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ScienceDirect
Journal of Ethnopharmacology 112 (2007) 482–489
Journal of ETHNO-PHARMACOLOGY
www.elsevier.com/locate/jethpharm

Amazonian plants from Peru used by Quechua and Mestizo to treat malaria with evaluation of their activity

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Received 22 December 2006; received in revised form 10 April 2007; accepted 13 April 2007
Available online 24 April 2007

Abstract

Indigenous Quechua and Mestizo populations from distinct areas in Loreto, Peru, were interviewed about traditional medication for the treatment of malaria. An ethnographic survey concerning the native theory of illness aetiology in the specific case of malaria permitted the elaboration of an efficient ethnopharmacological enquiry. The survey took place on three main zones corresponding to villages on the Napo and the Pastaza rivers (for the Quechua), and in the surroundings of Iquitos (for the Mestizos) and led to the collection of 14 plants. Serial extractions in hexane, dichloromethane, and methanol were performed on the different parts of the plants collected. The extracts were then tested for antiplasmodial activity *in vitro*. Seven plants displayed antiplasmodial activity (IC_{50} from 2 to 25 μg/mL) and usually low cytotoxicity, indicating their antiplasmodial specificity. The results give scientific validation to the traditional medical knowledge of Quechua and Mestizo populations from Loreto and confirm a source of potentially active plants.

Keywords: Malaria; Loreto; *In vitro* screening; Quechua; Ethnomedicine; Peru

1. Introduction

Malaria remains one of the most important infectious diseases in the world affecting hundreds of millions of people and causing up to 3 million deaths annually (mostly children). Difficulties in getting access to treatment, for both economic and geographical reasons, result in increased mortality. Besides this fact, in many cases, drug effectiveness is lost due to parasite resistance. In this context, safe, effective and accessible new treatments have become a matter of priority. Plant remedies seem to be the most convenient solution because of their accessibility and diversity in tropical regions. This study focused on the province of Loreto, which is classified by the WHO as a grade III zone for its high frequency of chloroquine resistant malaria. An ethnopharmacological study was performed in three locations regarding three groups of population: Quechua of the Pastaza, Quechua of the Napo, (fieldwork period between 2003 and 2004), and the Mestizo population living around Iquitos (fieldwork achieved during 2005). These specific groups were chosen firstly, according to their ability to use medicinal plants and to transmit the knowledge. The Pastaza and Napo populations belong to the same cultural group (Quechua) and share a very similar set of medicinal knowledge, although they live in separate territories and ecosystems (see map). The Pastaza populations are also geographically isolated from medical infrastructures which reinforces the necessity for a better transmission of medicinal

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doi:10.1016/j.jep.2007.04.009

knowledge. Secondly, the comparison with Mestizo medicinal knowledge offers valuable insights into the persistence of traditional knowledge in a context where access to Occidental remedies is easier, given the proximity with urban medical centers. The core of ethnographic data about the indigenous conception of illnesses and therapy gives an anthropological background to the analysis of the impact of malaria among these Amazonian groups. We underline the necessity of taking into account the context-dependence of native aetiological theories.

2. Material and methods

2.1. Ethnographic study

2.1.1. Ethnographic context

The study of lowland Quechua (Kichwa) ethnomedicine in Peru took place within a complex and interwoven ecological, cultural and historico-political context. Although the Quechua people are a minority in Peru (3000 people approximately for the Pastaza river and an average of 8000 people living in the Peruvian Napo region/27.3 million inhabitants in Peru), they occupy a large part of the Amazonian lowlands. Two groups of Quechua were studied:

- The *Kichwa* (whose autodenomination is "*Napu runa*" which means "people of the Napo") from the high Napo river. Their villages are located around a Dominical mission called Angoteros at the border with Ecuador;
- The *Quechua* (also autodenominated "*Inga*") are located near the high Pastaza river, an area very distant from Iquitos, the province's capital. This ethnological group is a composite of various indigenous populations, among which are the Achuar from the Jivaroan linguistic family, and the Andoan, a nearly extinct group belonging to the Zaparoan linguistic family.

The traditional economy relies on slash-and-burn horticulture, hunting fishing and gathering wild foods. This process requires and contributes to the maintenance of large forest areas, both primary and secondary, in various stages of regeneration. Medicinal plants are normally cultivated and collected in primary forest. Nevertheless, Balee (Balee, 1989) and Black (Black, 1978) showed, in a study involving North American Indians, that plant species distribution in Amazonian rainforest (in the so-called primary forest) was mainly influenced by the entropic impact of human settlements at various stages of history. Indeed medicinal plants are both transplanted and protected by indigenous people (Kohn, 1992). Lowland Quechua settlements are organized according to the pattern of a "horizontal archipelago", which underlines the mobility of people and the dynamics of migrations and exchanges (Uzendosky, 2004). Such human mobility explains that similar medicinal plants can be found in primary and secondary forests and used both in Napo and Pastaza regions. The plants may have been transplanted for human necessity from one habitat to another in a historical process, according to the group's logic of migration. The phenomenon of migration also accounts for a shared knowledge of plants among Mestizos living in the surroundings of Iquitos and lowland Quechua populations (Fig. 1).

2.2. Indigenous theory of illness and therapy

2.2.1. Indigenous theory of illness aetiology

The Quechua representation of various types of physical dysfunctions will be described here from an ethnographic approach for a better understanding of the native conception of malaria. It should be kept in mind that the concept of illness is obviously the result of an interpretation, and as such, it entails cosmological representations of the world and specific beliefs about the nature of the human being. The elementary assumption shared by many animist Amazonian cultures is the attribution of a common subjectivity or a "soul" to every being, including some plant and animal entities. According to this idea, elementary categories structuring social life are used to organize, in conceptual terms, the relations between human beings and natural species (Descola, 2005). Sickness in many ways reveals a disruption affecting the individual's soul and its relation with otherness, humans or non-humans. To give an example, symptoms of illness affecting young children are conceived by the Quechua as

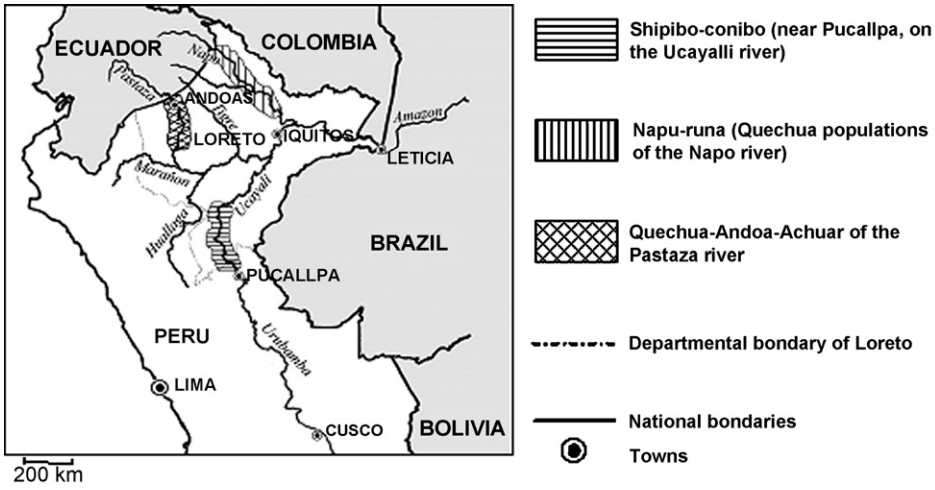

Fig. 1.

the capture of the soul by animals or spirits wishing to make their victim into kin, or to take revenge on their parents. Physical changes affecting the body are the visible indices of this process. According to our own data and those collected by Iglesias (Iglesias-Palacios, 1987), Quechua people from the Napo and Pastaza regions distinguish three types of illness categories:

- "*Unkuy*", considered to be due to natural causes like the excess of water (dampness), wind, heat or cold. It is also a generic term refering to physical disorder in general.
- "*Pahu*", due to supernatural origins or elements such as the encounter with mythical entities or spirits ("*supay*") whose promiscuity is thought to be dangerous, if a number of taboos and prescriptions are not respected. It provokes visible symptoms of illness or weakness.
- "*Wiruti*" (name of blowgun darts normally used in hunting, but also used to refer to an invisible projectile used by the shaman "*yachak*" to kill his enemy) or "*shitana*" (means "to blow") related to the ritual and shamanistic manipulation of power in order to harm. This category of illness can be classified as warfare sorcery; it is the physical consequence of underlying conflicts between distant kin from other villages or enemies from different ethnic groups.

These categories are not exclusive, a disease at first classified in category 1 "*unkuy*", for example can soon be attributed to category 3 "*shitana*", in specific circumstances, according to the variability of aetiological interpretation. It is interesting to note that from the first category to the third, emphasis is put on physicality and natural causes, or on the role of human or supernatural agency.

2.2.2. Indigenous theory of malaria

Malaria has specific indigenous terminologies used for its symptom description. Names of the diseases are related to the seriousness of the cases. For example "*chukchu unkuy*" ("shaking illness" in quechua) is said to occur when the disease does not kill the patient. It can also be qualified, according to the presence of fever, "*kalentura*" derived from the Spanish word "calendura". In this latter case it is supposed to be caused by the excess of "cold" elements. The principal symptoms cited by people questioned during enquiry were, in order of importance: (a) fever (in quechua, hot sensation: "*rupa*"); (b) shivering (in quechua, shaking body, "*chukchu aycha*"); (c) hurting heart or liver ("*shunku nanay*"), we point out that the heart "*shunku*" is considered by Quechua people as the main principle of animation and the locus of the soul; (d) strong headache and dizziness ("*uma muyurin*"); (e) weakness of vision ("*yana rikurin*", it looks dark).

In relation to the general ethno-classification of illness in regard to its presumed aetiology (see Section 2.2.1), malaria is classified in category (a) ("*chukchu unkui*") in 80% of the cases when it occurs in daily life without causing death. The minimization of the role of bad intentions determines the choice of using medicinal plants for curing, in the specific case of malaria. In this case, Quechua people think that malaria is caused by an excess of cold affecting the individual's breath "*samay*" (term also refering to a vital principle). This interpretation is inferred by the symptom of a suffering heart or liver (*shunku*) and the assumption that the individual's soul or "*samay*" lies in this place. The effect of medicinal plants is to make the patient "breath" again "*samachina*".

Malaria also appears, when it causes death or agony, in category (3), as the consequence of malevolent human entities manipulating their power "*wiruti*". A correlation exists between sharpness of the symptoms potentially leading to death, and the attribution of a predatory intention (an external cause) to the phenomenon. This native classification of malaria as a transformation caused by a "*wiruti*" inside the body has two consequences. At first the intervention of the shaman corresponds to an attempt to find a ritual interpretation and resolution for the trouble. Secondly, a political response can be chosen. Indeed an outburst of malaria in a village often leads to warfare when the victim's family wants to take a revenge on the presumed author of the crime. We noted, during fieldwork a coincidence between epidemics and the rise of socio-political tensions, sometimes ending in provisional dispersion of nucleated settlements or migrations to escape violence (community of Sabalo yaku, Pastaza, February 2005).

To sum up, the above study shows that a single disease, malaria, leads to the formulation of different aetiologies in native discourse and practice. The concept of illness is not considered as exclusively restricted to the relationship between individuals and their own bodies. It involves a network of social relations including non-human entities as well as different ethnic groups. The concept of body itself is the product of a social construction and transformation. Hence, every singular response adopted by indigenous people for facing malaria, such as shamanism, warfare or the use of medicinal plants, reveals the complexity and dynamics of a specific socio-political context. Because death or disease is never considered among these Amazonian groups as a mere biological fact (Taylor, 1993), medicinal knowledge is, in a logical way, always closely linked to politics.

2.2.3. Methodology of survey

The ethnopharmacological study and inquiry about the indigenous conception of the human body, illness and therapy were performed in autochthonous language, a variety of lowland Quechua dialect (classified by linguists as Quechua II-B). Plants classified by indigenous people as curing «malaria» were selected for our study when they fitted three of the five main symptoms described above [(a) fever, (b) shivering and (c) hurting heart or liver] or more. In other cases malaria was known and recognised under the same term "malaria" (in Spanish) in the area where populations were informed by medical brigades of the state. Ethnographic fieldwork and ethnopharmacological survey were partially undertaken during a long period (15 months overall between October 2003 and February 2005) with an interdisciplinary staff, in two main areas: the high Pastaza river and one of its affluents, the Capawari river, and the high Napo river, mainly with Quechua-Andoa-Achuar and Napu Runa groups (see Fig. 1). These areas were chosen for their distance from the Mestizo population of the province: the people there have been sufficiently in contact with the outside world to contract malaria (and other epidemic diseases), but at the same

time, they remain, especially for the case of the Pastaza population (in the villages most distant from Andoas) so isolated that they barely receive any help from the state sanitary infrastructures. As a consequence, most of these groups still possess and preserve the traditional knowledge about medicinal therapy, mainly as the only permanently available way of facing malaria.

Another part of the survey was undertaken in the surrounding area of Iquitos (Mestizo villages from the Nanay and the Amazon rivers), concerning a population informed about malaria by the medical brigades.

2.2.4. Nature of enquiry

Although some 30% of the *Napu runa* may be Spanish-speaking, only 15% of the indigenous population of the Pastaza area are, which made it necessary to elaborate specific structured interviews in quechua. Informants were chosen according to a "participate observation" methodology commonly used in ethnographic surveys. Men, women and children were equally questioned because of their equal access to natural medicines.

A detailed set of information was obtained concerning:

- Description of plant species used with their respective habitat and main qualities according to indigenous classification (phytonymy) emphasizing organoleptic properties.
- Context of plant collection (hunting expedition or specific search, moment of the day, season, ritual related to the event, etc.) and description of the part of the plant used as a remedy (e.g. bark, leaf, root, fruit)
- Mode of preparation or "recipe" (for instance decoctions or infusions).
- Effects of the remedy on the human body and indications of the recipes.

2.3. Plant material

Those plants which are indicated for three of the five main symptoms cited above (Section 2.2.3) were selected. Vernacular terminologies were reported (Rutter, 1990), (Duke, 1994), (Arevalo, 1994) and plants were collected with the agreement of the villagers and the presidents of the indigenous federations. The plants collected were then identified and deposited at the herbarium of the *Universidad Nacional de Amazonia Peruana* (UNAP, Iquitos) and after identification, a second collection was made in the primary and secondary forest close to Iquitos.

2.4. Methodology for extraction and in vitro screening

2.4.1. Plant extracts

The dried and pulverised parts of each plant (10 g) were successively extracted twice at 30 °C (3 h) with hexane, dichloromethane, and methanol to obtain three extracts. The extracts were filtered, and the solvents removed under reduced pressure. Two milligrams of each extract were weighed out exactly, then dissolved in DMSO to give a stock solution at 10 mg/mL used for the biological tests. Sixty-three extracts were tested.

2.4.2. Antiplasmodial screening

Parasites were cultured according to the method described by Trager and Jensen (Trager and Jensen, 1976) with modifications described by Benoit (Benoit et al., 1995). Briefly, parasites (FcB1-Columbia strain, considered to be chloroquine-resistant with an IC_{50} of 145 nM for chloroquine) were maintained on human red blood cells in RPMI 1640 medium (Cambrex, Emervainville, France) supplemented with 7.5% human serum and grown in a 5% CO_2 atmosphere. Cultures were synchronized every 48 h by 5% D-sorbitol lysis (Lambros and Vanderberg, 1979) (Merck-Germany). For *in vitro* antiplasmodial activity evaluation, the stock solutions of plant extracts were diluted in culture medium (10 and 50 μg/mL) and added to parasite culture (1% parasitaemia, 2% haematocrit). Parasite *in vitro* growth was followed by [^{3}H]-hypoxanthine (Amersham-France) incorporation as described by Desjardins (Desjardins et al., 1979) with modifications (Valentin et al., 1997). Inhibition values were plotted versus extract concentrations (average of two values) and the 50% inhibitory concentration (IC_{50}) was graphically determined by interpolation. The [^{3}H]-hypoxanthine incorporation, in the presence of extracts, was compared with that of control cultures without extract (the positive control being chloroquine (Sigma)).

2.4.3. Cytotoxicity evaluation

For the most active extracts (on *Plasmodium falciparum* culture), cytotoxicity was estimated on human breast cancer cells (MCF7). The cells were cultured in the same conditions as those used for *Plasmodium falciparum*, except that the 5% human serum was replaced by 5% foetal calf serum (Cambrex). Cells were distributed in 96-well plates at 2×10^4 cells/well in 100 μL of culture medium added to 100 μL of the same medium containing the plant extracts at various concentrations (the final concentrations in the wells were 1, 10, and 100 μg/mL). Cell growth was estimated by [^{3}H]-hypoxanthine incorporation after 24 and 72 h incubation as for the *Plasmodium falciparum* assay. The [^{3}H]-hypoxanthine incorporation, in the presence of extracts, was compared with that of control cultures without extract (positive control being doxorubicin (Sigma)).

3. Results

Species selected via the survey, as well as their medicinal uses, common names and their voucher specimen number from the herbarium of Iquitos are reported in Table 1.

All species reported were extracted and tested. Table 2 reports the antiplasmodial activities of the most active extracts, in comparison with their cytotoxicities. The CAR (cytotoxic/antiplasmodial ratio) was calculated and gave an estimation of the selectivity of the extracts on a chloroquine-resistant strain of *Plasmodium falciparum*.

4. Discussion

Sixty-three extracts obtained from 14 plants were tested on *Plasmodium falciparum*, and at least 7 plants displayed a potential antiplasmodial activity ($IC_{50} < 25$ μg/mL) (Willcox et

Table 1
Alphabetic list and traditional uses of the species investigated for *in vitro* antiplasmodial tests

Ingredient of the recipe: species (family), herbarium voucher	Part tested	Ethnic origin	Common name and traditional preparation of the recipe	Traditional uses
Abuta rufescens Aubl. (Menispermaceae), 021792	B.[a], L.[b]	Shipibo-conibo (Ucayali)	"*Pancha muca*, *Abuta macho*", decoction of the stem	Component of curare, against stomach ulcers and malaria
Cassia loretensis Killip & J. F. Macbr. Ex Killip. (Fabaceae), 029585:	B., L.	Quechua (Napo and Pastaza)	"*Waranka*", decoction of the bark	Antipyretic
Cyphomandra hartwegii (Miers) Walp. (Solanaceae), 028197	B., Fr.[c], L., R.[d]	Quechua (Pastaza)	"*Asna panka*", infusion of the leaves, steaming bath with fresh leaf infusion for children	Against liver pains and antipyretic
Eryngium foetidum L. (Apiaceae), 033895 (Garcia et al., 1999; Pino et al., 1997)	L.	Quechua (Pastaza and Napo)	"*Sacha kulantro*", decoction of the leaves	Against diarrhoea, stomach ache and malaria
Grias neuberthii J. F. Macbr. (Lecythidaceae), 033652	B.	Quechua (Napo, Pastaza,)	"*Pitun*" (Napo), "*Sacha mangua*" (Pastaza). Infusion of the stem, bark decoction	Purifying emetic, against inappetence, intestinal parasites and malaria
Lacistema aggregatum (P. J. Bergius) Rusby (Lacistemaceae), 027242	B., L.	Quechua (Pastaza), Mestizo (Iquitos)	"*Kapawari muyu*" (Pastaza)"*Huacapurana*" "*palometa huayo*", *trompo huayo*" (Mestizo). Maceration in rum or decoction of the wood	Against rheumatism and anti-pyretic.
Mikania congesta DC. (Asteraceae), 025548	L.	Quechua (Napo), Mestizo (Iquitos)	"*Yana anku*" (Napo), "*sanquillo*" (Mestizo). Infusion of the aerial parts	Laxative and antipyretic (Herz and Kulanthaivel, 1985).
Pagamea guianensis Aubl. (Rubiaceae), 035904	L.	Mestizo (Iquitos)	Dried stem decoction	Psychoactive genus, species selected for its bitter substance: "principio amargo"
Pithecellobium laetum Benth. (Fabaceae), 021229	A.p[e].	Quechua (Napo, Pastaza,), Mestizo (Iquitos)	"C*hunta ruku panka*" (Napo), "*sirimpachi*" (Pastaza), "*shimbillo*" (Mestizo), infusion of the aerial parts	Against malaria
Roucheria punctata (Ducke) Ducke (Linaceae), 033640	B.	Quechua (Napo)	"*Puma kaspi*, *Puma sacha*", bark decoction	Against diarrhoea and malaria
Sabicea villosa Willd. ex Roem. & Schult. (Rubiaceae), 024118	A.p.	Quechua (Napo)	"*Huasca mullaca*", infusion of the leaves	Against stomach ache, dysentery, and malaria
Verbena litoralis Kunth (Verbenaceae), 033670	A.p.	Quechua (Napo, Pastaza)	"*Pirpina*", infusion of the leaves	Antitussive, emetic, vermifuge, antipyretic
Virola calophylla (Spruce) Warb. (Myristicaceae), 035901	B., L.	Quechua (Napo-Pastaza)	"*Yura wapa*", inner bark decoction	Against fungi, bladder and stomach ailments, and malaria
Zygia latifolia (L.) Fawc. & Rendle (Fabaceae), 021217	A.p.	Quechua (Pastaza)	"*Yutsu*", infusion of the leaves, ingestion of the fresh bark resin	Purifying emetic, antipyretic and anti-malarial

[a] B.: stem bark.
[b] L.: leaves.
[c] Fr.: fruits.
[d] R.: roots.
[e] A.p.: aerial parts.

Table 2
Antiplasmodial activities (IC_{50} < 25 μg/mL) of the plant extracts

Active parts of the species	Extracts	*In vitro* activity on *Plasmodium falciparum* FcB1 (2)[a] IC_{50} (μg/mL)	Cytotoxicity MCF7 (2)[a] IC_{50} (μg/mL)	CAR[b] FcB1
Abuta rufescens Aubl. (Menispermaceae), bark and leaves	Bark CH_2Cl_2	2.3 ± 0.4[c]	22.5 ± 4.5	9.6
	Bark MeOH	3.8 ± 0.3	44.0 ± 3.0	11.5
	Leaves CH_2Cl_2	7.9 ± 0.1	36.5 ± 3.5	4.6
	Leaves MeOH	3.2 ± 0.2	>50.0	>15.0
Cyphomandra hartwegii (Miers) Walp. (Solanaceae), bark and leaves	Leaves CH_2Cl_2	10.0 ± 4.0	33.0 ± 0.5	3.3
Grias neuberthii J. F. Macbr. (Lecythidaceae), bark	Bark MeOH	22.0 ± 0.0	>50.0	>2.2
Lacistema aggregatum (P. J. Bergius) Rusby (Lacistemaceae), bark	Bark CH_2Cl_2	7.4 ± 2.5	41.0 ± 1.0	5.5
Pagamea guianensis Aubl. (Rubiaceae), leaves	Leaves CH_2Cl_2	18.5 ± 1.5	31.0 ± 3.0	1.7
Sabicea villosa Willd. ex Roem. & Schult. (Rubiaceae), aerial.parts	Aerial parts Hexane	5.3 ± 1.1	>50.0	>10.0
Virola calophylla (Spruce) Warb. (Myristicaceae), bark	Bark CH_2Cl_2	4.3 ± 0.4	35.5 ± 0.5	8.2
CQ[d]		0.14	>100.0	>500.0
Dox[e]		ND	0.4	ND

[a] Number of independent experiments.
[b] CAR cytotoxic/antiplasmodial (FcB1) ratio.
[c] Mean ± S.D.
[d] CQ, chloroquine; positive control for *Plasmodium falciparum* inhibition.
[e] Dox, doxorubicin; positive control for MCF7 inhibition.

al., 2004). This demonstrates that the ethnopharmacological approach is a powerful tool to select plants among the countless possible candidates. In the following discussion, we present the plants with the best activities on *Plasmodium falciparum*.

Abuta rufescens Aubl. (Menispermaceae), bark and leaves: The leaves of this plant are used by the Shipibo-Conibo people to produce curare and the bark decoction is employed against stomach ulcers, liver pains, diabetes, and malaria (Arevalo, 1994). The good-to-moderate antiplasmodial activity of the leaves and bark extracts (dichloramethane and methanol) with an IC_{50} from 2.3 to 7.9 μg/mL, can be explained by the presence of isoquinoline, azafluoranthene and oxoaporphine alkaloids isolated from the stem (Cava et al., 1975). Another species, *Abuta grandifolia* Mart. has been previously studied for its antiplasmodial activity due to its bisbenzylisoquinoline content (Steele et al., 1999). However, the species *Abuta rufescens* had never been tested for its antiplasmodial activity.
Cyphomandra hartwegii (Miers) Walp. (Solanaceae), leaves and roots: The fruits are edible and the leaves are used as an antipyretic by the Quechua from Pastaza. Its common name in quechua is "*Asna panka*" which means "bad-smelling leaf". Various parts of the plant were tested *in vitro* and the best antiplasmodial activity (IC_{50}:10 μg/mL) was obtained with the dichloromethane extract of the leaves. To our knowledge, this species has never been chemically or biologically studied and was the first of its genus to be tested on *Plasmodium falciparum*.
Grias neuberthii J. F. Macbr. (Lecythidaceae), bark: The stem infusion of this species is used by the Quechua (Pastaza) as a purifying emetic, against inappetence and malaria. Its name "*sacha mangua*" means mango from the wild forest. This species has never been chemically studied and we show that it displays a weak antiplasmodial activity (IC_{50} of the methanol extract: 22.0 μg/mL).
Lacistema aggregatum (P. J. Bergius) Rusby (Lacistemaceae), bark: The bark is commonly used macerated in rum "*huacapurana*" against rheumatism and as an antipyretic. To date, this species had not been chemically investigated. The dichloromethane extract of the bark displayed a good antiplasmodial activity (IC_{50}: 7.4 μg/mL) with a low cytotoxicity (IC_{50}: 41.0 μg/mL).
Pagamea guianensis Aubl. (Rubiaceae), leaves: This species belongs to a genus traditionally used for its psychoactive activity (like *Pagamea macrophylla*) (De Smet, 1985). *Pagamea guianensis* was selected for its bitter flavour "principio amargo" that is usually considered to be due to the presence of anti-infectious substances (Shepard, 2004). This species had never been chemically or biologically studied and showed a weak antiplasmodial activity (IC_{50}: 18.5 μg/mL for the CH_2Cl_2 leaf extract).
Sabicea villosa Willd. ex Roem. & Schult. (Rubiaceae), aerial parts: The leaf infusion of this plant is used against stomach disorders, dysentery and malaria. This plant has been studied for its anti-radical-type action (Pauly, 2000). However, the chemical composition of this species has not been described yet. The good antiplasmodial activity of the hexane extract of the aerial part (IC_{50}: 5.3 μg/mL) and its very low cytotoxicity (IC_{50}: >50 μg/mL) stressed the interest of this species for further phytochemical studies.
Virola calophylla (Spruce) Warb. (Myristicaceae), bark: This plant, named "*yura wapa*" (white *Kumala*), is used by the Quechua of the Napo as a bark decoction against external

infections and malaria. The genus *Virola* has usually been described as a source of hallucinogenic snuff. The antiplasmodial activity of the essential oil of another species of this genus (*Virola surinamensis*) has already been described (Lopes et al., 1999). Neolignans (Alvarez et al., 1987), dihydrochalcones (Constanza et al., 1998) and alkaloids (tryptamine and β-carboline derivatives) (Miles et al., 1987) have been isolated from the species *Virola calophylla*. However, no study concerning the antiplasmodial activities has been published, despite a good activity of the extract (IC_{50} of the dichloromethane bark extract: 4.3 μg/mL).

Among all the species tested in this study, three (*Abuta rufescens*, *Eryngium foetidum*, and *Grias neuberthii*) have been recently cited for their use in traditional medicine against malaria (Kvist et al., 2006). However, the IC_{50} antiplasmodial values of these species are reported for the first time in this study.

Chloroquine was used as positive control of the sensitivity of susceptible *Plasmodium falciparum* strains (FcB1). Comparison of the plant extract activities with those of chloroquine (which is 20 times more active) does not indicate that the bio-active compounds of the plant extracts have a low activity but that they are diluted in the whole extracts.

The dichloromethane extracts of *Cyphomandra hartwegii*, *Lacistema aggregatum*, *Virola calophylla* and the hexane extract of *Sabicea villosa* were the most potent against *Plasmodium falciparum* (IC_{50}: from 4.3 to 10 μg/mL). The low cytotoxicities ($IC_{50} > 35.5$ μg/mL) and the selectivity of the plant extracts showed the medicinal potential of these species.

5. Conclusion

The high quantity of potentially active plants (7) in comparison with the total number of plants collected (14) gave scientific support to the indigenous knowledge and showed the suitability of this type of survey to identify bio-active plants. The interesting cytotoxic/antiplasmodial (FcB1) ratio, the high antiplasmodial activity and the low level of prior scientific knowledge concerning the plants, were the three main criteria used to select the most interesting parts of the plants, namely the leaves of *Cyphomandra hartwegii* (Solanaceae), the bark of *Lacistema aggregatum* (Lacistemaceae), the aerial parts of *Sabicea villosa* (Rubiaceae), and the bark of *Virola calophylla* (Myristicaceae). Bioactivity-guided chemical fractionation of these plants is underway, to isolate and identify potentially novel antiplasmodial molecules.

Acknowledgements

The authors are grateful to the botanist Juan Ruiz from the herbarium of Iquitos, the staff of the laboratory of phytochemistry of the UNAP and Peggy Rigou for their important collaboration in this study. We are also indebted to the Quechua people from the Napo and the Pastaza rivers (“ñukanchipa wawkikuna”, our brothers) with other people (Mestizo) from the surrounding area of Iquitos, who patiently shared their knowledge regarding their traditional medicines, courageously facing the ignorance of the observer, and without whom this study would not have been possible. We are also very grateful to Henry Godard, director of IFEA (Institut Français d’Etudes Andines) and our friend Elsy Huboux for their unconditionnal support during research in the field.

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