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Antiprotozoal activity of medicinal plants used by Iquitos-Nauta road communities in Loreto (Peru)

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ABSTRACT

Ethnopharmacological relevance: In the Peruvian Amazon, the use of medicinal plants is a common practice. However, there is few documented information about the practical aspects of their use and few scientific validation. The starting point for this work was a set of interviews of people living in rural communities from the Peruvian Amazon about their uses of plants. Protozoan diseases are a public health issue in the Amazonian communities, who partly cope with it by using traditional remedies. Validation of these traditional practices contributes to public health care efficiency and may help identify new antiprotozoal compounds.

Aims of study: to inventory and validate the use of medicinal plants by rural people of Loreto region.

Materials and methods: Rural mestizos were interviewed about traditional medication of parasite infections with medicinal plants. Ethnopharmacological surveys were undertaken in two villages along Iquitos-Nauta road (Loreto region, Peru), namely 13 de Febrero and El Dorado communities. Forty-six plants were collected according to their traditional use for the treatment of parasitic diseases, 50 ethanolic extracts (different parts for some of the plants) were tested *in vitro* on *Plasmodium falciparum* (3D7 sensitive strain and W2 chloroquine resistant strain), *Leishmania donovani* LV9 strain and *Trypanosoma brucei gambiense*. Cytotoxic assessment (HUVEC cells) of the active extracts was performed. Two of the most active plants were submitted to preliminary bioguided fractionation to ascertain and explore their activities.

Results: From the initial plants list, 10 were found to be active on *P. falciparum*, 15 on L. *donovani* and 2 on the three parasites. The ethanolic extract from *Costus curvibracteatus* (Costaceae) leaves and *Grias neuberthii* (Lecythidaceae) bark showed strong *in vitro* activity on *P. falciparum* (sensitive and resistant strain) and L. *donovani* and moderate activity on *T. brucei gambiense*.

Conclusions: The Amazonian forest communities in Peru represents a source of knowledge on the use of medicinal plants. In this work, several extracts with antiprotozoal activity were identified. This work contributes to validate some traditional uses and opens subsequent investigations on active compounds isolation and identification.

1. Introduction

Infections caused by parasitic protozoa take an enormous toll on human health. Their prevalence is higher in tropical and equatorial countries, where the major number of deaths is due to malaria (World Health Organization, 2016a). Although significant progresses have been made in the past decades, malaria is still considered by the World Health Organization as the first parasitic disease with 229 000 deaths in 2016 in 91 countries. Chagas disease, African trypanosomiasis and leishmaniasis are classified as some of the 17 most important neglected diseases by the WHO (Simarro et al., 2012; World Health Organization, 2015a). In the absence of effective vaccines, global antiparasitic strategy relies on a multi-faceted approach based on prevention (*i.e.* vector control and pharmacological prophylaxis), quick and reliable diagnostic procedures, treatment with effective antiprotozoal drugs and medicinal plants. Natural products remain an interesting source of

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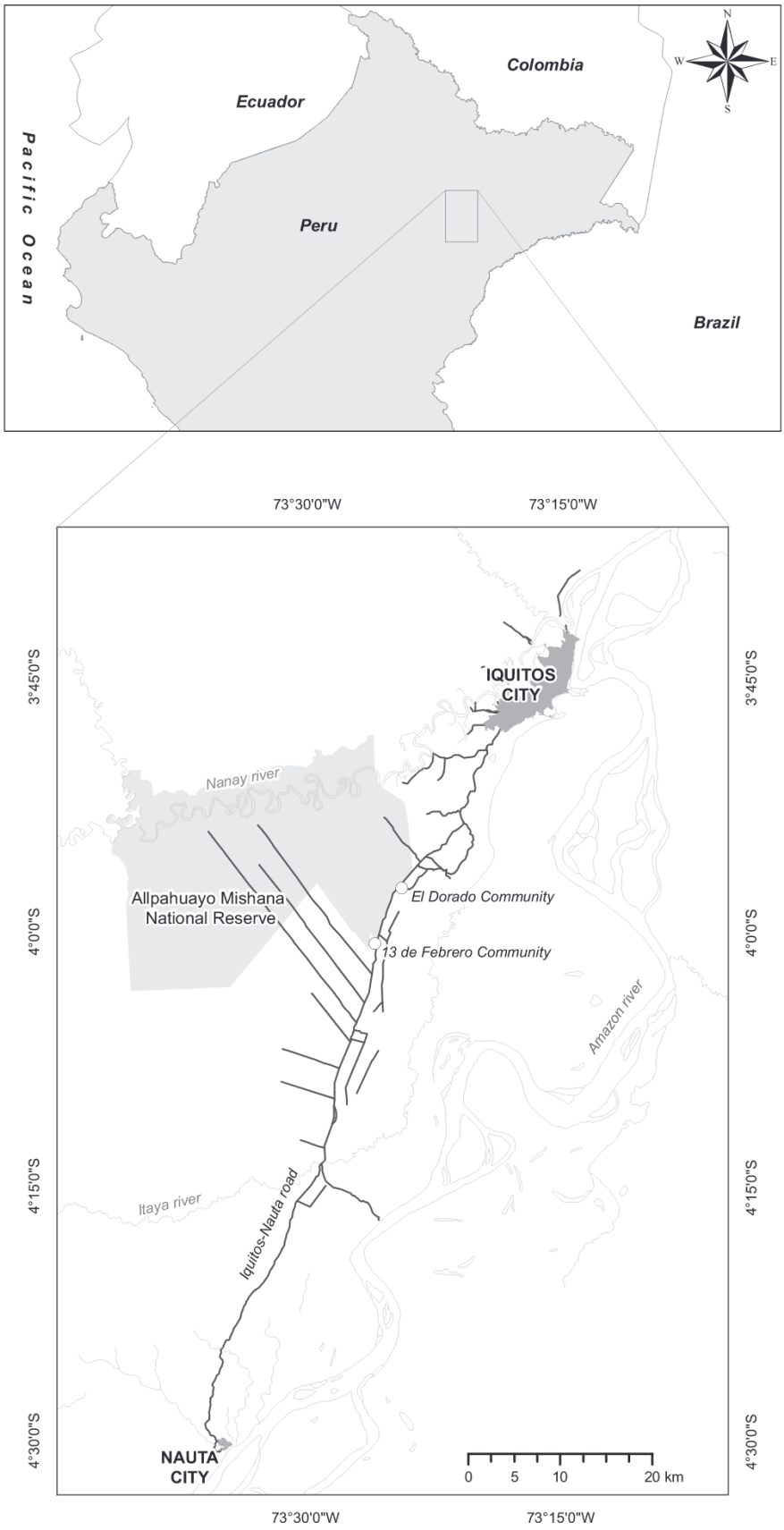

Fig. 1. Map of the place of ethnopharmacological survey.

research for the discovery of new drugs to fight NTDs (neglected tropical diseases) (Zucca et al., 2013).

Malaria is caused by the apicoplexan parasites *Plasmodium falciparum*, *P. vivax*, *P. malariae*, *P. ovale* and *P. knowlesi* (Centers for Disease Control and Prevention, 2017). It is transmitted by female anopheles mosquitoes, which bite mainly between dusk and dawn. The

most severe form is caused by *P. falciparum*; variable clinical symptoms include fever, chills, headache, muscular aching and weakness, vomiting, cough, diarrhea and abdominal pain, generalized convulsions, circulatory collapse and can be followed by coma and death if not treated. Initial symptoms, sometimes of mild intensity, may not be easy to recognize as being due to malaria (World Health Organization, 2016a).

Leishmania spp., *Trypanosoma cruzi* and *T. brucei* are protozoan parasites from the Kinetoplastidae family transmitted to mammalian hosts *via* their insect vectors, sandflies (*Phlebotomus* spp.), triatominae (such as *Triatoma infestans*), and tsetse flies (*Glossina* spp.), respectively (McCall and McKerrow, 2014). These parasites partly share similar structural and biochemical features. Recent analysis of their genome sequences identified common metabolic pathways, allowing a better understanding about their pathogenesis and leading to further investigations on biochemical/molecular drug targets (Barrett and Croft, 2012). Chagas disease caused by *T. cruzi*, human African trypanosomiasis caused by *T. brucei gambiense* and *T. brucei rhodesiense*, as well as different forms of leishmaniasis, caused by various species of *Leishmania*, are categorized amongst the most important NTDs (World Health Organization, 2016b).

Altogether, these diseases predominantly affect populations living in poverty and in close proximity with the vectors of disease-causing agents. The effects of NTDs are devastating: over 1 billion people in 149 countries suffer from one or more neglected diseases with millions of others at risk, and economic repercussions of these diseases can be just as damaging as their health effects. These diseases are 'neglected' primarily because there is no financial incentive to develop drugs for a patient population that cannot afford them (Klug et al., 2016; World Health Organization, 2015b). In Peru malaria, leishmaniasis and trypanosomiasis are the 3 priority health problems for the ministry of health due to increased transmission and risk factors. (Ministerio de Salud del Perú, 2015). These parasitic diseases are especially of public health concern in the Peruvian Amazon, mainly in the poor areas of the Loreto region. For example, of all the cases of malaria in Peru, 94.6% were found in the Loreto region (mostly *P. vivax*) (Ministerio de Salud del Perú, 2016).

Peru is considered to be one of the 12 megadiverse countries, with an estimated flora of approximately 25 000 species. Approximately 10% of the world flora grow in Peru, 30% of the species being endemic. It is estimated that approximately 5 000 Peruvian plants are used by the population for 49 different purposes and uses (Bussmann and Sharon, 2014). Plants traditionally used for the treatment of diseases need to be tested and scientifically validated to corroborate their uses and to know their toxicity.

The aims of this work were to firstly inventory the use of medicinal plants by rural mestizo people, and secondly evaluate the antiprotozoal activity *in vitro* of 50 extracts from 46 medicinal plants. Plants in this work were selected for their antiprotozoal use during an ethnopharmacological survey in Loreto-Peru (communities located along Iquitos-Nauta road).

2. Materials and methods

2.1. Ethnopharmacological survey

The surveys were taken between January and December 2008 in the communities of 13 de Febrero (Coordinates UTM 673903, 9555301; elevation 140 m.a.s.l.; zone 18) and El Dorado (Coordinates UTM 677057, 9562698; elevation 140 m.a.s.l.; zone 18) in km 33 and 25 of the Iquitos – Nauta road in the province of Maynas, located in the region of Loreto, Peru (Fig. 1). These surveys were realized as part of the project "Exploration and evaluation of bioactive substances and natural products: Record and documentation of traditional knowledge on useful plants in native communities of the Peruvian Amazonia". This project was realized in accordance with the Instituto de Investigaciones de la Amazonía Peruana (IIAP) guidelines pertaining to ethnopharmacological studies, and a detailed presentation of the project, following a standard procedure of IIAP, was given to the participants before obtaining their informed consent. 75 individuals were selected per community for the survey, with the purpose of getting to know the main use of medicinal plants, including the use against malaria and "uta" (local name for leishmaniasis). From these plants, a list was made of the plants recognized by at least 5 persons within the community to be used against malaria and uta. Later, the species were verified *in situ* and collected, either in backyards or in wild areas close to their homes. The plants referred by the informants were then identified and deposited at the National Herbarium of the San Marcos University, Lima, Peru. Collected information were divided in categories according to their uses against malaria, leishmaniasis or other diseases.

After botanical identification a second collection was realized in December 2015 at: 1) Area surrounding the Iquitos – Nauta road (Coordinates UTM 678002, 9560826; elevation 140 m.a.s.l; zone 18, District of San Juan, Province of Maynas, Region of Loreto); 2) Arboretum of the Research Center Jenaro Herrera "Jose Lopez Parodi" (Coordinates UTM 640726, 9458471; elevation 110 m.a.s.l; zone 18, District of Jenaro Herrera, Province of Requena, Region of Loreto, Peru).

2.2. Extracts preparation

Five grams of each dried and ground plant part were twice soaked 24 h in 15 mL of ethanol 96%, filtrated and evaporated under reduced pressure, below 40 °C. Dried extracts were stored at −4 °C until use. For active extracts or barks, tannins were removed by filtering 50 mg of extract through 150 mg of polyamide (MN-polyamide SC 6, particle size 0.05–0.16 mm). Elution was made using ethyl acetate: methanol 1:1. For *in vitro* bioassays, the extracts were solubilized in DMSO at a starting concentration of 10 mg/mL. For extracts with significant activity on all parasites, liquid-liquid fractionation was performed using solvents of increasing polarity (cyclohexane, dichloromethane, ethyl acetate, butanol and water).

2.3. Parasite culture

2.3.1. Plasmodium falciparum

The chloroquine-sensitive 3D7 *P. falciparum* strain (clone of the NF54) and W2 chloroquine-resistant strain were obtained from Malaria French National Reference Center (CNR Paludisme, Hôpital Bichat Claude Bernard, Paris). The strains were maintained in O^+ human erythrocytes in albumin supplemented RPMI medium under continuous culture using the candle-jar method (Trager and Jensen, 1976). The parasites were synchronized to the ring stage by repeated sorbitol treatment (Lambros and Vanderberg, 1979).

2.3.2. Leishmania donovani

The MHOM/ET/67/HU3 strain of L. *donovani* also called LV9 was maintained routinely in special pathogen-free (SPF) female Golden hamsters (Charles Rivers Ltd., UK) by passage every 12 weeks. Parasites were maintained as promastigote forms in M-199 medium (Sigma) supplemented with 40 mM HEPES, 100 mM adenosine, 0.5 mg/L hemin, 10% fetal bovine serum (FBS) at 25 °C in a dark environment. Differentiation of promastigotes into axenic amastigotes was achieved by diluting 1×10^6 promastigotes in stationary growth phase in 5 mL of axenic amastigote medium (promastigote medium supplemented with 2 mM $CaCl_2$, 2 mM $MgCl_2$, pH adjusted to 5.5) one day before

Table 1
Medicinal plants collected. Plant names are those given as accepted name on the Tropicos database of Missouri Botanical Garden.

Name Voucher reference (Location: CIN= Iquitos – Nauta road; JH = Jenaro Herrera community) vernacular name	Traditional indications	Traditional recipes
ARACEAE		
Dieffenbachia seguine (Jacq.) Schott (syn. *Dieffenbachia picta* Schott)	1. Mycosis;	1. Wash affected area using an infusion of dried leaves (2 leaves per liter of water). Also, the steam of the infusion can be applied on the affected area;
PV002PA (CIN) **patiquina, hoja blanca**	2. Rheumatism;	2. Apply the leaves on the painful area (2 leaves), by placing a gauze or a thin fabric between the leaves and the skin. Repeat the procedure for three days. Do not get the area wet for at least four days;
	3. Uta.	3. The stem is used topically against uta.
Dracontium spruceanum (Schott) G.H. Zhu (syn. *Dracontium loretense* K. Krause)	1. Snake bite;	1. Mash 1 tuber, wrap the paste in a *bijao* (*Calathea* spp) or banana leaf, and apply on the snake bite. Alternatively, grate the tuber and apply directly on the snake bite, and at the same time drink the paste diluted in a cup of cold, boiled water;
PV003P (CIN) **jergón sacha, hierba del jergón, fer de lance**	2. Hernia;	2. Boil the petioles and tuber in water until a thick liquid is obtained. Apply directly on the affected area;
	3. Wounds / uta.	3. Sun-dry the tuber, grind it, sift it and apply the powder on the wound (previously washed), until the wound is well covered, three times a day, until the wound is healed.
ARECACEAE		
Iriartea deltoidea Ruiz & Pav.	1. Cleaning of the stomach;	1–3. Grate 1 seed or roots and drink it directly with water every hour during 3 days;
PV004R (CIN) **pona**	2. Malaria;	
	3. Children's night terrors;	
	4. Aphrodisiac.	4. Macerate the roots (5 g approximately) in sugar cane alcohol (*aguardiente*). Alternatively, boil it in water, drink before sexual intercourse.
ASTERACEAE		
Tessaria integrifolia Ruiz & Pav.	1. Odontalgia;	1. Chew the bark until pain is alleviated;
PV038 (CIN) **pájaro bobo**	2. Asthma;	2–4. Boil 4 g of the aerial parts for 15 minutes, let it rest overnight, take as a common drinking water during the day;
	3. Fever;	
	4. Urinary tract infection;	
	5. Flatulence.	5. Boil ½ handful of leaves (approximately 10 g) per liter of water. Drink a cup before every meal.
BIGNONIACEAE		
Handroanthus impetiginosus (Mart. ex DC.) Mattos (syn. *Tabebuia impetiginosa* (Mart. ex DC.) Standl)	1. Malaria;	1. Boil 10 g of the bark in 1 l of water, take as common drinking water during the day;
PV043 () **tahuari**	2. Uta;	2. Boil 200 g of the bark in 1 l of water. Wash the affected area and apply as a compress until cicatrization of the ulcers;
	3. Diabetes.	3. Boil 25 g of the bark in 1 l of water, take a cup two times a day during five days.
Handroanthus serratifolius (Vahl) S.O. Grose (syn. *Tabebuia serratifolia* (Vahl) G. Nicholson)	1. Malaria;	1. Boil 10 g of the bark in 1 l of water, take a cup two times a day during five days;
PV005C (CIN) **tahuari, palo arco**	2. Uta;	2. Boil 200 g of the bark in 1 l of water. Wash the affected area and apply as a compress until cicatrization of the ulcers;
	3. Diabetes.	3. Boil 25 g of the bark in 1 l of water, take a cup two times a day during five days.
CELASTRACEAE		
Maytenus macrocarpa (Ruiz & Pav.) Briq.	1. Malaria;	1, 2. Macerate 20 g of bark in *aguardiente* for 15 days, drink a small cup of 30 mL before bathing;
	2. Common cold	
PV006C (CIN) **chuchuhuasi, chuchuhuasha**	3. Uta;	3. Boil 200 g of the bark in 1 l of water. Wash the affected area with it and apply the mixture of bark powder with decoction as a plaster until the cicatrization of the ulcer;
	4. Stomach ache	4. Boil 5 g of bark, drink three times a day.
COSTACEAE		
Costus curvibracteatus Maas (syn. *Costus scaber* Ruiz & Pav.)	1. Fever;	1. Grate the stem pulp or crush the leaves, apply the paste on the head and wrap with a fabric every morning to lower the fever. Extract the juice of the stem and leaves, drink it three times a day;
PV037T (CIN)	2. Pertussis.	2. Scratch the stem and extract the juice, drink it three times a day.
PV037H (CIN) **caña agria**		
ELAEOCARPACEAE		
Sloanea schomburgkii Spruce ex Benth. (syn. *Sloanea verrucosa* Ducke)	1. Malaria;	In a large pan containing water make a decoctions of 20 g of bark and 20 g of leaves. This decoction is used to take a bath once a day in order to reduce fever.
PV007C (JH) **sepanchina**	2. Fever, inflammation.	
EUPHORBIACEAE		
Alchornea castaneifolia (Humb. & Bonpl. ex Willd.) A. Juss.	1. Antirheumatic;	1. Boil 50 g of the bark and root in 1 l of water, take in on an empty stomach;
PV041H (CIN) **ipururo**	2. Aphrodisiac;	2, 3. Boil the leaves in water, drink it every night;
	3. Fever;	
	4. Bite of stingray, joint pain.	4. Apply a plaster of the grinded leaves on the affected area.
Alchornea triplinervia (Spreng.) Müll. Arg.	1. Diarrhea.	In a large pan containing boiling water, add 10 g of bark during 30 minutes. A small cup is drunk 2 times, after breakfast and lunch.
PV008C (JH) **zancudo caspi, mojarra caspi**	2. Fever.	
Hevea guianensis Aubl. (syn. *Hevea cuneata* Huber)	1. Malaria;	On an empty stomach, take once a day a quarter of a spoon (children) to a full spoon (adult) of latex.
PV009C (CIN) **shiringa**	2. Fever.	
FABACEAE		
Albizia niopoides (Spruce ex Benth.) Burkart	Fever.	Boil 5 g of the bark in a large pan containing water. Drink a spoon every night and wash all the body with a humid towel in order to decrease fever.
PV047 (CIN) **pashaco**		

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Table 1 *(continued)*

Name Voucher reference (Location: CIN = Iquitos – Nauta road; JH = Jenaro Herrera community) vernacular name	Traditional indications	Traditional recipes
Bauhinia herrerae (Britton & Rose) Standl. & Steyerm. PV040 (JH) **pata de vaca**	Fever, inflammation.	Boil 5 g of the bark in a large pan containing water. Drink a spoon every night and wash all the body with a humid towel in order to decrease fever.
Campsiandra angustifolia Spruce ex Benth. PV010C (JH) **huacapurana**	1. Rheumatism, joint pain; 2. fever; 3. Diarrhea; 4. Liver.	1, 2. Macerate 100 g of the bark in 1 l of *aguardiente* for 15 days. Drink a cup every morning before shower; 3. Boil 50 g of the bark in water, drink one cup; 4. Macerate 200 g in 1 l of *aguardiente* for 7 days, filter it and add honey.
Copaifera paupera (Herzog) Dwyer PV011C (JH) **copaiba**	1. Wounds; 2. Throat pain; 3. Ulcers, uta; 4. Herpes.	1. Apply the oil (exsudate) of the fruit (obtained directly or after boiling extraction), on the affected area; 2. Mix one tablespoon of honey and three drops of copaiba oil (exsudate), apply on the wound; 3. Take five drops of oil (exsudate) diluted in a tablespoon of warm water, on an empty stomach, for seven days; 4. Mix one part of copaiba oil (exsudate) and one part of *andiroba* oil (*Carapa guianensis* Aubl.), apply on the affected area, leaving it for 30 minutes. Apply twice a day.
Inga sertulifera DC. (syn. *Inga coriacea* (Pers.) Desv.) PV012C (JH) **tuna congona, simbillo**	Malaria.	Boil 5 g of the bark in 1 l of water. Drink one cup of the beverage in the mornings for 3 day.
Abrus arboreus Vell. (syn. *Ormosia arborea* (Vell.) Harms) PV042 (CIN) **huayruro**	1. Hemorrhoids; 2. Malaria, fever.	Roast 7 seeds and 5 g of bark, smash and boil, take the steam in a sitz bath for 15 nights.
Ormosia costulata (Miq.) Kleinhoonte (syn. *Ormosia coccinea* (Aubl.) Jacks.) PV013C (CIN) PV013H (CIN) **huayruro**	1. Hemorrhoids; 2. Malaria, fever.	Roast 7 seeds and 5 g of bark, smash and boil, take the steam in a sitz bath for 15 nights.
Swartzia simplex (Sw.) Spreng. PV014C (CIN) **porotillo**	Malaria.	In a pan boil 50 g of bark in 1 l of water. Take as drinking water during the day, when thirsty.
Tachigali polyphylla Poepp. (syn. *Tachigali poeppigiana* Tul.) PV015C (JH) **tangarana de altura**	1. Intestinal parasites; 2. Fever.	1. Boil approximately 5 g of fresh leaves, take as drinking water during the day; 2. In a pan, boil 100 g of bark in 1 l of water. Take as drinking water during the day, when thirsty.
GENTIANACEAE		
Chelonanthus alatus (Aubl.) Pulle (syn. *Irlbachia alata* (Aubl.) Maas) PV016PA (CIN) **campanita**	1. Fever; 2. Body pain; 3. Rheumatism; 4. Antimalarial; 5. Headache; 6. Diabetes; 7. Liver damage; 8. Wounds; 9. Uta.	Boil 20–30 g of fresh leaves in 1 l of water, drink the beverage three times a day.
LECYTHIDACEAE		
Grias neuberthii J.F.Macbr. PV017C (JH) **sacha mango, sacha mangua**	1. Depurative; 2. Diarrhea, emetic, enema, laxative, purgative; 3. Uterine bleeding; 4. recovering after giving birth; 5. Sinusitis; 6. Fever.	1–5. Boil 20–30 g of leaves per liter of water, drink it when thirsty; 4, 6. Boil 20–30 g of the bark per liter of water, drink it when thirsty.
Gustavia angustifolia Benth. (syn. *Gustavia angusta* Ruiz ex O. Berg) PV018C (CIN) **chopé**	1. Emetic. 2. Purgative. 3. Fever	1, 2. Make an infusion with the leaves, using one leaf per liter of water. Drink the beverage as drinking water; 3. Make an infusion with 5 g of bark per liter of water. Drink the beverage as drinking water.
LINACEAE		
Roucheria columbiana Hallier f. (syn. *Roucheria punctate* (Ducke) Ducke) PV019C (JH) **puma sacha, puma caspi**	Malaria	Boil 5 g of the bark in one liter of water. Drink one cup every morning for three days.
MELIACEAE		
Cedrela odorata L. PV020C (CIN) **cedro**	1. Fever; 2. Snake bite; 3. Diarrhea; 4. Malaria; 5. Spasms; 6. Uta.	1. Boil the bark, take as drinking water during the day; 2. Scratch the bark, boil until a red color is obtained, sweeten and take a cup. Wash the wound with the left over adding a teaspoon of salt; 3–4. Boil the bark (20 g per liter of water). Drink a cup with every main meal; 5. Slightly heat two leaves over direct fire. Apply on the abdomen as a cataplasm; 6. Boil the bark (20 g), wash the wounds with it.
Guarea guidonia (L.) Sleumer (syn. *Guarea trichilioides* L.) PV021C (JH) **requia colorada**	Malaria.	Boil 5 grams of well grated bark in one liter of water, simmer it. Drink one cup every morning for three days.
MORACEAE		
Clarisia biflora Ruiz & Pav. PV045 (CIN) **chimicua**	1. Common cold; 2. Feverish chill; 3. Rheumatism and arthritis; 4. Body pain; 5. Hernia.	Macerate 5 g of the bark in *aguardiente*. Take a small cup (30 mL) before breakfast.
Clarisia racemosa Ruiz & Pav.	1. Common cold;	Macerate 5 g of the bark in *aguardiente*. Take a small cup (30 mL) before

(continued on next page)

Table 1 *(continued)*

Name Voucher reference (Location: CIN = Iquitos – Nauta road; JH = Jenaro Herrera community) vernacular name	Traditional indications	Traditional recipes
PV022C (CIN) **mashonaste**	2. Feverish chill; 3. Rheumatism and arthritis; 4. Body pain; 5. Hernia.	breakfast.
Ficus insipida Willd. PV023C (CIN) **ojé, doctor ojé**	1. Odontalgia; 2. Intestinal parasites; 3. Malaria; 4. Uta; 5. Rheumatism.	1. Apply the fresh latex topically using a piece of cotton; 2, 3. Dilute a cup of the latex in orange juice or sugared water. Drink every three days; 4. Apply directly the latex on the wound for three or more days, until cicatrization is observed; 5. Apply the latex as a plaster on the affected area.
MYRISTICACEAE		
Virola calophylla (Spruce) Warb. PV024C (CIN) **cumala blanca**	1. Antifungal; 2. Stomachache; 3. Malaria, fever; 4. Hallucinogenic.	1–3. Boil 5 g of the bark in one liter of water. Drink one cup every morning for three days.
Virola surinamensis (Rol. ex Rottb.) Warb. PV046 (CIN) **cumala colorada**	1. Stomach cramps; 2. Wound healing; 3. Digestive; 4. Dyspepsia; 5. Erysipelas; 6. Contusions; 7. Uta; 8. Hallucinogenic.	1–7. Boil 5 g of the bark in one liter of water. Drink one cup every morning for three days.
OLACACEAE		
Minquartia guianensis Aubl. (syn. *Minquartia punctata* (Radlk.) Sleumer) PV042 (CIN) **huacapú**	1. Hepatitis; 2. Malaria, fever; 3. Rheumatism.	1–2. Boil 50 g of the bark in 1 l of water. Drink four times a day; 3. Macerate 200 g of the bark in 1 l of *aguardiente*, add honey. Drink one cup on an empty stomach for a month.
PASSIFLORACEAE		
Passiflora quadrangularis L. PV026H (JH) **tumbo**	1. Therapeutic abortion; 2. Malaria, fever.	Boil 20 leaves (approximately 30 g) per liter of water. Boil until water is reduced to a third. Take three cups a day: the first one on an empty stomach, the second one hour later, and the third one, three hours after the first one.
PIPERACEAE		
Piper coruscans (Miq.) C. DC. (syn. *Piper amazonicum* (Miq.) C. DC.) PV048 (CIN) **cordoncillo**	1. Malaria 2. Fever; 3. Purgative.	Crush 50 g of aerial parts in 1 l of water and prepare an infusion. Take as drinking water.
POLYGONACEAE		
Triplaris weigeltiana (Rchb.) Kuntze (syn. *Triplaris americana* L.) PV027C (JH) **tangarana del bajo**	1. Stomach ache; 2. Diarrhea; 3. Swelling; 4. Body pain; 5. Bone-ache; 6. Vomiting; 7. Anemia.	Make an infusion with the bark and leaves (50 g of each in 1 l of water). Drink as drinking water after each meal.
RUBIACEAE		
Calycophyllum multiflorum Griseb. (syn. *Calycophyllum spruceanum* (Benth.) Hook.f. ex K.Schum.)	1. Anemia;	1. Boil the leaves and bark (5 g each) for one hour in water with ripe fruits of *huito* (*Genipa americana* L.). Drink this beverage in the morning and the afternoon for five days;
PV028C (JH) **capirona**	2. Uta.	2. Scrape the bark, apply on the affected area. Renew it every day.
Capirona decorticans Spruce PV029C (CIN) **capirona**	1. Acariasis and psoriasis. 2. Malaria. 3. Uta.	1, 3. Make an infusion with 3 g of the bark and wash the affected area with it; 2. The same infusion can be taken as drinking water.
Isertia hypoleuca Benth. PV030H (CIN) **ocuera blanca**	1. Alterations of the menstrual flow. 2. Malaria.	Make an infusion with 50 g of leaves in 1 l of water, drink the beverage three times a day.
Ladenbergia oblongifolia (Humb. ex Mutis) L. Andersson (syn. *Ladenbergia magnifolia* (Ruiz & Pav.) Klotzsch) PV031C (JH) **cascarilla verde**	Malaria.	Boil 10 g of the bark in 1 l of water, take this beverage three times a day.
Cephaelis tomentosa (Aubl.) Vahl (syn. *Psychotria poeppigiana* Müll.Arg.) PV032H (CIN) **oreja de diablo, boca pintada**	1. Earache; 2. Hemorrhoids, 3. Lung diseases; 4. Uterine bleeding; 5. Vomiting; 6. Fever.	1. Drop the liquid contained inside the flower into the ears; 2–6. Drink the leaves infusion.
SAPOTACEAE		
Prieurella prieurii (A. DC.) Aubrév. (syn. *Chrysophyllum prieurii* A. DC.) PV033C (JH) **cotoquinilla**	1. Wound healing; 2. Uta;3. Malaria.	1. Use the leaves to disinfect wounds locally; 2. Crush the leaves, apply on the wound, let it rest during the night so as “the germs stick to the milk”; 3. Boil the leaves in water, drink the beverage as a common drinking water during the day.
Pouteria guianensis Aubl. (syn. *Pouteria caimito* (Ruiz & Pav.) Radlk.) PV034C (JH)	1. Wound healing; 2. Diarrhea; 3. Uta;	1. Use the leaves to disinfect wounds locally; 2. Crush the leaves, add some water, drink this beverage twice a day; 3. Crush the leaves, apply on the wound, let it rest during the night so as “the

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Table 1 *(continued)*

Name Voucher reference (Location: CIN = Iquitos – Nauta road; JH = Jenaro Herrera community) vernacular name	Traditional indications	Traditional recipes
PV034H (JH) PV034R (JH) **caimito**	4. Malaria.	germs stick to the milk"; 4. Boil the leaves, drink the beverage as a common drinking water during the day.
SIMAROUBACEAE		
Simarouba amara Aubl. PV035C (CIN) **huamansana, marupa**	1. Skin conditions; 2. Amoebiasis; 3. Cancer; 4. Weakness; 5. Diarrhea, dyspepsia, emetic; 6. Fever; 7. Nervousness; 8. Malaria.	Boil 10 g of the bark in 1 l of water during 15 to 30 minutes. Take this beverage three times a day.
TILIACEAE		
Apeiba tibourbou Aubl. PV036C (CIN) **peine de mono**	1. Asthma; 2. Intestinal parasites. 3. Body pain.	Boil 10 g of the bark in 1 l of water, drink the beverage as common drinking water during the day.
VERBENACEAE		
Stachytarpheta cayennensis (Rich.) Vahl PV044 (CIN) **sacha verbena, verbena negra**	1. Ulcer healing; 2. Fever; 3. Chronic hepatitis; 4. Diuretic; 5. Arthritis; 6. Anti-inflammatory; 7. Diabetes; 8. Renal problems.	Crush the fresh leaves into a paste, mix it with a few drops of lime and cooking oil, take a teaspoon in the morning on an empty stomach. Repeat three times.

treatment. Axenic amastigotes were grown at 37 C in 5% CO_2 (Balaraman et al., 2015).

2.3.3. Trypanosoma brucei gambiense

T. b. gambiense trypomastigotes (FéoITMAP/1893 strain) were maintained in HMI9 medium (Iscove's medium: Dulbecco's medium supplemented with 36 mM $NaHCO_3$, 1 mM hypoxanthine, 0.05 mM bathocuproine, 0.16 mM thymidine, 0.2 mM 2-mercaptoethanol, 1.2 mM L-cysteine, 10% heat-inactivated fetal bovine serum, 100 IU/mL penicillin and 100 µg/mL streptomycin) (Pomel et al., 2015).

2.4. In vitro antiprotozoal activity

2.4.1. In vitro antiplasmodial activity on Plasmodium falciparum

A 2.5% (V/V) erythrocytes suspension with 1% parasitemia (number of infected red blood cells per 100 red blood cells) was incubated with the compounds. Compound concentrations ranged between 48.5 nM and 100 µM, obtained by serial dilution, in duplicates. After 44 h of incubation at 37 °C, the plates were subjected to 3 freeze-thaw cycles to achieve complete hemolysis. The parasite lysis suspension was diluted 1:5 in lysis buffer. Monitoring of *in vitro* susceptibility uses the concentration that inhibits 50% of the parasite's growth (IC_{50}). Parasite growth was determined by using SYBR® Green I, a dye with marked fluorescence enhancement upon contact with parasite DNA. Incorporation of SYBR® Green I (Applied Biosystems, France) in parasite DNA was measured using the Mastercycler epRealplex (Eppendorf, France) according to the following program to increase the SYBR® Green I incorporation: 90 °C (1 min), decrease in temperature from 90 °C to 10 °C (during 5 min), followed by fluorescence reading. Untreated infected and uninfected erythrocytes were used as controls and chloroquine diphosphate (Sigma, France) as reference drug (Komlaga et al., 2016). IC_{50} was calculated by IC-estimator online software (http://www.antimalarial-icestimator.net).

2.4.2. In vitro antileishmanial activity on Leishmania donovani axenic amastigotes

Amastigote forms were suspended to obtain 10^7 cells/mL. Final concentrations obtained by serial dilution in triplicates ranged between 780 nM and 100 µM for pure compounds or 780 ng/mL and 100 µg/mL for extracts. Viability of amastigotes was assessed using the SYBR® Green I incorporation method (see above). Parasites were lysed following direct PCR-cell genotyping without DNA isolation protocol (Euromedex, France). 10 µL of lysed parasite solution was added to 40 µL of PCR-Cell reagent containing the SYBR® green I in a qPCR plate of 96 wells, and the contents were mixed. Fluorescence was measured with Mastercycler epRealplex (Eppendorf, France) and compared to those from the range obtained with different known parasite densities. Miltefosine was used as reference compound. The antileishmanial activity was expressed as IC_{50} in µM (concentration of drug inhibiting 50% of the parasite growth, comparatively to the controls treated with the excipient only) (Balaraman et al., 2015). IC_{50} was calculated by IC-estimator online software (http://www.antimalarial-icestimator.net).

2.4.3. In vitro antileishmanial activity on intramacrophagic amastigote form

The mouse monocyte/macrophage cell line RAW 264.7 was maintained in DMEM (Applied Biosystems, France) supplemented with 10% heat-inactivated fetal bovine serum. RAW 264.7 cells were seeded into a 96-wells microtitration plate at a density of 1,00,000 cells/well in 100 µL. After incubation in a 5% CO_2 incubator at 37 °C for 24 h, the culture medium was replaced with 100 µL of fresh DMEM containing a suspension of amastigote forms of 10^6 cells/mL. After incubation in a 5% CO_2 incubator at 37 °C for 24 h (the time needed by the parasite to infect the macrophage) the culture medium was replaced with 100 µL of fresh DMEM containing the test compounds for a new incubation period of 48 h. Final concentrations obtained by serial dilution in duplicates ranged between 780 nM and 100 µM for pure compounds or between 780 ng/mL and 100 µg/mL for extracts. After incubation, cells

Table 2
In vitro activity of extracts.

FAMILY and *Specie*	Plant part	*P. falciparum* 3D7 strain, IC_{50} ± SD, μg/mL	L. *donovani* LV9 strain (axenic amastigote form), IC_{50} ± SD, μg/mL	L. *donovani* LV9 strain (intramacrophagic amastigote form), IC_{50} ± SD, μg/mL	*Trypanosoma brucei gambiense* IC_{50} ± SD, μg/mL
Abrus arboreus	B	> 10	10.56 ± 0.01	> 20	> 10
Albizia niopoides	B	> 10	> 20	NT	> 10
Alchornea castaneifolia	L	> 10	> 20	NT	> 10
Alchornea triplinervia	B	0.38 ± 0.03	> 20	NT	> 10
Apeiba tibourbou	B	> 10	30.5 ± 3.25	1.71 ± 0.14	> 10
Bauhinia herrerae	L	> 10	> 20	NT	> 10
Calycophyllum multiflorum	B	> 10	> 20	NT	> 10
Campsiandra angustifolia	B	> 10	> 20	NT	> 10
Capirona decorticans	B	> 10	22.98 ± 0.79	0.69 ± 0.11	
Cedrela odorata	B	> 10	6.12 ± 0.67	1.49 ± 0.33	> 10
Cephaelis tomentosa	L	> 10	54.96 ± 4.58	6.39 ± 0.77	> 10
Chelonanthus alatus	AP	> 10	> 20	NT	> 10
Clarisia biflora	B	> 10	1.82 ± 0.26	10.34 ± 3.58	> 10
Clarisia racemosa	B	> 10	1.63 ± 0.08	> 20	> 10
Copaifera paupera	B	> 10	> 20	NT	> 10
Costus curvibracteatus	S	> 10	> 20	NT	> 10
Costus curvibracteatus	L	1.39 ± 0.3	31.68 ± 4.96	0.39 ± 0.07	3.84 ± 0.08
Dieffenbachia seguine	AP	0.7 ± 0.04	13.6 ± 1.53	> 20	> 10
Dracontium spruceanum	T	> 10	6.44 ± 0.72	3.21 ± 0.99	> 10
Ficus insipida	B	> 10	0.4 ± 0.01	3.04 ± 0.38	> 10
Grias neuberthii	B	0.04 ± 0.01	15.91 ± 2.17	2.84 ± 0.57	2.93 ± 0.22
Guarea guidonia	B	> 10	18.37 ± 4.76	1.39 ± 0.1	> 10
Gustavia angustifolia	B	> 10	> 20	NT	> 10
Handroanthus impetiginosus	B	> 10	> 20	NT	> 10
Handroanthus serratifolius	B	> 10	9.52 ± 1.18	1.55 ± 0.55	> 10
Hevea guianensis	B	7.41 ± 3.58	> 20	NT	> 10
Inga sertulifera	B	> 10	> 20	NT	> 10
Iriartea deltoidea	R	> 10	> 20	NT	> 10
Isertia hypoleuca	L	> 10	24.98 ± 3.69	1.51 ± 0.18	> 10
Ladenbergia oblongifolia	B	> 10	> 20	NT	> 10
Maytenus macrocarpa	B	1.49 ± 0.51	15.26 ± 0.9	19.92 ± 1.43	> 10
Minquartia guianensis	B	> 10	3.14 ± 0.15	> 10	> 10
Ormosia costulata	B	> 10	> 20	NT	> 10
Ormosia costulata	L	> 10	> 20	NT	> 10
Passiflora quadrangularis	L	> 10	> 20	NT	> 10
Piper coruscans	AP	1.36 ± 0.06	4.66 ± 0.59	6.1 ± 0.45	> 10
Pouteria guianensis	B	> 10	13 ± 0.34	14.25 ± 1.78	> 10
Pouteria guianensis	L	> 10	> 20	NT	> 10
Pouteria guianensis	R	> 10	> 20	NT	> 10
Prieurella prieurii	B	> 10	> 20	NT	> 10
Roucheria columbiana	B	> 10	> 20	NT	> 10
Simarouba amara	B	> 10	> 20	NT	> 10
Sloanea schomburgkii	B	1.52 ± 0.12	> 20	NT	> 10
Stachytarpheta cayennensis	AP	> 10	49.25 ± 3.87	0.95 ± 0.12	> 10
Swartzia simplex	B	0.12 ± 0.05	17.21 ± 3.37	13.3 ± 1.49	> 10
Tachigali polyphylla	B	0.04 ± 0.01	> 20	NT	> 10
Tessaria integrifolia	L	> 10	4.99 ± 1.27	0.51 ± 0.09	> 10
Triplaris weigeltiana	B	> 10	> 20	NT	> 10
Virola calophylla	B	> 10	0.38 ± 0.12	0.7 ± 0.06	> 10

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Table 2 *(continued)*

FAMILY and *Specie*	Plant part	*P. falciparum* 3D7 strain, IC_{50} ± SD, µg/mL	*L. donovani* LV9 strain (axenic amastigote form), IC_{50} ± SD, µg/mL	*L. donovani* LV9 strain (intramacrophagic amastigote form), IC_{50} ± SD, µg/mL	*Trypanosoma brucei gambiense* IC_{50} ± SD, µg/mL
Virola surinamensis	B	> 10	> 20	NT	> 10
Chloroquine		0.0062 ± 1.6(20 ± 5.15 nM)	NT	NT	NT
Miltefosine		NT	0.26 ± 0.08(0.65 ± 0.08 µM)	2.49 ± 0.45(6.1 ± 0.45 µM)	NT
Pentamidine		NT	NT	NT	0.0004 ± 0.00007(0.0012 ± 0-.0002 µM)

AP = aerial parts; B = bark; L = leaves; R = roots; S = stems; T = tuber; NT = not tested; NA = not active.

were visualized using an inverted microscope to check their morphology or the presence of parasites outside. The medium was subsequently removed and replaced by lysis buffer and the plates were subjected to 3 freeze-thaw cycles. Parasite growth was quantified by using SYBR® Green I incorporation following the protocol described above using uninfected or infected macrophages as control and miltefosine as reference compound (Balaraman et al., 2015). IC_{50} was calculated by IC-estimator online software (http://www.antimalarial-icestimator.net).

2.4.4. In vitro evaluation on bloodstream form of Trypanosoma brucei gambiense

Two-fold serial dilution of the compounds were performed in 100 µL of HMI9 medium in 96-wells microplates. Parasites were then added to each well (200 µL of a suspension of 4.10^4 cells/mL). After 72 h of incubation at 37 °C in 5% CO_2, 20 µL of 450 µM resazurin was added to each well and further incubated for 6 h at 37 °C in 5% CO_2. In living cells, resazurin is reduced in resorufin. This conversion is monitored by measuring the absorbance at specific wavelengths of resorufin (570 nm) and resazurin (600 nm) using a Multiskan MS microplate reader (Labsystems, France). Compounds activity was expressed as IC_{50}. Pentamidine di-isethionate was used as the reference compound (Pomel et al., 2015).

2.4.4.1. Cytotoxic activity. For determination of cytotoxicity IC_{50}, human umbilical vein endothelial cells (HUVECs) were cultured in Dulbecco's Modified Eagle medium/nutrient mixture F-12 (DMEM-F12) medium in the presence of 10% fetal bovine serum (FBS) plus 1% streptomycin and incubated in 5% CO_2 at 37 °C. The cytotoxicity of extracts (starting concentration of 10 mg/mL) was evaluated using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) colorimetric method (Mosmann, 1983). HUVECs were seeded in a 96-well plate at 15,000 cells per well and incubated for 24 h until cells reached > 80% confluence. After discarding the old medium, the cells were incubated in the medium containing test solutions at eight concentrations ranging from 0.78 to 100 µg/mL. After 24 h incubation, 20 µL MTT (5 mg/mL) was added to each well and cells were incubated for another 3 h. Finally, the culture medium containing MTT solution was removed and the formazan crystals were dissolved in 100 µL DMSO. Absorbance was read with an Eppendorf plate reader at 546 nm. CC_{50}, defined as extract concentration that reduced the number of viable cells by 50%, was calculated using GraphPad Prism Software (Version 5.0, San Diego, CA, USA) (Komlaga et al., 2016).

The mouse monocyte/macrophage cell line RAW264.7 was maintained in DMEM supplemented with 10% heat-inactivated fetal bovine serum. RAW 264.7 cells were seeded into a 96-well plate at a density of 5.10^3 cells/well in 100 µL of DMEM. After incubation in a 5% CO_2 incubator at 37 °C for 24 h, the culture medium was replaced with 100 µL of fresh DMEM containing the compounds to test. The number of living cells was determined by the Trypan blue dye exclusion assay, after additional 48 h incubation: the cells were suspended with a cell scraper, incubated with Trypan blue, and counted using a hemocytometer. The results are expressed in MTC (Maximum Tolerated Concentration), the maximum concentration in µM for which no toxicity was observed (Balaraman et al., 2015). CC_{50}, defined as extract concentration that reduced the number of viable cells by 50%, was calculated using GraphPad Prism Software (Version 5.0, San Diego, CA, USA).

3. Results

3.1. Ethnopharmacological survey

Communities where the surveys took place are groups locally called “Mestizos”, living in a rural habitat but not far from urbanized areas. They are settled on the merge of the Allpahuayo-Mishana National Reserve, characterized by rare ecosystems (white sand soils, argileous

Table 3
Antiplasmodial activity on *P. falciparum* 3D7 and W2 strain and cytotoxicity on HUVEC cells of most actives extracts filtrated on polyamide (IC_{50} < 10 µg/mL).

Species	*P. falciparum* 3D7 (extract w/o tannins) IC_{50} ± SD, µg/mL	Effect of filtration (activity increasing factor)	*P. falciparum* W2 IC_{50} ± SD, µg/mL	Cytotoxicity (% of living cells)
Alchornea triplinervia	0.62 ± 0.08	0.6	0.72 ± 0.07	100.0 ± 8.4
Costus curvibracteatus	NF	NF	1.16 ± 0.02	9.5 ± 0.8
Dieffenbachia seguine	NF	NF	0.01 ± 0.01	62.2 ± 6.4
Grias neuberthii	0.03 ± 0.01	1.3	0.03 ± 0.01	100.0 ± 19.7
Hevea guianensis	0.08 ± 0.62	92.6	4.94 ± 0.5	88.8 ± 6.4
Maytenus macrocarpa	0.02 ± 0.01	74	0.02 ± 0.01	44.1 ± 8.0
Piper coruscans	NF	NF	2.33 ± 0.26	43.6 ± 8.7
Sloanea schomburgkii	0.36 ± 0.08	4.2	0.7 ± 0.01	90.3 ± 8.1
Swartzia simplex	0.07 ± 0.01	1.7	0.77 ± 0.19	100.0 ± 10.3
Tachigali polyphylla	2.51 ± 0.21	0.02	2.66 ± 0.2	86.1 ± 5.2
Chloroquine	0.0062 ± 1.6 (20 ± 5.15 nM)	–	0.1 ± 0.02 (0.31 ± 0.06 µM)	NT

NT = not tested.

hills, marshes and floodable terraces) showing particular biodiversity. These communities live from traditional agriculture, fishery and animal farming within the reserve. Therefore, they have a daily contact with the rich biodiversity surrounding them, in addition to the traditional knowledge they inherited from their ancestors, parents or relatives. As their habitat, their culture interfaces traditional customs and urban education. As a consequence, their use of medicinal plants is exposed in some extent to modern medical concepts and this context may account for fewer biases in terms of unmatching disease concepts. People were interrogated with the medical concepts of “malaria” and “uta”, but during the interviews it appeared that they had their own way to relate the symptoms. They related malaria by: fever after mosquito bite (principally during rainy season when mosquito abundance is very high), headache, body discomfort/body pain (tertiary fever, shivering described as “scrapie”). In case of leishmaniasis, they related it by: exposed wounds in the body, fever, body discomfort/body pain (“They don’t wanna go to work”). In some cases, individuals would directly recognize the symptoms and use the terminology of malaria and uta.

Plants with their traditional indications and recipes are shown in Table 1. We collected 46 plants of 24 botanical families. One plant part was collected for each plant, except *Costus curvibracteatus* (stems and leaves), *Ormosia costulata* (bark and leaves) and *Pouteria guianensis* (bark, leaves and roots). Fabaceae is the most important family collected (9 species), followed by Rubiaceae (5), Euphorbiaceae, Moraceae (3 species of each family), Araceae, Bignonaceae, Lecythidaceae, Meliaceae, Myristicaceae, Olacaceae, Sapotaceae (2 species of each family) and other families with only one collected species.

3.2. Antiprotozoal activity

From these collections, 50 ethanolic extracts were made and tested. The plant part collected (bark, leaves, roots or tuber) was the plant part used traditionally (excepted for *C. tomentosa*). The protocols of use of the plants were very diverse, with some traditional preparations described as maceration in a local cane sugar alcohol (aguardiente). We chose cold ethanol extraction as a way to obtain most of the plants metabolites.

The protozoans species used for the screening have limited epidemiological and clinical relevance: *Plasmodium falciparum*, *Leishmania donovani* and *Trypanosoma brucei gambiense* were tested whereas *P. vivax*, *Leishmania* (*Viannia*) complexes of *L. braziliensis,* L. *peruviana* and L. *guyanensis* that are all associated with cutaneous leishmaniasis and *T. cruzi* are prevalent species in Peru and more widely Latin America (Marsden, 1986; Schwartz et al., 2006; Alroy et al., 2015). As a reminder, for malaria and leishmaniasis the treatment is the same whatever the species or the strain. For trypanosomiasis the treatment differs depending on the species. For all these parasites, development of drug resistance depends on the area and also impacts the choice of the treatment drug. In our case, several aspects including availability, reproducibility and ease of cultivation dictated the species and strains used for the screening. The fact they do not match the species actually affecting interrogated people has an impact on the ethnopharmacological relevance, but does not weakens the pharmacological outcome of the screening, especially the difference of selectivity among parasites. IC_{50} values for antiplasmodial, antileishmanial, antitrypanosomal activities of each extract are shown in Table 2. Ten plants were active on 3D7 *P. falciparum* chloroquine-sensitive strain with an IC_{50} < 10 µg/mL. Several of these extracts displayed a strong activity < 1 µg/mL: barks of *T. polyphylla* and *G. neuberthii* were the most active extracts at 0.04 ± 0.01 µg/mL. These extracts were more active than the reference compound chloroquine (IC_{50} = 0.13 µg/mL ± 0.01). Antileishmanial assays on L. *donovani* LV9 intramacrophagic amastigotes showed an IC_{50} < 10 µg/mL for 15 extracts (Table 2). Among them, 5 plants showed an activity < 1 µg/mL: *C. curvibracteatus* (0.39 ± 0.07), *T. integrifolia* (0.51 ± 0.09), *C. decorticans* (0.6 ± 0.11), *V. calophylla* (0.7 ± 0.06), *S. cayennensis* (0.95 ± 0.12). Only two plants showed moderate activity in the antitrypanosomal assay: *G. neuberthii* and *C. curvibracteatus* with IC_{50} of 3.84 ± 0.08 and 2.93 ± 0.22 µg/mL, respectively. Noteworthy, *G. neuberthii* and *C. curvibracteatus* displayed antiprotozoal activity against the three parasites tested.

Plants with mild or strong antiplasmodial activity (IC_{50} < 10 µg/mL) were tested on the chloroquine resistant *P. falciparum* W2 strain. When plant part was likely to contain tannins, the extracts were filtered on polyamide before testing their activity on W2 parasites and HUVEC cells. (Table 3). Six extracts showed an IC_{50} < 1 µg/mL on W2 strain: *D. seguine*, *M. macrocarpa, G. neuberthii*, *S. schomburgkii*, *A. triplinervia* and *S. simplex*, the most active being *D. seguine* (0.01 ± 0.01 µg/mL), *M. macrocarpa* (0.02 ± 0.01 µg/mL) and *G. neuberthii* (0.03 ± 0.01 µg/mL). Filtration on polyamide did in some cases remove a significant part of the extract but did not increase the activity, suggesting that tannins were not responsible of the activity. The cytotoxic assay of *A. triplinervia*, *S. simplex* and *G. neuberthii* extracts revealed no cytotoxicity on HUVEC cells (100% healthy cells). However, *C. curvibracteatus* extract presented a strong cytotoxicity (9% healthy cells).

G. neuberthii and *C. curvibracteatus* were fractionated by liquid/liquid partitioning. For *G. neuberthii*, antiplasmodial activity was concentrated in the ethyl acetate fraction (IC_{50} = 0.58 ± 0.07 µg/mL) whereas antileishmanial activity was concentrated in the cyclohexane and butanol fractions with IC_{50} of 1.93 ± 0.32 and 1.30 ± 0.11 µg/mL, respectively. For *C. curvibracteatus* antiplasmodial and antileishmanial activities were concentrated in water with IC_{50} of 3.02 ± 0.4 and 8.47 ± 1.58 µg/mL, respectively (Table 4).

Table 4
Antiprotozoal activity for the fractionation of *G. neuberthii* and *C. curvibracteatus*.

Species	Solvent	*P. falciparum* 3D7 strain IC_{50} ± SD, µg/mL	HUVEC cells CC_{50} ± SD, µg/mL	selectivity index CC_{50}/ IC_{50}	LV9 L. *donovani* IC_{50} ± SD, µg/mL	RAW 264.7 macrophages CC_{50} ± SD, µg/mL	selectivity index CC_{50}/ IC_{50}
Grias neuberthii	Cyclohexane	39.82 ± 2.89	> 100	> 2.51	1.93 ± 0.32	62.31 ± 4.29	32.28
	Dichloromethane	> 100	> 100	> 1	> 100	> 100	> 1
	Ethyl acetate	0.58 ± 0.07	12.40 ± 0.8	21.38	29.68 ± 2.87	10.37 ± 1.23	0.35
	1-butanol	56.35 ± 4.02	50.83 ± 2.96	0.9	1.30 ± 0.11	47.96 ± 3.54	36.89
	Water	> 100	18.51 ± 1.75	0.19	45.19 ± 2.54	9.79 ± 1.88	0.22
Costus curvibracteatus	Cyclohexane	42.43 ± 2.97	> 100	2.36	30.65 ± 3.03	> 100	3.26
	Dichloromethane	40.15 ± 2.51	13.75 ± 2.4	0.34	14.73 ± 2.30	12.23 ± 1.26	0.83
	Ethyl acetate	30.14 ± 4.71	> 100	> 3.32	12.12 ± 1.4	34.85 ± 2.41	2.88
	1-butanol	46.18 ± 9.3	> 100	> 2.17	> 100	> 100	> 1
	Water	3.02 ± 0.4	17.78 ± 3.09	5.89	8.47 ± 1.58	11.50 ± 0.86	1.36
Chloroquine		0.0062 ± 1.6 (20 ± 5.15 nM)	NT		NT	NT	
Miltefosine		NT	NT		2.49 ± 0.45 (6.1 ± 0.45 µM)	NT	

NT = not tested.

4. Discussion

People living in the Peruvian Amazon use edible/medicinal plants on a daily basis, either by cultivating them in their houses backyards or finding them near the community (Bussmann and Sharon, 2014). Loreto is the biggest but least populated region of Peru, mostly because of its inaccessibility due to multiple rivers surrounding it. The knowledge on medicinal plants in this region is very little explored and reported in research papers. Some reports exist on the use of medicinal antiprotozoal plants in Loreto, along with validation of their biological activities (Kvist et al., 2006; Ruiz et al., 2011). In our study, it is interesting to note that none of the plants used traditionally in this area were endemic from Peru. This is not surprising, as Amazonian forest overlaps several countries and is not a biodiversity hotspot by itself. Endemic plants, including those in Peru, are usually located in specific ecosystems like particularly dry or wet areas. As a consequence, the plants described in our study are most likely available for other communities or in other countries. In spite of this low specificity, 17% of the plants used traditionally in our survey were never reported as medicinal plants in the scientific literature. This observation highlights the interest of ethnopharmacological studies. Indeed, many traditional uses are probably under-reported, and their inventory can help establishing common aspects of different cultures when it comes to health and diseases. On the other hand, even if a plant is common to a wide geographic area, its use as medicine may be very specific of one particular area or community, thus representing a knowledge to preserve. Noteworthy, among the 46 plants collected, 42 are specific from Latin America, as checked on the Missouri Botanical Garden's “Tropicos” website. Only three plants can be also found in Africa, and one in Australia. During such studies, one of the major drawbacks is the discrepancy between medical and traditional nosologies. In our study the main criterion on which plants were selected as potential antiprotozoal agents was their use against fever. Fever is indeed caused by malaria and leishmaniasis, but can also be due to other etiologies, and an antipyretic remedy may not be antiprotozoal. Therefore, we can expect the *in vitro* validation to be somewhat disappointing, not to mention clinical validation. In our case, on the 46 plants collected, only 22 were active on one or several parasites *in vitro*. In terms of specificity, among these 22 active plants, only 5 were explicitly described traditionally as specifically active on malaria or leishmaniasis (uta), whereas most of them are used against fever, suggesting their use whatever the cause of the infection. However, although *Plasmodium* and *Leishmania* are both protozoan parasites, current treatments of these diseases differ and imply different molecules and mechanisms. Our *in vitro* data clearly indicate that among the active extracts, it is possible to distinguish four groups: one group of 12 extracts specifically active on L. *donovani* with an IC_{50} < 10 µg/mL, without any activity on *P. falciparum* (3D7 strain); one group of 5 extracts specifically active on *P. falciparum* with an IC_{50} < 10 µg/mL, without any activity on L. *donovani*; one group of 5 extracts simultaneously active on *P. falciparum* and L. *donovani* with an IC_{50} < 20 µg/mL; one group of 2 plants simultaneously active on *P. falciparum*, L. *donovani* and *T. b. gambiense* The clearcut specificity of many extracts for one or the other parasite is somewhat surprising.

A first group can be constituted by extracts showing specific antileishmanial activity. Among antileishmanial extracts, only one was described in the ethnopharmacological survey as specific of uta (*Dracontium spruceanum*), although it is not among the most active ones on L. *donovani*. It belongs to a group of extracts with IC_{50} comprised between 1 and 10 µg/mL, including *Apeiba tibourbou*, *Cedrela odorata*, *Cephaelis tomentosa, Ficus insipida, Guarea guidonia*, *Handroanthus serratifolius* and *Isertia hypoleuca*. Some extracts showed an IC_{50} < 1 µg/mL: *Capirona decorticans*, *Stachytarpheta cayennensis*, *Tessaria integrifolia* and *Virola calophylla*. In order to identify most interesting extracts, we also took into consideration mechanism of action besides activity levels. Leishmania are cellular parasites. Nevertheless, axenic conditions (parasite without host cells) being easier to grow, this model has been

the usual format of biological assays for nearly a decade (*ca* 1997–2007) until intracellular conditions have been developed around 2008. Intracellular assays are more relevant as protein expression, drug susceptibility, membrane hindrance and acidic environment of the phagolysosome is closer to the *in vivo* conditions. It has been shown that axenic assays are more susceptible to drugs and yield more false-positive results (De Rycker et al., 2013). As a rule, hits in axenic assays must be confirmed by intracellular assays. Compounds or extracts must be active on axenic as well as intracellular assays to be of interest. In our case, among the 23 extracts active on the axenic form, only 19 are still active on the intracellular form. Among them, 2 extracts are less active on the intracellular parasites, 7 have an equivalent activity on both forms and 10 are between 5 and 80 times more active on the intracellular form. One could consider that cytotoxicity could account for such a biased interpretation, but the type monitoring of the assay (fluorescence levels compared to those of healthy macrophages) is supposed to avoid confusing antiparasitic effect with unspecific cytotoxic effect. Therefore, a higher specific activity on intracellular forms suggests an activation of the antileishmanial effect by the host cell, which is particularly interesting. Therefore, a subgroup of 3 extracts can be identified, being simultaneously i) specific against leishmania, ii) more active on intracellular from and iii) active with an IC_{50} < 1 μg/mL (intracellular form): *Capirona decorticans*, *Stachytarpheta cayennensis* and *Tessaria integrifolia*. *Capirona decorticans* is an important timber tree in the Peruvian Amazon. The bark of this tree is used by Chayauitas community to cure symptoms of cutaneous leishmaniasis (Estevez et al., 2007). In this previous work, a negligible antileishmanial activity on L. *amazonensis* was reported (IC_{50} > 100 μg/mL). In our work, the bark extract had a strong activity on L. *donovani* (IC_{50} = 0.69 ± 0.11 μg/mL). *Stachytarpheta cayennensis* displayed a strong antileishmanial activity on L. *donovani* intramacrophagic amastigote form (IC_{50} = 0.95 ± 0.12 μg/mL). In the literature, antileishmanial activity (L. *amazonensis*) was recently demonstrated for the butanolic fraction of an aqueous extract and this activity was associated with selective inhibition of the parasite arginase (Maquiaveli et al., 2016). Antimalarial as well as anti-inflammatory and anti-ulcerogenic activities were previously described, authors suggesting that these activities could be attributed to the main arylpropanoid and iridoid constituents (verbascoside, isoverbascoside and ipolamiide) (Froelich et al., 2008; Penido et al., 2006). *Tessaria integrifolia* was reported in Southern America with other traditional uses than those reported in our work. In the Andes, it is used against liver and kidney diseases (Monigatti et al., 2013). In Central Amazon, Yanesha population use it against liver and kidney pain, urinary inflammation, prostatic pain and throat pain (Valadeau et al., 2010). In our work, the extract of this species showed a strong leishmanicidal activity (IC_{50} = 0.51 ± 0.09 μg/mL) close to miltefosine (0.44 nM, 0.18 μg/mL). Eudesmane-type sesquiterpenoids, with a strong cytotoxic activity against cancer cells, were identified in *T. integrifolia* (Minakawa et al., 2012; Ono et al., 2000). A last extract can also be considered of interest in this group: *Virola calophylla*. It is not significantly more active on intracellular forms, but is shows a strong activity on L. *donovani* intramacrophagic amastigote form (IC_{50} = 0.70 ± 0.06 μg/mL). *Virola calophylla* is a tree widely used as traditional medicine in the Peruvian Amazon (Rengifo, 2007). Neolignans were isolated from this plant, however their antiprotozoal activity has not been tested (Martinez et al., 1990).

In the second group of 5 plants specifically active on *P. falciparum*, none was traditionally described as specific of malaria. *Alchornea triplinervia* presented a strong activity on *P. falciparum* (IC_{50} = 0.38 ± 0.03 μg/mL). In Brazil, leaves and aerial parts are used traditionally in tea form to treat gastric disturbances. Ethyl acetate fraction showed an intense inhibition of H_2O_2 and NO production. It is supposed that such biological effect of *A. triplinervia* was related to the presence of phenolic compounds in the plant leaves (Mascia Lopes et al., 2010). *Dieffenbachia seguine* was very active on *P. falciparum* 3D7 strain and its activity was 70 times higher on W2 strain (IC_{50} = 0.01 ± 0.01 μg/mL). *D. seguine* is used in the Peruvian Amazon as medicinal plant (Kvist et al., 2006; Rengifo, 2007), in spite of it toxicity (Arditti and Rodriguez, 1982; Fochtman et al., 1969; Ladeira et al., 1975). Chemistry of this species has been little explored so far (Walter and Khanna, 1972). *Hevea guianensis* displayed a strong activity on *P. falciparum* 3D7 after tannins filtration (IC_{50} = 0.08 ± 0.62 μg/mL). Until now, no work related to the antiprotozoal activity and chemistry of this species existed, though studies are reported on the Hevea genus and principally on the *H. brasiliensis* latex (Jacob et al., 1993). To our knowledge, the two species *Tachigali polyphylla* and *Sloanea schomburgkii* were never reported as medicinal plants. For *T. polyphylla*, the removal of tannins decreased the activity by a factor 60, suggesting that tannins are responsible for a significant part of the activity. For *S. schomburgkii*, the bark extract displays a strong activity (IC_{50} = 0.7 μg/mL on W2), which is obviously not borne by tannins.

Noteworthy, among the 10 extracts tested on 3D7 as well as W2 strains (Table 3), 2 of them are less active on W2 (*H. guianensis* and *S. simplex*), most of them have equivalent activity on both strains and one of them is more active on W2 (*D. seguine*). This variety of results suggests that the assays have been properly performed and are discriminating. For extracts less active on W2, we can assume that their mechanism of action is similar to chloroquine or that the compounds are recognized by the parasite's efflux pumps. For extracts similarly active on 3D7 and W2, we assume that the mechanism(s) of action is/are different from quinolines. The extract more active on W2 is very intriguing and we cannot explain it.

In the third group of 5 plants either active on *P. falciparum* or L. *donovani*, the most active was the bark of *Maytenus macrocarpa,* which is commonly used in Peruvian traditional medicine, with a significant antiplasmodial activity equivalent on 3D7 and W2 strain (IC_{50} = 0.02 ± 0.01 μg/mL). Surprisingly, the removal of tannins led to a 50 times increase of antiplasmodial activity. Activity on L. *donovani* was not as good though not negligible (IC_{50} = 19.9 μg/mL). This is not in accordance with a previous report on an hydro-alcoholic extract of the bark of this species, which had no activity on chloroquine resistant strain FCR-3 of *P. falciparum* (Ruiz et al., 2011). Dammaranes, nor-triterpenes, sesquiterpene polyol esters and lignans were isolated from *M. macrocarpa*, and some of these compounds showed an activity on cancer cells (Chávez et al., 2000, 1999; Spivey et al., 2002; Torpocco et al., 2007). Aerial parts of *Piper coruscans* are used as infusion in the Peruvian Amazon to treat malaria. Our study demonstrated that *P. coruscans* extract is active on *P. falciparum* 3D7 strain (IC_{50} = 1.36 ± 0.06 μg/mL), however this activity decreases two times when tested against W2 strain. Cyclopentenediones from *P. coruscans* displaying significant antifungal activity against *Candida albicans* (IC_{50} < 2 μg/mL) have been isolated (Li et al., 2004). The bark extract from *Swartia simplex* after filtration exhibited a strong antiplasmodial activity on 3D7 strain (IC_{50} = 0.07 ± 0.01 μg/mL), however was 10 time less active on W2 strain. Antiprotozoal activity of this species was not explored so far. Nevertheless diterpens displaying strong activity against *C. albicans* have been isolated from the roots of *S.* simplex and molluscicidal saponins have been isolated from the leaves (Borel et al., 1987; Favre-Godal et al., 2015).

Finally, according to our biological results (Table 2), the two most interesting extracts found in this work are *Costus curvibracteatus* (Costaceae) leaves and *Grias neuberthii* (Lecythidaceae) bark. These plants were active *in vitro* on the three tested parasites. As shown in Table 3, tannins cannot be responsible for any non-specific antiplasmodial activity. Also, concerning their antileishmanial activity, *G. neuberthii* is 6 times more active on the intracellular form without showing any cytotoxicity, confirming that the surprising increase of activity on the intracellular form is not due to an unspecific effect on the host cells. *C. curvibracteatus* is cytotoxic and this may account for the unique, 2-fold higher activity on intracellular form.

The tropical monocotyledonous family Costaceae belongs to the “gingers family” (Zingiberaceae, Costaceae, Cannaceae, Marantaceae).

Costus, the principal and largest genus, is pantotropical with its greatest diversity centered in the neotropics (*ca* 40 species); 25 species occur in tropical Africa and about 5 species in southeastern Asia (Specht et al., 2001). In Amazon the leaves of *C. curvibracteatus* are used against parasitic pains and inflammation (Rengifo, 2007). No chemical studies had been carried out on its constituents, however saponins, flavonols diglycosides and polysaccharides were identified in rhizomes, leaves and stems of *C. spitacus* (Da Silva et al., 2000, 1999; Pereira da Silva and Paz Parente, 2003). In other articles, anti-inflammatory, anti-nociceptive and toxicity activities were mentioned for *C. spitacus* and *C. pulverulentus* (Alonso-Castro et al., 2016; Quintans Junior et al., 2010). From the phylogenetic closeness between Costaceae and Zingiberaceae families we can assume a chemotaxonomic proximity. Two germacradiene alcohols isolated from *Reneilmia cincinnata* were reported to be active *in vitro* on *P. falciparum* D6 and W2 strains (Tchuendem et al., 1999). Diterpenes active on *Trypanosoma brucei brucei* were isolated from *Curcuma aromatica* (Schmidt et al., 2012). Two antiparasitic diterpenes have been isolated from *Aframonum spectrum*, one of them presenting a strong activity on L. *donovani* (IC_{50} = 5.7 μM) and a moderate activity on *T. brucei brucei* (IC_{50} = 35.7 μM) (Cheikh-Ali et al., 2011). The strong antiprotozoal activity of *C. curvibracteatus* initial extract on *P. falciparum* (IC_{50} = 3.02 ± 0.04 μg/mL) and L. *donovani* (IC_{50} = 8.47 ± 1.58 μg/mL) was concentrated in the aqueous fraction (Tables 2, 3). From the bibliographic references cited above, we can infer the presence of saponins in the aqueous extract. Such compounds would support the strong cytotoxicity observed against healthy cells (9.5 ± 0.8%) and therefore a selectivity index < 10 (Table 4).

Lecythidaceae family, known as the "Brazil nut family", is considered as the third most abundant phylogenic group of the Amazon with about 25 genera and 489 species (Mori et al., 2007, 1990). People in the Peruvian Amazon consume the fruit of *G. neuberthii* because of its very fatty mesocarp. Bark and leaves are traditionally used against leishmaniasis, malaria and other diseases (Rengifo, 2007). To our knowledge, there are neither studies carried out on the antiprotozoal activity from the *G. neuberthii* bark nor compounds isolated from this species. Phytochemical studies of Lecythidaceae plants are still scarce and are restricted to 21 species in thirteen genera (Oliveira et al., 2012). Chemical constituents identified in plants of the Lecythidaceae family include monoterpenoids, neoclerodane diterpenoids, sesquiterpenoids, pentacyclic triterpenoids and saponins, steroids, alkaloids, phenolic compounds, flavonoids, ellagic tannins, depsides with phenol-ester linkage like gustastatin (Oliveira et al., 2012; Pettit et al., 2004; Ragasa et al., 2012, 2011). Several pharmacological activities of extracts from plants of this family have been reported, such as antinociceptive, antibacterial, antitumoral, anti-inflammatory, antifungal, antileishmanial, antioxidant, hepatoprotective and cytotoxic (Bomba et al., 2015; Oliveira et al., 2012). Another study focused on the cytotoxic activity of the ethanol extract of *Lecythis pisonis* on colon cancer cells HCT-8 highlighting the presence of triterpenoids in the extract (Oliveira et al., 2012). The most representative species of Lecythidaceae family is *Bertholletia excelsa* (Brazil nut or *castanheira*), a typical plant from South America. The internal bark of the fruits is used in traditional medicine for the treatment of malaria (Oliveira et al., 2012). Phytochemical analysis of a stem bark extract from *B. excelsa* afforded several fractions with weak *in vitro* activity (75.4% growth inhibition of *T. cruzi* at 500 μg/mL) (Schmidt et al., 2012). In our study, bioguided fractionation of *G. neuberthii* revealed that ethyl acetate fraction concentrates the antiplasmodial activity and the butanol fraction concentrates the antileishmanial activity (Table 4). These results can be compared with previous studies where hydro-ethanolic extracts of *G. neuberthii* fruits were inactive against *P. falciparum* FCR-3 and 3D7 strains (Kvist et al., 2006; Ruiz et al., 2011). The low and medium polarity compounds conferring activity to the extracts could be terpenes, flavonoids or alkaloids. Moreover, no toxicity on healthy cells was found, giving a good selectivity index to these extracts (*P. falciparum* = 21.4 and L. *donovani* = 36.9), making them interesting for further exploration.

5. Conclusions

Results from our ethnopharmacological surveys and *in vitro* results corroborated the relevance of several of the traditional treatments based on the use of plants. The list of plants presented in this work provides a selection of candidates for antiprotozoal research, with the aim of isolating active components. The traditional use of plants and methods for treating diseases is part of the richness of the mixed culture of rural mestizos. These communities easily share this knowledge, making it a resource that should be respected and preserved with the creation of indigenous pharmacopeias. *Grias neuberthii* (Lecythidaceae) and *Costus curvibracteatus* (Costaceae) have a high potential of providing active principles due to their strong antiprotozoal activity shown on this work. Further isolation and structural identification of active compounds will be performed in our laboratory.

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