) Codd. (Apocynaceae) were found to have an interesting antiplasmodial activity (IC₅₀ < 10 µg/ml) with a good SI. A second antiplasmodial test was performed after a new collection of these plants and the results obtained was similar (data not shown). This preliminary work allowed us to select these plants for further phytochemical studies.

DISCUSSION

The analysis of traditional medicine led to numerous new active molecules, and in the fight against malaria, a better knowledge of traditional medicine will help in the discovery of new active molecules (Houël et al., 2009). As stated by other authors, (Rasoanaivo et al., 2004), extracts that presented an IC₅₀ under 20 µg/ml could be considered as potential sources for antimalarials while those that presented an IC₅₀ < 10 µg/ml have to be included in further investigations (mostly research of new active principles). In the present study, some extracts of at

least six plants among the 19 tested reached this value ($IC_{50} < 10 \mu\text{g/ml}$). Climatic variations might explain the notable differences in the antiplasmodial activity of medicinal plants harvested at two different seasons. During September in Kenya, plants experiencing drought leading to poor growth and reduced principle stock, which likely accounts for the lower antiplasmodial activity. On the other hand, the 'rainy season' falls in October and November, i.e. just after the dry season. Therefore, plants which were in a stationary phase took advantage of water abundance to grow quicker and consequently saved high quality antiplasmodial principles. In addition the low activity of some extracts could be explained by the differences in the extraction methods which differed between traditional (Wilcox et al., 2004). Thus, in the following discussion, our attention will be focused mainly on these plants.

Acokanthera oppositifolia (Lam) (Apocynaceae) (part tested: stems). In our study, the dichloromethane extract from the stems displayed a moderate activity on both strains of *P. falciparum* with IC_{50} values of $9.82 \mu\text{g/ml}$ (FcB1) and $8.95 \mu\text{g/ml}$ (F32). These extracts also presented a high cytotoxicity ($IC_{50} = 4.60 \mu\text{g/ml}$) and a low SI (< 1). The presence of cardiac glycosides in *Acokanthera* species (Kingston & Reichstein, 1974; Pieri et al., 1992; Hanna et al., 1998) could explain the cytotoxicity and antiplasmodial activities observed with the dichloromethane stem extract (Laphookhieo et al., 2004; Wang et al., 2007).

A. chinense (Lour.) Harms (Alangiaceae) (part tested: aerial parts). In the present antiplasmodial evaluation, the dichloromethane extract was moderately active only against FcB1 strain ($IC_{50} = 6.15 \mu\text{g/ml}$) whereas the methanolic extract was active against both strains and especially against the CQ-resistant one with $IC_{50} = 2.81$ (FcB1) and $6.36 \mu\text{g/ml}$ (F32). The selectivity indexes of the dichloromethane and methanol extracts were respectively 14.39 and 12.27 with FcB1 strain and no cytotoxic activities on VERO cells (IC_{50} 88.5 and $34.5 \mu\text{g/ml}$) was observed. The chemical profiles of *Alangium* species are well documented. A tetrahydroisoquinoline monoterpene glycoside closely related to the ipecac alkaloids (Itoh et al., 1991) and diverse benzyl, phenolic or flavonoid glycosides (Itoh et al., 2001) that were isolated from *A. chinense* might contribute to its biological activities (Bravo et al., 1999; Murakami et al., 2001; Salib and Michael, 2004).

C. farinosa Forssk. (Capparaceae) (Part tested: aerial parts). In the present investigation, the dichloromethane extract showed good to moderate antiplasmodial activities on both strains (FcB1 $IC_{50} = 3.05 \mu\text{g/ml}$ and F32 $IC_{50} = 6.25 \mu\text{g/ml}$). This might confirm its traditional use against fever. However, the IC_{50} values on VERO cells are higher than $100 \mu\text{g/ml}$ (dichloromethane extract), giving a rather high SI. Spermidine alkaloids (cadabicine and cadabicine diacetate), and an eudesmolide sesquiterpene lactone (cabacilone) were previously

isolated from the stem bark of *C. farinosa* (Viqar Uddin et al., 1985; Viqar Uddin et al., 1987). Even if the antiplasmodial properties of these compounds had never been studied, they could be responsible for the antiplasmodial activity, observed, even if the presence of other active molecules could not be excluded.

S. coffaeoides Baill. (Apocynaceae) (part tested: leaves, stems and root). In the evaluation of antiplasmodial activity, the leaves the stems and the roots methanol extracts of *S. coffaeoides* were considered to be inactive, whereas good to moderate antiplasmodial activities were obtained with the *n*-hexane and dichloromethane extracts of the stems and the roots. Indeed, stems and roots *n*-hexane extracts were moderately active on the sensitive strain (F32) ($IC_{50} = 8.0$ and $6.50 \mu\text{g/ml}$ respectively). Dichloromethane stems and roots extracts displayed also moderate activities on both strains (IC_{50} values ranging from 4.80 to $9.70 \mu\text{g/ml}$) with a better activity of the roots on the resistant strain ($IC_{50} = 4.80 \mu\text{g/ml}$). Moreover, the *n*-hexane extracts and the dichloromethane roots extract were moderately cytotoxic (SI = 3.52), whereas the IC_{50} on the VERO cells line for the dichloromethane stems extract was higher (SI values: 6.18 and 8 for FcB1 and F32 strains, respectively). Preliminary biological study had prompted use to isolate the schizogane alkaloids from the stems and the roots of *S. coffaeoides* in order to evaluate their antiplasmodial and cytotoxic properties (Le Lamer et al. 2008). Thus, the good antiplasmodial activity noticed for the dichloromethane extracts of *S. coffaeoides* should be attributed to minor schizogane alkaloids.

S. zeyheri Warb (Flacourtiaceae) (part tested: aerial parts). A moderate activity was detected for the dichloromethane aerial part extract of this plant, but only on the CQ-sensitive strain ($IC_{50} = 7.50 \mu\text{g/ml}$). In addition, no cytotoxicity was noticed on the VERO cells line, even at concentrations higher than $100 \mu\text{g/ml}$. To date, there are no data about the chemical content and the biological properties of *S. zeyheri*. Previous phytochemical investigations on *Scolopia chinense* (Lour.) Clos (Lu et al., 2008) and on *Scolopia spinosa* Warb. (Shaari and Waterman., 1994) revealed the presence of various secondary metabolites, such as flavonoid glycosides and a series of phenolic glycosides, representatives of which are found on Flacourtiaceae (Mosaddick et al., 2007). Therefore, it is not excluded that this kind of compounds might be involved in the present antiplasmodial activity (Bravo et al., 1999). This interesting result combined with a very low cytotoxicity deserves further phytochemical investigations.

Toddalia asiatica Lam. (Rutaceae). Our investigation confirmed these results: besides a weak *in vitro* antiplasmodial activity on the F32 strain for the hexane extract, depicted by an IC_{50} of $10.0 \mu\text{g/ml}$, a moderate activity was noticed for the dichloromethane extract on both strains ($IC_{50} = 5.0$ and $5.75 \mu\text{g/ml}$ on FcB1 and F32 respectively). This dichloromethane extract was not cytotoxic

on VERO cell line ($IC_{50} = 59 \mu\text{g/ml}$) giving SI values of 11.8 and 10.26 for FcB1 and F32 respectively.

CONCLUSION

This study permitted to identify some potentially compounds involved in observed antiplasmodial activities. Many people living in developing countries use plants, often in combination, for the health care management of malaria. There is now evidence that some whole plant extracts can be more active than single compounds, as a result of synergy and positive interactions between different constituents in the extracts, compared to a single product.

The three main criteria used to select the most interesting parts of the plants were (i) a high antiplasmodial activity, (ii) an interesting cytotoxic/antiplasmodial (FcB1; F32) ratio and (iii) a low level of prior scientific knowledge concerning these plants. Therefore, one can consider that the roots of *Caddaba farinosa* and *A. chinense* and the aerial parts of *S. zeyheri* will have to be analyzed for further phytochemical studies.

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DECLARATION OF CONFLICT OF INTEREST

The authors wish to declare that there is no conflict of interest.

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