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SCREENING OF TRADITIONALLY USED PLANTS FOR *IN VIVO* ANTIMALARIAL ACTIVITY IN MICE**Esther Innocent¹, Mainen J. Moshi¹, Pax J. Masimba¹, Zakaria H. Mbwambo², Modest C. Kapingu¹, Appolinary Kamuhabwa³.**

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Abstract

Aqueous ethanol (80%) extracts of six plants used traditionally for treatment of malaria, *Vepris glomerata* (F.Hoffm.) Engl (Rutaceae), *Maranthus floribunda* (Bak.) F.White (Chrysobalanaceae), *Strophanthus eminii* Asch. & Pax ex Pax (Apocynaceae), *Cassia abbreviata* Oliv. (Leguminosae) and *Caesalpinia bonducella* L. Fleming (Fabaceae) were screened for antimalarial activity to establish validity of their claims. The extracts exhibited antimalarial activity in the 4-day Peter's suppressive antimalarial assay in mice inoculated with red blood cells parasitized with *Plasmodium berghei*. The extracts gave ID₅₀ values of 42.8, 111.0, 639.3 and 1560 mg/kg body wt for *C. bonducella*, *C. abbreviata*, *T. furialis* and *S. eminii*, respectively. The ID₅₀ values for *V. glomerata* and *M. floribunda* were above 2400 mg/kg body wt, above which point solubility was a problem. All the tested extracts were innocuous to the mice, up to 2400 mg/kg body wt, suggesting they may be safe for short-term use.

Keywords: Antimalarial activity, *Plasmodium berghei*, traditional medicines

Introduction

Malaria is the leading cause of morbidity and mortality in sub-Saharan Africa, especially in young children and pregnant women (UNICEF, 2004). Compounding the problem are other factors that include environmental changes, the collapse of health systems in areas of civil strife and war, resistance of malaria parasites to affordable anti-malarial drugs and limitations in national health services (Nsimba, 2006). Recently, a number of studies and campaigns have been done for the adoption of artemisinin combination therapies (ACTs) (Sutherland et al., 2005; Attaran, 2004; Duffy and Mutabingwa, 2005), which have now been adopted in a number of countries including Tanzania. However, the slowly eliminated partner drugs in ACTs may soon or later, be susceptible to development of resistance in high endemic settings (Kremsner and Krishna, 2004; Talisuna et al., 2004). Signs of *in vitro* resistance to some artemisinins (Jambou et al., 2005; Uhlemann et al., 2005; Sisowath et al., 2007), already being observed emphasize the need to increase efforts to develop new antimalarials, to provide future therapeutic options.

This study aimed to validate six traditionally used plants, in Tabora and Dar es Salaam (Tanzania), for their efficacy in the treatment of malaria as further follow-up in their development and

use for managing malaria infections. The current study reports the *invivo* activity of aqueous ethanolic extracts of the plants.

Materials and Methods

Solvents

Dimethyl sulphoxide (DMSO) was purchased from Sigma (Poole, Dorset, UK), ethanol from Fisher Scientific UK Ltd (Bishop Meadow Road, Loughborough, Leicestershire, LE 11 5RG, UK). Carboxymethylcellulose (CMC) was purchased from BDH chemical Ltd (Poole, UK) and Saline was purchased from Claris Lifescience Ltd, (Ahmedabad-382 213, India).

Plant information and collection details

Five of the plants were collected in September, 1999 in Tabora and one is among collections done in the Coast region in 2006 (Table 1), and all were identified by Mr. Selemani Haji (Botany Department, University of Dar es Salaam). Voucher specimens are kept in the Herbarium of the Institute of Traditional Medicine, MUHAS.

Preparation of plant extracts

Air-dried powdered plant materials (150-200g) were extracted with 80% ethanol (1L), for 72h at room temperature, twice, to ensure exhaustive extraction. The filtrates were concentrated under reduced pressure, *in vacuo*, followed by removal of residual water by freeze-drying. Extract yields of between 0.25-7.5% were obtained. The extracts were stored in a freezer, at -20°C, until needed for testing. On test day the extracts were suspended in a 3:7 mixture of DMSO and 1% carboxymethylcellulose (CMC).

In vivo antiplasmodial testing

Parasite strain

In vivo antimalarial testing in mice was done using chloroquine sensitive strain of *Plasmodium berghei berghei* (Obtained from the National Institute for Medical Research, NIMR). The parasite stock was maintained by continuous re-infection in the mice.

Animals

Male and female Theiller's original white albino mice (20-32 g), used in the study were kept in an air-conditioned room, and fed *ad libitum* with food and water during the whole period of the study. The study obtained approval from the University Ethical Review Committee, and in accordance to the National Guidelines for handling laboratory animals.

Inoculum

Four donor mice were injected with a chloroquine sensitive strain of *Plasmodium berghei*, and parasitaemia allowed to build up for three days, at which point blood smears were taken. Blood smears were taken on the third day, and the mice only used after ensuring that 30-40 % parasitaemia was attained. After anaesthesia with chloroform, 0.5ml of blood were drawn through the eye vein with heparinized syringe, transferred into a screw capped sterile plastic tube and then topped up to 14 ml with normal saline. Each mouse was injected intra-peritoneally (ip) with 0.2ml of the suspension (10^7 infected red blood cells).

Four day suppression test

Peters' 4-day suppressive test against *P. berghei* infection was used (Peters et al, 1975). After 3-hrs of infection with parasites, the mice were randomly assigned into treatment groups of five. One group was given 5 ml/kg body wt 30% DMSO in 1% CMC, one group was given chloroquine (10 mg/kg body wt) and the remaining

groups were given between 25 - 2400 mg/kg body wt of 80% ethanolic extracts of the plants, with intermediate doses interpolated using a log scale. The extracts were administered orally once daily for four consecutive days.

Determination of parasitaemia

Percentage suppression of parasitaemia was calculated using the following formula:

$$\% \text{ Suppression} = \frac{\text{Parasitaemia in negative control} - \text{Parasitaemia in study group}}{\text{Parasitaemia in negative control}}$$

Calculations for ID₅₀

The mean results of percentage suppression of parasitaemia against the logarithms of doses were plotted using the Fig P computer program (Biosoft Inc, USA), which also gives the regression equations. The regression equations were used to calculate ID₅₀ values.

Acute toxicity testing

An acute assessment of toxicity was performed to ascertain the lethality and potency of the extracts. Mice were divided into groups of 10, and starved for 24 hrs, before the experiment began with only water allowed. On the next day the two groups were given solvent and 2400mg/kg body wt, respectively. The mice were observed for 24 h. Assessment was only done for survival at the 24 h time point.

Results and Discussion

Table 1 shows the general information on the plants used in this study and the reasons that prompted us to test them for antimalarial activity. The antimalarial activity of *Tragia fuliaris*, *Maranthes floribunda*, *Vepris glomerata* and *Strophanthus eminii* are reported for the first time while the *invitro* antimalarial activity of *Cassia abbreviata* and *Caesalpinia bonducella* are available in the literature (Spencer et al., 1947; Addae Kyereme and Wright, 1997; Mukerji et al., 1943; Weenen et al., 1990). It was noted that the most active extract was *Caesalpinia bonducella* root extract (ID₅₀ 42.8 mg/kg body wt), followed by *Cassia abbreviata* leaf extract (ID₅₀ 111.0 mg/kg/wt). *Tragia furialis* (ID₅₀ = 639.3 mg/kg/wt) has good *invivo* antimalarial activity to support traditional claims. The ID₅₀ values for *Vepris glomerata* and *Maranthes floribunda* extracts were above 2400 mg/kg body wt, and could not be estimated due to difficulty of solubility at higher concentrations. Chloroquine used as a positive control caused 93% suppression of parasitaemia at 10 mg/kg body wt. Four previous studies reported negative antimalarial results for extracts of *Caesalpinia bonducella* (Spencer et al., 1947; Addae Kyereme and Wright, 1997; Mukerji et al., 1943; Weenen et al., 1990), while one study reported weak antimalarial activity of dried stem ethanol extract (Simonsen et al., 2001). The present results suggest that the root 80% ethanol extract has potential to yield a compound/s with much higher activity. This is, however, the only study to use the *invivo* model to assess antimalarial activity of this plant. There are three previous studies on *in vitro* antimalarial activity of extracts of *Cassia abbreviata* leaves (Connelly et al., 1996) and roots (Gessler et al., 1996; Weenen et al., 1990). The leaf extract was, previously reported to have weak activity against *Plasmodium falciparum* (Connelly et al., 1996), while for the root only an ethyl acetate extract was active against *Plasmodium falciparum* (Gessler et al., 1994).

The current results are the only reported *in vivo* results, that support the therapeutic claims by traditional healers, and the ID₅₀ value recorded is good enough to warrant further studies to identify active fractions or compounds and in line with current practice, to explore possibility for use in a combination regime with other plant extracts or established antimalarial compounds. The extracts of the six plants used in this work were not toxic to mice following single dose administration up to 2400 mg/kg body wt; the highest dose used in this work. These results are in agreement with brine shrimp results, which showed that the 80% ethanol extracts of these plants exhibited low toxicity (Moshi et al., 2006).

Table 1. General information of the six plant materials

Species Name (Family)	Voucher No.	Vernacular name (Tribe):	Ethnomedical uses:	Part used and preparation
<i>Cassia abbreviata</i> Oliv.(Caesalpiniceae)[IMPP 002-0037,	Mlundalunda /mzoka (Nyamwezi);	HIV/AIDS, malaria;	Fresh roots are dried, extracted and drunk three times a day
<i>Maranthes floribunda</i> (Bak) F. White (Chrysobalanaceae)	[IMPP 002-0137;	Msundwi (Nyamwezi)	Malaria, psychosis, epilepsy	Dried root powder is mixed with warm water or porridge and taken 2-3 times a day.
<i>Strophauthus eminii</i> Asch. Pax (Apocynaceae)	IMPP 002-0013/002-0096	Ifumya (Nyamwezi);	Malaria, epilepsy;	The roots are powdered, and the powder administered by sniffing.
<i>Tragia fuliaris</i> Boj (Euphorbiaceae)	IMPP 002-0126/002-0071	Mpugambu/ Kaboroja (Nyamwezi);	Malaria, aphrodisiac, paralysis	Dried roots extracted with cold water and one cup taken three times a day for three days.
<i>Vepris glomerata</i> (F.Hoffm) Engl. (Rutaceae)	IMPP 002-0042	Mlungusikiti (Nyamwezi);	Psychosis, malaria, epilepsy, stroke;	Dried root powder is extracted with cold water or mixed with tea and drunk thrice a day for 3 days.
<i>Caesalpinia bonducella</i> L.(Fabaceae)	ADSK 12	Msolo (Zaramo);	Malaria, diabetes, gastrointestinal problems, convulsions, leprosy;	Powdered dry leaves are boiled with water and taken 3 times a day.

Conclusion

The extracts of six plants used in traditional medicine to treat malaria exhibited *in vivo* antimalarial activity, but three had very weak activity. *Caesalpinia bonducella* root and *Cassia abbreviata* leaf ethanol extracts were the most promising for further work

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