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A search for natural bioactive compounds in Bolivia through a multidisciplinary approach Part I. Evaluation of the antimalarial activity of plants used by the Chacobo Indians

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Abstract

Thirty extracts of plants traditionally used by the Chacobos, a native community living in the Amazonian part of Bolivia, were screened in vitro and/or in vivo for antimalarial activity. Two of the four species designated as antimalarial, *Geissospermum laeve* and *Maquira coriacea*, displayed rather good activity, corroborating their traditional uses. However, they did show a rather high toxicity in vivo. Among twelve species used to cure symptoms relevant to malaria, five showed good activity: *Apuleia leiocarpa*, *Bauhinia guianensis*, *Nectandra cuspidata*, *Sparattanthelium amazonum*, *Tanaecium jaroba*. Two species, *Qualea paraensis* and *Sclerolobium* aff. *guianense*, used to treat scabies, showed interesting antimalarial activity in vivo; three other species (*Iryanthera laevis*, *Prunus amplifolia*, *Pterocarpus* aff. *amazonum*) used for various medicinal purposes, apparently not related with a *Plasmodium* infection, also showed antimalarial activity. Finally, one species (*Derris amazonica*) used as a piscicide displayed good in vitro activity, in the same way as one Annonaceae, *Guatteria* aff. *schomburgkiana*, used for construction purposes.

Keywords: *Plasmodium*; Antimalarial agents; Traditional medicine; Bolivia; Medicinal plants; Chacobo

1. Introduction

The endemic region of malaria in Bolivia covers an area of 821 346 km^2, i.e. 75% of the country's

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land surface. More than three million people are exposed to this disease, and a recent epidemiological study (Ministerio de Desarrollo Humano, 1996) showed a spectacular rise of cases: from 9774 in 1981 to 46 911 in 1995. This deterioration is concentrated in the Amazonian areas, where the vector *Anopheles darlingi* and two *Plasmodium* species, *P. falciparum*, responsible for the most severe cases of malaria (7.2% of the cases), and *P. vivax* (92.8% of the cases) coexist. Under Amazonian climatic conditions both vector and parasite find an environment for persistent transmission all year round.

The Chacobos are a Bolivian Amazonian ethnic group, of almost seven hundred persons, living in high-ground forest between Yata and Benicito in Beni (Fig. 1). They are exposed to malaria and have developed various plant-based remedies to treat this disease and to alleviate the symptoms associated with it, such as, fever, digestive troubles, splenomegaly and headache. Our purpose was to evaluate the relevance of these preparations, and of other non-malaria remedies selected on ethnopharmacological-chemotaxonomic bases.

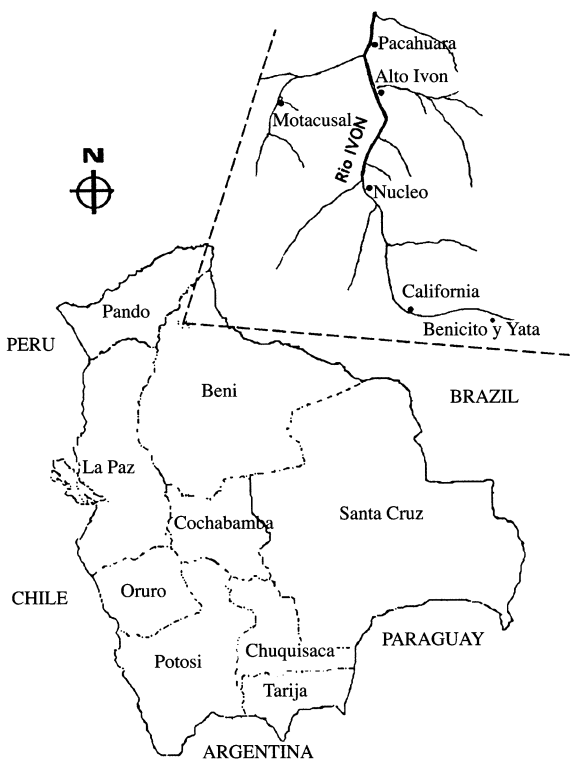

Fig. 1. Map of Bolivia and location of the Chacobo communities, between Benicito and Yata in the Department of Beni.

2. Materials and methods

2.1. Ethnobotanical survey

The ethnobotanical survey was undertaken with the agreement of the communities involved. Two different methodologies were utilized. A permanent plot of one hectare in a high-ground forest was established. All trees, lianas, and palms ≥ 10 cm diameter at breast height (dbh) were tagged, and at least four herbarium samples were collected for each tree. The data collected included height, dbh, Chacobo and/or other local names, and traditional uses. Also, species were collected in different ecological sites with the help of informants willing to participate. Voucher herbarium specimens were collected for each plant designated by the informant as useful, and the Chacobo name of the plant was recorded, as well as its other names, and uses.

Voucher specimens were deposited in the Herbario Nacional de Bolivia in La Paz. A first determination of the identity of the specimens was performed by the ethnobotanists of the team with the help of specialists from the herbarium. Duplicate specimens were sent to specialists relevant to the plant family of interest.

2.2. Selection of plants

A total of 156 different species were indicated by the Chacobo to be used to cure or alleviate various diseases and symptoms (Bergeron, 1992; Bergeron et al., 1997). Of this number, 30 species were selected for biological evaluation. The details of their traditional use are presented in Table 1.

2.3. Treatment of plants samples

All parts of the plant (20–25 g) selected for biological assay were ground, submitted to a maceration process with ethanol-water (70–30%) for

48 h at 25°C and protected from sunlight. The aqueous-ethanolic solution obtained was evaporated under vacuum and the residue directly assayed against *Plasmodium*.

Table 1
Traditional uses of species selected for antimalarial investigation

Species (family), vernacular name, voucher number	Traditional Chacobo use (Bergeron, 1992; Bergeron et al., 1997	Preparation, posology (Bergeron, 1992; Bergeron et al., 1997)	Part of plant tested
Alibertia edulis (L. Rich.) A. Rich (Rubiaceae) Tusa SB 073	Digestive problems	Cut a ripe fruit in 4 parts, and boil in one liter of water for 15 min. Drink when thirsty	Fruit
Amburana cearensis (Fr. Allem.) A.C. Smith (Fabaceae) Quixono SB 467	Alleviate headache	The stembark is crushed and applied directly on the head	Stembark
Apeiba tibourbou Aublet (Tiliaceae) Moxoqüe SB 744	Alleviate headache	The leaves are crushed in water and then applied in the form of a paste	Stembark
Apuleia leiocarpa (J. Vogel) Macbr. (Fabaceae) Mani SB 765	Fever	One handful of grated stembark is boiled in 2 liter of water, until reduced to 1 liter. Drink when thirsty, until fever calms down	Stembark
Arrabidaea sp. (Bignoniaceae) Nishirajoxo SB 307	Digestive problems, diarrhea	Drink one glassful of sap exuding from the liana, 3 times a day	Stembark
Bauhinia guianensis Aublet (Fabaceae) Nishipara SB 869	Diarrhea, stomachache	Boil in 2 liter of water one handful of grated stembark, until reduced to 1 liter. Drink half-a-cup 3× per day	Stembark
Cedrela fissilis Vell. (Meliaceae) Itsa SB 483	Malaria, vomiting, diarrhea	One handfull of grated trunkbark is boiled in 2 liter of water until reduced to 1 liter. Drink 3 cups a day, until the symptoms disappear	Stembark
Coutarea hexandra (Jacq.) Schum. (Rubiaceae) Jihuimoca SB 33	Malaria, vomiting, fever	One handfull of grated trunkbark is boiled in 2 liter of water until reduced to 1 liter. Drink half-a-cup 3× a day, until the symptoms disappear	Stembark
Derris amazonica Killip (Fabaceae) Capëhitsa SB 880	Fish poison	Fresh roots and fresh stems are crushed and thrown into a pond	Stems and leaves
Derris sp. (Fabaceae) Jënëhaxahua SB 688	Fish poison	Fresh roots and fresh stems are crushed and thrown into a pond	Stembark
Duguetia spixiana C. Martius (Annonaceae) Xahuisi SB 728	Construction		Stembark
Geissospermum laeve (Vellozo) Miers (Apocynaceae) Jihuimoca SB 701	Used to cure malaria, vomiting, liver pain	500 g of grated trunkbark is boiled for 15 min in 2 liters of water. Drink one cup, two times a day, until symptoms disappear	Stembark
Guatteria aff. *schomburgkiana* Mart. (Annonaceae) Ahuabaca SB 15	Construction		Stembark
Iryanthera laevis Mkgf. (Myristicaceae) Mëquënobita SB 727	Lesions in the mouth	Apply the sap exuding from the trunkbark directly on the lesions	Stembark
Licania intrapetiolaris Spr. ex Hook. (Chrysobalanaceae) Pacachësti SB 175	Skin problems	The stembark is crushed, burned, and then applied on the skin as a poultice	Stembark

Table 1 (*Continued*).

Species (family), vernacular name, voucher number	Traditional Chacobo use (Bergeron, 1992; Bergeron et al., 1997	Preparation, posology (Bergeron, 1992; Bergeron et al., 1997)	Part of plant tested
Maquira coriacea (Karst.) C.C. Berg (Moraceae) Huápamo SB 691	Malaria, fever	One handful of grated trunkbark and one handful of grated rootbark are boiled in 2 liters of water, until reduced to one liter. Drink half-a-cup, 3 times a day until the symptoms disappear	Stembark
Mascagnia macrophylla Rusby (Malpighiaceae) Ascana SB 258	Diarrhea	Stembark juice is drunk 3 times a day to cure diarrhea	Stembark
Mussatia hyacinthina (Standl.) Sandw. (Bignoniaceae) Boa SB 726	Diarrhea and stomachache	One handful of leaves are prepared like a tea. Drink 1 full-cup 3 times a day	Leaves
Nectandra cuspidata Nees (Lauracaeae) Yobini SB 528	Stomachache	One handful of grated stembark is boiled in 2 liters of water, until reduced to 1 liter. Drink 3 cups a day, until the pain calms down	Stembark
Piper darienense C. DC. (Piperaceae) Nibosa SB 694	Used to alleviate tooth pain	Apply the crushed root directly on aching tooth, or chew the root	Roots
Prunus amplifolia Pilger (Rosaceae) Jihuirononopa SB 628	Used to alleviate pain caused by insect bite and pain as a result of rheumatism pain	The stembark is crushed in a paste which is applied to the painful area	Stembark
Pterocarpus cf. *amazonum* (Benth.) Amsh (Fabaceae) Capanahahuati SB 152	Used as cicatrizant of burns	The stembark is pulverized, applied on the skin and covered with a cloth	Stembark
Qualea paraensis Ducke (Vochysiaceae) Nihipëpëcho SB 164	Scabies	The stembark is boiled in water, and the water is used in the form of a bath	Stembark
Sclerolobium aff. *guianense* Benth. (Fabaceae) Xabahuasicano SB 381	Scabies	The stembark is boiled in water, and the water is used in the form of a bath	Stembark
Serjania sp. (Sapindaceae) Carihanahaxa SB 692	Fish poison	Fresh roots and fresh stems are crushed and thrown into a pond	Stems
Sparattanthelium amazonum Mart. (Hernandiaceae) Nishitsanona SB 819	Stomachache, vomiting, diarrhea	One handful of grated stembark is boiled in 2 liters of water, until reduced to 1 liter. Drink when thirsty, until diarrhea and vomiting stop	Stembark
Tabebuia serratifolia (Vahl) Nicholson (Bignoniaceae) Nisho SB 626	Used to cure fever	One handful of grated stembark is boiled in 2 liters of water, until reduced to 1 liter Drink when thirsty, until fever calms down	Stembark
Tanaecium jaroba Sw. (Bignoniaceae) Nishiratëquëya SB 266	Diarrhea, vomiting	One handful of grated stembark is boiled in 2 liters of water, until reduced to 1 liter. Drink when thirsty, until diarrhea and vomiting stop	Stembark
	Inflammation, or swelling after a trauma	Apply grated stembark as a poultice on the swelling. Change it until complete cure	
Vataireopsis sp. (Fabaceae) Canamashia SB 473	Scabies	The ashes of the stembark are applied on the skin	Stembark
Xylopia cuspidata Diels (Annonaceae) Xahui-xahuira SB 729	Construction		Stembark

2.4. Biological tests

The in vivo antimalarial activity of plant extracts were determined by the classical 4-day suppressive test (Peters and Robinson, 1992) against *Plasmodium berghei* NK65 and *P. vinckei* 279BY strains.

Swiss male mice (Charles River, France), of a mean body weight 20 ± 2 g, were infected with 10^8 parasitized cells in 0.9% saline, on day 0. Groups of five mice were treated intraperitoneally from day 0–3 with increasing doses (100 mg/kg up to 1 g/kg) of plant extracts. The malaria-suppressive effect was estimated on day 4, by examination of Giemsa-stained thin blood smears made from the tails of the treated mice, compared with a control group of mice treated only with the solvent of the extract (saline, dimethyl sulfoxide or Tween). Each test also included a positive control with a *Cinchona calisaya* Wedd. (Rubiaceae) bark extract (from 50 to 250 mg/kg in saline), collected by one of us, in the Department of Sud Yungas (Bolivia). Chloroquine (Sigma-USA) was also used as standard (5 mg/kg). The stained thin blood smears were examined under $1000\times$ magnification, and the percentage of parasitized red blood cells was counted on at least 9000 red blood cells for each concentrations. Percent growth inhibition of the parasite was calculated by the following formula:

$$\frac{(\text{parasitaemia in control} - \text{parasitaemia with drugs})}{\text{parasitaemia in control}} \times 100 = \%\ \text{of inhibition}$$

For the in vitro tests, cultures of the Indo (chloroquine resistant) and F32-Tanzania (chloroquine sensitive) strains of *P. falciparum* kindly provided by Dr Fandeur T. (Pasteur Institute, Cayenne, France) were maintained according to the method of Trager and Jensen (1976), on glucose-enriched RPMI 1640 medium supplemented with 10% human serum at 37°C. A total of 50 μl of dimethyl sulfoxide (DMSO) were added to the plant extracts, which were dissolved in RPMI 1640 medium with the aid of mild sonication in a sonicleaner bath (Branson Ltd), and then diluted as required in culture medium.

The final DMSO concentration was never greater than 0.1%. One hundred and fifty microlitres of total culture medium with the diluted extract and the suspension of infected human red blood cells (0+ group, 5% haematocrit, 1% parasitaemia), were distributed into 96-well microtitre plates. All tests were performed in triplicate. After 24 h of incubation at 37°C in a candle jar incubator, the medium was replaced by fresh medium with the diluted extract, and incubation was continued for a further 48 h. On the 3rd day of the test, a blood smear was taken from each well and parasitaemia counted.

The parasitaemia for each well was obtained and the percentage inhibition of parasitaemia for each concentration of extract was calculated in relation with the control. IC_{50} values were determined graphically by plotting concentration versus percentage inhibition. Each test also included an untreated control with solvent and a positive control with a *Cinchona calisaya* Wedd. (Rubiaceae) bark extract. Chloroquine (Sigma, USA) was also used as control.

3. Results

The results of biological evaluation are presented in Table 2.

3.1. In vitro results

Twenty eight ethanolic plant extracts were evaluated in vitro using chloroquine-sensitive and chloroquine-resistant *P. falciparum* strains (F32 and Indo, respectively). It was considered that if the extracts displayed an IC_{50} less than 5 μg/ml, the antimalarial activity was very good; from 5 to 10 μg/ml the antimalarial activity was good; over 10 μg/ml the extract was considered inactive. The IC_{50} of *Cinchona calisaya* stembark extract used as the standard drug in these assays was 0.2 μg/ml against both strains. The IC_{50} of Chloroquine (excluded from Table 2) was 6.3 ng/ml for the F32 strain and 74 ng/ml for the Indo strain.

Three plant extracts (*Geissospermum laeve*, *Mascagnia macrophylla*, *Sparattanthelium amazonum*) showed very good antimalarial activity against both strains with IC_{50} values ranging from 1.7 to 3.1 μg/ml, comparable to the activity of

Table 2
Antimalarial activities of the selected species[a]

Voucher	Scientific name	Family	In vitro		In vivo			
			F32 IC_{50} μg/ml	Indo IC_{50} μg/ml	Dose mg/kg 4 days	*P. vinckei* % inhibition	Dose mg/kg 4 days	*P. berghei* % inhibition
LR 232	*Cinchona calisaya*	Rubiaceae	0.2	0.2			250	91
LR 232	*Cinchona calisaya*	Rubiaceae					100	41
LR 232	*Cinchona calisaya*	Rubiaceae					50	36
SB 765	*Apuleia leiocarpa*	Fabaceae	50	50			250	53
SB 765	*Apuleia leiocarpa*	Fabaceae					100	31
SB 869	*Bauhinia guianensis*	Fabaceae	>100	28	250	Toxic		
SB 869	*Bauhinia guianensis*	Fabaceae			50	84		
SB 880	*Derris amazonica*	Fabaceae	3.2	18				
SB 701	*Geissospermum laeve*	Apocynaceae	3.1	2	50	41	100	Toxic
SB 701	*Geissospermum laeve*	Apocynaceae					50	36
SB 015	*Guatteria aff schomburgkiana*	Annonaceae	19	4			>500	36
SB 727	*Iryanthera laevis*	Myristicaceae	40	40			100	59
SB 691	*Maquira coriacea*	Moraceae	50	9	250	Toxic	100	36
SB 691	*Maquira coriacea*	Moraceae			100	92		
SB 258	*Mascagnia macrophylla*	Malpighiaceae	1.7	2.2	472	58		
SB 528	*Nectandra cuspidata*	Lauraceae	>100	>100	250	83	250	61
SB 628	*Prunus amplifolia*	Rosaceae	>100	>100			250	66
SB 152	*Pterocarpus amazonum*	Fabaceae	>100	>100	250	98		
SB 164	*Qualea paraensis*	Vochysiaceae	>100	>100	250	Toxic		
SB 164	*Qualea paraensis*	Vochysiaceae			100	67		
SB 381	*Sclerolobium aff guianense*	Fabaceae	10	10	200	82		
SB 819	*Sparanttanthelium amazonum*	Hernandiaceae	2	2	250	57	250	Toxic
SB 266	*Tanaecium jaroba*	Bignoniaceae	20	20	250	78	250	70

[a] Toxic: death of more than half of the animals.

Artemisia annua and of some Simaroubaceae plants (O'Neill et al., 1985). One extract (*Guatteria* aff. *schomburgkiana*) showed very good activity (IC_{50} 4 μg/ml) on the Indo strain and was inactive on the F32 strain, while one extract (*Derris amazonica*) showed very good activity on the F32 strain and was inactive on the Indo strain. One extract (*Maquira coriacea*) displayed good activity on the Indo strain. These results indicate that six extracts displayed good antimalarial in vitro activity against the sensitive or against the resistant strains, representing 21% of the screened extracts. However, none of the extracts was as active as the bark extract of *Cinchona calisaya*.

3.2. In vivo results

Twenty eight ethanolic extracts were tested in the in vivo model of the rodent malaria *P. vinckei petteri* 279 BY and sixteen in the *P. berghei* NK65 model. The in vivo results were classified as follows: at the dose of 500 mg/kg/day, if the extract displayed a percent growth inhibition equal or greater than 50%, the antimalarial activity was considered moderate; at the dose of 250 mg/kg/day, if the percent growth inhibition was equal or greater than 50%, the antimalarial activity was considered good; at the dose of 100 mg/kg/day if the percent growth inhibition was equal to 50%,

the antimalarial activity was considered very good. The *Cinchona calisaya* bark extract, used as a positive standard for these assays displayed 91% inhibition at 250 mg/kg/day, while 5 mg/kg of chloroquine inhibited 100% of the parasite growth.

In the *P. vinckei* model, three plant extracts displayed very good activity (*Bauhinia guianensis*, *Maquira coriacea*, *Qualea paraensis*) and five plant extracts displayed good activity (*Nectandra cuspidata*, *Pterocarpus amazonum*, *Sclerolobium* aff. *guianense*, *Sparattanthelium amazonum*, *Tanaecium jaroba*).

In the *P. berghei* model, one plant extract displayed very good activity (*Iryanthera laevis*) and four plant extracts displayed good activity (*Nectandra cuspidata*, *Tanaecium jaroba*, *Apuleia leiocarpa*, *Prunus amplifolia*). It should be pointed out that only three species, *Nectandra cuspidata*, *Tanaecium jaroba*, and *Geissospermum laeve* exhibited good activity against both strains, the former also showed very good in vitro activity.

4. Discussion

Apuleia leiocarpa (J. Vogel) Macbr. (Fabaceae): at 250 mg/kg the percent growth inhibition reached 53% and fell to 31% at 100 mg/kg against *P. berghei*, demonstrating good antimalarial activity. The extract was inactive in vitro. *A. leiocarpa* has also been reported to have anti-inflammatory activity (Ruppelt et al., 1991). This observation was confirmed by Mitaine (1998) because in the petroleum ether fraction, β-amyrin, a triterpene with anti-inflammatory and sedative properties (Kweifio-Okai et al., 1994) was found. The use of this species by the Chacobos to reduce any kind of fevers appears to be justified by the presence of β-amyrin (though a complementary antipyretic test should be performed) and also by its antimalarial properties, thus, helping to reduce the fevers as a result of malaria attacks.

Research for the active antimalarial compounds is in progress, justified by the activity displayed in vivo.

Bauhinia guianensis Aublet (Fabaceae): the ethanolic stembark extract of this species was inactive against both Indo and F32 strains ($IC_{50} > 10$ μg/ml). At 50 mg/kg it showed very good activity against the *P. vinckei* strain (84%) but was toxic at 250 mg/kg. No other biological activities were reported for *Bauhinia guianensis*, but the strong in vivo antimalarial activity justifies further investigation of this species for active principle(s). This extract should also be tested at lower doses.

Derris amazonica Killip (Fabaceae): it was decided to investigate the antimalarial potential of *Derris* species, after it was shown (Sauvain, 1989), in work performed in French Guyana, that the genus *Tephrosia*, which is botanically close to *Derris*, displayed good activity against *Plasmodium*.

The extract of *Derris amazonica* showed stronger activity against the F32 strain (IC_{50} 3.2 μg/ml) than against the Indo (IC_{50} 18 μg/ml). The genera *Derris* and *Tephrosia* are known to contain rotenones and related compounds which are potent pesticides and piscicides (Moretti and Grenand, 1982), thus explaining their traditional use as 'fish poisons'. From a Brazilian species of *Derris*, *D. sericea*, Buckingham (1998) isolated prenylated chalcones, such as 2′-hydroxy-4′-prenyloxychalcone, which displayed moderate toxicity (LD_{50} of 275 mg/kg in mice by the intraperitoneal route). As indicated previously, chalcones and their derivatives are potentially active against malaria. Therefore, it is possible that the in vitro activity of *Derris amazonica* is related to the presence of chalcone compounds, but this hypothesis still has to be proven.

Geissospermum laeve (Vellozo) Miers (Apocynaceae): the stembark extract showed very good activity in vitro (IC_{50} 3.1 μg/ml against the F32 strain and 2 μg/ml against the Indo strain), but was inactive on *P. berghei*, and was highly toxic at 100 mg/kg. *G. laeve* is known to contain flavopereirin, a monoterpene indole alkaloid. Its derivative, 5,6-dihydroflavopereirin displayed a marked activity against *P. falciparum* in vitro at an IC_{50} of 3.02 μmoles (Wright et al., 1996). Further studies are required to confirm that the antimalarial activity detected is as a result of the presence of flavopereirin derivatives. The toxicity of the ethanolic stembark extract in mice presents some

contradiction with the famous antimalarial reputation of this species in the Amazonian region (Grenand et al., 1987). Therefore, it is worthwhile to investigate this species, in order to determine if the compounds responsible for the antimalarial activity are the same as those responsible for the toxicity in mice.

Guatteria aff. *schomburgkiana* Mart. (Annonaceae): the ethanolic stembark extract did not display any activity against the F32 strain (IC_{50} 19 μg/ml), but was active on the Indo strain (IC_{50} 4 μg/ml). This extract was inactive on *P. berghei*. Species belonging to the family Annonaceae are well known to contain acetogenins (Rupprecht et al., 1990) and isoquinolines (Leboeuf et al., 1982) with antimalarial properties (Sahpaz et al., 1994). Previously Cortés et al. (1984) isolated from the stembark of this species two 7-methylaporphines (guattescine type) characteristic of the genus *Guatteria*. Though this extract did not show antimalarial activity in vivo, it should be interesting to verify if the in vitro activity is as a result of this type of compound.

Iryanthera laevis Mkgf. (Myristicaceae): the ethanolic stembark extract of this species was inactive in vitro (IC_{50} 40 μg/ml against both strains), while against P. *berghei* it displayed very good antimalarial activity, reducing the parasitaemia by 59% at 100 mg/kg. Previous studies showed the presence of chalcones in the stembark, confirming the results of Garzon et al. (1987). Chalcones and derivatives are potentially active against malaria (Chen et al., 1994; Li et al., 1995) which have been shown to inhibit glutathione reductase (Elliott et al., 1992), a key enzyme whose inhibition increases the intra-erythrocytic level of H_2O_2, responsible for the oxidative stress leading to the death of *Plasmodium* (Ginsburg, 1990). The antimalarial activity observed could be explained by the presence of these molecules in *I. laevis* stembark. Further investigations are planned.

Maquira coriacea (Karst.) C.C. Berg (Moraceae): the stembark extract of this species was active in vitro against the chloroquine-resistant strain (IC_{50} 9 μg/ml) and inactive against the sensitive F32 strain (IC_{50} 50 μg/ml). The extract decreased dramatically (92% at 100 mg/kg) the parasitaemia of *P. vinckei*-infected mice and was inactive against *P. berghei*. At 250 mg/kg the extract showed high toxicity, and killed all the treated mice. Using *Maquira* species from Peru, *M. calophylla* (Poepp. et Endl.) C.C. Berg., Rovinski and Sneden (1984), Rovinski et al. (1987) isolated various cytotoxic compounds, including maquiroside A, cannogenol and some furanocoumarins. The good activity shown in vivo corroborates the traditional use of this plant by the Chacobos, although it seems to be a toxic species which should be used with caution. These results make this species an interesting candidate for further investigation, if it can be established that the active compounds are not the same as those responsible for the toxic effects. This extract should also be tested at lower doses.

Mascagnia macrophylla Rusby (Malpighiaceae): the stembark extract showed very good activity in vitro (IC_{50} 1.7 μg/ml against the F32 strain and 2.2 μg/ml against the Indo strain). It displayed moderate antimalarial activity against *P. vinckei* (58% at 472 mg/kg). Therefore, *Mascagnia macrophylla* is worth investigating for its antimalarial principle(s).

Nectandra cuspidata Nees (Lauraceae): the stembark extract produced very good in vivo antimalarial activity (83% against *P. vinckei*, and 61% against *P. berghei*, at 250 mg/kg). No activity was observed in vitro. Böhlke et al. (1996), isolated costaricine, a bisbenzylisoquinoline alkaloid, from *N. salicifolia* trunk bark which was active against *P. falciparum* chloroquine-sensitive and chloroquine-resistant strains in vitro. These results justify further biological and chemical investigation of the stembark extract of *N. cuspidata*, if the extract still demonstrates antimalarial activity at lower doses.

Prunus amplifolia Pilger (Rosaceae): the ethanolic stembark extract was inactive in vitro ($IC_{50} >$ 100 μg/ml) while the in vivo antimalarial activity was good (66% inhibition at 250 mg/kg on *P. berghei*).

This result and the large number of biological activities encountered for the genus *Prunus* make this species worthwhile for further investigation.

Pterocarpus aff. *amazonum* (Benth.) Amsh. (Fabaceae): at 250 mg/kg, the ethanolic stembark

extract showed good antimalarial activity (98%) against *P. vinckei*. It was inactive against both *Plasmodium* strains in vitro. Due to the in vivo antimalarial activity against *P. vinckei*, and because some *Pterocarpus* species such as *P. rohrii* Vahl are highly valued antimalarial remedies in the Amazon region (Schultes and Raffauf, 1990), *P.* aff. *amazonum* is considered to be a species worthwhile for further investigation.

Qualea paraensis Ducke (Vochysiaceae): the ethanolic stembark extract of *Q. paraensis* was inactive in vitro. In vivo, it reduced substantially the parasitaemia of *P. vinckei*-infected mice (67% at 100 mg/kg).

Other studies (Brandao et al., 1985), showed that various extracts of *Q. grandifolia* C. Martius tested against *P. berghei*-infected mice (100 mg/kg, intragastrically) did not show any antimalarial activity.

Sclerolobium aff. *guianense* Benth. (Fabaceae): the ethanolic stembark extract displayed good antimalarial activity against *P. vinckei* (82% at 200 mg/kg), but was inactive in vitro. No antimalarial properties have been indicated for members of this genus, and no chemical investigations have been performed on *Sclerolobium* aff. *guianense*. It is suggested that, if the response level is still good at lower doses, the stembark of this species is worthy for further biological and chemical investigations.

Sparattanthelium amazonum Mart. (Hernandiaceae): the stembark extract of *Sparattanthelium amazonum* showed very good activity in vitro (IC_{50} 2 μg/ml against both strains). It was active (52%) in vivo at 250 mg/kg against *P. vinckei*, but was toxic at 250 mg/kg against *P. berghei*-infected mice. Therefore, the traditional Chacobo uses of this species and of related *Sparattanthelium* sp. (Boom, 1987) against diarrhea, stomach ache and vomiting, might be linked to its antiparasitic activity. This species is worth investigating for its antimalarial principle(s).

Tanaecium jaroba Sw. (Bignoniaceae): the stembark extract of this species displayed good activity in vivo against both *Plasmodium* strains (around 70% at 250 mg/kg, nearly equaling the activity of *Cinchona calisaya* bark extract). In vitro it was inactive. These results should be followed by further investigation, first of all testing the extract at lower doses in order to confirm the observed activity, and if still active, identifying the compound(s) responsible for the antimalarial activity.

5. Discussion and conclusion

Of the 30 species tested in vivo against *P. vinckei* and/or *P. berghei*, eleven (36%) displayed an interesting in vivo antimalarial activity, and 21% of the extracts tested in vitro proved to be active. This discrepancy is a common aspect of the antimalarial studies as the metabolic processes are not present in culture. Of the four species designated by the Chacobos to be used against malaria, two showed good antimalarial activity, in vitro and in vivo, therefore, corroborating the traditional Chacobo use. Among the twelve species used traditionally against complaints possibly related to malaria attack, five extracts appear to justify further antimalarial investigation. One extract displayed only good in vitro activity, namely *Mascagnia macrophylla*, while three extracts were active only in vivo. One extract, *Sparattanthelium amazonum*, displayed both in vivo and in vitro activity, making this species a priority for further investigation. Two species used against scabies, in the form of a bath, showed interesting antimalarial in vivo activity, in the same way as four other species used for various medicinal purposes, apparently not related with a *Plasmodium* infection.

Finally, one species (*Derris amazonica*) used as a piscicide displayed good in vitro activity, in a similar manner as one Annonaceae, *Guatteria* aff. *schomburgkiana*, used for construction purposes.

These results however, must be considered as preliminary in nature and need further confirmation. First of all, some doubts will always remain about the accuracy of the uses of the species traditionally employed by the Chacobo Indians for treating malaria, or any kind of disease. In the traditional Chacobo medicine, when somebody gets sick, a first treatment with one particular plant is administered; if the person does not get well after few days under this treatment, another plant/treatment is administered until the symptoms disappears.

Therefore, it is sometimes hard to deduce as which plant that is really effective against the symptoms, and this is the reason why it seems more appropriate to link those species used for a specific disorder and to test them sequentially, in order to try to correlate this information with the traditional use. Secondly, the efficacy of medicinal plants, and especially those for the treatment of malaria, might also be related with the immunizing potential of the people treated. In cases of *Plasmodium* infections, it is well-known that people living in endemic areas develop a certain immunity against this parasite. Therefore, it is possible that a low antimalarial activity observed in our non-immune mice is sufficient for treating immunized people, or it can also be assumed that some species act as immuno-modulators.

Finally, the lack of in vivo activity of some extracts might be as a result of the route of administration (intraperitoneal) performed in our tests, interfering with parameters such as absorption rate, delivery process, metabolism process, or the absence of another compound that should be present in the alimentary diet (Ginsburg et al., 1988). Indeed, the methodology should follow the traditional oral route of administration, because passage through the peritoneal area shunts the tremendous alterations caused by the digestive tract, and therefore avoids structural alterations, possibly essential for the antimalarial activity. It should also be pointed out however, that the intraperitoneal route provides some assurances concerning good reproducibility, in terms of the amount of delivered drug, and is the easiest route of administration in screening tests.

The tests performed in the laboratory do not mirror the conditions for the administration of the medicinal plants in situ namely, using dried material instead of fresh, using ethanolic solvents instead of water, using different routes of administration, testing the extracts in vitro or in vivo on mice, and using *Plasmodium* species which are not the ones encountered in humans, though *P. berghei* has been described as a *P. vivax*-like species (Landau and Boulard, 1978).

However, we consider that the selection of species based on ethnopharmacological investigation, coupled with chemotaxonomic-biological data, and followed by biological tests, is a very fruitful method to assess the possible antimalarial activity of plants, eventually leading to the development of new antimalarial compounds.

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References

Bergeron, S., 1992. Approche Préliminaire de la Connaissance du Système de Classification Botanique Chacobo. Editions Institut Français des Etudes Andines, Lima, Peru.

Bergeron, S., Ortiz, J., Ortiz, B., Soria, C., 1997. El uso de las Plantas por los Chacobos (Alto Ivón, Beni, Bolivia). Editions IBIS Dinamarca, La Paz, Bolivia.

Böhlke, M., Guinaudeau, H., Angerhofer, C.K., Wongpanich, V., Soejarto, D.D., Farnsworth, N.R., et al., 1996. Costaricine, a new antiplasmodial bisbenzylisoquinoline alkaloid from *Nectandra salicifolia* trunk bark. J. Nat. Prod. 59, 576–580.

Boom, B., 1987. Ethnobotany of the Chacobo Indians, Beni, Bolivia. Adv. Econ. Botany 4, 1–68.

Brandao, M., Botelho, M., Krettli, E., 1985. Antimalarial experimental chemotherapy using natural products. Cien. Cultura 37, 1152–1163.

Buckingham, J., 1998. Dictionary of Natural Products on CD-ROM, Version 6.2.2. Chapman & Hall, London.

Chen, M., Theander, T.G., Christensen, S.B., Hviid, L., Zhai, L., Kharazmi, A., 1994. Licochalcone A, a new antimalarial agent, inhibits in vitro growth of the human malaria parasite *Plasmodium falciparum* and protects mice from *Plasmodium yoelii* infection. Antimicrob. Agents Chemother. 38, 1470–1475.

Cortés, D., Ramahatta, A., Dadoun, H., Cavé, A., 1984. La Bélémine, intermédiaire biogénétique potentiel des méthyl-

7 aporphines. Compte-Rendus Hebdomadaires Séances l'Acad. Sci. 299, 311–314.

Elliott, A.J., Scheiber, S.A., Thomas, C., Pardini, R.S., 1992. Inhibition of glutathione reductase by flavonoids. A structure-activity study. Biochem. Pharmacol. 44, 1603–1608.

Garzon, L., Cuca, L.E., Martinez, J.C., Yoshida, M., Gottlieb, O.R., 1987. Flavonolignoid from the fruit of *Iryanthera laevis*. Phytochemistry 26, 2835–2837.

Ginsburg, H., Landau, I., Baccam, D., 1988. Effect of cholesterol-rich diet on the susceptibility of rodent malarial parasites to chloroquine chemotherapy. Life Sci. 42, 7–10.

Ginsburg, H., 1990. Some reflexions concerning host erythrocyte-malarial parasite interrelationships. Blood cells 16, 225–235.

Grenand, P., Moretti, C., Jacquemin, H., 1987. Pharmacopées Traditionelles en Guyane. Editions ORSTOM, Paris, France.

Kweifio-Okai, G., De Munk, F., Rumble, B.A., Macrides, T.A., Cropley, M., 1994. Antiarthritic mechanisms of amyrin triterpenes. Res. Commun. Mol. Pathol. Pharmacol. 85, 45–55.

Landau, I., Boulard, Y., 1978. Life Cycles and Morphology. Rodent Malaria. Academic Press, pp. 53–84.

Leboeuf, M., Cavé, A., Bhaumik, P.K., Mukherjee, B., Mukherjee, R., 1982. The phytochemistry of the Annonaceae. Phytochemistry 21, 2783–2813.

Li, R., Kenyon, G.L., Cohen, F.E., Chen, X., Gong, B., Dominguez, J.N., et al., 1995. In vitro antimalarial activity of chalcones and their derivatives. J. Med. Chem. 38, 5031–5037.

Ministerio de Desarrollo Humano, Secretaria Nacional de Salud, Dirección Nacional de Vigilancia y Control de Enfermedades y Riesgos. Departamento Nacional de Control de Vectores, 1996. Informe de Actividades del Programa de Vigilancia y Control de la Malaria. Secretaria Nacional de Salud Edition, La Paz, Bolivia.

Mitaine, C., 1998. personal communication. Laboratoire de Pharmacognosie UPRESA 6013 CNRS, Centre de Recherche en Physico-chimie et Biotechnologies, Moulin de la Housse-BP 1039-51687 Reims cedex 2-France.

Moretti, C., Grenand, P., 1982. Les Nivrées ou plantes ichtyotoxiques de la Guyane Française. J. Ethnopharmacol. 6, 139–160.

O'Neill, M., Bray, D.H., Phillipson, J.D., Warhust, D.C., 1985. Plants as source of antimalarial drugs. Part I. In vitro test method for the evaluation of crude extracts from plants. Planta Med. 61, 394–398.

Peters, W., Robinson, B.L., 1992. The chemotherapy of rodent malaria. XLVII. Studies on pyronaridine and other Mannich base antimalarials. Ann. Trop. Med. Parasitol. 86, 455–465.

Rovinski, J.M., Sneden, A.T., 1984. Furanocoumarins from *Maquira calophylla*. J. Nat. Prod. 47, 557.

Rovinski, J.M., Tewalt, G.L., Sneden, A.T., 1987. Maquiroside A, a new cytotoxic cardiac glycoside from *Maquira calophylla*. J. Nat. Prod. 50, 211–216.

Ruppelt, B.M., Pereira, E.F., Gonçalves, L.C., Pereira, N.A., 1991. Pharmacological screening of plants recommended by folk medicine as anti-snake venom. Analgesic and anti-inflammatory activities. Mem. Inst. Oswaldo Cruz 86, 203–205.

Rupprecht, J.K., Hui, Y.H., McLaughin, J.L., 1990. Annonaceous acetogenins: a review. J. Nat. Prod. 53, 237–278.

Sahpaz, S., Bories, C., Loiseau, P.M., Cortes, D., Hocquemiller, R., Laurens, A., et al., 1994. Cytotoxic and antiparasitic activity from *Annona senegalensis* seeds. Planta Med. 60, 538–540.

Sauvain, M., 1989. Etudes de Plantes Antiparasitaires du Plateau des Guyanes en Amazonie: Antipaludiques et Antileishmaniens. Doctorat de l'Université de Paris Sud, Châtenay Malabry, France.

Schultes, R.E., Raffauf, R.F., 1990. The Healing Forest. Dioscorides Press, Portland, Oregon, USA.

Trager, W., Jensen, J.B., 1976. Human malaria in continuous culture. Science 193, 673–675.

Wright, C.W., Phillipson, J.D., Awe, S.O., Kirby, G.C., Warhust, D.C., Quetin-Leclercq, J., et al., 1996. Antimalarial activity of cryptolepine and some other anhydrium bases. Phytother. Res. 10, 361–363.