ANTIMALARIAL ACTIVITY OF SOME PLANTS TRADITIONALLY USED IN MOZAMBIQUE

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Abstract

Developing countries, where malaria is endemic, depend strongly on traditional medicine as a source for inexpensive treatment of this disease. However, scientific data to validate the antimalarial properties of these herbal remedies are scarce. Consequently, it is important that antimalarial medicinal plants are investigated, in order to establish their efficacy and to determine their potential as sources of new antimalarial drugs.
In this study, we evaluated the claimed antimalarial properties of fifty eight crude extracts from fifteen plants used in traditional medicine against malaria and fever, mainly from Southern African regions. The air-dried powdered plant parts (roots, leaves, seeds or bark) or whole plants were extracted, sequentially, with solvents of increased polarity (*n*-hexane, dichloromethane, ethyl acetate and methanol). Schizontocidal activity was measured using a standard in *vitro* assay, with 3D7 *Plasmodium falciparum* strain. From the 58 extracts tested, two of them showed a significant activity (IC_{50} < 5 µg/mL), and 34.5 % showed a moderate activity (10 < IC_{50} < 50 µg/mL). A bioassay-guided fractionation of the most active extracts is ongoing.

Key words: Traditional medicine*;* Medicinal plants*;* Malaria; Antimalarial; *Plasmodium falciparum.*

1. INTRODUCTION

Malaria is a major parasitic disease in the world, especially in Africa. It is responsible for 500 million new cases and 2 to 3 millions deaths every year, mostly among children under five years and pregnant women (WHO, 2008). *Plasmodium falciparum* the most widespread etiological agent for human malaria has become increasingly resistant to standard antimalarials *e.g.* chloroquine and antifolates. Consequently, new drugs or drug combinations are urgently needed today for the treatment of malaria. These drugs should have novel modes of action or be chemically different from the drugs in current use.

In Africa and elsewhere, plant extracts are still widely used in the treatment of malaria and other ailments, and up to 80% of the African population use traditional medicines for primary health care (WHO, 2002). Since little scientific data exist to validate antimalarial properties of these medicinal plants, it is important that their claimed

antimalarial properties are investigated, in order to establish their efficacy and determine their potential as sources of new antimalarial drugs (such as, artemisinin isolated from *Artemisia annua*).

In the present work, we have evaluated the *in vitro* antimalarial activity of fifteen plants uses in traditional medicine against malaria and/or fever.

2. MATERIAL AND METHODS

2.1. Plant materials

Plants were obtained from Mozambique and Portugal. The information of the plant material is summarized in Table 1. The species collected in Mozambique were identified locally and voucher specimens (Table 1) have been deposited at the herbarium of Instituto de Investigação Agronómica of Mozambique The remaining species were collected in Portugal and identified by Dr. Teresa Vasconcelos from the Instituto Superior de Agronomia, University of Lisbon, Portugal, where voucher specimens (Table 1) were deposited.

2.2. Preparation of extracts

Different plant parts (roots, leaves, seeds, and bark) or the entire plant, from selected species (Table 1), were dried at room temperature. Crude plant extracts were prepared by submitting 15-50g of air-dried powdered plant material to a sequential extraction procedure with 150-500 mL of *n*-hexane, dichloromethane (DCM), ethyl acetate (EtOAc), and methanol (MeOH) for 48 h, at room temperature. After filtration, the extracts were fully dried, under reduced pressure at 40-45 ºC, by using a *Büchi* rotatory evaporator, and then stored at low temperature (4ºC) until their use in antimalarial assays.

2.3. *In vitro* culture of *Plasmodium falciparum* and antimalarial activity

The *in vitro* activity against *Plasmodium falciparum* intraerythrocytic stages of crude extracts was evaluated by means of the Mark III test, as developed by the WHO (WHO, 2001). Briefly, the 3D7 *P. falciparum* strain (susceptible to chloroquine) was cultivated in recently collected erythrocytes as host cells in RPMI 1640 medium (Gibco) containing 25 mM HEPES (Sigma) and 6.8 M hypoxanthine (Sigma) supplemented with 0.5% AlbuMAX II (Invitrogen). Cultures were kept at 37ºC under an atmosphere of 5% O_2, 3–5% CO_2, and N_2. Drug-sensitivity assays were carried out on 96-well micro-plates. Crude extracts were dissolved in ethanol or dimethyl sulfoxide (DMSO) and diluted in RPMI. The micro-plates were titrated with two-fold serial dilutions of each extract as well

as the control. The strain was plated (in duplicate) using a synchronized culture at ring-stage parasites, with a parasitaemia between 0.6–0.8%. Each well received 10 µL of parasite-loaded erythrocytes, 5% haematocrit, and 90 µL of the different drug dilutions. The plates were incubated at 37ºC for 20-24 h, after confirmation of presence of mature schizonts in control wells, without drug. At the end of this period, the blood from each well was harvested, and a thick film was prepared. The films were fixated with acetone, and stained for 60 min in *Giemsa* stain at a dilution of 1 vol-% in buffered H_2O (pH 6.8). Three independent optical-microscopy readings of the number of schizonts with three or more nuclei were carried out in 200 parasitized red cells for each dilution and duplicate. Growth inhibition was expressed as percent of the number of schizonts for each concentration, compared with untreated controls. Mean IC_{50} values were calculated from dose-response curves (percentage of schizonts vs. logarithm of drug concentration) by linear interpolation.

Table 1. Plant material data

Plant species	Family	Plant part	Voucher number
[a]***Acacia karroo* Hayne**	Fabaceae	Aerial parts	111/2008
[b]***Aloe parvibracteata* Schonland**	Aloaceae	Leaves	113/2008
[c]***Bridelia cathartica* Bertol.f.**	Euphorbiaceae	Roots	24 SM
[d]***Cassia abbreviate* Oliv.**	Fabaceae	Stem Bark	26 SM
[c]***Cassia occidentalis* L.**	Fabaceae	Roots	29 SM
[e]***Crossopteryx febrifuga (Afzel. ex G. Don) Benth***	Rubiaceae	Aerial parts	29 189
[f]***Leonotis leonurus* (L.) R.Br**	Lamiaceae	Aerial parts	562/2005
[d]***Momordica balsamina* L.**	Cucurbitaceae	Aerial parts	30 SM
[b]***Parkinsonia aculeata* L.**	Caesalpiniaceae	Aerial parts	574/2003
[a]***Pittosporum tobira* (Thunb.) W.T. Aiton**	Pittosporaceae	Aerial parts	116/2008
[b]***Plumbago auriculata* Lam.**	Plumbaginaceae	Aerial parts	269/2004
[f]***Senna didymobotrya* Fresen.**	Fabaceae	Twigs	115/2008
[a]***Schefflera actinophylla* (Endl.) Harms**	Araliaceae	Leaves	114/2008
[c]***Tabernaemontana elegans* Stapt.**	Apocynaceae	Leaves	23 SM
[c]***Trichilia emetica* Vahl**	Meliaceae	Seeds	25 SM

Plants collected in: [a]Garcia da Horta Garden (Lisbon, Portugal, January 2006), [b] Parque botânico da Tapada da Ajuda (Lisbon, Portugal. August, 2005), [c] Maputo (Mozambique, March 2006), [d] Gaza (Mozambique, January 2006), [e] Inhambane (Mozambique, April 2006), [f]Algarve (Portugal, July 2005).

3. RESULTS AND DISCUSSION

The main goal of this work was to investigate the potential antimalarial properties of some plants used in traditional medicine, mostly in Mozambique, against malaria and/ or fever, and providing scientific validation for their use. Therefore, selection of plants was carried out based mainly on an ethnobotanical approach (JANSEN and MENDES, 1982, 1983, 1990, 1991; MADUREIRA, 2002). Some species, namely *Aloe parvibracteata***,** *Pittosporum tobira*, *Plumbago auriculata* and *Schefflera actinophylla* were selected based on a quimiotaxonomic approach (CLARKSON, 2004).

Fifty eight extracts from fifteen plants species, belonging to several families, were screened for their potential antimalarial properties against cloroquine-sensitive *P. falciparum* strain (3D7). The extracts were prepared by sequentially extracting the plant material with *n*-hexane, dichloromethane, ethyl acetate and methanol (see experimental section). The results are summarized in Table 2. The antimalarial activity of extracts was defined according to the IC_{50} values obtained. An extract showing an IC_{50} value $\leq$ 5 µg/mL was classified as highly active. Extracts with IC_{50} values $\geq$ 10 µg/mL and $\leq$ 50 µg/mL were considered moderately active and those with IC_{50} values > 50 µg/mL inactive. As can be observed in Table 2, the lowest IC_{50} values were found for the ethyl acetate extracts of *Momordica balsamina* (IC_{50} = 1.0 ± 0.1 µg/mL) and *Pittosporum tobira* (IC_{50} = 4.8 ± 1.8 µg/mL). Significant IC_{50} values were also found for the *n*-hexane extracts of *Cassia occidentalis* (IC_{50} 19.3 ± 2.0 µg/mL), and *Parkisonia aculeata* (IC_{50} 24.5 ± 2.9 µg/mL). The remaining species have showed a moderate/week antiplasmodial activity or were inactive.

Momordica balsamina Linn. (Cucurbitaceae) a climber extensively cultivated in many tropical and subtropical regions of the world has been used in sub-Saharan Africa as food (FLYMEN, 2007). This plant is traditionally used in Mozambique to treat vomiting believed to be associated with fever (BANDEIRA, 2001; VELE, 2000). Previous studies on this plant have resulted in the isolation and identification of two phenylpropanoid esters, rosmarinic acid and five pimarane diterpenes (DE TOMMASI,

1991 and 1996). Antimalarial activity for *Momordica balsamina* (CLARKSON, 2004; BENOIT-VICAL, 2006) and other species of *Momordica* genus, was previously described (KAOU, 2008; KÜLTÜR, 2007; FROELICH, 2007; NGUYEN-POUPLIN, 2007; WAAKO, 2005; MÉNAN, 2006; HOUT, 2006).

Table 2. *In vitro* antimalarial activity (IC_{50} values) of the plant extracts against *Plasmodium falciparum* 3D7.

Plant Species	Extract IC_{50} (µg/ mL)			
	***n*-hexane**	**DMC**	**EtOAc**	**MeOH**
Acacia karroo	99.0 ± 17.3	60.0 ± 12.3	70.2 ± 1.9	>100
Aloe parvibracteata	>100	>100	>100	>100
Bridelia cathartica	99± 20.2	>100	44.0 ± 1.3	>100
Cassia abbreviata	>100	40.0 ± 1.8	>100	>100
Cassia occidentalis	19.3± 2.0	59.9 ± 15.3	31.9 ± 4.6	88.2 ± 2.2
Crossopteryx febrifuga	–	44.4 ± 3.1	–	>100
Leonotis leonurus	> 100	45.4 ± 9.6	38.4 ± 4.7	> 100
Momordica balsamina	> 100	35.5 ± 2.9	1.0 ± 0.1	46.9 ± 2.3
Parkinsonia aculeata	24.5 ± 2.9	26.3 ± 2.9	36.4 ± 14.1	54.9 ± 1.7
Pittosporum tobira	34.4 ± 2.9	44.6 ± 2.4	4.8 ± 1.8	> 100
Plumbago auriculata	45.9 ± 3.3	40.2 ± 1.6	53.8 ± 3.1	80.0 ± 15.1
Senna didymobotrya	57.6 ± 22.3	92.0 ± 21.4	>100	56.0 ± 9.9
Schefflera actinophylla	32.5 ± 0.5	36.3 ± 3.9	41.7 ± 4.7	> 100
Tabernaemontana elegans	59.0 ± 8.4	26.9 ± 5.3	>100	>100
Trichilia emetica	> 100	> 100	> 100	> 100

Pittosporum tobira is an ornamental shrub or small tree common on the Mediterranean coast. In previous studies, this plant was reported to contain some terpenoids, sesquiterpenoids, and carotonoids, but no antimalarial activity was demonstrated (D'ACQUARICA, 2002; OGIHARA, 1989; FUJIWARA and MAOKA,

2001; FUJIWARA, 2001). A saponin mixture, obtained from the leaves of this specie showed antibiotic activity (D'ACQUARICA, 2002). A high antimalarial activity was also shown for *Pittosporum viridiflorum* (CLARKSON, 2004) corroborating the interest of *Pittosporum* genus as a potential source of antimalarials.

Like *Pittosporum tobira* (Thumb.) W.T. Aiton, *Acacia karroo* Hayne, *Aloe parvibracteata* Schonland, *Crossopteryx febriguga* (Afzel. ex G. Don) Benth, *Plumbago auriculata* Lam., *Schefflera actinophylla* (Endl.) Harms, *Tabernaemontana elegans* Stapt. and *Trichilia emetica* (seeds) were also screened for the first time for their antimalarial activity in this work. Among these plants, *Acacia karroo* (IC_{50} = 60 ± 12.3 µg/mL), *Crossopteryx febrifuga* (IC_{50} = 44.4 ± 3.1 µg/mL), and *Tabernaemontana elegans* (IC_{50} = 26.9 ± 5.3 µg/mL), widely used to treat malaria in developing countries, namely Mozambique, revealed moderate or no significant activity. However, it is important to note that these plants are frequently used to treat fever, generally associated to malaria. Therefore, an explanation for their lack of *in vitro* antimalarial inactivity could be that these plants may act as antipyretics or may enhance the immune system, rather than having direct antiparasitic activity (PHILLIPSON and WRIGHT, 1991).

Another explanation is that these plants could contain prodrugs non-active by themselves. In this case, these precursors of the active compounds have to be metabolized *in vivo* into active antimalarials, a major limitation in this study.

As illustrated by the results, a number of plants did not display an effective antimalarial activity, though some of these had previously been described as having antimalarial activity. For example, the ethanol extract from the stem of *Bridelia cathartica* Bertol.f (Marracuene, Mozambique) caused a 50% inhibition of parasite growth at an incubation concentration of 0.05 µg/mL (JURG, 1991). In addition, extracts (DCM/MeOH; 1:1) from twigs of *Senna didymobotrya, Parkinsonia aculeata* as welll as from *Leonotis leonurus* showed a significant antimalarial activity against *P. falciparum* D10 strain (IC_{50} < 10 µg/mL) (CLARKSON, 2004). Furthermore, the ethanol extract from the leaves of *Cassia occidentalis*, showed an high *in vitro* antimalarial activity against a *P. falciparum* chloroquine-sensitive strain (IC_{50} < 3 µg/mL) (TONA, 2004).

The regions and periods of the year of plant collection are known to play an important role in the variation of the type of compounds found in plants as well as their

concentration. Therefore, they may be responsible for the different results obtained in our study.

This study has highlighted two promising plants for further antimalarial investigations. The determination of their active compounds is on going.

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