



Antiplasmodial and cytotoxic activities of medicinal plants traditionally used in the village of Kiohima, Uganda

Damien Lacroix^a, Soizic Prado^{a,*}, Dennis Kamoga^b, John Kasenene^b, Jane Namukobe^c, Sabrina Krief^d, Vincent Dumontet^e, Elisabeth Mouray^a, Bernard Bodo^a, Florence Brunois^f

^a Molécules de Communication et Adaptation des Micro-Organismes, FRE 3206 CNRS/MNHN, Muséum National d'Histoire Naturelle, 57 rue Cuvier, 75005 Paris, France

^b Department of Botany, P.O. Box 7062, Makerere University, Kampala, Uganda

^c Chemistry Department, Makerere University, Box 7062, Kampala, Uganda

^d Eco-Anthropologie et Ethnobiologie, MNHN-CNRS, 43 rue Buffon, 75005 Paris, France

^e Centre de recherche de Gif, Institut de Chimie des Substances Naturelles, CNRS – Avenue de la Terrasse 91198 Gif-sur-Yvette, France

^f Laboratoire d'Anthropologie Sociale, Collège de France, 52 rue Cardinal Lemoine, 75005 Paris, France

ARTICLE INFO

Article history:

Received 23 April 2010

Received in revised form 26 October 2010

Accepted 3 November 2010

Available online 11 November 2010

Keywords:

Traditional medicine

Uganda

Malaria

Plasmodium falciparum

ABSTRACT

Aim of the study: In Uganda, malaria is the most common disease and Ugandan people largely rely on traditional medicine. In this context, we carried out an ethnobotanical study on the Kiohima village, located close to the Kibale National Park in South-Western Uganda and investigated *in vitro* the antiplasmodial and cytotoxic activities of selected medicinal plants.

Materials and methods: Seventy-five plants-using adults (men and women) were interviewed to find out their plant use. From these information, 48 plants used in traditional medicine were identified and according to their reported uses and to bibliographic data, several parts of 28 plants (leaves, barks, roots), were selected and collected for biological evaluations. These samples were dried, extracted with ethyl acetate and the crude extracts were assayed for *in vitro* antiplasmodial and cytotoxic activities at 10 µg/mL.

Results: One third of the screened plants showed a significant antiplasmodial activity with inhibition greater than 50% at 10 µg/mL.

Conclusion: These results may indicate a possible explanation of the use of some medicinal plant against malaria in the village of Kiohima and have also allowed to highlight a plant with potent antimalarial activity: *Citropsis articulata* root barks.

© 2010 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

Malaria, the major parasitic infection in many tropical and sub-tropical regions, is still the largest burden on public health of developing countries (Arrow et al., 2004). Annually, 600 million new infections occur worldwide, and at least 1 million of these infections are fatal. Despite the existence of an array of available antimalarial drugs, the control of this ancient infection is increasingly threatened by the emergence of drug-resistant strains of the malaria parasite, *Plasmodium* (Craft, 2008). In view of these developing resistances, most of these drugs, when used in monotherapy are rapidly losing their efficacy. The re-emergence of malaria as a public health problem calls for the discovery and development of new antimalarial drugs. The use of medicinal plants, such as the South American “quinine bark” *Cinchona succirubra* and the Chinese “Ginghao” *Artemisia annua*, have a long tradition of use in the treatment of malaria (Schwikkard and Van Heerden, 2002). Identifi-

fication of the major active metabolites of these plants, quinine and artemisinin, gave rise to the development of numerous antimalarial drugs. Nowadays, 11 of the 15 drugs included in the WHO malaria therapeutic scheme are natural products or derivatives (Bourdy et al., 2008).

In Uganda, malaria is the most common disease and accounts for 25–40% of out-patient attendance at health facilities, and 20% of in-patient admissions. It also kills at least 9 to 14% of all in-patients. Children aged five years and below as well as pregnant women are the most affected. In Uganda, more than 200 children die daily from this disease (Tabuti, 2008) and Ugandan people largely rely on traditional medicine (Ssegawa and Kasenene, 2007). In this context, we carried out an ethnobotanical study in the Kiohima village, located close to the Kibale National Park in South-Western Uganda in order to better know the plants used and their active components. Thus, in the course of this investigation, 75 plants-using adults (men and women) were interviewed to find out their plant use. From this information, 48 specimens of plants were collected and according to their reported uses and to bibliographic data, several parts (leaves, barks, roots) of 28 plants were selected for biological evaluations. These samples were dried, extracted with ethyl acetate

* Corresponding author. Tel.: +33 01 40 79 31 19; fax: +33 01 40 79 31 35.
E-mail address: sprado@mnhn.fr (S. Prado).

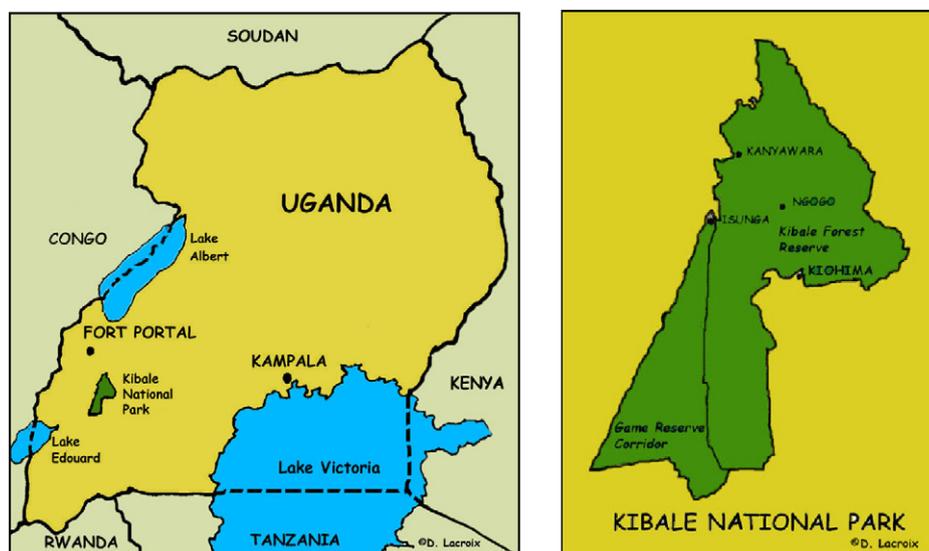


Fig. 1. Maps of Uganda showing the location of the area of study.

and the crude extracts were assayed for *in vitro* antiplasmodial and cytotoxic activities at 10 $\mu\text{g}/\text{mL}$.

2. Materials and methods

2.1. Field program

This ethnopharmacological study was conducted by two of us (DL and FB) in September–October 2008 for 50 days in the village of Kiohima. This village, located close to the Kibale National Park in the South-West part of Uganda ($0^{\circ}26' \text{N}$; $30^{\circ}23' \text{E}$) is about one thousand meters above the sea level and is surrounded by a typical rainforest (Fig. 1). The Kiohima population numbers about 3000 people and is composed of two groups of people. The Batooros, the local population of Kiohima who speak Rutooro and the Bakigas, a migrant population originating from the region of Kabale (South-east of Uganda, about 300 km south of Kibale National Park), and who speak Rukiga. This latter group of population, mainly constituted of farmers and foresters, moved to Kiohima 40 years ago because of the reduction of the lands and forests of their original dwelling. During their stay the investigators have lived daily with the native people, learnt the sociological organization of the village so as to choose the best interpreters and source of information. Because of the coexistence of two different populations speaking two languages (Rutooro and Rukiga), and also with their different traditional cultures, we asked successively for two translators in the course of the discussion with the villagers. This coexistence prompts us to compare the knowledge of each group within the village population and also to find whether they have a common use of plants. Information was obtained from 75 adults of the two populations (41% Batooros and 59% Bakigas) who have been taught in the use of plants for healing or treating different diseases such as malaria, angina and diarrhea. Among them, three types of people could be distinguished, the so-called “witch-doctors” (3 interviewed including 2 Batooros), the so-called “good-doctors” (4 interviewed including 3 Batooros) and the villagers (68 interviewed). The first type, “the witch-doctors”, is able to treat bewitched peoples. We have observed that they generally ask the spirit of the dead to know which plant can be used to treat the patient which corroborate with previous observation of et al. Tabuti et al. (2003). The second type, the “good-doctors” or “herbalists”, have the knowledge of the medicinal plants and are able to treat usual organic diseases. The third type, composed of

all the villagers, but mainly women with children, have some limited knowledge on medicinal plants. The survey was conducted by way of discussions with people from different socio-cultural groups (protestant and catholic, young and old, men and women, chairman and pastor, farmers and forestry worker). First, they were queried on their origin, then their general knowledge of the biodiversity (animals and plants) and finally, they were interviewed for their specific affinity toward plants, animal and for their medicinal or hunting knowledges. To better understand the transmission of such knowledges (either from family tradition, inside a group of one of the two populations or between the two groups) the actual source of the knowledge was also investigated.

2.2. Plant collection and identification

The collecting campaign was realized within the framework of the “Collaborative phytochemical research using selected flora and fauna species in Uganda” (Memorandum of Cooperation between the Centre National de la Recherche Scientifique (CNRS), the Muséum National d’Histoire Naturelle (MNHN), Makerere University (MU) and the Uganda Wildlife Authority (UWA). It was conducted inside the Kibale National Park by DK and JK (MU) and in the village of Kiohima by DL and FB (MNHN).

For each selected and collected plant, herbarium specimens were prepared and kept at the Herbarium of the Makerere University (MU) at Kampala (Uganda) and where they were identified by two of us (DK and JK).

2.3. Plant extraction

The collected plants were dried at the Makerere University Biological Field Station, Fort Portal and grounded at the Makerere University, Kampala. Extraction of different parts of plants was achieved following a standardized protocol at the ICSN. Thus, the powdered parts of plants (30–100 g) were extracted at MU by JN using ethyl acetate (200 mL, 3 times at room temperature) or at ICSN with a Dionex automated extractor using ethyl acetate (four runs each, 100 bar, 40°C). The extracts were concentrated *in vacuo* at 40°C to yield crude extracts. A 100 mg aliquot of each crude extract was dissolved in AcOEt-MeOH (1:1), filtered on a polyamide cartridge and evaporated to dryness. The extracts were then dissolved in DMSO and tested *in vitro* at a concentration of 10 $\mu\text{g}/\text{mL}$ for antiplasmodial and cytotoxic activities.

Table 1
Medicinal plants used in Kiohima area by Batooros (Rutooro name) and Bakigas (Rukiga name).

Scientific name	Family	Rutooro name	Rukiga name	Human use	Part	Preparation
<i>Acanthus pubescens</i> (Thomson ex Oliv.) Engl.	Acanthaceae	Etojo	NU	Worms/denutrition	Roots	Decoction
<i>Albizia coriaria</i> Welw. ex Oliv.	Fabaceae	Omusesa	NU	Diarrhea	Bark	Infusion
<i>Albizia grandibracteata</i> Taub.	Fabaceae	Omorongo	Omushobeya	Back pain, malaria	Leaves	Decoction
<i>Antiaris toxicaria</i> Lesch.	Moraceae	Mumaka	Mukede	Worms	Bark chewed	Bark chewed
<i>Bidens pilosa</i> L.	Asteraceae	NU	Enyabalashana	Cicatrisation	Leaves Whole plant	Leaves juice Infusion
<i>Blighia unijugata</i> Baker	Sapindaceae	Omwataibare	NU	Worms	Leaves	Infusion
<i>Bridelia micrantha</i> (Hochst.) Baill.	Phyllanthaceae	Ombarakasa	NU	Construction	Leaves	Infusion
<i>Brugmansia candida</i> Pers.	Solanaceae	Omoboyu	NU	Psychosis	Leaves	Infusion
<i>Capsicum frutescens</i> L.	Solanaceae	Kambrali	NU	Food condiment	Fruit	Added to food
<i>Celtis africana</i> burm.f.	Cannabaceae	Ektemazi	NU	Witchcraft, worms, syphilis	Bark	Decoction
<i>Citropsis articulata</i> (Spreng.) Swingle and M.Kellerm.	Rutaceae	Katimboro	NU	Men sexual weakness	Root barks	Root barks powdered and mixed with water
<i>Clerodendron ugandense</i> Prain	Lamiaceae	Eksekeseke	Echeche	Stomach pain, laxative	Leaves	Leaves juice mixed with water
<i>Coffea arabica</i> L.	Rubiaceae	NU	Omwani	Aerophagia, cough	Leaves	Decoction
<i>Crassocephalum vitellinum</i> (Benth.) S.Moore	Asteraceae	Echomoro	NU	Vaginal dryness	Entire plant	Decoction
<i>Cupressus lusitanica</i> Mill.	Cupressaceae	Kapurusi	NU	Magic plant to keep away the bad spirits	Leaves	Smoke of burned leaves
<i>Dracaena fragrans</i> (L.) Ker Gawl.	Asparagaceae	Omugorogoro	NU	Ear pain Denutrition	Leaves Bark	Rainwater from the leaves Decoction Leaves juice
<i>Diospyros abyssinica</i> (Hiern) F.White	Ebenaceae	Omohoko	NU	Tropical ulcer	Leaves	Leaves juice
<i>Eucalyptus</i> sp.	Myrtaceae	Kaletusi	NU	Cough	Leaves	Decoction
<i>Erythrina abyssinica</i> Lam. ex DC.	Fabaceae	Omoko	Echuko	Cough, malaria	Bark	Decoction
<i>Fagara macrophylla</i> (Oliv.) Engl.	Rutaceae	Omubakampungo	NU	Abortive	Entire Plant	Decoction
<i>Fagaropsis angolensis</i> (Engl.) Dale	Rutaceae	NU	Omumara	Stomach pain	Roots	Decoction
<i>Flacourtia indica</i> (Burm.f.) Merr.	Salicaceae	NU	Omunyeye	Snake bite	Bark	Decoction
<i>Funtumia latifolia</i> (Stapf) Stapf	Apocynaceae	Omongogwekende	Ekinyamagosi	Musical instrument	Fruit	Attached to the ankle in marriage
<i>Galinsoga ciliata</i> (Raf.) S.F.Blake	Asteraceae	NU	Kahunika	Increase milk production	Fruit	Decoction with black salt
<i>Harrisonia abyssinica</i> Oliv.	Rutaceae	Omurarike	NU	Vegetable	Arial parts	Cooked in water
<i>Hoslundia opposita</i> Vahl.	Lamiaceae	Orutataima	NU	Worms, diarrhea	Leaves	Leaves juice mixed with water
<i>Ipomoea batatas</i> L.	Convolvulaceae	Akarandura	NU	Cough fever	Flowers leaves	Infusion leaves juice
<i>Leonotis nepetifolia</i> (L.) R.Br.	Asteraceae	Echumuchumu	NU	Vegetable	Rhizomes	Cooked in water
<i>Mangifera indica</i> L.	Anacardiaceae	Omuyembe	NU	Diuretic	Leaves	Leaves juice
<i>Markhamia lutea</i> (Benth.) K.Schum.	Bignoniaceae	Omusambya	NU	Cough	Leaves	Leaves decoction
<i>Monodora myristica</i> (Gaertn.) Dunal	Annonaceae	Omuho	Omugema	Malaria, asthma, syphilis	Leaves	Leaves juice mixed with water
<i>Nicotiana tabacum</i> L.	Solanaceae	NU	Echimani	Witchcraft	Bark	Decoction
<i>Parinari excelsa</i> Sabine	Chrysobalanaceae	Ibura	Omubura	Snake bite	Leaves	Leaves juice applied on bite
<i>Phoenix reclinata</i> Jacq.	Arecaceae	Ekindo	NU	Witchcraft	Bark	Decoction
<i>Physalis minima</i> L.	Solanaceae	Entutu	NU	Mat fabrication	Leaves	Leaves plaited
<i>Phytolacca dodecandra</i> L'Her.	Phytolaccaceae	Omohoko	NU	Cough	Leaves	Infusion
<i>Plantago palmata</i> Hook.f.	Plantaginaceae	NU	Embatabata	Constipation	Leaves	Juice of four leaves
<i>Plumeria rubra</i> L.	Apocynaceae	NU	NU	Fracture	Roots	Dried roots powdered and mixed with butter as a plaster
				Protection against thunder	Entire plant	Grown in front of the houses

Table 1 (Continued)

Scientific name	Family	Rutooro name	Rukiga name	Human use	Part	Preparation
<i>Pseudospondias microcarpa</i> (A.Rich.) Engl.	Anacardiaceae	Babigamba	Nimpyata	Witchcraft	Fruit	Keeped in the pocket to get power
<i>Psidium guajava</i> L.	Myrtaceae	Ipera	Ipera	Aerophagia, laxative	Leaves	Infusion
<i>Rubus pinnatus</i> Willd.	Rosaceae	Encherere	NU	Cold	Leaves	Chew the leaves with black salt
<i>Solanum betaceum</i> Cav.	Solanaceae	Akatunda	NU	Cough	Leaves	Leaves juice mixed with salt
<i>Spathodea campanulata</i> P. Beauv.	Bignoniaceae	Omunyara	NU	Vaginal dryness	Bark	Decoction
<i>Tagetes minuta</i> L.	Asteraceae	Mokazi murofa	Mokazi murofa	Headache, articulation, pain, epilepsy	Leaves and flowers	Leaves and flowers rubbed on the head
<i>Teclea nobilis</i> Delile	Rutaceae	Omuzo	NU	Spears Malaria	Branch Aerial part	Decoction
<i>Vernonia amygdalina</i> Delile	Asteraceae	Ekiivilisi	Omuvilisi	Malaria worms	Leaves roots	Decoction
<i>Vernonia ugandensis</i> S. Moore	Asteraceae	Ektokotoko	Echuganyanja	Against women sterility	Leaves	Infusion
<i>Warburgia ugandensis</i> Sprague	Canellaceae	Omuharumi	Omwiha	Fever, gonorrhoea, syphilis, worm	Bark	Bark decoction

NU: not used.

2.4. Biological evaluations

2.4.1. Antiplasmodial assay

The antiplasmodial activity was evaluated against the chloroquine-resistant FcB1/Colombia strain of *Plasmodium falciparum*. The test was performed using the method of Desjardins et al. (1979). Extracts and pure compounds were diluted in DMSO at 20 µg/mL with culture medium to the expected concentrations in 96-well microplates. Asynchronous parasite cultures were then added (1% parasitemia and 1% final hematocrite), and plates were maintained for 24 h at 37 °C in a candle jar. [³H]hypoxanthine (0.5 µCi) was subsequently added to each well, and parasites were maintained for an additional 24 h. After a cycle of freezing and thawing, the cells were harvested from each well onto glass fiber filters, and the dried filters were counted in a scintillation counter. The growth inhibition for each well was determined by comparison of the radioactivity incorporated into the treated culture with that in the control culture maintained on the same plate. Quinine was used as positive control. Experiments were performed in triplicate.

2.4.2. Cytotoxicity on mammalian cells and selectivity index

The human tumor cell lines KB (mouth epidermoid carcinoma) and the human diploid embryonic lung cells MRC-5 were seeded into 96-well microplates at 2000 cells per well. The cytotoxicity assays were performed according to published procedures (Pierre et al., 1991; Tempete et al., 1995). Taxotere® was used as a control compound. Experiments were performed in triplicate.

3. Results and discussion

A total of 48 medicinal plants belonging to 28 families of angiosperms used traditionally by one or the two populations of villagers of Kiohima for medicinal purpose were recorded in an ethnobotanical investigation, identified and characterized for their traditional utilization (Table 1). From these, 28 plants were selected, from their medicinal uses or bibliographic data, to evaluate their biological activity against *Plasmodium falciparum* (Table 2). From a general point of view, it was observed that a larger number of plants were used by the Batooros than by the Bakigas. Indeed as shown in Table 1, a total of 40 medicinal plants are employed by

the Batooros whereas 21 plants are used by the Bakigas. This can be explained by the fact that Batooros being the ancestral local population have a better knowledge of the local plants as compared to the Bakigas who are newly arrived population in the Kiohima area. However, the Bakiga population who came from a forest region has a large medicinal plant knowledge, especially the ones working as sawyers in the forest. This can explain the specific use of some medicinal plants by the Bakigas and not by the Batooros such as *Bidens pilosa*, *Fagaropsis angolensis*, *Coffea arabica*. Nevertheless, the majority of plants used by the Bakiga are common with the ones used by the Batooros, which suggested that the Bakigas have rapidly adapted to their new environment. From the 48 identified plant parts, eight (16.7%) are used to treat symptoms related to malaria (fever, cough, shaking, muscle pain). Among these plants, some are very often used in other African countries against malaria. It is notably the case of *Erythrina abyssinica* which is one of the most widely used in African traditional medicine for the treatment of malaria and microbial infections (Yenesew et al., 2004). The aromatic essential oil of *Tagetes minuta*, is also used against malaria, but mainly for its insecticidal activity (Hamill et al., 2000; Batish et al., 2007; Lehman et al., 2007). Leaves of *Vernonia amygdalina* are commonly employed to treat malaria, not only in Kiohima village but more generally in Uganda (Tabuti, 2004) as well as in other East-African countries such as Congo and Zimbabwe (Tona et al., 2004; Mbatchi et al., 2006).

Our investigation also underlined the common use of leaves of *Markhamia lutea* against malaria in the village of Kiohima. Surprisingly, the anti-parasitic activity of the leaves has not yet been investigated until our recent work on the chemistry and Antitrypanosomal activity of cycloartane triterpenoids (Lacroix et al., 2009). The same observation was made for the use of the leaves of *Albizia grandibracteata* against malaria, which was also uncovered in this study. Interestingly, relatively few examples were found in the literature for this use (Krief et al., 2005). The aerial part of *Teclea nobilis* are also used for the treatment of malaria, like in East-Africa (Kuria et al., 2001; Bussmann et al., 2006; Kareru et al., 2007). The leaves of *Warburgia ugandensis* are used against fever in the Kiohima village. This observation corroborates with the previous ones by M. Adriaens in the Rwenzori region (Adriaens, 2004); the stem bark of this plant has been widely used in East African ethnomedicine for the treatment of many diseases such as stomach-

Table 2
Antiplasmodial and cytotoxic activities of selected parts of plants.

Scientific name	Plant part	% Inhibition <i>P. falciparum</i> FcB1 10 µg/mL	% Inhibition KB cells 10 µg/mL	% Inhibition MRC5 cells 10 µg/mL
<i>Acanthus pubescens</i>	Leaves	28.3 ± 3.2	7.0 ± 5.0	5.0 ± 7.0
<i>Albizia grandibracteata</i>	Leaves	22.0 ± 4.6	21.0 ± 10.0	18.0 ± 8.0
<i>Antiaris toxicaria</i>	Bark	36.4 ± 4.1	96.0 ± 0.0	85.0 ± 1.0
<i>Bidens pilosa</i>	Aerial parts	45.8 ± 6.2	24.0 ± 17.0	14.0 ± 2.0
<i>Blighia unijugata</i>	Leaves	2.3 ± 1.0	4.0 ± 3.0	9.0 ± 3.0
<i>Celtis africana</i>	Bark	37.5 ± 12.9	22.0 ± 18.0	17.0 ± 5.0
<i>Citropsis articulata</i>	Roots bark	77.0 ± 13.0	43.0 ± 5.0	12.0 ± 9.0
<i>Coffea arabica</i>	Leaves	39.7 ± 12.4	20.0 ± 4.0	4.0 ± 3.0
<i>Crassocephalum vitellinum</i>	Leaves	40.6 ± 9.2	26.0 ± 2.0	6.0 ± 4.0
<i>Dracaena fragrans</i>	Leaves	34.76 ± 7.0	44.0 ± 1.0	3.0 ± 3.0
<i>Diospyros abyssinica</i>	Leaves	51.3 ± 8.8	1.0 ± 2.0	6.0 ± 5.0
<i>Erythrina abyssinica</i>	Bark	83.6 ± 16.0	89.0 ± 2.0	75.0 ± 7.0
<i>Fagaropsis angolensis</i>	Bark	42.3 ± 11.0	16.0 ± 4.0	27.0 ± 12.0
<i>Funtumia latifolia</i>	Leaves	68.1 ± 9.0	57.0 ± 2.0	22.0 ± 4.0
	Bark	29.3 ± 11.2	13.0 ± 10.0	4.0 ± 3.0
<i>Harrisonia abyssinica</i>	Leaves	35.7 ± 17.8	32.0 ± 3.0	13.0 ± 8.0
<i>Hoslundia opposita</i>	Leaves	66.2 ± 7.8	61.0 ± 4.0	41.0 ± 5.0
	Flower	48.4 ± 6.0	67.0 ± 7.0	64.0 ± 3.0
<i>Leonotis nepetifolia</i>	Leaves	27.0 ± 0.4	36.0 ± 10.0	17.0 ± 8.0
<i>Markhamia lutea</i>	Leaves	70.8 ± 11.0	4.0 ± 5.0	3.0 ± 0.0
<i>Monodora myristica</i>	Bark	42.1 ± 18.0	26.0 ± 7.0	11.0 ± 6.0
<i>Parinari excelsa</i>	Bark	66.5 ± 9.9	54.0 ± 1.0	21.0 ± 9.0
<i>Phytolacca dodecandra</i>	Leaves	25.8 ± 7.2	29.0 ± 4.0	17.0 ± 5.0
<i>Pseudospondias microcarpa</i>	Leaves	30.8 ± 15.2	6.0 ± 9.0	03.0 ± 4.0
	Bark	24.5 ± 10.8	7.0 ± 6.0	07.0 ± 6.0
<i>Rubus pinnatus</i>	Leaves	20.8 ± 13.4	38.0 ± 9.0	3.0 ± 4.0
<i>Spathodea campanulata</i>	Bark	28.9 ± 6.2	27.0 ± 3.0	6.0 ± 5.0
<i>Tagetes minuta</i>	Leaves	61.0 ± 1.8	NT	NT
<i>Teclea nobilis</i>	Bark	54.7 ± 5.7	36.0 ± 2.0	22.0 ± 2.0
<i>Vernonia amygdalina</i>	Leaves	97.8 ± 0.2	99.0 ± 1.0	83.0 ± 2.0
<i>Warburgia ugandensis</i>	Leaves	26.5 ± 10.5	45.0 ± 5.0	33.0 ± 7.0
Chloroquine	–	98.1 ± 0.3	NT	NT
Taxotere	–	NT	93.0 ± 0.3	NT

NT: not tested.

ache, constipation, toothache, cough, fever, muscle pains, weak joints and general body pains (Wube et al., 2005). Moreover, its use against malaria has been observed in Kenya (Irungu et al., 2007).

Surprisingly, some plants commonly used to treat malaria in other East-African countries were selected and collected during the present study, but mainly for other purposes (diseases) than malaria or symptoms associated with. This is the case for *Hoslundia opposita* which is used against malaria in Tanzania (Achenbach and Frey, 1992; Gessler et al., 1994), as well as *Harrisonia abyssinica* (Chhabra et al., 1993; Muthaura et al., 2007).

Some other plants, devoid of malaria employment in Uganda, were collected in the course of this investigation because they have been described in traditional preparations against malaria in other East-African countries. Nevertheless, the use of these plants seems to be less common than those described above. It is notably the case for *Leonotis nepetifolia* (Tabuti, 2008), described as a plant used against malaria in Uganda or *Parinari excelsa* used by traditional healers in Tanzania (Gessler et al., 1995) as well as *Phytolacca dodecandra* (Wilson and Gebre, 1979; Tadege et al., 2005).

The second part of this study deals with the biological evaluation of the selected plants used in the Kiohima village. Thus, 31 several parts (leaves, barks, wood), of the 28 selected plants were extracted with ethyl acetate and the resulting crude extracts were then assayed for both their antiplasmodial activity against *P. falciparum* FcB1 strain and for their cytotoxicity against KB and MRC5 cells (Table 2). Among the 31 parts of plant extracts, around a third (32%) presented more than 50% of inhibition on the growth of *P. falciparum* FcB1 at 10 µg/mL. It is thus interesting to note that the plants used traditionally in Kiohima village against malaria presented a good activity against *P. falciparum* except for the leaves of *A. grandibracteata* and *W. ugandensis* which had relatively modest activity (22 and 27% inhibition at 10 µg/mL, respectively). Nevertheless these results were of interest because no cytotoxicity was

measured at this concentration. However, cytotoxicity on KB cell line of the bark of *W. ugandensis* has been highlighted (Xu et al., 2009). The most active extract was that of the leaves of *V. amygdalina*, but in this case a strong cytotoxicity was observed on the two cell lines (KB and MRC5) at 10 µg/mL and this may account for the antiplasmodial activity against *P. falciparum*. Vernangulides A and B, two sesquiterpene lactones, have been previously isolated from this plant and described to be responsible for the antiplasmodial activity (IC₅₀ of 2 µg/mL), nevertheless their selectivity index is very low (from 2 to 5.3) (Pedersen et al., 2009). The leaves extract of *Markhamia lutea* showed 71% inhibition of *P. falciparum* at 10 µg/mL, a significant activity was assigned to some of the cycloartane triterpenes which showed antiparasitical activity not only against *P. falciparum*, but also *Leishmania donovani* and *Trypanosoma brucei* (Lacroix et al., 2009).

Some plants also traditionally used against malaria as previously reported in the literature showed a good activity in our assays against *P. falciparum* FcB1. In particular, the bark extract of *P. excelsa* and the leaves of *H. opposita* displayed respectively 66.5 and 66.2% inhibition at 10 µg/mL. However, these parts of plants were also significantly cytotoxic against the two cell lines tested (average of 50% at 10 µg/mL).

It is noteworthy that one plant used in traditional medicine for other purposes than malaria has a significant antiplasmodial activity. Thus, the EtOAc extract of the roots of *Citropsis articulata* a plant well known and used in Uganda as a sexual stimulant, showed at 10 µg/mL, a *P. falciparum* inhibition growth (77%) associated with a moderate cytotoxicity (43% on MRC5 cell line).

4. Conclusion

Among the 28 plants selected from this ethnopharmacological investigation, collected in Kiohima village and evaluated for their

inhibition on *P. falciparum* growth, one third of them showed a significant antiplasmodial activity with inhibition greater than 50% at 10 µg/mL and with a low cytotoxicity for most of them. These results might help to explain the use of these medicinal plants against malaria in the village of Kiohima in Uganda. It is noteworthy that most of the plants used to treat malaria in Kiohima area, are also used for the same purpose in other districts or countries in East-Africa. Indeed, this study has highlighted a promising plant, *C. articulata* for further antimalarial investigations and the determination of its active constituents. Nevertheless, this medicinal knowledge is frail and could be lost if the population is no longer in contact with the surrounding biodiversity. In the last few decades, the creation of National Parks to preserve the biodiversity is often associated with a restriction of its accesses by local people. Thus as the population may gradually lose the contact with the medicinal wild plants and by the same way their knowledge, this issue may question the merits of this interdiction for the sake of biodiversity. It would be thus very important to identify and select the really efficient wild plants in order to cultivate them in medicinal plants gardens, which would be then both plants and traditional knowledges conservatories.

Acknowledgements

The French “Ministère de la Recherche” is acknowledged for a fellowship for one of us (DL), the “Yves Rocher” company for its generous contribution to this project. The National Academy of Medicine is also gratefully acknowledged for the attribution of the Price Hugues Gounelle de Pontavel to DL. We wish to thank Didier Sergent for extraction of part of plants with a Dionex automated extractor. We also wish to thank Pr. Grellier for the access to its parasitological laboratory and Geneviève Aubert for cytotoxicity measurements. This work was done within the framework of the “Collaborative phytochemical research using selected flora and fauna species in Uganda” (CNRS, MNHN, MU and UWA) program and the authors are grateful to the Uganda Wildlife Authority (UWA) and to the Uganda National Council for Science and Technology (UNCST) for authorization to conduct scientific research in Uganda. DL and FB are very grateful to the local traditional healers and villagers that have been very welcoming.

References

- Achenbach, H., Frey, D., 1992. Constituents of tropical medicinal plants. Cycloartanes and other terpenoids and Phenylpropanoids from *Monocyclanthus vignei*. *Phytochemistry* 31, 4263–4274.
- Adriaens, M., 2004. Family Medicinal Plant Gardens in the Rwenzori Region. Mariam Press, Uganda.
- Arrow, K.J., Panosian, C., Gelband, H., 2004. Saving Lives. Buying Time. Economics of Malaria Drugs in an Age of Resistance. The National Academies Press, Washington.
- Batish, D.R., Arora, K., Singh, H.P., Kohli, R.K., 2007. Potential utilization of dried powder of *Tagetes minuta* as a natural herbicide for managing rice weeds. *Crop Protection* 26, 566–571.
- Bourdy, G., Willcox, M.L., Ginsburg, H., Rasoanaivo, P., Graz, B., Deharo, E., 2008. Ethnopharmacology and malaria: new hypothetical leads or old efficient anti-malarials? *International Journal for Parasitology* 38, 33–41.
- Bussmann, R.W., Gilbreath, G.G., Solio, J., Lutera, M., Lutuluo, R., Kunguru, K., Wood, N., Mathenge, S.G., 2006. Plant use of the Maasai of Sekenani Valley, Maasai Mara, Kenya. *Journal of Ethnobiology and Ethnomedicine* 2, 1–7.
- Chhabra, S.C., Mahunnah, R.L.A., Mshiu, E.N., 1993. Plants used in traditional medicine in Eastern Tanzania. Angiosperms (Sapotaceae to Zingiberaceae). *Journal of Ethnopharmacology* 39, 83–103.
- Craft, J.C., 2008. Challenges facing drug development for malaria. *Current Opinion in Microbiology* 11, 428–433.
- Desjardins, R.E., Candfield, C.J., Haynes, J.D., Chulay, J.D., 1979. Quantitative assessment of antimalarial activity in vitro by a semiautomated microdilution technique. *Antimicrobial Agent and Chemotherapy* 16, 710–718.
- Gessler, M.C., Nkunya, M.H.H., Mwasumbi, L.B., Heinrich, M., Tanner, M., 1994. Screening tanzanian medicinal plants for antimalarial activity. *Acta Tropica* 56, 65–77.
- Gessler, M.C., Tanner, M., Chollet, J., Nkunya, M.H.H., Heinrich, M., 1995. Tanzanian medicinal plants used traditionally for the treatment of malaria—in vivo antimalarial and in vitro cytotoxic activities. *Phytotherapy Research* 9, 504–508.
- Hamill, F.A., Apio, S., Mubiru, N.K., Mosango, M., Bukunya-Ziraba, R., Maganyi, O.W., Soejarto, D.D., 2000. Traditional herbal drugs of southern Uganda. *Journal of Ethnopharmacology* 70, 281–300.
- Irungu, B.N., Rukunga, G.M., Mungai, G.M., Muthaura, C.N., 2007. In vitro antiplasmodial and cytotoxicity activities of 14 medicinal plants from Kenya. *South African Journal of Botany* 73, 204–207.
- Kareru, P.G., Kenji, G.M., Gachanja, A.N., Keriko, J.M., Mungai, G., 2007. Traditional medicines among the Embu and Mbeere peoples of Kenya. *African Journal of Ecology* 4, 75–86.
- Krief, S., Thoison, O., Sevenet, T., Wrangham, R.W., Lavaud, C., 2005. Triterpenoid saponin anthranilates from *Albizia grandibracteata* leaves ingested by primates in Uganda. *Journal of Natural Products* 68, 897–903.
- Kuria, K.A.M., De Coster, S., Muriuki, G., Masengo, W., Kibwage, I., Hoogmartens, J., Laekeman, G.M., 2001. Antimalarial activity of *Ajuga remota* Benth (Labiatae) and *Caesalpinia volkensii* Harms (Caesalpinaceae): in vitro confirmation of ethnopharmacological use. *Journal of Ethnopharmacology* 74, 141–148.
- Lacroix, D., Prado, S., Deville, A., Krief, S., Dumontet, V., Kasenene, J., Mouray, E., Bories, C., Bodo, B., 2009. Hydroperoxy-cycloartane triterpenoids from the leaves of *Markhamia lutea*, a plant ingested by wild chimpanzees. *Phytochemistry* 70, 1239–1245.
- Lehman, A.D., Dunkel, F.V., Klein, R.A., Ouattara, S., Diallo, D., Gamby, K.T., N'Diaye, M., 2007. Insect management products from Malian traditional medicine—establishing systematic criteria for their identification. *Journal of Ethnopharmacology* 110, 235–249.
- Mbatshi, S.F., Mbatshi, B., Banzouzi, J.T., Bansimba, T., Ntandou, G.F.N., Ouamba, J.M., Berry, A., Benoit-Vical, F., 2006. In vitro antiplasmodial activity of 18 plants used in Congo Brazzaville traditional medicine. *Journal of Ethnopharmacology* 104, 168–174.
- Muthaura, C.N., Rukunga, G.M., Chhabra, S.C., Omar, S.A., Guantai, A.N., Gathirwa, J.W., Tolo, F.M., Mwitari, P.G., Keter, L.K., Kirira, P.G., Kimani, C.W., Mungai, G.M., Njagi, E.N.M., 2007. Antimalarial activity of some plants traditionally used in treatment of malaria in Kwale district of Kenya. *Journal of Ethnopharmacology* 112, 545–551.
- Pedersen, M.M., Chukwujekwu, J.C., Lategan, C.A., van Staden, J., Smith, P.J., Staerk, D., 2009. Antimalarial sesquiterpene lactones from *Distephanus angulifolius*. *Phytochemistry* 70, 601–607.
- Pierre, A., Kraus-Berthier, L., Atassi, G., Cros, S., Poupon, M.F., Lavielle, G., Berlion, M., Bizzari, J.P., 1991. Preclinical antitumor activity of a Vinca alkaloid derivative, S 12363. *Cancer Research* 51, 2312–2318.
- Schwikkard, S., Van Heerden, F.R., 2002. Antimalarial activity of plant metabolites. *Natural Products Report* 19, 675–692.
- Ssegawa, P., Kasenene, J.M., 2007. Medicinal plant diversity and uses in the Sango bay area, Southern Uganda. *Journal of Ethnopharmacology* 113, 521–540.
- Tabuti, J.R.S., 2004. The traditional medicine practitioners (TMPs) and attitudes of the rural community of Bulamogi County (Uganda) towards traditional medicine: preliminary findings. *African Journal of Ecology* 42, 40–41.
- Tabuti, J.R.S., 2008. Herbal medicines used in the treatment of malaria in Budiope country, Uganda. *Journal of Ethnopharmacology* 116, 33–42.
- Tabuti, J.R.S., Dhillion, S.S., Lye, K.A., 2003. Traditional medicine in Bulamogi county, Uganda: its practitioners, users and viability. *Journal of Ethnopharmacology* 85, 119–129.
- Tadeg, H., Mohammed, E., Asres, K., Gebre-Mariam, T., 2005. Antimicrobial activities of some selected traditional Ethiopian medicinal plants used in the treatment of skin disorders. *Journal of Ethnopharmacology* 100, 168–175.
- Tempete, C., Werner, G.H., Favre, F., Rojas, A., Langlois, N., 1995. In vitro cytostatic activity of 9-demethoxyprothramycin B. *European Journal of Medicinal Chemistry* 30, 647–650.
- Tona, L., Cimanga, R.K., Mesia, K., Musuamba, C.T., De Bruyne, T., Apers, S., Hernans, N., Van Miert, S., Pieters, L., Totte, J., Vlietinck, A.J., 2004. In vitro antiplasmodial activity of extracts and fractions from seven medicinal plants used in the Democratic Republic of Congo. *Journal of Ethnopharmacology* 93, 27–32.
- Wilson, R.T., Gebre, M.W., 1979. Medicine and magic in Central Tigre: a contribution to the ethnobotany of the Ethiopian plateau. *Economic Botany* 33, 29–34.
- Wube, A.A., Bucar, F., Gibbons, S., Asres, K., 2005. Sesquiterpenes from *Warburgia ugandensis* and their antimycobacterial activity. *Phytochemistry* 66, 2309–2315.
- Xu, M., Litaudon, M., Krief, S., Martin, M.T., Kasenene, J., Kiremire, B., Dumontet, V., Gueritte, F., 2009. Ugandensis A, a new drimane-type sesquiterpenoid from *Warburgia ugandensis*. *Molecules* 14, 3844–3850.
- Yenesew, A., Induli, M., Derese, S., Midiwo, J.O., Heydenreich, M., Peter, M.G., Akala, H., Wangui, J., Liyala, P., Waters, N.C., 2004. Anti-plasmodial flavonoids from the stem bark of *Erythrina abyssinica*. *Phytochemistry* 65, 3029–3032.