Ethnopharmacological evaluation of medicinal plants used against malaria by quilombola communities from Oriximiná, Brazil

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ABSTRACT

Ethnopharmacological relevance: Malaria is the most important parasitic disease in the world, including in the Amazon region, due to its high incidence. In addition, malaria is difficult to control because of the geographical characteristics of the endemic Amazon region. The quilombola communities of Oriximina, located in remote rainforest areas, have extensive experience with medicinal plants due to their close contact with and dependence on local biodiversity as a therapeutic resource. To search for active bioproducts against malaria, based on in vitro tests using blood culture-derived parasites and plants selected by an ethno-directed approach in traditional quilombola communities of Oriximiná, in the Amazon region of Brazil.

Materials and methods: Ethnobotanical data were collected from 35 informants in the quilombola communities of Oriximiná, Brazil, by a free-listing method for the survey of species locally indicated to be effective against malaria and related symptoms. Data were analyzed by salience index (S) and major use agreement. The activity of extracts from 11 plants, selected based on their Salience values (four plants with $S > 1$; seven plants with $S < 0.1$), was measured in vitro in cultures of W2 clone Plasmodium falciparum parasites resistant to chloroquine.

Results: Thirty-five ethnospecies comprising 40 different plants belonging to 23 botanical families and 37 genera were listed as antimalarials by the ethno-directed approach. Among these, 11 species selected based on their S values were assayed against P. falciparum. The most active plant extracts, with an IC_{50} as low as 1.6 μg/mL, were obtained from Aspidosperma rigidum (Apocynaceae), Bertholletia excelsa (Lecythidaceae) and Simaba cedron (Simaroubaceae), all of which displayed an S value > 1.

Conclusion: A strong correlation between the consensus of the informants from quilombola communities living in a malaria endemic area and the salience index indicating antiplasmodial activity was observed, where the ethnospecies mostly cited to be effective against malaria produced the most active plant extracts in vitro. It was also evident from the data that these groups approached the treatment of malaria with an holistic view, making use of purgative, depurative, emetic and adaptogen plants.

1. Introduction

Malaria affects millions of people in tropical and subtropical regions of the world and is a major source of human suffering and high mortality; in 2012, there were approximately 207 million malaria cases and 627 thousands malaria-related deaths (WHO, 2012). The disease is characterized by the typical symptomatology: high and intermittent fever accompanied by malaise, headache, body pain, tiredness, weakness, vomit, intense sweating, anemia, jaundice, and swelling of the spleen and liver (Kotepui et al., 2015). Five species of Plasmodium, transmitted by Anopheles spp. mosquitoes, cause human malaria, but in Brazil, two species are predominant: Plasmodium falciparum, the most virulent, and Plasmodium vivax, the most prevalent (87%) (Brazil, 2014). In addition, P. vivax may cause a late-stage relapsing disease due to the remaining liver parasites in latency (Breman, 2001; Lacerda et al., 2014), named hipnozoites, which make it more difficult to control

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the species. A negative impact on social development, correlated social inequalities and increased risk of developing malaria are described among the poorest population as being more susceptible to the disease symptoms compared to the wealthiest local populations (Junior et al., 2014).

There are a vast number of drugs available as antimalarials; however, malaria treatment has been hampered by the appearance and dispersion of drug-resistant parasites that require drug associations with artemisinine derivatives. This compound was originally derived from the plant species *Artemisia annua,* employed for millennia in Chinese medicine against fever and hemorrhoids (Krettli, 2009). *P. falciparum* parasites are vastly resistant to chloroquine, as well to pyrimethamine and sulfadoxine (WHO, 2012). Cases of therapeutic failure of *P. vivax* malaria after cloroquine treatment have been reported in Brazil, and this parasite is already resistant in other parts of the world (Chehuan et al., 2013; Price et al., 2009; Suwanarusk et al., 2007)

The principal antimalarial treatment, effective against human malaria appeared approximately 1770 and contained infusions of bark from the *Cinchona* sp. trees, a plant native to the Peruvian Amazon. After many years of empirical phytotherapy, the alkaloid quinine was identified in this plant species by French chemists as being responsible for the plant activity (in Garnham, 1966). *Cinchona* plants were historically used against fever and malaria until the Second World War, when chloroquine was synthesized and used to treat the disease. This was one of the first synthetic antimalarials based on the quinine molecule (Sweeney, 2000). Other drugs were synthesized for the treatment of malaria, including amodiaquine, mefloquine, primaquine, derivatives of the quinoline moiety. More recently, the search for new drugs has been based on the exploitation of biodiversity including studies in the Amazon region (Brandão et al., 1985; Brandão et al., 1992a; Carvalho et al., 1991), and the ethnopharmacological approach has gained an increased attention (Willcox et al., 2004).

The vast Amazonian biodiversity as well as the traditional knowledge of the forest by people living in the region composed primarily by *caboclo-river* dwellers, indians, *quilombolas* and other traditional groups (Oliveira et al., 2010b) are potential sources for the discovery of new therapeutic agents. Malaria has been eradicated from most of Brazil but remains endemic in the Amazon, where vast collections of water make the control of mosquitos difficult. There are only a few ethnodirected studies for this region focusing on the bioprospecting of antimalarial plants (Spencer et al., 1947; Brandão et al., 1985; Ruiz et al., 2011; Krettli et al., 2001).

The municipality of Oriximiná, located in northern Brazil, Pará State, has 33 *quilombola* communities that are ethnic and racial groups according to criteria of self-attribution. These communities have their own historical background and are endowed with specific territorial relations with presumption of black ancestry related to the historical resistance to oppression suffered (Oliveira et al., 2010b). These communities have sprung up in remote areas of the Amazon forestry after the escape of African slaves from farms planting cocoa, coffee and cotton (Oliveira, 2009; Oliveira et al., 2012). The *quilombolas* are endowed with extensive experience in the use of medicinal plants, as they have centuries of close contact and dependence of local biodiversity as a means of livelihood and as a therapeutic resource. This fact makes the traditional communities attractive for conducting an ethnodirected study for medicinal plants used against malaria and related diseases. The aim of the present work was to select plants used medicinally to treat malaria and fever by the ethnopharmacological approach in the *quilombola* communities (maroons) of Oriximiná and to evaluate the *in vitro* antiplasmodial activity of certain plants with cultural relevance in these communities.

2. Materials and methods

2.1. Characterization of the search area

The city of Oriximiná located in State of Pará, northern Brazil, is bordered by Suriname, Guyana and French Guiana to the north, the cities of Faro, Juruti, and Óbidos to the South and East, and the States of Amazonas and Roraima to the West. It has an area of 107.603 km² and is the second largest municipality in the Brazilian territory. According to the 2010 census, Oriximiná has 62,963 inhabitants, which includes 40,182 in urban areas and 22,781 in rural areas (IBGE, 2010). Currently, there are 33 known *quilombola* communities in Oriximiná that are divided into eight territories (Água Fria, Boa Vista, Trombetas, Erepecuru, Alto Trombetas, Jamari/Último Quilombo, Moura, and Ariramba) that, together, encompass more than 600,000 ha (Fig. 1). The *quilombolas* are represented by their association, called the "Associação de Comunidades Remanescentes de Quilombos do Município de Oriximiná" or ARQMO (Remaining of the *Quilombo* Communities Association from Oriximiná City). In this work, five communities representing two "quilombola" areas were chosen: Bacabal and Arancuã-de-Cima from the Trombetas region and Serrinha, Jauari, and Pancada, from the Erepecuru region (Fig. 1).

2.2. Ethnopharmacological data collection

This work received authorization for access to the traditional knowledge associated with bioprospecting by the Directing Council of Genetic Heritage (Conselho de Gestão do Patrimônio Genético-CGEN) through Resolution no. 213 (06.12.2007), published in the Federal Official Gazette of Brazil on 27 December 2007 (Oliveira et al., 2010b).

The selection of the interviewees by the "snowball method" began with the search for key informants who were respected people in the community, such as the community coordinator, matriarch or patriarch, and/or community health agents. These individuals could indicate and lead us to the local specialists, who were "quilombolas", with wide experience in the use of medicinal plants as extractives, woodsmen, healers, faith healers, prayer ladies, midwives, and "puxadores" or "puxadoras" (traditional chiropractors). For data acquisition, four ethnobotanical field trips were performed between the period of June 2006 (after signing the Prior Informed Consent between UFRJ and ARQMO) until September 2008. Each field worker had a residence period of 30–60 days in the communities that were studied.

Ethnobotanical data were collected through semi-structured interviews, participating observation and walk-in-the-woods. The formularies applied included socio-economic data (sex, age, professional, level of schooling, monthly family income, number of residents) and the medicinal plant information (common name, therapeutic indications, doses, preparation methods, counter-indications) including where to obtain the plant. Thirty-five quilombolas from the 5 communities studied (20 women and 15 men) were interviewed. They were between 19 and 87 years old. They survived mainly by fishing, hunting, and subsistence farming, and their only source of income was the extraction of the Pará nut ("Brazil nut"), which is available for only a few months of the year.

Quantitative data analysis techniques, such as the salience index (S) and corrected major use agreement (MUAc), were also applied.

2.2.1. Free-list and salience index (S)

The free-list technique can identify items within an emic category or a cultural domain, and it offers a direct method to obtain data easily and simply (Thompson and Juan, 2006). It has also been used as an exploratory technique for bioprospecting (Oliveira
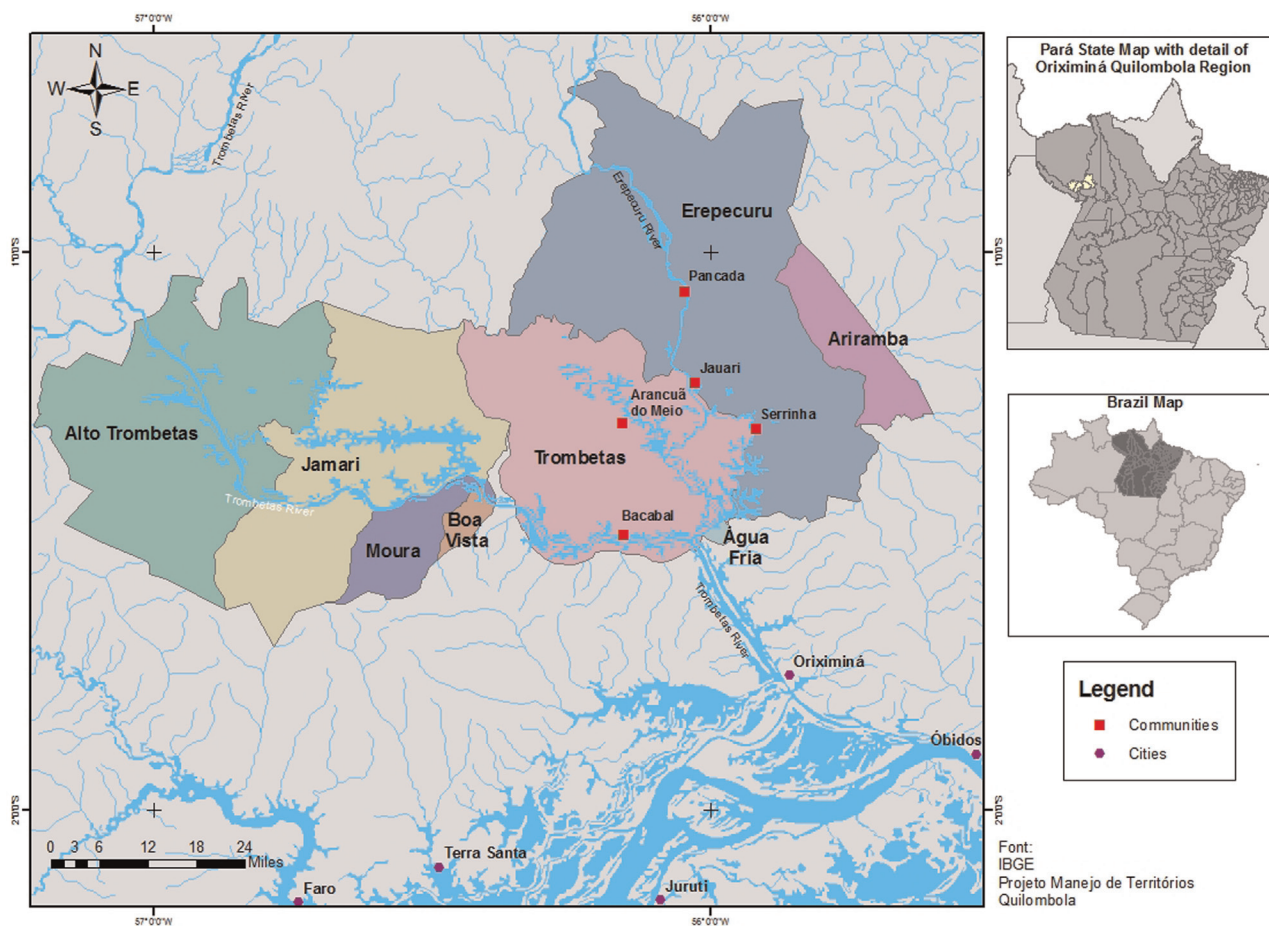
Fig. 1. Map of the eight areas of Oriximiná comprising 33 maroon communities located in Pará State, Brazil, with emphasis on the map for the 5 communities studied.

et al., 2011a, 2012). Using this technique, a direct list of medicinal plants known and used by the informers was obtained with the aim of searching for specific information about this cultural domain within the communities and its diffusion. Additionally, an ethnodirected inquiry regarding the plants used against malaria and related symptoms and disorders (high fever, hepatitis, liver and jaundice) was conducted. These emic terms used locally were surveyed in a previous study performed in Oriximiná City in 2004 (Oliveira, 2004) through participant observation and ethnographic techniques. Altogether, these approaches indicate the most important cultural elements and the order of their importance (Albuquerque and Lucena, 2004). The *S* was calculated using the program ANTHROPAC 4.0 (Analytic Technologies, USA)

2.2.2. Major Use Agreement (MUA)

The Major Use Agreement (MUA) is a quantitative technique used to evaluate the agreement between the main uses cited by various informants that have been applied by our group with ethnopharmacological purposes (Oliveira et al., 2006; Oliveira et al., 2012). MUA is determined as the ratio between the number of informers who independently cited the species for a major use (MU) and the total number of informers who mentioned that species for any use (total uses, TU_s) (Amoroso and Gély, 1988).

$$MUA = \frac{MU}{TU_s} \times 100$$

A correction factor (CF) was applied to calculate the corrected MUA (MUAc), given by the formula: MUAc = MUA × CF. The

correction factor is the ratio between the number of informers citing each species for any use (TUs) and the highest number of informers citing the most-cited species. In this study, *Dipteryx odorata* was the most-cited species and was cited by 24 informers (TU_{s+}) (Oliveira et al., 2011a).

$$CF = \frac{TU}{TU_{s+}}$$

2.3. Selection of the plant species and the preparation of the extracts

From the free listing, a total of 11 species were selected for testing against malaria according to their *S* value (high/low) (Table 2). Air-dried and powdered plant materials were used for the preparation of extracts (Table 2). The samples were extracted exhaustively by maceration with ethanol 96°GL at room temperature. After filtration and concentration under reduced pressure, the residues were sequentially extracted by liquid–liquid partition between water and hexane, dichloromethane, ethyl acetate and n-butanol, in this order. The organic extracts were concentrated under reduced pressure. The aqueous extract from the barks of *Ampeloziziphus amazonicus* was obtained by the quilombola traditional method of preparation, as previously described by Oliveira et al. (2011b), while the sap of *Bertholletia excelsa* was obtained from a piece of the inner bark, known as "envira", which was removed, dampened in water and twisted to get a concentrated juice. Both aqueous extracts were freeze-dried.

Table 1
Ethnospecies listed in order of the salience values for malaria (and related symptoms and diseases) and the respective MUA and MUAc.

Species	Ethnospecies	Voucher number	Used part	Indications to "malaria and related diseases and symptoms"	FR (%)	S	TI	MUA (%)	MUAc (%)	Antiplasmodial activity in the literature[a]	Uses against malaria and fever previously described in the literature[a]	Continents
Ampelozizyphus amazonicus Ducke[b] Rhamnaceae	Saracuramirá	INPA 224161	Bark	**Malaria (12)**, liver (5), depurative (2); purgative (1); anemia (1), lack of appetite (1)	50	0.36	12	100.0	50.0	PF− [1,2]; OBS: prophylatic against PG+ [3] and sporozoites PB+ [5]	[1,4,6] OBS: the prophytatic use is also highlighted by [1,2,7]	South America
Aspidosperma excelsum Benth.[b] (Syn. *A. marcgravianum*) and *Aspidosperma rigidum* Rusby[b] Apocynaceae	Carapanaúba	INPA 224692; INPA 224704	Bark	**Malaria (10)**, liver (7); fever (1); migraine (1), body pain (1)	36.4	0.251	10	100.0	41.7	PF+ Against *Aspidosperma* spp. [8,9,10,11]	[12,13,10]	South America
Geissospermum argenteum Woodson[b] Apocynaceae	Quinarana	INPA 224162	Bark	Liver (7), **malaria (5)**, hepatitis (2)	40.9	0.249	8	62.5	20.8	PF+[14,15]	[13,16]	South America
Simaba cedron Planch.[b] Simaroubaceae	Pau-paratudo	INPA 223283	Bark	**Malaria (4)**	18.2	0.148	6	66.7	16.7	PF+[17]	[12,17,18]	South America, North America
Bertholletia excelsa Bonpl[b] Lecythidaceae	Castanheira	INPA 224171	Internal rind of the fruit	**Malária (2)**, jaundice	9.1	0.124	16	6.3	4.2	Not found	[19]	South America
Operculina hamiltonii (G. Don) D.F. Austin & Staples.[b] (Syn. *O. alata*) Convolvulaceae	Batatão, batata-de-purga	INPA 223281	Tubercle	Purgative (7), blood depurative (5), hepatitis (1), **malaria (2)**	9.1	0.091	10	20.0	8.3	Not found	Not found	South America
Machaerium ferox (Benth.) Ducke[b] Fabaceae	Saratudo	INPA 233440	Stem	**Malaria (1)**; jaundice (1)	9.1	0.082	14	7.1	4.2	PF+[10]	Not found	South America
Bidens bipinnata L.[c] Asteraceae	Picão, carrapicho	INPA 223287	Roots	Malaria (3); liver (3)	13.6	0.08	8	37.5	12.5	PF+ and PB+ against *B. pilosa* [20,21]	[3,13, 19, 22, 23]	South America, Africa
Carapa guianensis Aubl.[b] Meliaceae	Andiroba	INPA 223282	Seeds	**Malaria (2)**	9.1	0.08	16	12.5	8.3	PF-[10]	[6,13, 19]	South America, Africa, Asia
Phyllanthus caroliniensis Walter[b] *Phyllanthus orbiculatus* Rich.[b], *Phyllanthus stipulatus* (Raf.) G. L.[b] Euphorbiaceae	Quebra-pedra	INPA 224168, INPA 224134, INPA 233363	Whole plant	**Malaria (2)**; jaundice (1)	9.1	0.07	11	18.2	8.3	PB+[24,25,26]	[13,26,27]	South America, Africa, Asia
Luffa operculata Cogn.[b] Curcubitaceae	Cabacinha	INPA 224139	Fruit	Vomitory (3), **malaria (2)**	9.1	0.069	9	22.2	8.3	Not found	[17, 19]	South America
Cedrela odorata L.[b] Meliaceae	Cedro	INPA 223380	Bark	**Malaria (2)**	9.1	0.061	5	40.0	8.3	PF+[2,10]PF− [28]	[13,2,10]	South America, Africa
Physalis angulata L.[c] Solanaceae	Gamapu, camapu	INPA 224149	Roots	Liver (3); anemia (2); hepatitis (2); **malaria (1)**	9.1	0.056	5	20.0	4.2	PF+[10,29,41]	[13,30]	South America, Africa
Senna occidentalis Link.[b] Fabaceae	Paramagioba	INPA 223302	Roots	**Malaria (1r)**; anemia (1)	4.5	0.045	2	50.0	4.2	PF+[10,21,23,25]	[13, 19, 23,27,31]	South America, Africa, Asia
Carica papaya L.[c] Caricaceae	Mamão-macho	INPA 224694	Yellow leaves	**Malaria (1)**; liver; to clean the intestine; nausea; to stop vomit; uneasiness; anemia; problem in the spleen (1)	4.5	0.045	14	7.1	4.2	PF+[32,[33]	[13, 18,19,30,33]	South America, Africa, Asia
Croton sacaquinha Croizat [b] Euphorbiaceae	Sacaquinha, piaçoca	INPA 224660	Bark	Liver (3); **malaria (1)**; migraine (1)	4.5	0.042	3	33.3	4.2	Not found	Not found	South America
Citrus×aurantium L.[b] Rutaceae	Laranja-da-terra, laranjeira	INPA 224695	Peel fruit	Liver (2); **malaria (2)**; anemia (1); weakness (1); headache (1); migraine (1);	4.5	0.038	10	20.0	8.3	PF+ [10]	[10,13,23,30]	South America, Africa
Cinnamomum verum J.Presl[c] Lauraceae	Canela	INPA 224156	Leaves	Weakness (5); headache (2); migraine (1); liver	4.5	0.034	7	28.6	8.3	Not found	[13,23,27]	South America, Africa

Table 1 (*continued*)

Species	Ethnospecies	Voucher number	Used part	Indications to "malaria and related diseases and symptoms"	FR (%)	S	TI	MUA (%)	MUAc (%)	Antiplasmodial activity in the literature[a]	Uses against malaria and fever previously described in the literature[a]	Continents
				(2); **malaria (2)**								
Mangifera indica L.[c] Anacardiaceae	Manga-grande, mangueira	INPA 224636	Bark	**Malaria (1)**	4.5	0.032	8	12.5	4.2	PF− [10,29]	[10,13 23,27,30]	South America, Africa, Asia
Spilanthes acmella (L.) L.[b] Asteraceae	Jambu, jambuí	INPA 223275	Aerial parts	Liver (8); **malaria (1)**;hepatitis (1); uneasiness (1); nausea (1); fever (1)	9.1	0.03	12	8.3	4.2	PF+ [34]	[35]	South America
Gymnanthemum amygdalinum (Delile) Sch.Bip. ex Walp (Syn: *Vernonia condensata* Baker)[c] Asteraceae	Figatil	INPA 224659	Leaves	Liver (6); **malaria (1)**; migraine (1);	4.5	0.03	10	10.0	4.2	PF+ [21,36,37]	[35]	South America, Asia
Uncaria guianensis (Aubl.) J.F.Gme[b] and *Ferdinandusa rudgeoides* (Benth.) Wedd.[b] Rubiaceae	Unha-de-gato	INPA 224608; INPA 223278	Bark	Anemia (2); **malaria (1)**	4.5	0.027	12	8.3	4.2	PF− [10,28]	[41]	South America
Plectranthus barbatus (Andrews) Benth.[c] Lamiaceae	Melhoral, boldo	INPA 224638	Leaves	Liver (7); ressaca (1); migraine (1); **malaria (1)**; anemia (1)	4.5	0.027	10	10.0	4.2	PF− [38]	[35]	South America, Africa
Croton cajucara Benth.[b] Euphorbiaceae	Sacaca	INPA 224661	Bark	Liver (4), **malaria (1)**; hepatitis (1)	4.5	0.023	4	25.0	4.2	Not found	[13, 19]	South America
Ruta graveolens L.[c] Rutaceae	Arruda	INPA 224600	Aerial parts	Fever (4), high fever that makes onesquirm all "faz a pessoa encarangar" (1), **malaria (1)**; headache (1), body pain (4), to prevent diseases (2), weakness (1)	4.5	0.023	19	5.3	4.2	Not found	[12,35]	South America
Parahancornia fasciculata (Poir.) Benoist[b] And *Brosimum* sp.[b] Moraceae	Amapá-amargo	INPA 224693	Latex	**To prevent malaria (1)**; to strengthen the blood (1)	4.5	0.023	7	14.3	4.2	Not found	[39]	South America
Citrullus lanatus (Thunb.) Matsum. & Nakai[c] Curcubitaceae	Melância	Without Voucher Number	Seeds	**Malaria (1)**	4.5	0.023	2	50.0	4.2	Not found	[40]	South America
Sesamum indicum L.[c] Pedaliaceae	Gergelim	INPA 224675	Seeds	**Malaria (1)**	4.5	0.023	11	9.1	4.2	Not found	Not found	South America
Euterpe oleraceae Mart.[b] Arecaceae	Açaí	INPA 224644	Roots	anemia (8); hepatitis (5); liver (2); jaundice (1); weakness (1); **malaria (1)**	9.1	0.019	13	7.7	4.2	PF− [10,28]	[10,13,18]	South America
Dalbergia riedelii (Benth.) Sandwith[b] Fabaceae	Verônica	INPA 224158	Bark	**Malaria (1)**, anemia (1)	4.5	0.018	6	16.7	4.2	Not found	Not found	South America
Quararibea guianensis Aubl.[b] Malvaceae	Inajarana	INPA 233369	Bark	**Malaria (2)**, hepatitis (1), anemia (1), liver (1)	9.1	0.013	3	66.7	8.3	Not found	Not found	South America
Jatropha curcas L.[c] Euphorbiaceae	Peão-branco	INPA 224670	Seeds	Purgative (4), **malaria (1l)**, vomitory (1), lack of appetite (1)	4.5	0.011	23	4.3	4.2	PF+ [23]	[17,23,27]	South America, Africa, Asia
Artemisia vulgaris L.[c] Asteraceae	Anador	INPA 224615	Aerial parts	Headache (4); fever (1); **malaria (1)**; body pain (1)	4.5	0.011	4	25.0	4.2	PF+ [17,25]	[13,25]	South America, Asia
Endopleura uchi (Huber) Cuatrec.[b] Humiriaceae	Uxi-liso	INPA 224690	Bark	**Malaria (1)**; liver diseases (1); anemia (1)	4.5	0.009	14	7.1	4.2	Not found	Not found	South America
Himatanthus sucuuba (Spr. Ex Müll.Arg.) W.[b] Apocynaceae	Sucuuba	INPA 224149	Latex	**Malaria (1l)**; fortifier (1l)	4.5	0.006	16	6.3	4.2	PF− [10,28]	[6,10]	South America

S=Salience Index; TI=total of the interviewed that cited the ethnospecies; MUA=Major Use Agreement; MUAc=Corrected MUA; FR=Frequence; PF=*Plasmodium falciparum in vitro*; PB=*Plasmodium berghei in vitro*; PG=*Plasmodium gallinaceum in vivo*; (+)=active or partially active; (−)=inactive
In the column "**Malaria and related diseases and symptoms**" the Major Use Agreement and Corrected MUA is calculated only for the indication highlighted in bold, while between parentheses is the number of times that the plant

2.4. In vitro activity of the extracts against P. falciparum

The *in vitro* blood schizonticide activity of the compounds was evaluated against *P. falciparum* (clone W2, chloroquine resistant) cultured in human erythrocytes as described (Trager and Jensen, 1976), using synchronized rings in sorbitol (Lambros et al., 1979). Parasitized cells were incubated with the test and control compounds at various concentrations in triplicate. The results were compared with those of control cultures cultivated in the absence of drugs. The anti-*P. falciparum* effects of the drugs were measured using the [3H]-hypoxanthine incorporation assay (Desjardins et al., 1979). The half-maximal drug inhibitory response (IC_{50}) was estimated by curve-fitting using software from the OriginLab Corporation (Northampton, MA, USA). All results were compared with parasite growth in the drug-free controls. The specific conditions for such tests are in details in a previous work (Aguiar et al., 2014).

3. Results

3.1. Ethnopharmacological survey

The thirty-five interviewed individuals reported 254 ethnospecies and a total of 2508 use indications. Among these, 233 plant species were identified, belonging to 211 genera and 72 botanical families (Oliveira et al., 2012). A specific survey was also conducted to assess medicinal plants used by the "quilombolas" to treat malaria and related symptoms and diseases by the free-list ethnodirected method. In this way, thirty-five ethnospecies were surveyed as possible antimalarial plants, comprising forty different plant species belonging to 23 botanical families and 37 genera (Table 1).

Some botanical families stand out for having a greater number of ethnospecies used for the treatment of malaria and related diseases such as Asteraceae and Euphorbiaceae (4 ethnospecies), Apocynaceae and Fabaceae (3 ethnospecies), Meliaceae, Rutaceae and Curcubitaceae (2 ethnospecies) (Fig. 2). However, two families deserve special attention: Rhamnaceae, represented by only one species, *A. amazonicus* ("saracuramirá"), which has the highest salience index against malaria and other related symptoms ($S=0.36$), a high major use agreement (MUA=100%; MUAc=50%) and frequency of use (Fr=50%), and Apocynaceae, which includes the widely used *Aspidosperma* spp. ("carapanaúba") ($S=0.25$; Fr=36.4; MUA=100%; MUAc=41.7%) and *Geissospermum argenteum* ("quinarana") ($S=0.25$; Fr=40.9; MUA=62.5%; MUAc=20.8%), that appear in the second and third places in Table 1, respectively, following *A. amazonicus*.

Most of the species noted in this survey are native (70%), according to the List of Species of the Brazilian Flora (2014) (Fig. 3a); whereas, the non-native species (30%), known as naturalized, subspontaneous or cultivated, may have been introduced over centuries in the Amazon region (Corrêa, 1984). The most medicinally employed plant parts were the barks (34%), followed by leaves, and roots or seeds, all accounting for 11% of the used parts (Fig. 3b).

Another interesting result from this survey is the fact that 27 ethnospecies (77.1%) cited during the interviews have already been cited previously in the literature as being useful against malaria (Table 1). Curiously, at least 11 ethnospecies (31.4%) used by the quilombolas of Oriximiná against malaria, fever and liver disorders are also used in African countries for the same purpose. This is the case for *Citrus aurantium, Plectranthus barbatus, Cedrela odorata, Physalis angulata, Cinnamomum verum,* plants of the genera *Bidens* and *Phyllanthus, Carica papaya, Mangifera indica, Jatropha curcas,* and *Senna occidentalis*. The last four
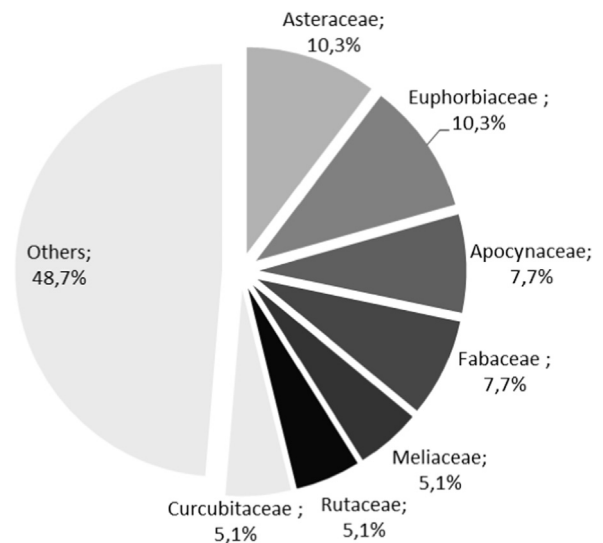

Fig. 2. Percentage of ethnospecies used against malaria (and related diseases and symptoms) according to their botanical families in the *quilombola* communities from Oriximiná, Brazil.

mentioned species, and species of the genus *Phyllanthus*, are still used for this purpose in at least three continents (see Table 1 and references therein).

Elsewhere, at least 22 ethnospecies (62.9%) from the ethnodirected survey have already been tested against *Plasmodium*, and 15 of them (42.9%) showed weak to strong antiplasmodial activity as shown in Table 1.

3.2. Antiplasmodial activity

As a result of the information acquired, 16 extracts encompassing 11 ethnospecies obtained from the ethnodirected approach were assayed *in vitro* against the blood forms of *P. falciparum* in culture (Table 2). In this selection, we included plants (ethnospecies) with higher Salience values ($S > 01$) such as saracuramirá, carapanauba, pau-para-tudo and castanheira, as well as plants with the lowest **S** (< 0.1) such as batatão, saratudo, andiroba, manga grande, unha de gato, açai and uxi-liso. In the screening, 9 samples from 4 ethnospecies showed *in vitro* antiplasmodial activity (active or partially active), representing 36.4% of the ethnospecies assayed, with $IC_{50} < 20$ μg/mL. Four extracts stood out as having the best antiplasmodial activity: the aqueous extract of the bark from *Simaba cedron* (IC_{50} 1.6 μg/mL), the dichloromethane and butanol extracts from the barks of *Aspidosperma rigidum* Benth. (IC_{50} 2.5 μg/mL), the ethanol extracts from the fruit peel ("casca do ouriço") and the bark of *B. excelsa* Kunth (IC_{50} 2.0–4.5 μg/mL).

Moreover, the extracts of *Machaerium ferox A. amazonicus, Carapa guianensis, Euterpe oleraceae, M. indica, Operculina hamiltonii, Uncaria guianensis* and *Endopleura uchi* were partially or inactive with $IC_{50} \geq 20$ μg/mL.

4. Discussion

In Brazil, the transmission of malaria is concentrated in the Amazon region, which represents 99% of the cases with an average of over 230,000 cases per year (MS/UFPA, 2009). The Municipality of Oriximiná is an area where the population has a medium to high risk of contracting malaria, as it has a large geographical area with several indigenous tribes, and other rural communities such as *quilombolas* and *riverine-caboclos*, that suffer with this disease.
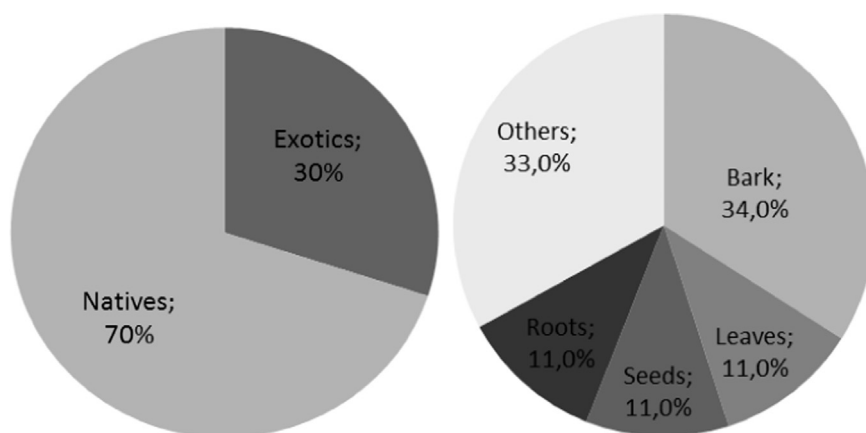
Fig. 3. (a) Percentage of the native *vs.* non-native species and (b) percentage of the medicinal parts of the ethnospecies used in the *quilombola* communities from Oriximiná, Brazil.

Table 2
In vitro activity against *P. falciparum*, W2 strains, of selected medicinal plants used against malaria and related symptoms by the *quilombola* population of Oriximiná, Brazil.

Ethnospecies/ Salience index	Part used	Extract	Antiplasmodial activity IC_{50} (µg/ml)	Activity[a]
Pau-para-tudo *Simaba cedron* (S=0.148)	Bark	Aqueous	1.6[b]	Active
Carapanaúba *Aspidosperma rigidum*	Bark	Ethanolic	6.0 ± 0.0	Active
		Dicloromethane	2.5 ± 0.7	Active
(S=0.251)		Butanolic	2.5 ± 0.7	Active
		Aqueous	10.2	Active
Castanheira	Fruit peel	Ethanolic	4.5 ± 0.7	Active
Bertholletia excelsa	Bark	Ethanolic	2.0 ± 0.0	Active
(S=0.124)	Bast	Sap	7.5 ± 3.5	Active
Saratudo *Machaerium ferox* (S=0.082)	Stalk	Ethanolic	20[b]	PA
Saracuramirá *Ampelozizyphus amazonicus* (S=0.360)	Bark	Aqueous	>50[b]	Inactive
Manga grande *Mangifera indica* (S=0.032)	Bark	Ethanolic	>50[b]	Inactive
Batatão *Operculina hamiltonii* (S=0.091)	Potato	Aqueous	>50[b]	Inactive
Andiroba *Carapa guianensis* (S=0.08)	Seeds	Oil	>50[b]	Inactive
Unha-de-gato *Uncaria guianensis* (S=0.027)	Bark	Aqueous	>50[b]	Inactive
Açai *Euterpe oleraceae* (S=0.019)	Roots	Ethanolic	>50[b]	Inactive
Uxi-liso *Endopleura uchi* (S=0.009)	Bark	Aqueous	>50[b]	Inactive
Chloroquine	Control antimalarial		0.059 ± 0.036	**Active**

[a] Samples with $IC_{50} \leq 10$ µg/mL are considered active; between 10 and 25 µg/mL partially active (PA) an ≥ 25 µg/mL inactive as defined for crude extracts in previous work (Krettli et al., 2009).
[b] Results of one experiment.

This suggests that over the centuries, people in this region sought forest resources for the treatment of diseases, including malaria, resulting in knowledge of a therapeutic arsenal that was traditionally experienced and empirically proven effective in many cases (Oliveira et al., 2012).

In this survey, some peculiarities could be observed in relation to the biodiversity and the traditional knowledge. The first was the high incidence of native plants among the ethnospecies surveyed. Among these, 7 plants listed in Table 1 have never been cited in ethnopharmacological studies focusing on antimalarial plants: *Operculina hamiltonii*, *Machaerium ferox*, *Croton sacaquinha*, *Sesamum indicum*, *Dalbergia riedelli*, *Quaribea guianensis* and *Endopleura uchi*. This situation may reflect the geographical isolation of these communities in an area of vast biodiversity, associated with the limited number of ethnodirected studies for malaria in the Amazon, especially in this region of Pará State.

The non-native species (30%) surveyed, such as *P. barbatus*, *S. indicum*, *Citrulus lanatus* and *Artemisia vulgaris*, are already naturalized in the Amazon region and were likely introduced there centuries ago by African slaves, Portuguese settlers and religious missionaries. This influence could be observed, as 31.4% of the ethnospecies surveyed are also used in some African countries for the same purpose, while 11.4% are also used against malaria in at least 3 continents. This situation demonstrates that the broad knowledge of local biodiversity by the quilombola people added to the knowledge acquired for some exotic species.

Another important observation is the widespread use of barks as the principal plant part used as antimalarials (34.0%), which could be related to the vast local biodiversity in an area substantially conserved of the Amazon Rain Forest of the *quilombolas lands*. This area is characteristic by high treetops with an abundance of trees from which the leaves are not employed at the expense of barks, making these results different from other studies (Caraballo et al., 2004; Koudouvo et al., 2011). The last point comprises the large number of families and genera that represent the forty plant species surveyed, with almost one distinct genus per species. This fact highlights the great therapeutic potential of the arsenal in these communities that are represented by distinct natural products with antiplasmodial activity.

For some plant families, the activity is related with specific compounds already shown to have antiplasmodial activity; this is the case of the indole alkaloids in Apocynaceae (Coutinho et al., 2013; Spencer et al., 1947), the diterpene phorbol esters in Euphorbiaceae, the acridone alkaloids in Rutaceae, the quassinoids in Simaroubaceae, and the sesquiterpene lactones and polyacetylenes in Asteraceae (Andrade-Neto et al., 2004; Carvalho and Krettli, 1991a; Wright an Phillipson, 1990). The botanical families highlighted in this study are also found in other countries such as in West and South Africa (Clarkson et al., 2004; Zirihi et al., 2005) and are also used against malaria.

The rational approach that uses traditional knowledge, or ethnopharmacology, as a tool has proven to be the most promising approach for identifying compounds for antimalarial drugs (Brandão et al., 1985; Carvalho and Krettli, 1991a]). Accordingly, in the present study it was assumed that the higher frequency, salience index and MUAc, would lead to higher antiplasmodial activity. Although only small number of samples was tested in the present study, it showed that 36.4% of the assayed ethnospecies are active against *P. falciparum in vitro* in accordance with the ethnopharmacological data surveyed towards malaria and its related symptoms and diseases. A correlation between ethnobotanical analyses and positive biological results was found in an ethno-directed study with antimycobacterial plants used against tuberculosis (Oliveira et al., 2011a), and another was observed for plants used for memory disturbances that inhibit acetylcholinesterase enzyme (Oliveira et al., 2012) in these communities.

In the present ethno-directed survey, *A. amazonicus* was the species with the highest salience index, frequency and MUAc, as previously shown by Oliveira et al. (2011b). However, extracts and fractions from this plant were shown to be totally inactive against blood forms of *P. falciparum in vitro* as well as in mice with experimental malaria by Brandão et al. (1985) , Carvalho et al. (1992) and Krettli et al. (2001). Not surprisingly, the prophylactic use of this plant against malaria has been described (Brandão et al., 1985; Carvalho et al., 1992; Silva et al., 2009). Such ethnopharmacological activity was confirmed in studies using the sporozoite forms that initiate malaria when inoculated in a vertebrate host by a mosquito bite. The experimental model first used was *P. gallinaceum* avian malaria (Krettli et al., 2001). In addition, as shown by Andrade-Neto et al. (2008), in cultures infected with sporozoites, the parasite development was abrogated in the presence of plant ethanolic extracts. The authors confirmed that mice treated with *A. amazonicus* prior to inoculation with sporozoites of *Plasmodium berghei* had a delay in the onset of parasitemia and displayed reduced mortality, compared to non-treated control mice.

Moreover, there are ethnobotanical studies that indicate the depurative, stimulatory, energetic and revitalizing properties of *A. amazonicus* (Oliveira et al., 2011b, 2012; Santos et al., 2005). The high cultural importance of this species in these communities, demonstrated by the high salience index, could be related to an adaptogenic effect. According to Panossian et al. (1999), "adaptogens constitute a new class of metabolic regulators (of a natural origin) that increase the ability of the organism to adapt to environmental factors and to avoid damage from such factors". In this sense, an important evidence of the immunomodulatory properties of *A. amazonicus* that suggests an adaptogen effect has been demonstrated by Peçanha et al. (2013), which could be related to the dammarane saponins previously described in this species (Brandão et al., 1992b, 1993).

Aspidosperma spp. and *Geissospermum* from the Apocynaceae family are the species with the highest salience index against malaria and related disorders after *A. amazonicus*. As expected by ethnopharmacological data, *A. rigidum* bark extracts tested showed antiplasmodial activity with an IC$_{50}$ of 2.5 μg/mL. Species of the *Aspidosperma* genus are commonly known in the North of Brazil as "carapanaúba", which means mosquito's tree. In this region, the decoction (teas) made from its barks are widely used in the "quilombola's" communities to treat malaria, liver diseases (hepatitis), ameba, fever and as a tonic for the nerves (Oliveira et al., 2012). Among the *Aspidosperma* species known as "caparanaúba" are *A. auriculatum, A. carapanauba, A. desmanthum, A. discolor, A. excelsum* (syn. *A. marcgravianum*), *A. nitidum, A. oblongum* and *A. vagarsii* (Pereira et al., 2007; Ribeiro et al., 1999). The diversity of species often known by the same vulgar name

("carapanauba") that are used for the same purpose (malaria) suggests that the morphological aspects that characterize the ethnospecies are sufficient to also characterize its therapeutic use, independently of which species is collected. However, the most cited species in the Amazon used as a remedy against malaria is *A. nitidum*, which has proven to be the most active species based on its specific activity (Penna-Coutinho et al., 2013), superior than the other species less cited as antimalarial, such as *A. ollivaceum* (Chierrito et al., 2014). Other species tested showed variable *in vitro* activity against the *P. falciparum* with IC$_{50}$ values ranging from 0.019 to 42 μM (Brandão et al., 1985; Carvalho et al., 1992; Mitaine-Offer et al., 2002; Paula et al., 2014; Torres et al., 2013). The biological activity of the genus has been attributed to indole alkaloids (Frederich et al., 2008; Vieira et al., 2013). Oliveira et al. (2010a) showed evidence of the indole alkaloid activity on the digestive vacuole of the parasite, a potential target for the alkaloid's action.

G. argenteum, named "quinarana", also from Apocynaceae family, and other species of the same genus (*G. sericeum* and *G. leave*), are common in the Amazon region. Previous surveys among the inhabitants of endemic areas of malaria, refer to them as useful to treat fever, malaria and liver diseases (Bertani et al., 2012; Brandão et al., 1992a; Ming et al., 1997; Muñoz et al., 2000). Moreover, in the study of Bertani et al. (2005) it was highlighted the main use of this species to prevent malaria in the French Guiana. Furthermore, it was demonstrated that the extract from *G. argenteum* bark macerated in rum was able to impair the intrahepatic cycle of the parasite and displayed poor activity *in vivo* and *in vitro* against the blood forms (Bertani et al., 2005). Species of the genus *Geissospermum* are also rich in indole alkaloids (Bertani et al., 2005; Mbeunkui et al., 2012).

B. excelsa, known as castanheira or brazil nut, is the most versatile species used by the quilombola communities, as described by Oliveira et al. (2012). It is used to treat 30 different disorders, grouped into 10 categories of the International Classification of Diseases, ICD-10 (WHO, 2010). Its antimalarial use seems restricted and is cited by only two individuals, resulting in a low frequency and MUAc. However, *B. excelsa* presented an interesting salience index due to its great cultural importance to the informants that cited this plant among the first species. A correlation of restricted knowledge of this could be showed by the experimental demonstration of its high antiplasmodial activity *in vitro* (IC$_{50}$ between 2.5 and 7.5 μg/ml). Anti-parasite activity of *B. excelsa* was also demonstrated previously against *Trypanosoma cruzi* (Campos et al., 2005).

The species *S. cedron,* known as "pau-para-tudo" ("stick for everything"), presented an interesting activity for the tea of barks with an IC$_{50}$=1.6 μg/ml. Several quassinoids have been isolated from the barks of this species (Hitotsuyanagi et al., 2001; Ozeki et al., 1998), among them cedronine, with an IC$_{50}$ of 0.25 μg/ml *in vitro* against strains of *P. falciparum* resistant and susceptible to chloroquine, and active against *P. vinkei in vivo*, with an oral dose of 1.8 mg/kg/day (Moretti et al., 1994). The authors claim that this species is also used by the "quilombolas" (*Marrons* or *creole*) of the French Guiana, who call it "quinquine of Cayenne".

Although very active, the limited use of *S. cedron* by the quilombolas compared to *Aspidosperma* species, *Geissospermum argentum* and *A. amazonicus*, may be related to several factors. First, the quassinoids have a very persistent and unpleasant bitter taste. Second, quassinoids are known for their toxicity and cytotoxicity (Phillipson et al., 1993; Vieira and Braz-Filho, 2006), especially for quassinoids having a basic skeleton of 20 carbons. Cedronine, for example, has a C19 backbone and is non-cytotoxic to B lymphocytes; but it is still much more cytotoxic than chloroquine (Moretti et al., 1994). Furthermore, almost all other present quassinoids have the basic skeleton C20. Cedronolactone A exhibited high

cytotoxicity *in vivo* (IC$_{50}$ 0.0074 μg/ml). This result points to the questionable safety of the plant for its internal use. In the communities from the Erepecuru River, curiously, the interviewees claim to only implement a topical use for *S. cedron* to combat itching and other skin disorders; this plant was cited against malaria only in the Trombetas River communities.

Some species presented a low number of indications and low salience index as antimalarials between the informants in this work. The plant species also presented little or insignificant activity like *M. ferox* (partially active – IC$_{50}$=20 μg/ml) (Table 2). The ethnopharmacological data are in agreement with previous studies with Peruvian plants where *Macherium floribunda* presented low activity, and *M. indica*, *C. guianensis*, *E. oleraceae* and *U. guianensis* were also found to be inactive (Kvist et al., 2006).

Other species cited by quilombolas in the ethnobotanical survey are widely used against fever and were not included in this work, because they were not cited in the specific (ethno-directed) free-list. For example, *Euphorbia thymifolia* L., *Zea mays* L. and *Sambucus* sp. have been used against fever related to infectious diseases caused by viruses, such as measles and chickenpox. Other examples are *Allium sativum* L., *Leucas martinicensis* (Jacq.) R.Br., *Eryngium foetidum* L., *Citrus limon* (L.) Burm.f. and *Annona montana* (Macfad.) R.E.Fr. which are used for fever only in the event of cold and flu (Oliveira, 2009).

The indiscriminate use of large-scale screening procedures that prioritize quantity over quality tends to overestimate the potential antimalarial as a result of the selection of "tree useful against fever", according to Bourdy et al. (2008). Thus, to select species using "enlarged symptomatic criteria" causes an undesirable increase in the number of species to be tested.

From the ethnomedicine perspective, it is also important to stand out in these quilombola communities that the treatment of malaria does not necessarily take into account a possible antiplasmodial activity in order to prevent, treat and cure disease. In these communities, knowledge about the existence of a malaria causal agent is a recent happening, while the plants reported mostly already have secular use. As malaria is regarded as a disease that affects the individual as a whole, it is necessary to treat all symptoms of the illness (fever, swelling of the liver and spleen, headache, body pain, uneasiness, nausea, flatulence) as well as to cleanse and strengthen the body for healing. To purify the body, it is common to use purgative, blood depurative and emetic plants, while for strengthening the body, plants are used to strengthen the blood and improve the organism as a whole to try to prevent disease in general and relapses of malaria.

Emetics and purgatives are widely used traditionally with the aim to "clean inside the body", due to the concept that the disease is an entity within the body (Geest and Whyte, 1989; Montagner and Rao, 1991). In the popular vision, it is necessary to stimulate/promote the excretory functions of the ill body with the aim of flushing out the internal cause of illness. To purify the organisms by the use of vomitories, purgatives and blood depuratives, in the "quilombola" communities the plants "saracuramirá", "batatão", "cabacinha", "pinhão-branco" and "mamão-macho" are frequently used. In the literature, the depurative use of *A. amazonicus* is well described (Ducke and Martinez, 1994; Santos et al., 2005) as well as the depurative/purgative action of *Operculina alata* (Fleming-Moran, 1992; Ming et al., 1997). *J. curcas* also has a purgative effect described in the literature (Ducke and Martinez, 1994; Ming et al., 1997) together with *Luffa operculata* used as vomitory/purgative (Berg, 1993; Ducke and Martinez, 1994; Ming et al., 1997).

The "holistic" view of traditional antimalarial treatments was also discussed as complex mixtures prepared and used, in many cases, as carefully selected for a specific patient on a particular occasion. Therefore, negative results about antiplasmodial activity are minimized by several authors. Bourdy et al. (2008), for example, describes the social context of the traditional use, saying that "in the real life, it is rare that only one species is used in a cure. It is more likely that remedies are made from several ingredients or administered sequentially". Kirby (1996) suggests, "traditional remedies may be effective in patients who have been previously exposed to the disease and hence have some degree of immunity to malaria". Clarkson et al. (2004), in turn, highlighted that "some plants act as antipyretics or immune stimulants to relieve the symptoms of the disease rather than having direct antiparasitic activity". Elsewhere, this author considers that some plant extracts need to be modified *in vivo* before activity is exhibited.

5. Conclusions

The "*quilombolas*" from Oriximiná are located in a region with a high incidence of malaria which allowed people to experience and learn about plants useful against malaria and its related symptoms. These plants are represented by a large number of genera and families showing a diversity of chemical profiles, which increases the chance of finding active species. The most used species are native, showing the intimacy of these traditional communities with the local biodiversity. Interestingly, in the case of exotic species, many of them have been used against malaria in Africa and, in some cases, in the Asian continent. The African origin of these communities could have some relationship with this fact. Furthermore, for some species it seems to be a traditional knowledge diffuse about the antimalarial use worldwide. In some cases the expected antiplasmodial activity was verified *in vitro* and/or *in vivo*, but not in others such as for *M. indica*.

In the ethnodirected approach used in this study it was shown the antiplasmodial potential of some species, especially *A. rigidum* and *B. excelsa*. However, for other species such as *A. amazonicus* this activity has not been demonstrated. It is important to note the holistic vision of treatment in these communities that comprises the use of more than one species sequentially, especially purgative, depurative, emetic and adaptogen plants, used in the treatment to recover the health of individuals. Therefore, in many cases, the lack of antiplasmodial activity could be expected.

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