Antiplasmodial activity of extracts of selected medicinal plants used by local communities in western Uganda for treatment of malaria

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Abstract

This study investigated the antiplasmodial activity of ten medicinal plants used to treat malaria in Southwestern Uganda. The study plants were *Bothlioclines longpipes* (Olive and Hiern), N.E.Br., *Toddalia asiatica* (L.) Lam., *Maesa lanceolata* Forssk., *Indigofera emerginella* steud. Ex A. Rich., *Lantana trifolia* L., *Vernonia lasiopus* O. Hoffm., *Trimmeria bakeri* Gilg., *Rhus natalensis* Bernh. ex. Krauss *Erythrophleum pyrifolia* and *Conyza* sp. Dry powdered plant material was extracted by sequential cold maceration using petroleum ether, chloroform and ethanol solvents respectively. Extracts were subjected to *in vitro* antiplasmodial screening against wild strains of *Plasmodium falciparum* using the nitro-tetrazolium blue-based lactate dehydrogenase assay. The chloroform extract of *M. lanceolata* (EC_{50} 1.60 $\mu g\ ml^{-1}$.), showed the highest antiplasmodial activity followed by *R. natalensis* (EC_{50} 1.80 $\mu g\ ml^{-1}$). Other extracts with significant activity were the chloroform leaf extract of *Bothriocline longipes* (EC_{50} 3.66 $\mu g\ ml^{-1}$) and the petroleum ether root extract of *T. bakeri* (EC_{50} 3.955 $\mu g\ ml^{-1}$).

Key words: antimalarial, *Plasmodium falciparum*, traditional medicine, Uganda

Introduction

Malaria is an important public health problem in Uganda with over 90% of the country being highly endemic. The disease exerts an untold socio-economic burden on the population. The treatment of malaria is confounded by the challenges of widespread resistance of the malaria parasites to cheap and affordable antimalarial drugs (Kilama, 2005). Antimalarial drug resistance has spread and intensified over the years leading to a dramatic decline in the efficacies of the antimalarial drugs (Marsh, 1998; Bloland & Ettling, 1999). Faced with these reduced efficacies there is urgent need to develop new antimalarial drugs.

Traditional medicinal plants have contributed significantly to current malaria therapy. The first effective drug treatment against malaria was quinine, which was extracted from the cinchona tree. The structure of quinine was used to synthesize antimalarials, like chloroquine and primaquine. The importance of plants as effective antimalarials was further reinforced by the isolation of artemisinin from the Chinese medicinal plant, *Artemisia annua* (family Asteraceae). Artemisinins are presently the most effective drug against multi-drug resistant strains of *Plasmodium falciparum*. Several other pharmacologically active antimalarial compounds have been isolated from plants and are at different stages of development (Brandao *et al.*, 1997; Valentin *et al.*, 1997; Ogwal-Okeng, 1998; Waako, Smith & Folb, 2005b; Waako *et al.*, 2005a, 2007; Sebisubi, 2007).

A number of surveys have been carried out in Uganda to document the use of herbal medicines (Adjanohoun *et al.*, 1993; Mubiru *et al.*, 1994; Bukenya-Ziraba *et al.*, 1997; Ogwal-Okeng, 1998; Tabuti, Dhillion & Lye, 2003; Katuura *et al.*, 2007). However, few studies have been undertaken to investigate the antimalarial efficacy and safety of plants claimed to have antimalarial therapeutic value (Willcox, 1999; Waako *et al.*, 2005a,b). The need to carry out systematic scientific analyses of herbal medicine aimed at testing plants and to characterize the active principles cannot be over-emphasized. This study evaluated antiplasmodial activity of ten priority plants traditionally used in western Uganda for the treatment of malaria.

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All authors declare no conflicts of interest

Materials and methods

Plant material

Study species were selected on the basis of the extent of use, from amongst species that had been identified in an earlier ethnobotanical study (Katuura *et al.*, 2007). Mature plants parts without defects or parasitic infection were collected. Voucher specimens for all these species are archived at the Makerere University Herbarium.

Preparation and extraction of plant material

The plant samples (Table 1) were dried at room temperature to a constant weight. The pulverized materials (200 g) were extracted by cold maceration through sequential solvent extraction, using petroleum ether (fraction 1), chloroform (fraction 2) and ethanol (fraction 3). The extracts were concentrated to dryness under reduced pressure using a rotary evaporator (BUCHI scientific equipments [BUCHI Analytical Inc., Newcastle, USA]). The weight of the dry extract was expressed as a percentage of the total mass of dry plant powder. The crude extracts were then kept under refrigeration until required for antiplasmodial activity testing.

Table 1 Medicinal plants traditionally used for treatment of malaria (Katuura *et al.*, 2007)

Scientific name, family, local name, voucher number	Parts analysed
Lantana trifolia L., Verbanaceae, Omuhuki, EKM 010	Arial part
Toddalia asiatica (L.) Lam., Rutaceae, Kabakura, EKM 030	Leaves, roots
Vernonia lasiopus O. Hoffm., Asteraceae, Omujuma, EKM 003	Leaves
Indigofera emerginella Steud. ex A. Rich., Papilionaceae, Omunyazabashumba, EKM 050	Leaves, roots
Bothriocline longipes (Oliv. & Hiern), N.E. Br., Asteraceae, Ekyoganyanja, EKM 015	Leaves
Maesa lanceolata Forssk., Myrsinaceae, Omuhanga, EKM 045	Leaves
Trimeria bakeri Gilg., Flacourtiaceae, Omwatanshare, EKM 019	Leaves
Conyza sp., Asteraceae, Oruheza, EKM 016	Whole plant
Rhus natalensis Bernh. ex Krauss, Anarcardiaceae, Omesheshe, EKM 013	Leaves
Erythrophleum pyrifolia, Ceasalpinaceae, Omurama, EKM 047	Leaves, roots

Evaluation of antiplasmodial activity

Culture media was made up of RPMI 1640 (Bio Whittaker, Sigma-Aldrich, South Africa) medium supplemented with plasma (obtained from Nakasero blood bank, Kampala, Uganda), hypoxanthine(44 mg l^{-1}), *N*-(2-hydroxyethl)-piperazine-*N*-2-ethosulphonic acid (HEPES) (6 g l^{-1}), sodium bicarbonate (2.1 g l^{-1}) and gentamycin (50 mg l^{-1}). The reagents used for lactate dehydrogenase assay included nitroblue tetrazolium (NBT) (1.96 nM) and phenizine ethosulphate (PES) (0.24 mM) solution in millipore water. The malstat reagent was made from TritonX 100 (1 ml l^{-1}), 3-acetyl pyridine adenine dinucleotide (APAD) (0.33 g l^{-1}) and TRIS EDTA buffer, pH 7.4 (3.3 g l^{-1}) in millipore water. Chloroquine diphosphate was used as a positive control and D-sorbital as a synchronizer. Reagents were obtained from South Africa Technology Inc. Group O+ blood was obtained from Nakasero blood bank, Uganda. A 7520 Plate reader (Cambridge) was used for Elisa reading at Joint Medical Research centre, Uganda. The test parasites were obtained from blood of male and female adult patients at the malaria assessment centre Mulago hospital in Uganda, with acquired *P. falciparum* infection. Donor selection was based on a history of having not used any drug since infection and high parasitemia of 2–3%. Blood samples were obtained by venipuncture.

Preparation of plant extracts for the assay was based on experience that crude extracts with significant antiplasmodial activity have a 50% inhibitory concentration (IC_{50}) of less than 10 μg ml^{-1}. Stock solutions of the crude extracts were made so as to test a dose range of 200 μg ml^{-1} and below of extract dissolved in culture medium. For extracts that did not dissolve readily, a solvent of 1% dimethyl sulphur oxide in culture medium was used. The solutions were maintained under sterile conditions at 4°C until used for testing. For chloroquine an initial concentration of 4 μg ml^{-1} was achieved by dissolving the drugs in culture medium.

Antiplasmodial assay

Antiplasmodial activity of the plant extract was assessed by its ability to inhibit the maturation of the plasmodium parasites. Plasmodial lactate dehydrogenase (pLDH) enzyme, which uses 3-APAD as a coenzyme in a reaction leading to the formation of pyruvate from L-lactate, was used to study the susceptibility of the parasite to the

extract *in vitro*. The activity of this enzyme was determined by the colorimetric method according to Makler *et al.* (1993).

A thin blood smear stained with giemsa was examined under the microscope to determine the number of schizonts. Blood was then washed with wash media under a centrifuge (centrifuge at 1000 rpm for eight minutes) and the supernatant including the white blood cells removed. Unused blood samples were incinerated at the National referral hospital, Mulago.

The parasites were maintained according to a modified Trager & Jensen (1976) protocol. Approximately, 50 ml of complete culture media was added to 100 μl of blood in a 250 ml culture bottle. The mixture was then maintained in a candle jar for the duration of the experiment. Culture media was changed after 24 h. Parasite cultures were synchronized by washing with D-sorbital at the beginning of incubation. Sensitivity assays were initiated by adjusting the initial parasitaemia to 2% with normal type A+ human red cells of haematocrit 2%. The suspensions from extracts were then dispensed in duplicate per a single extract at 200 μl per well into 96-well, U-bottomed microtitre plates and serially diluted. To obtain serial double dilution's on the 96-well plate, 100 μl of complete culture media was added to all wells except the third column. To the third column, 200 μl of the working extract solution was added using a multipipette dispenser. Approximately, 100 μl of extract was transferred from the third, through, to the last column. To the first column, 100 μl of nonparasitized blood of 2% haematocrit was added and this acted as a control, while to other wells 100 μl of erythrocytes suspension in culture medium with 2% haematocrit and 2%parasitemia were added. The plates were placed in a candle jar and gassed for 2 min, then placed in a 37°C incubator for 48 h.

At the end of the incubation, the set-up was carefully resuspended and 25 μl removed for analysis of pLDH activity. To each well containing 100 μl of complete media, 15 μl of the blood suspension and 20 μl of a mixture of NBT and phenazine ethosulphate (20 : 1) was added. The plates were kept away from light for 10 min to develop. The pLDH reduced tetrazolium to blue formazan, which was detected by measuring the optical density (OD) of each well by a 7520 plate reader (Bio-Tek Instruments, Cambridge Technology Inc., MA, USA) at 650 nm.

A column of nonparasitized erythrocytes at 2% haematocrit served as a blank while wells with parasitized erythrocytes with no drug were set and represented normal parasite growth. Chloroquine diphosphate was used as a positive control.

Data analysis

The percentage parasite growth inhibition (GI) was determined using the formula:

$$\mathrm{GI} = 100 - \left(\frac{\mathrm{OD}_t}{\mathrm{OD}_c} \times 100\right),$$

where OD_t is the optical density at each concentration and OD_c the optical density of the negative control.

Prism Graph pad Version 3 (Graph pad software, San Diego, CA, USA) computer program was used to analyse the data. Percentage survival at each concentration was determined by expressing absorbance at each concentration as a percentage of the absorbance corresponding to wells with normal growth. EC_{50} was obtained as an inverse of the log concentration of the extracts.

Results

The chloroform extracts of *Maesa lanceolata* and *Rhus natalensis* had the highest activity against *P. falciparum* (IC_{50} = 1.6 μg ml^{-1}) and (IC_{50} = 1.8 μg ml^{-1}) respectively. Other extracts with significant activity were the chloroform extract of *Bothriocline longipes* (IC_{50} = 3.6 μg ml^{-1}) petroleum ether extract of *Trimmeria bakeri* (IC_{50} = 3.9 μg ml^{-1}), ethanol extract of *Indigofera emerginella* (IC_{50} = 5.8 μg ml^{-1}), petroleum ether extract of *Toddalia asiatica* (IC_{50} = 6.6 μg ml^{-1}), ethanolic extract of *R. natalensis* (IC_{50} = 6.6 μg ml^{-1}) and the chloroform extract of *Conyza* sp (IC_{50} = 9.1 μg ml^{-1}) (Table 2).

The ethanolic extract of *M. lanceolata, Conyza* sp., *T. bakeri* and *Vernonia lasiopus*, petroleum ether extract of *Lantana trifolia,* chloroform extract of *T. asiatica*, petroleum and chloroform extracts of *I. emerginella* showed moderate antiplasmodial activity (10–50 μg ml^{-1}). The rest of the extracts had no antiplasmodial activity with inhibitory concentrations above 50 μg ml^{-1}.

Discussion and conclusion

This study showed that extracts of several medicinal plants used in traditional treatment of against malaria/fever in western Uganda, had antiplasmodial activity against the blood stage of *P. falciparum*. A number of other studies

Table 2 The antiplasmodial activity of extracts from selected plants used in traditional treatment of malaria

	IC_{50} (μg ml^{-1})		
Species	PE	CL	ET
Maesa lanceolata	>50.0	1.6	11.4
Erythrophleum suaveolens	>50.0	>50.0	>50.0
Conyza Sp.	>50.0	9.1	20.5
Rhus natalensis	>50.0	1.8	6.6
Lantana trifolia	13.2	>50.0	>50.0
Toddalia asiatica	6.6	22.4	>50.0
Bothriocline longipes	>50.0	3.7	50.0
Trimmeria bakeri	3.9	>50.0	33.2
Indigofera emerginella	38.0	25.3	5.8
Vernonia lasiopus	43.9	>50.0	>50.0

PE, petroleum ether extract; CL, chloroform extract; ET, ethanolic extract.
The results are mean values of experiments carried out in triplicate.

have reported antiplasmodial activity of extracts from medicinal plants of Uganda (Ogwal-Okeng, 1998; Waako *et al.*, 2005a, 2007). The activity of extracts could be improved on further fractionation to yield extracts with better antiplasmodail activity. Lack of *in vitro* antiplasmodial activity in some plants may have been due to the methods of extraction used, which were not the same as those used in traditional practice. Furthermore, the specific traditional preparation techniques often employ mixtures of plants (Adjanohoun *et al.*, 1993; Tabuti *et al.*, 2003). This means that further approaches can still be followed in investigation of these plants.

Our study relied on an *in vitro* system, it is known, however, that some compounds that show *in vitro* activity may not possess *in vivo* activity due to pharmacokinetic and immunological factors (Waako *et al.*, 2005a,b). This calls for further studies to investigate the efficacy and safety of these plants in an *in vivo* system.

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