In vitro and in vivo anti-malarial activity of plants from the Brazilian Amazon

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Abstract

Background: The anti-malarials quinine and artemisinin were isolated from traditionally used plants (Cinchona spp. and Artemisia annua, respectively). The synthetic quinoline anti-malarials (e.g. chloroquine) and semi-synthetic artemisinin derivatives (e.g. artesunate) were developed based on these natural products. Malaria is endemic to the Amazon region where Plasmodium falciparum and Plasmodium vivax drug-resistance is of concern. There is an urgent need for new anti-malarials. Traditionally used Amazonian plants may provide new treatments for drug-resistant P. vivax and P. falciparum. Herein, the in vitro and in vivo antimalarial activity and cytotoxicity of medicinal plant extracts were investigated.

Methods: Sixty-nine extracts from 11 plant species were prepared and screened for in vitro activity against P. falciparum K1 strain and for cytotoxicity against human fibroblasts and two melanoma cell lines. Median inhibitory concentrations (IC50) were established against chloroquine-resistant P. falciparum W2 clone using monoclonal anti-HRPII (histidine-rich protein II) antibodies in an enzyme-linked immunosorbent assay. Extracts were evaluated for toxicity against murine macrophages (IC50) and selectivity indices (SI) were determined. Three extracts were also evaluated orally in Plasmodium berghei-infected mice.

Results: High in vitro antimalarial activity (IC50 = 6.4–9.9 µg/mL) was observed for Andropogon leucostachyus aerial part methanol extracts, Croton cajucara red variety leaf chloroform extracts, Miconia nervosa leaf methanol extracts, and Xylopia amazonica leaf chloroform and branch ethanol extracts. Paulinia cupana branch chloroform extracts and Croton cajucara red variety leaf ethanol extracts were toxic to fibroblasts and or melanoma cells. Xylopia amazonica branch ethanol extracts and Zanthoxylum djalma-batistae branch chloroform extracts were toxic to macrophages (IC50 = 6.9 and 24.7 µg/mL, respectively). Andropogon leucostachyus extracts were the most selective (SI >28.2) and the most active in vivo (at doses of 250 mg/kg, 71 % suppression of P. berghei parasitaemia versus untreated controls).

Conclusions: Ethnobotanical or ethnopharmacological reports describe the anti-malarial use of these plants or the antimalarial activity of congeneric species. No antimalarial activity has been demonstrated previously for the extracts of these plants. Seven plants exhibit in vivo and or in vitro anti-malarial potential. Future work should aim to discover the anti-malarial substances present.

Keywords: Plasmodium falciparum, Plasmodium berghei, Antiplasmodial, Cytotoxic, Anacardium occidentale, Andropogon leucostachyus, Croton cajucara, Paulinia cupana, Xylopia amazonica, Zanthoxylum djalma-batistae

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Background
In the Amazon region, the occurrence of malaria is related to demographic, ecological, socio-economic, and cultural changes, especially in the first half of the twentieth century. In this period, relatively few plants from this region were used to treat this disease [1, 2]. Over time, the co-existence of traditional populations with this disease caused a search for new therapeutic resources in the Amazonian environment, especially among plants, to treat the symptoms of malaria. Nowadays, this traditional knowledge, available through ethnopharmacological studies, is the most often used means to target plants for the discovery of new bioactive substances. The ethnopharmacological approach has led to the saving of time and financial resources as compared to other approaches, such as chemosystematic or random plant selection [3, 4]. The chemosystematic approach for the selection of anti-malarial plants for study is also valid especially where ethnopharmacological studies have shown a plant family, or a particular genus, to contain anti-malarial extracts and chemical constituents.

Natural products are the origin of approximately two-thirds of all drugs introduced in the past 30 years [5]. Plants are recognized as important sources of antiprotozoal compounds for the development of drugs against many tropical diseases, including malaria. Examples of anti-malarial natural products are (1) quinine, present in the Peruvian Cinchona spp., (2) quassinoids and limonoids in plants of the Simaroubaceae and Meliaceae families, respectively, and, (3) artemisinin from Artemisia annua, among others [6, 7].

The Amazon region is megadiverse. Screening for in vitro and in vivo anti-malarial activity of extracts of traditionally used plants from this region is a strategy for the discovery of new anti-malarial substances [8–10]. Studies on the anti-malarial activity of plant species from countries of the Amazon region such as Bolivia [11–15], Brazil [16–29], Colombia [30], French Guiana [31–34], and Peru [35–38] have demonstrated the potential of local traditional medicinal practices as sources of potent extracts and anti-malarial substances.

Krettli and collaborators performed ethnobotanic surveys of anti-malarial plants across the Brazilian Amazon region and applied the ethnopharmacological approach to the study of these plants for the first time [1, 39]. Ethnopharmacology provided a relatively large number (4 in 22 plants or 18%) of plants exhibiting extracts with in vivo efficacy against Plasmodium berghei compared to an approach based on random selection of plants (two active plants in 273 tested or 0.7%) [40, 41].

In the present work, after a systematic literature search, 11 Amazonian plants were selected based on their use as anti-malarials or based on the proven anti-malarial activity of the plant genus. No previous report on the activity against Plasmodium parasites was found for the selected plant species. Their extracts were assayed for in vitro and in vivo anti-malarial activity and cytotoxicity. The aim of this study was to discover Amazonian plant extracts exhibiting important in vitro and in vivo anti-malarial activity as a first step towards bioguided isolation of active principles. The plant species studied are shown in Table 1.

Methods
Collection, identification and processing of plant materials
Initially, library and online (Web of Knowledge, Scopus, Scifinder Scholar, among others) surveys of the literature on plants traditionally used as anti-malarials were performed using the convenient search term ‘antimalarials from plants’. Also, a survey of plant species exhibiting proven anti-malarial properties according to previous laboratory studies was performed. Registry (No. 33110-1) for the collection of plant materials was performed online through the Brazilian Government’s Authorization and Information in Biodiversity System (SISBIO), Chico Mendes Biodiversity Conservation Institute (ICMBio), Ministry of Environment (MMA). Collection of the different parts of 11 Amazonian species was performed based on ethnobotanic information, where available. These were generally the more readily collected parts of each plant species (Table 1). Authorization for collection of plant materials at the National Institute for Amazon Research (INPA) Adolfo Ducke Forest Reserve, located in the municipality of Manaus, was obtained prior to collection. Plant materials from Embrapa Amazônia Ocidental’s live plant collections were prepared by Dr. Francisco Célio Maia Chaves. No in vitro and/or in vivo anti-malarial activity data were available for these plant species in the literature. Collection was performed from August to October 2012 in Amazonas State, Brazil. Vouchers were deposited and identified at the INPA and Federal Agro-technical School of Manaus (EAFM) herbariums. Prior to extraction, plant materials were dried in the shade at ambient temperature (average of ca. 27°C) for 72 h and then further dried in a circulating air oven at 40°C for 7 days. The dry plant materials were ground and stored at −20°C until extraction was performed.

Extraction of plant materials
Ethanol (or methanol), water and chloroform extracts were prepared from each plant part (Table 1). Water extracts were prepared by infusing dry, ground plant materials in boiling de-ionized water (100°C, 3 × 15 min) or when indicated in the literature, a decoction was prepared by boiling plant materials for 1 h under reflux. Ethanol, chloroform and methanol extractions were performed using
Table 1 Information on plant species, voucher specimens, traditional remedies and ethnomedicinal sources indicating anti-malarial use

| Species                  | Family               | Accession number | Common name            | Remedy                      | Source                  |
|--------------------------|----------------------|------------------|------------------------|-----------------------------|-------------------------|
| Anacardium occidentale   | Anacardiaceae        | INPA 57941       | Cajueiro               | Bark, leaves, fruit infusions, decoction (10 drops 2 × day of trunk bark alcohol extract) | [58, 68, 82, 96, 109, 113] |
| Andropogon leucostachyus | Poaceae              | INPA 250467      | Capim-colchão          | Whole plant decoction       | [58]                    |
| Cidemia hirta            | Melastomataceae      | INPA 250466      | Caiuia                 | Not found
| Croton cajucara          | Euphorbiaceae        | EAFM 315         | Sacaca                 | Not found
| Denis floribunda         | Fabaceae             | INPA 15562       | Timbó                   | Branches                  | [58]                    |
| Miccion nervosa          | Melastomataceae      | INPA 250467      | Miraúba                | Decoction (part not specified) | [58]                    |
| Parkia nitida            | Fabaceae             | INPA 152124      | Faveira                 | Not specified              | [58]                    |
| Paullinia cupana         | Sapindaceae          | INPA 122001      | Guaraná                | Leaves, branches, roots, seeds | [58, 63, 96, 97] |
| Stigmaiphyllon sinuatum  | Malpighiaceae        | INPA 205629      | Cipó asa de gafanhoto  | Leaves decoction            | [58]                    |
| Xylopia amazonica       | Annonaceae           | INPA 183108      | Envira sarassará        | Not found
| Zanthoxylum djmal-mbaritaet | Rutaceae             | INPA 210077      | Tamanqueira             | Not found

* Cidemia hirta is the species cited as being in use by traditional peoples of the Peruvian Amazon [38]

b Fruit and trunk bark macerates and infusions of these Xylopia spp. are used as anti-malarials: Xylopia aethiopica, Xylopia aromatica, Xylopia brasiliensis, Xylopia emarginata, Xylopia frutescens, Xylopia grandiflora, Xylopia hypolampra, Xylopia longifolia, Xylopia parviflora, Xylopia phloiodora, Xylopia staudei, Xylopia xylopoides [30, 58, 80–85, 125, 126]

c These Zanthoxylum spp. are used as anti-malarials: Zanthoxylum armatum, Zanthoxylum caribaem, Zanthoxylum chalybeum, Zanthoxylum chinoperone, Zanthoxylum gilletii, Zanthoxylum hermaphroditum, Zanthoxylum leprieuri, Zanthoxylum pentandrum, Zanthoxylum perrottetti, Zanthoxylum rhoifolium, Zanthoxylum rubescens, Zanthoxylum tingassuiuba, Zanthoxylum tshikanimposa, Zanthoxylum usambarensce, Zanthoxylum zanthoxyloides. Leaf, fruit, trunk bark and root bark decoctions are used [33, 58, 59, 84, 85, 100–105, 107–110, 127–129].

In vitro culture of Plasmodium falciparum

Chloroquine-resistant (CQR) Plasmodium falciparum W2 clone and K1 strain were used for in vitro antimalarial studies. The Trager and Jensen [42] in vitro culture technique was used with modifications [17]. Parasites were cultivated in type A+ erythrocytes and culture medium (Roswell Park Memorial Institute or RPMI-1640) enriched with 10 % human serum (complete medium) and maintained at 37 °C under an atmosphere of 5 % carbon dioxide, 5 % oxygen and 90 % nitrogen.

Monitoring of parasite growth was performed in 24 h during the daily refreshing of culture medium. Parasitaemia was calculated as a percentage based on the viable parasitic forms observed by counting at least 2000 erythrocytes.

Screening for in vitro antimalarial activity against Plasmodium falciparum K1 strain

Parasite quantification by optical microscopy is a traditional and reliable technique in the performance of in vitro antimalarial assays [43]. In vitro antimalarial screening was performed according to a previously published procedure [17]. Each extract (1.0 mg) was dissolved in dimethyl sulfoxide (DMSO) to provide a stock solution (5.0 mg/mL). Test solutions of each extract were prepared by diluting stock solution in RPMI-1640 culture medium and 20 μL of each test solution were introduced into the wells of a 96-well plate. Test plates were incubated at 37 °C for 48 h under an atmosphere of 5 % oxygen, 5 % carbon dioxide and 90 % nitrogen. After this period, thin blood smears of the contents of each well were stained with Panótico® (Laborclin, Pinhais, Paraná, Brazil) for evaluation of the parasitaemia using an optical microscope. The parasitaemia was expressed as a percentage of the viable erythrocytic parasite forms observed in 2000 RBCs. Parasite inhibition was expressed as a percentage of the growth of untreated (negative) controls. All assays were performed in triplicate [45]. Extracts that inhibited parasite growth by ≥80 % at concentrations of 50 μg/mL were considered active.
were further evaluated using the procedure described below.

**In vitro antiplasmodial activity assessed by anti-HRPII ELISA**

*Plasmodium falciparum* histidine-rich protein II (HRPII) may be quantified as an indicator of cell growth through the enzyme-linked immunosorbent assay (ELISA)-sandwich technique [46–48]. This technique has been applied to the screening of a variety of crude plant extracts and fractions [49–52] and is a valid method for the screening of plant extracts providing similar IC50 values to traditional methods [49, 50].

Extracts exhibiting anti-malarial potential in the screening procedure described above against *P. falciparum* K1 strain were further evaluated to determine the concentrations that inhibit 50 % of parasite growth (IC50) using histidine rich protein II antibody (anti-HRPII) ELISA [47] with slight modifications [49]. Briefly, each extract was dissolved in DMSO using an ultrasound bath to form stock solutions (10 mg/mL). Each stock solution was serially diluted in culture medium to provide seven dilute samples. Each dilute sample (20 μL) was applied to a 96-well test plate in triplicate. A suspension of sorbitol-synchronized pRBCs was adjusted to 0.05 % parasitaemia and 1.5 % haematocrit and placed in 96-well plates containing the test and control drugs providing final (in well) extract concentrations of 100–0.13 μg/mL. The plates were incubated for 72 h. After 24 h, the contents of the six control wells (parasites in drug-free medium) were harvested in microtubes and frozen for later use to further exclude the background value (i.e., the production of HRPII during the first 24 h of incubation) by subtracting the average value obtained from these wells from that of the wells containing the test and control drugs. After 72 h of incubation, the plates were frozen and thawed twice to lyse the erythrocytes.

To perform the test, a clean plate (Maxysorp, Nunc, Denmark) was first coated with 100 μL of the primary antibody anti-HRPII (MPFM ICLLAB-55A®, Stuart, FL, USA) at 1.0 μg/mL. Following overnight incubation at 4 °C, the antibody solution was discarded and replaced with 100 μL of 3,3′,5,5′-tetramethylbenzidine (TMB) chromogen (KPL, Gaithersburg, MD, USA) and was incubated for 10 min at room temperature in the dark. The reaction was stopped with 50 μL/well of 1 M sulfuric acid and the absorbance was immediately read at 450 nm on a spectrophotometer (SpectraMax® 340PC384, Molecular Devices, Sunnyvale, CA, USA). Three separate experimental determinations were performed and the average readings were plotted and the IC50 values were determined from the plots.

**Screening for in vitro cytotoxicity (cell viability test)**

The cytotoxicity of extracts was evaluated in vitro against one non-neoplastic cell line (MRC-5-human fibroblast) purchased from the American Type Culture Collection (ATCC) and two melanoma cell lines (SK-MEL-19 and SK-MEL-28) donated by Dr. María S Soengas (CNIO, Madrid, Spain). The cells were cultivated in 96-well plates (0.5 × 10^4 cells per well) and then were treated with extract in DMSO at a single concentration (50 μg/mL) over 72 h. The Alamar Blue™ assay was performed after 72 h following a standard procedure [53].

**Murine macrophage culture**

J774 cells (murine macrophages) were obtained from the Cell Bank of Rio de Janeiro, Brazil and were maintained in Dulbecco’s Modified Eagle Medium (DMEM), which was supplemented with 10 % fetal bovine serum (FBS), penicillin (100 U/mL) and streptomycin (100 U/mL). The cells were incubated at 37 °C in a humidified atmosphere containing 5 % of CO2.

**Determination of in vitro toxicity against murine macrophages (cell viability test)**

The cytotoxicity was determined by the Alamar Blue method as described by Nakayama and co-workers [54]. Briefly, adherent cells (5 × 10^3 cells/well) were grown in 96-well tissue culture plates and exposed to extracts (1.56–200 μg/mL) for 48 h. After incubation, Alamar Blue solution (10 μL of 0.4 % Alamar Blue (resazurin) in water) was added and the cells were incubated for 3 h at 37 °C. Fluorescence was measured (excitation at 545 nm and emission at 595 nm) and expressed as a percentage of the cells in the control after background fluorescence was subtracted. Doxorubicin was used as a positive control of cell death. The assays were performed in triplicate. The IC50 values and their 95 % confidence intervals (95 % CI) were obtained by non-linear regression using the Graphpad program (Intuitive software for science, San Diego, CA, USA).
of plant extracts are shown in Table 2. Extracts exhibit IC50 values in the range 25 to 50 μg/mL were considered moderately active. Extracts exhibiting IC50 values in the range 10 to 25 μg/mL were considered highly active. The parasitaemia was determined in blood smears that were characterized by random counting of 2000–4000 erythrocytes when parasitaemia was low (≤10%) or up to 1000 erythrocytes when parasitaemia was higher. Mortality was monitored daily in all groups during a period of 4 weeks after inoculation.

Animals and ethical approval
Adult BALB/c mice (25 ± 3 g weight) were used for the in vivo anti-malarial tests and received water and food ad libitum. In vivo tests were performed following the Guidelines for Ethical Conduct in The Care and Use of Animals of the National Institute for Amazon Research (INPA). This work was authorized by INPA’s Commission for the Ethical Use of Animals (CEUA 029/2013).

In vivo anti-malarial test
Three extracts exhibiting in vitro antiplasmodial activity in the above assay were tested for oral activity against the Plasmodium berghei NK65 strain in mice with blood-induced infections maintained by successive passages from mouse to mouse. The protocol used was based on the Peters [55] four-day suppressive test with slight modifications [21]. In each experiment, the animals were divided into groups of five individuals and treated for four successive days with extract (250 mg/kg/day) starting 24 h after inoculation with P. berghei (1 × 10^6 parasitized erythrocytes/animal). In each experiment, positive and negative control groups received chloroquine (10 mg/kg/day) and vehicle (2% DMSO), respectively. Each sample was tested in at least two independent experiments. On days 5 and 7 after infection, blood smears from all mice were prepared, stained with the Panótico® system (Laborclín, Pinhais, Paraná, Brazil) and examined under a microscope. The parasitaemia was determined in blood smears that were characterized by random counting of 2000–4000 erythrocytes when parasitaemia was low (≤10%) or up to 1000 erythrocytes when parasitaemia was higher. Mortality was monitored daily in all groups during a period of 4 weeks after inoculation.

Results
In vitro antiplasmodial activity, cytotoxicity and selectivity of extracts
A total of 69 chloroform, ethanol, methanol, and water extracts from different parts of the 11 plant species were prepared. Screening against P. falciparum K1 strain revealed 32 extracts that exhibited antiplasmodial potential (IC50 ≤50 μg/mL). These extracts were further evaluated in vitro against the CQR P. falciparum W2 clone to establish accurate IC50 values. The in vitro IC50 values of plant extracts are shown in Table 2. Extracts exhibiting IC50 values ≤10 μg/mL were considered to be active. Those exhibiting IC50 values in the range 10 to ≤25 μg/mL were considered moderately active. Extracts exhibiting IC50 values >25 μg/mL were considered inactive. Andropogon leucostachyus aerial part methanol extracts, Croton cajucara red variety leaf chloroform extracts, Miconia nervosa leaf methanol extracts and Xylopia amazonica leaf chloroform and branch ethanol extracts were the most active (7% of all extracts). Moderate activity (22% of all extracts) was observed for Clidemia bullosa DC branch chloroform extract and decoction, Croton cajucara white variety leaf chloroform and ethanol and bark ethanol extracts, Croton cajucara red variety leaf ethanol extract, Miconia nervosa bark and leaf chloroform extracts and leaf decoction, Paullinia cupana fruit and branch chloroform extracts, Xylopia amazonica leaf decoction and branch chloroform extract and Zanthoxylum djalma-batistae leaf decoction and branch chloroform extract. Seventy-one per cent of the extracts were inactive. All extracts prepared from Anacardium occidentale, Derris floribunda, Parkia nitida and Stigmaphyllum sinuatum were inactive in vitro against P. falciparum as were Croton cajucara red variety bark and Paullinia cupana leaf extracts.

During initial screening, those extracts that reduced in vitro cell viability to less than 10% of that of untreated controls after 72 h were considered to be cytotoxic. Thus, only Paullinia cupana branch chloroform extract was toxic to MRC-5 cells (6.8% viability after 72 h). This same extract was toxic to SK-MEL-19 and SK-MEL-28 melanoma cells (7.3 and 6.6% viability, respectively). Croton cajucara red variety leaf ethanol extract was also toxic to SK-MEL-28 cells. All other extracts did not significantly inhibit proliferation of MRC-5, SK-MEL-19 or SK-MEL-28 cells (viability >50% at concentrations of 50 μg/mL).

Thirty extracts were evaluated for in vitro toxicity against murine macrophages to establish IC50 values and selectivity parameters (Table 2). Twenty-three extracts were essentially non-toxic to murine macrophages (IC50 values >50 μg/mL). However, Xylopia amazonica branch ethanol and Zanthoxylum djalma-batistae branch chloroform extracts (IC50 = 6.9 and 24.7 μg/mL, respectively) exhibited significant cytotoxicity (IC50 <25 μg/mL) to macrophages. Andropogon leucostachyus aerial part methanol extracts exhibited the greatest selectivity index (SI >28.2) (Table 2).

In vivo activity of extracts
Three extracts exhibiting in vitro antiplasmodial activity were assayed for in vivo anti-malarial activity and the results are presented in Table 3. Andropogon leucostachyus extract was the most active in vivo exhibiting 71% suppression of P. berghei parasitaemia on the fifth day. Xylopia amazonica leaf extracts exhibited 52% suppression on the fifth day of infection and low in vivo parasite suppression was observed for Croton cajucara red variety leaf extracts (Table 3).

Discussion
Several strategies are available for the discovery of new anti-malarial drugs. In vitro screening for inhibitory
Table 2 In vitro median inhibitory concentrations (IC₅₀) against *Plasmodium falciparum* strains, toxicity to murine macrophages (IC₅₀) and selectivity indices (SI) of plant extracts

| Plant species                  | Part       | Extract | P. falciparum IC₅₀ | Macrophages IC₅₀ (µg/mL) | SI   |
|--------------------------------|------------|---------|--------------------|--------------------------|------|
|                                |            |         | µg/mL ± SD         |                          |      |
| *Anacardium occidentale*        | Bark       | CHCl₃   | 36.6 ± 17.7        | >200                      | >5.5 |
|                                |            | EtOH    | >50                |                          |      |
|                                |            | H₂O(i)  | >50                |                          |      |
|                                | Leaf       | CHCl₃   | 43.9 ± 10.8        | >200                      | >4.6 |
|                                |            | EtOH    | >50                |                          |      |
|                                |            | H₂O(i)  | 45.0 ± 5.0         | >200                      | >4.4 |
| *Andropogon leucostachyus*     | Aerial part| CHCl₃   | >50                |                          |      |
|                                |            | H₂O(d)  | 45.4 ± 0.4         | >200                      | >4.4 |
|                                |            | MeOH    | 7.1 ± 3.3          | A                         | >200 |
|                                | Leaf       | CHCl₃   | >50                |                          |      |
|                                |            | H₂O(d)  | 26.2 ± 3.1         | >200                      | >6.2 |
|                                |            | MeOH    | >50                |                          |      |
|                                | Branch     | CHCl₃   | 13.5 ± 2.7         | >200                      | >14.8|
|                                |            | H₂O(d)  | 21.2 ± 4.0         |                          |      |
|                                |            | MeOH    | >50                |                          |      |
| *Clidemia bullosa*             | Leaf       | CHCl₃   | >50                |                          |      |
|                                |            | H₂O(d)  | 26.2 ± 3.1         | >200                      | >7.6 |
|                                |            | MeOH    | >50                |                          |      |
|                                | Branch     | CHCl₃   | 13.5 ± 2.7         | >200                      | >14.8|
|                                |            | H₂O(d)  | 21.2 ± 4.0         |                          |      |
|                                |            | MeOH    | >50                |                          |      |
| *Croton cajucara* (white variety-WV) | Bark    | CHCl₃   | 29.1 ± 6.3         | 43.1 (27.4–67.8)          | 1.5  |
|                                |            | EtOH    | 17.2 ± 6.6         | MA 127 (498–321)          | 7.4  |
|                                |            | H₂O(i)  | >50                | I                         |      |
|                                | Leaf       | CHCl₃   | 11.3 ± 3.4         | MA >200                   | >17.7|
|                                |            | EtOH    | 16.3 ± 4.5         | MA >200                   | >12.3|
|                                |            | H₂O(i)  | >50                | I                         |      |
| *Croton cajucara* (red variety-RV) | Bark    | CHCl₃   | 32.2 ± 5.7         | >200                      | >6.2 |
|                                |            | EtOH    | >50                | I                         |      |
|                                |            | H₂O(i)  | >50                | I                         |      |
|                                | Leaf       | CHCl₃   | 6.4 ± 1.2          | A 40.6 (32.6–50.6)        | 6.3  |
|                                |            | EtOH    | 13.3 ± 2.3         | MA >200                   | >15.0|
| *Derris floribunda*            | Bark       | CHCl₃   | >50                | I                         |      |
|                                |            | H₂O(i)  | >50                | I                         |      |
|                                |            | MeOH    | >50                | I                         |      |
|                                | Leaf       | CHCl₃   | 47.4 ± 1.6         | >200                      | >4.2 |
|                                |            | H₂O(i)  | 27.5 ± 7.5         | I                         |      |
|                                |            | MeOH    | >50                | I                         |      |
| *Miconia nervosa*              | Bark       | CHCl₃   | 13.3 ± 2.0         | MA 46.6 (43.1–50.4)       | 3.5  |
|                                |            | H₂O(d)  | >50                | I                         |      |
|                                |            | MeOH    | >50                | I                         |      |
|                                | Leaf       | CHCl₃   | 12.4 ± 4.1         | MA 70.6 (62.7–79.7)       | 5.7  |
|                                |            | H₂O(d)  | 10.2 ± 2.5         | MA >200                   | >19.6|
|                                |            | MeOH    | 9.9 ± 3.2          | A 95.9 (71.0–130)         | 9.7  |
| *Parkia nitida*                | Bark       | CHCl₃   | >50                | I                         |      |
|                                |            | H₂O(d)  | >50                | I                         |      |
|                                |            | MeOH    | >50                | I                         |      |
| *Paullinia cupana*             | Leaf       | CHCl₃   | >50                | I                         |      |
|                                |            | H₂O(i)  | >50                | I                         |      |
|                                |            | MeOH    | >50                | I                         |      |
activity against *P. falciparum* and identification of traditionally used plant extracts exhibiting IC₅₀ values less than 10 µg/mL are important first steps in the search for new anti-malarial plant extracts. Similar approaches have led to the identification of extracts for chemical composition studies and the discovery of potent plant natural products, such as artemisinin and nimbulide [56, 57].

Table 2 continued

| Plant species | Part | Extract | P. falciparum IC₅₀ μg/mL ± SD | Macrophages IC₅₀ μg/mL (95 % CI) | SI² |
|---------------|------|---------|-----------------------------|----------------------------------|-----|
| *Fruit*       |      |         |                             |                                  |     |
|               | CHCl₃| 19.3 ± 6.4 | MA >200                     | >104                             |     |
| H₂O(i)        | >50  | I       | –                           | –                                |     |
| MeOH          | >50  | I       | –                           | –                                |     |
| *Branch*      |      |         |                             |                                  |     |
|               | CHCl₃| 19.3 ± 5.5 | MA 62.9 (53.9–73.4)         | 3.3                              |     |
| H₂O(i)        | >50  | I       | –                           | –                                |     |
| MeOH          | >50  | I       | –                           | –                                |     |
| *Stigmaphyllon sinuatum* | Leaf | CHCl₃ | >50                         | –                                | –   |
|               | EtOH | >50     | I                           | –                                | –   |
| H₂O(i)        | >50  | I       | –                           | –                                |     |
| *Xylopia amazonica* | Leaf | CHCl₃ | 7.3 ± 1.8                   | A 33.9 (29.6–38.9)               | 4.6 |
|               | H₂O(d) | 10.5 ± 3.3 | MA >200                 | >190                             |     |
| MeOH          | >50  | I       | –                           | –                                |     |
| *Branch*      |      |         |                             |                                  |     |
|               | CHCl₃| 19.5 ± 3.1 | MA 29.2 (19.6–43.5)       | 1.5                              |     |
| H₂O(d)        | >50  | I       | –                           | –                                |     |
| EtOH          | 9.8 ± 1.8  | A 6.9 (0.4–12.1) | 0.7                            |     |
| *Zanthoxylum djalma-baristae* | Leaf | CHCl₃ | 40.2 ± 3.2                   | A >200                          | 4.6 |
|               | H₂O(i) | 15.6 ± 2.9 | MA >200                 | >128                             |     |
| MeOH          | >50  | I       | –                           | –                                |     |
| *Branch*      |      |         |                             |                                  |     |
|               | CHCl₃| 17.4 ± 1.3 | MA 24.7 (18.6–32.9)      | 1.4                              |     |
| H₂O(i)        | 32.5 ± 7.9 | I >200 | >6.2                         | >9.2                             |     |
| MeOH          | 21.8 ± 3.7 | I >200 | >9.2                         | >9.2                             |     |
| *Controls*    |      |         |                             |                                  |     |
|               | DMSO | –       | –                           | –                                | –   |
| Chloroquine diphosphate | 0.23 ± 0.03 | A – | –                            | –                                | –   |
| Doxorubicin   | –    | 0.63 (0.59–0.68) | –                             | –                                | –   |

EtOH ethanol, MeOH methanol, H₂O(i) infusion, H₂O(d) decoction, SD standard deviation, 95 % CI 95 % confidence interval, – not evaluated, not determined

² All extracts were screened at 50 and 5 mg/mL against *P. falciparum* K1 strain using optical microscopy in three separate experiments. Accurate IC₅₀ values were determined using seven concentrations of extract against *P. falciparum* W2 strain using the HRP2-ELISA method

³ Antiplasmodial effect based on IC₅₀: A active (IC₅₀ ≤ 10 µg/mL), MA moderately active (10 < IC₅₀ ≤ 25 µg/mL) and I inactive (IC₅₀ > 25 µg/mL)

⁴ SI = IC₅₀(murine macrophage)/IC₅₀(P. falciparum)

Table 3 Parasitemia suppression versus untreated controls and survival in mice infected with *Plasmodium berghei* after oral administration of plant extracts for four days

| Plant                  | Part       | Extract | Dose (mg/kg/day) | % parasite ± SD (% suppression) | Avg. survival ± SD (days) |
|------------------------|------------|---------|------------------|---------------------------------|--------------------------|
|                        |            |         |                  | Day 5                           | Day 7                    |

| Andropogon leucostachyus | Aerial part | MeOH | 250  | 0.49 ± 0.10 (71) | 1.12 ± 0.07 (48) | 19 ± 2 |
| Croton caucara RV        | Leaf       | CHCl₃| 250  | 1.4 ± 0.27 (19) | 2.2 ± 0.15 (0)   | 23 ± 3 |
| Xylopia amazonica        | Leaf       | CHCl₃| 250  | 0.82 ± 0.22 (52) | 1.87 ± 0.26 (11) | 20 ± 2 |
| Controls                |            |       | 10   | 0.15 ± 0.04 (91) | 0.14 ± 0.04 (93)  | 31 ± 4 |
|                         |            |       |      | 1.74 ± 0.21     | 2.12 ± 0.31      | 22 ± 1 |

CHCl₃ chloroform, MeOH methanol, SD standard deviation
In traditional medicine, *Andropogon leucostachyus* whole plant decoctions are ingested as a treatment for malaria [58]. In this work, *Andropogon leucostachyus* aerial part decoctions exhibited low in vitro activity. Methanol extraction was the most efficient process for concentrating the in vitro (IC$_{50} = 7.1 \pm 3.3$ µg/mL against *P. falciparum*) anti-malarial activity and selectivity of *Andropogon leucostachyus*. In fact, *Andropogon leucostachyus* aerial part methanol extracts exhibited the highest selectivity index (SI = 28.2) of all extracts evaluated herein. The concentration of anti-malarial components in these extracts is further attested to by the in vivo result (71 % suppression of *P. berghei*). Interestingly, the leaf decoctions of a related species, *Andropogon schoenanthus*, are ingested (with large amounts of sugar) to treat malaria fevers. Inhalation of the vapours from the boiling decoction is also used to treat malaria [59]. Very little is known about the chemical composition of *Andropogon leucostachyus*. C-glycosylflavones, the O-methyl flavone tricin and the flavanol luteoforol have been described in the leaves of *Andropogon leucostachyus* [60]. No anti-malarial activity has been reported for these flavonoids in the literature. In silico docking studies have explored the potential of tricin as a parasite dihydrofolate reductase inhibitor however it was found to interact less favorably with this enzyme than other compounds [61].

*Croton cajucara* is a cultivated plant that has red and white varieties (a reference to the coloration of young leaves). It occurs in Bolivia, Brazil, Guyana and Venezuela. *Croton cajucara* trunk bark or leaf infusions are used in traditional medicine to treat malaria according to many sources [62–68]. Herein, *Croton cajucara* extracts of both varieties were active or moderately active in vitro. Red variety leaf chloroform extract exhibited the highest in vitro inhibitory activity against *P. falciparum* W2 clone (IC$_{50} = 6.4 \pm 1.2$ µg/mL). This extract was further evaluated for in vivo oral activity against *P. berghei* in infected mice, however, it exhibited low in vivo anti-malarial activity (Table 3). Synergism among the bioactive components that comprise an extract could explain in vitro activity (79–86 % parasitaemia suppression at doses of 27–81 mg/kg/day) against *P. berghei* in rodents [70].

Also, *Croton mubango* stem bark water extracts inhibited *P. falciparum* in vitro (IC$_{50} = 3.2$ µg/mL) and suppressed *P. berghei* ANKA by 77 % at oral doses of 200 mg/kg/day [71]. Significant dose dependency in the suppression of *P. berghei* in mice has been observed for *Croton macrostachyus* water and methanol extracts (200, 400 and 600 mg/kg) [72]. Similar results were obtained for crude extracts and chloroform, methanol and water fractions of this same species wherein the chloroform fraction exhibited the best result [73].

A number of antiplasmodial diterpenes have been isolated from *Croton* species. 8,9-secoakaurane was isolated from *Croton kongensis* and inhibited *P. falciparum* K1 strain (IC$_{50} = 1–2.8$ µg/mL) [74] and geranyl geraniol was isolated from *Croton lobatus* extracts and inhibited *P. falciparum* (IC$_{50} = 3.7$ µM) [64]. Steenkrotin A, was isolated from *Croton steenkampianus* leaf ethanol extracts and exhibited IC$_{50} = 15.8, >30, 9.4$ and 9.1 µM against *P. falciparum* D10, D6, Dd2 and W2 clones, respectively [66].

*Miconia nervosa* is used traditionally in the treatment of malaria as a decoction as are *Miconia laevigata* and *Miconia willdenowii* [58, 62, 75, 76]. Herein, *Miconia nervosa* leaf extracts exhibited in vitro activity against *P. falciparum* W2 clone. No previous report on the antiplasmodial activity of extracts of a species of *Miconia* is available in the literature. Interestingly, other species from this genus, *Miconia fallax* and *Miconia stenoschyla*, are known to produce triterpene compounds that inhibit the protozoa *Trypanosoma cruzi* [77].

*Xylopia amazonica* was revealed herein as a plant whose crude extracts have anti-malarial potential. Two of its extracts were active and a third was moderately active. Among these, the leaf chloroform extracts exhibited good in vitro antiplasmodial activity (IC$_{50} = 7.3$ µg/mL) and no significant toxicity to human fibroblasts or melanoma cells was observed. Notwithstanding, these extracts exhibited low selectivity (SI = 4.6), which is an indication that chloroform extraction concentrates specific toxicity to *P. falciparum* and murine macrophages. Interestingly, cytotoxicity has been observed for the extracts of *Xylopia aromatica* trunk chloroform–methanol extracts against NCI-H460, KM-12 and SF-268 cell lines and cancer cell line RPMI-8226 [78]. Also, *Xylopia aromatica* wood hexane extracts exhibited IC$_{50} = 5–20$ µg/mL against several tumour cell lines (SF-295, HCT-8, MDA-MB-435 and HL-60) [79].

In several countries, the macerated or infused fruit and/or trunk bark of at least a dozen *Xylopia* species are used to treat malaria [30, 58, 80–85]. The extracts of several of these plants exhibit in vitro antiplasmodial activity according to previous studies. *Xylopia philoiodora* and *Xylopia aethiopica* extracts inhibit *P. falciparum* in vitro
(IC₅₀ = 18 µg/mL) [80]. *Xylopia emarginata* leaf [81], root bark, trunk bark and wood [86] extracts exhibit IC₅₀ = 3–11 µg/mL against *P. falciparum* Palo Alto or FCB1 strains. The ethanol extract of the aerial parts of *Xylopia aromatica* strongly inhibit *P. falciparum* in vitro (IC₅₀ < 1 µg/mL) [30] while root, root bark and trunk bark hexane extracts do so to a lesser extent (IC₅₀ = 4.7, 6.8 and 15.3 µg/mL, respectively) against *P. falciparum* FCB1 strain [86].

Besides the in vitro antiplasmodial activity observed, *Xylopia amazonica* leaf chloroform extracts administered orally were able to suppress (52%) *P. berghei* in mice at daily doses of 250 mg/kg herein. For a related species, *Xylopia aromatica*, it was found that aerial part ethanol extracts strongly inhibited *P. falciparum* in vitro (IC₅₀ < 1 µg/mL) but were inactive in vivo [30].

*Xylopia amazonica* is known to produce kaurene diterpenes and aporphine alkaloids. From the wood and or bark, the diterpenes beyerene, *ent*-kauran-16β-ol, *ent*-kauro-16-en-19-oic acid (kaurenoic acid) and 4-epikaurenoic acid have been isolated [87, 88] as have the aporphine alkaloids liriodenine, dicentrinone [87], oxoglaucaic, (+)-glaucine, lirioferine and (+)-laurotetanine [87, 88].

Importantly, several of the natural products isolated from *Xylopia amazonica* have been isolated from other plant species and found to exhibit in vitro antimalarial activity. Thus, (+)-laurotetanine (isolated from *Nectandra salicifolia*) exhibited IC₅₀ values of 3.9 and 2.5 µg/mL against *P. falciparum* D6 and W2 parasite clones [89]. Dicentrinone from another species inhibited *P. falciparum* K1 strain (IC₅₀ = 1.2 µg/mL) [90]. Liriodenine inhibited *P. falciparum* D6, D10 and W2 clones (IC₅₀ = 1.3, 4.1 and 2.4 µg/mL, respectively) [91, 92] whereas oxoglaucaic exhibited low activity [92]. The diterpene *ent*-kauro-16-en-19-oic acid was not active against chloroquine-sensitive *P. falciparum* D10 clone (IC₅₀ = 31.8 µg/mL) [93].

*Clidemia hirta* known as soap bush or Koster’s curse, is used as an anti-malarial among the traditional peoples of the Peruvian Amazon [38]. Herein, three *Clidemia bullosa* extracts exhibited moderate in vitro antimalarial activity. There is no information referring specifically to the traditional anti-malarial use of this species. However, *Clidemia bullosa*, *Clidemia hirta* and another species are closely related and occur together [94]. No information is available on the chemical composition of *Clidemia bullosa*, however, recent work on the chemical composition of the related species, *Clidemia hirta*, revealed the presence of hydrolysable tannins, derivatives of ellagic acid and the triterpene arjunolic acid [95].

Most *Paulinia cupana* (guaraná) extracts did not exhibit antimalarial activity. Bark and fruit chloroform extracts of this plant exhibited only moderate antiplasmodial activity. Guaraná extracts are widely consumed in the form of soft drinks and other beverages in Brazil. This plant is used in anti-malarial remedies in different locations in Latin America [58, 63, 96, 97]. In the branch bark, catechin and epicatechin have been detected [98]. These compounds may contribute, together with other compounds, to the moderate antimalarial properties observed [99].

*Xanthoxylum djalma-batistae* leaf infusion and branch chloroform extracts exhibited moderate antimalarial activity (IC₅₀ = 15.6 ± 2.9 and 17.4 ± 1.3 µg/mL, respectively) herein. More than a dozen *Zanthoxylum* species are reported to be used as anti-malarials in several countries [33, 59, 84, 85, 100–110] and antimalarial activity has been observed for extracts of these plants. Thus, *Zanthoxylum chalybeum* extracts inhibit *P. falciparum* in vitro (IC₅₀ < 10 µg/mL) [100, 102, 104, 107]. Also, *Zanthoxylum usambarenses* trunk bark and trunk wood methanol extracts inhibit *P. falciparum* NF54 strain (IC₅₀ < 5 µg/mL) [103].

There is no information on the chemical composition of *Zanthoxylum djalma-batistae*. Antimalodial benzophenanthidine alkaloids such as nitidine are found in *Zanthoxylum* and other Rutaceae species. Nitidine has been isolated from *Zanthoxylum usambarenses* and *Zanthoxylum rhoifolium* [33, 111] and exhibits submicromolar IC₅₀ values against *P. falciparum* [33, 112].

Use of cashew tree (*Anacardium occidentale*) trunk bark, leaves or fruit in the treatment of malaria symptoms is practiced by traditional peoples in Brazil, Colombia, Nigeria and Peru [58, 68, 82, 96, 113]. *Anacardium occidentale* leaves are reported to contain anacardic acid and cardol [58, 114]. Anacardic acid is believed to alter parasite gene expression and inhibit parasite development in vitro through enzyme inhibition. In vitro this compound was inactive (IC₅₀ = 30.4–34.8 µM) against a number of *P. falciparum* strains (3D7, D10, 7G8 and Dd2) [115]. *Anacardium occidentale* extracts were in general inactive in the present study.

Surprisingly, *Derris floribunda* extracts were inactive herein despite the traditional use of timbó as an anti-malarial and previous reports on the antimalarial activity of *Derris* species. *Derris amazonica* extracts have been previously shown to inhibit *P. falciparum* F32 strain in vitro (IC₅₀ = 3.2 µg/mL) [15] and lupifolin has been isolated from *Derris trifoliata* seed pod extracts and inhibits *P. falciparum* D6 and W2 strains (IC₅₀ = 2.6–3.7 µg/mL) [116].

In animal models of malaria, large experimental variability of the results is associated with drug, parasite and host interactions. For ethical reasons, the numbers of animals used may not be increased to more accurately
characterize the antiparasitic effects. Despite this low experimental reproducibility, the murine malaria model used herein is an important tool in anti-malarial drug discovery and development programmes.

Methods and criteria vary among research groups that investigate the anti-malarial potential of plants using rodent models. Extract doses of 300–650 mg/kg/day providing 47.0–84.5 % parasitaemia suppressions have been considered evidence of important anti-malarial activity [22, 117–119]. Also, extracts providing >60 % suppression of parasitaemia at oral doses of 100–250 mg/kg/day in the rodent model have been deemed active or highly active and suppression >30 % at these doses has been deemed moderate activity [120–123]. In the present work, oral doses of 250 mg of plant extract per kg of body weight per day were used for evaluation of plant extracts and detection of relevant parasitaemia suppression (>30 %) on the fifth and or seventh days in the rodent model.

Herein, significant in vivo oral suppression of P. berghei by Andropogon leucostachyus and Xylopia amazonica extracts was demonstrated. Significant differences were not observed in the mean survival of animals treated with these extracts and untreated controls. This is true even for Andropogon leucostachyus extracts that were responsible for the largest suppression of parasitaemia observed. In the antiplasmodial extracts tested in vivo, the substances responsible for the suppressive activity may be present in low amounts and may exhibit short half-lives, thus not attaining the concentrations necessary for parasite suppression after the end of treatment. This together with rapid parasite metabolism may provide extract-treated and control groups exhibiting equal parasitaemia after only a few parasitic cycles, which for P. berghei is 24 h [124].

The in vitro inhibition of P. falciparum and selectivity demonstrated by several plant extracts and oral suppression of P. berghei by Andropogon leucostachyus and Xylopia amazonica extracts are significant findings. Bioguided fractionation of several of the extracts revealed in this study is now underway and should reveal the anti-malarial chemical constituents of these plant species in the future.

Conclusions
Anti-malarial plants used traditionally in the Amazon and closely related species should be investigated for their anti-malarial potential to increase the knowledge of the useful flora of this region and provide active extracts. The identification of antiplasmodial and cytotoxic, traditionally used species can be useful as an initial step in pharmacological evaluations that can lead to more rational use. Furthermore, antiplasmodial plant extracts are the starting point for bioguided isolation of new anti-malarial chemical constituents. It is always important to remember that among the clinically most relevant anti-malarials in use today are the synthetic quinolines (quinine analogues) and semi-synthetic artemisinin derivatives that comprise artemisinin-based combination therapy (ACT). Both of these classes of anti-malarials owe their origins to quinine and artemisinin discovered in traditionally used plants from the Peruvian Amazon region and China, respectively. Herein, 29 % of the extracts studied were active or moderately active in vitro. The choice of plants studied was based on the traditional use of the species (ethnobotanic approach) or on the traditional use and or proven anti-malarial activity of plants and chemical constituents from the same genus (chemosystematic approach). The large number of active extracts attests to the importance of the accumulated traditional knowledge of anti-malarial plants in the Amazon region. The toxicity of a few extracts to tumour and non-tumour cells should serve as an alert that further toxicological evaluation of these plant extracts is necessary. The few extracts evaluated for in vivo anti-malarial activity were those that exhibited optimal in vitro antiplasmodial activity. However, in vivo evaluation of extracts that correspond to traditional plant remedies, generally infusions, decoctions and tinctures, and fractions enriched in anti-malarial components is necessary as a means to more fully evaluate their anti-malarial potential. Bioguided chemical studies on the active extracts of Andropogon leucostachyus, Croton cajuca and Xylopia amazonica are now underway and should reveal the antiplasmodial components of these plants in the future.

Authors’ contributions
RBSL collected plants; processed plant materials, carried out extractions, quantified blood smears for in vitro antiplasmodial screening and drafted the manuscript; LFRS carried out the in vitro antiplasmodial screenings using optical microscopy, performed the IC50 determinations with the immunosay (with JPS and AUK), performed the in vivo antimalarial experiments and drafted the corresponding experimental, results and discussion sections of the manuscript, MRSM carried out in vivo screening of extracts; JQS performed the in vitro IC50 determinations using the immunosay; NSP carried out the in vivo antimalarial experiments; ELS and MCV performed the screening of all extracts for in vitro cytotoxicity; JMPS researched and wrote sections of the discussion on chemistry and biological activity; APAB evaluated extracts in macrophage toxicity assays, revised sections of manuscript; RCNA processed plant materials, carried out extractions; FCMC cultivated, harvested and processed plant materials; IPC and AUK carried out (with LFRS) the in vitro immunosays and critically reviewed and corrected the final manuscript; WPT participated in the coordination of the study; AMP conceived of the study, and participated in its design and coordination and drafted the manuscript. All authors read and approved the final manuscript.

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References

1. Brandão MGL, Grandi TSM, Rocha EMM, Sawyer DR, Krettli AU. Survey of medicinal plants used as antimalarials in the Amazon. J Ethnopharmacol. 1992;36:175–82.

2. Breitbach UB, Niehues M, Lopes NP, Faria JEQ, Brandão MGL. Amazonian medicinal plants described by C.F.P von Martius in the 19th century. J Ethnopharmacol. 2013;147:180–9.

3. Adebayo JO, Krettli AU. Potential antimalarial from Nigerian plants: a review. J Ethnopharmacol. 2011;133:289–302.

4. Calderon LA, Silva-Jardim J, Zuliani JP, Silva AA, Ciancaglini P, Silva LH, et al. Amazonian biodiversity: a view of drug development for leishmaniasis and malaria. J Braz Chem Soc. 2009;20:1001–23.

5. Newman DJ, Cragg GM. Natural products as sources of new drugs over the past 30 years from 1981 to 2010. J Nat Prod. 2012;75:311–35.

6. Schmidt TJ, Khalid SA, Romana JA, Alves TM, Buatier MW, Brun R, et al. The potential of secondary metabolites from plants as drugs or leads against protozoan neglected diseases—part I. Curr Med Chem. 2012;19:2178–275.

7. Schmidt TJ, Khalid SA, Romana JA, Alves TM, Buatier MW, Brun R, et al. The potential of secondary metabolites from plants as drugs or leads against protozoan neglected diseases—part II. Curr Med Chem. 2012;19:2176–228.

8. Kaur N, Jain M, Kaur T, Jain R. Antimalarial from nature. Bioorg Med Chem. 2009;17:3229–56.

9. Pohlitt AM, Lima RBS, Frausin G, Silva LF, Lopes SC, Moraes CB, et al. Amazonian plant natural products: perspectives for discovery of new antimalarial drug leads. Molecules. 2013;18:9219–40.

10. Garavito G, Rincon J, Arteaga L, Hata Y, Bourdy G, Gimenez A, et al. Antimalarial activity of synthetic cryptolepine analog. Phytomedicine. 2012;19:1049.

11. Andrade-Neto VF, Brandão MGL, Nogueira F, Rosário VE, Krettli AU. Ampeloxypophus amazonicus Ducke (Rhamnaceae), a medicinal plant used to prevent malaria in the Amazon Region, hampers the development of Plasmodium berghei sporozoites. Int J Parasitol. 2008;38:1505–11.

12. Dölabela MF, Oliveira SG, Nascimento JM, Peres JM, Wagner H, Póvoa MM, et al. In vitro antiparasomal activity of extract and constituents from Esenbeckia febrifuga, a plant traditionally used to treat malaria in the Brazilian Amazon. Phytomedicine. 2008;15:367–72.

13. Henrique MC, Nunomura SM, Pohlitt AM. Alkaloids indólicos de cascas de Aspidosperma vagasii e A. desmanthum. Quim Nova. 2010;33:284–7.

14. Pohlitt AM, Rocha LF, Pinto ACS, Pohlitt AM, Quignard EL, Vieira PRP, Tadei WF, et al. In vivo and in vitro antimalarial activity of 4-nerolidylcatechol. Phytother Res. 2011;25:1181–8.

15. Arita MI, Lobo LT, Costa JM, Costa LS, Rocha HA, Rocha e Silva LF, et al. In vitro and in vivo antimalarial activity of essential oils and chemical components from three medicinal plants found in northeastern Brazil. Planta Med. 2012;78:658–64.

16. Andrade-Neto VF, Montaia A, Morim RCN, Melo MRS, Henrique MC, Nunomura SM, et al. Comparative in vitro and in vivo antimalarial activity of the indole alkaloids ellipticine, olivacine, cryptolepine and a synthetic cryptolepine analog. Phytomedicine. 2012;19:71–6.

17. Pohlitt AM, Rocha e Silva LF, Henrique MC, Montoia A, Morim RCN, Nunomura SM, et al. Antimalarial activity of ellipticine. Phytomedicine. 2012;19:1049.

18. Andrade-Neto VF, Brandão MGL, Nogueira F, Rosário VE, Krettli AU. Ampeloxypophus amazonicus Ducke (Rhamnaceae), a medicinal plant used to prevent malaria in the Amazon Region, hampers the development of Plasmodium berghei sporozoites. Int J Parasitol. 2008;38:1505–11.

19. Dölabela MF, Oliveira SG, Nascimento JM, Peres JM, Wagner H, Póvoa MM, et al. In vitro antiparasomal activity of extract and constituents from Esenbeckia febrifuga, a plant traditionally used to treat malaria in the Brazilian Amazon. Phytomedicine. 2008;15:367–72.

20. Henrique MC, Nunomura SM, Pohlitt AM. Alkaloids indólicos de cascas de Aspidosperma vagasii e A. desmanthum. Quim Nova. 2010;33:284–7.

21. Rocha e Silva LF, Pinto ACS, Pohlitt AM, Quignard EL, Vieira PRP, Tadei WF, et al. In vivo and in vitro antimalarial activity of 4-nerolidylcatechol. Phytother Res. 2011;25:1181–8.

22. Mota MI, Arita MI, Lobo LT, Costa JM, Costa LS, Rocha HA, Rocha e Silva LF, et al. In vitro and in vivo antimalarial activity of essential oils and chemical components from three medicinal plants found in northeastern Brazil. Planta Med. 2012;78:658–64.

23. Andrade-Neto VF, Montaia A, Morim RCN, Melo MRS, Henrique MC, Nunomura SM, et al. Comparative in vitro and in vivo antimalarial activity of the indole alkaloids ellipticine, olivacine, cryptolepine and a synthetic cryptolepine analog. Phytomedicine. 2012;19:71–6.

24. Pohlitt AM, Rocha e Silva LF, Henrique MC, Montoia A, Morim RCN, Nunomura SM, et al. Antimalarial activity of ellipticine. Phytomedicine. 2012;19:1049.

25. Pohlitt AM, Santos EVM, Silva TC, Moskis SR, Nunomura SM, Struve L. A rare secoiridoid monoterpene and a xanthone from Tachia grandiflora Maguire & Weaver. Biochem Syst Ecol. 2012;44:267–9.

26. Torres ZES, Silveira E, Rocha e Silva LF, Lima ES, Vasconcellos MC, Uchoa DA, et al. Chemical composition of Aspidosperma del Markgr. Antiplasmodial activity of selected indole alkaloids. Molecules. 2013;18:6281–97.

27. Rocha e Silva LF, Lima ES, Vasconcellos MC, Aranha EP, Costa DS, Santos EVM, et al. In vitro and in vivo antimalarial activity and cytotoxicity of extracts, fractions and a substance isolated from the Amazonian plant Tachia grandiflora (Gentianaceae). Mem Inst Oswaldo Cruz. 2013;108:501–7.

28. Montaia A, Rocha e Silva LF, Torres ZES, Costa DS, Henrique MC, Lima ES, et al. Antiplasmodial activity of synthetic ellipticine derivatives and an isolated analog. Bioorg Med Chem Lett. 2014;24:2631–4.

29. Pereira TB, Rocha e Silva LF, Amorim RCN, Melo MRS, Souza RCZ, Eberlin MN, et al. In vitro and in vivo anti-malarial activity of limonoids isolated from the residual seed biomass from Carapa guianensis (andiroba) oil production. Malar J. 2014;13:317.

30. Garavito G, Rincon J, Arteaga L, Hata Y, Bourdy G, Gimenez A, et al. Antimalarial activity of some Colombian medicinal plants. J Ethnopharmacol. 2006;107:460–2.

31. Berti S, Bourdy G, Landau I, Robinson JC, Esteire P, Dehau E. Evaluation of French Guiana traditional antimalarial remedies. J Ethnopharmacol. 2005;98:45–54.

32. Vigneron M, Deparis X, Dehau E, Bourdy G. Antimalarial remedies in French Guiana: a knowledge attitudes and practices study. J Ethnopharmacol. 2005;98:351–60.
33. Jullian V, Bouri D, Georges S, Maurel S, Sauvain M. Validation of use of a traditional antimalarial remedy from French Guiana, Zanthoxylum rhoifolium Lam. J Ethnopharmacol. 2006;106:348–52.
34. Marti G, Epavrier V, Litaudon M, Grellet P, Guettte F. A new xanthone from the bark extract of Rheedia acuminata and antiplasmodial activity of its major compounds. Molecules. 2010;15:7105–14.
35. Kvet JP, Christensen SB, Rasmussen HB, Mejia K, Gonzalez A. Identification and evaluation of Peruvian plants used to treat malaria and leishmaniasis. J Ethnopharmacol. 2006;106:390–402.
36. Roumy V, Garcia-Pizcango G, Gutierrez-Chouquevica AL, Ruiz L, Julian V, Winterton P et al. Amazonian plants from Peru used by Quechua and Mestizo to treat malaria with evaluation of their activity. J Ethnopharmacol. 2007;112:482–9.
37. Valadeau C, Pabon A, Deharo E, Albán-Castillo J, Estevez Y, Lores FA, Ruiz L, Maco M, Cobos M, Gutierrez-Choquevilca AL, Roumy V. Plants of its major compounds. Molecules. 2010;15:7106–14.
38. Brandão MGL, Botelho MGA, Krettli AU. Antimalarial chemotherapy with natural products and chemically defined molecules. Mem Inst Oswaldo Cruz. 1991;86(suppl. II):181–4.
39. Carvalho LH, Krettli AU. Antimalarial activity of crude extracts from Brazilian plants studied in vivo in Plasmodium berghei-infected mice and in vitro against Plasmodium falciparum in culture. Braz J Med Biol Res. 1991;24:1113–23.
40. Trager W, Jensen JB. Human malaria parasites in continuous culture. Science. 1976;193:673–5.
41. Rieckmann KH, Sax LJ, Campbell GH, Mrema JE. Drug sensitivity of Plasmodium falciparum to several antimalarial drugs. J Comput Biol Bioinform Res. 2012;4:23–7.
42. Rocha e Silva LFR, Magalhães PM, Costa MRF, Alecrim MGC, Chaves MR. In vitro susceptibility of Plasmodium falciparum Welch field isolates to infusions prepared from Artemisia annua and other medicinal plants in Latin America—a bibliographic survey. Kew: The Royal Botanic Garden, 1997.
43. Hidalgo AF. Plants of use popular para o tratamento da malária e males associados da área de influência do Rio Solimões e Região de Manaus-AM. Ph.D. Thesis, State University of São Paulo (UNESP), Agronomy Department, Botucatu, 2003.
44. Atienza B, Bernard W, Philippe C. Antiplasmodial activity of constituents isolated from Croton lobatus. Pharm Biol. 2007;45:263–6.
45. Costa MP, Melgão H, Costas J, Infante CM, Francois JF. Antimalarial activity in Plasmodium falciparum in culture and in vivo. J Nat Prod. 2003;66:668–70.
46. Mohamed T, Enke B, Giday M. Evaluation of antimalarial activity of leaves of Acokanthera schimperi and Croton macrostachyus against Plasmodium berghei in Swiss albino mice. BMC Complement Altern Med. 2014;14:314.
47. Bantie L, Assese S, Teklehaimanot T, Engdawork E. In vivo antimalarial activity of the crude leaf extract and solvent fractions of Croton macrostachyus Hochst. (Euphorbiaceae) against Plasmodium berghei in mice. BMC Complement Altern Med. 2014;14:79.
48. Thongtan J, Kitthaipong P, Ruangrungsi N, Saeboonruangrueng J, Thetbaranony Y. New antymyocobacterial and antimalarial 8,9-seco-koakaurane diterpenes from Croton kongensis. J Nat Prod. 2003;66:659–70.
49. Li XC, Jacob Mr, Pasco DS, Eshofly HH, Ninrod AC, Walker LA et al. Phenolic compounds from Miconia myriaphora inhibiting Candida asparagine proteases. J Nat Prod. 2001;64:1262–5.
50. Serpelyoni MJ, Barcelos MGR, Mori PM, Yanagu K, Velagis V, Varanda AE, et al. Cytotoxic and mutagenic evaluation of extracts from plant species
of the Miconia genus and their influence on doxorubicin-induced mutagenicity: an in vitro analysis. Exp Toxicol Pathol. 2011;63:499–504.
77. Cunha WR, Crevelin EJ, Arantes GM, Crotti AEM, Silva MLA, Furtado NAIC, et al. A study of the trypanocidal activity of triterpenic acids isolated from Miconia species. Phytother Res. 2006;20:474–8.
78. Suffredini A, Pacienza MLB, Varella AD, Younes RN. In vitro cytotoxic activity of Brazilian plant extracts against human lung, colon and CNS solid cancers and leukemia. Fitoterapia. 2007;78:223–6.
79. Mesquita ML, Paula JE, Pessoa C, Moraes MO, Costa-Lotufo LV, Groungert R, et al. Cytotoxic activity of Brazilian cerrado plants used in traditional medicine against cancer cell lines. J Ethnopharmacol. 2009;123:439–45.
80. Boyom FF, Njoguana V, Zolfo PHA, Menut C, Bessiere JM, Gut J, et al. Composition and anti-plasmodial activities of essential oils from some Cameroonmedicinal plants. Phytochemistry. 2003;64:1269–75.
81. Fischer DCH, Guadalia NCA, Bachiega D, Carvalho CS, Lupo FN, Bonotto SV, et al. In vitro screening for antiplasmodial activity of isoquinoline alkaloids from Brazilian plant species. Acta Trop. 2004;92:261–6.
82. Dormugbe TO, Akinlure OR, Abinu JE, Fabeku PO. Medicinal plants useful for malaria therapy in Okeigbo, Ondo State, southwest Nigeria. Afr J Trad CAM. 2007;4:19–1. 83. Botsaris AS. Plants used traditionally to treat malaria in Brazil: the
84. Koudouvo K, Karou DS, Kokou K, Essien K, Aklikokou K, Glitho IA, et al. Tsabang N, Fokou PVT, Tchokouaha LRY, Noguem B, Bakarnga-Via
85. Mambu L, Paula JE, Espindola LS. In vitro antimalarial activity and toxicity of extracts of plants used in traditional malaria therapy in Menu and Kóf Districts of Kenya. J Ethnopharmacol. 2006;106:403–7.
86. Böhlke M, Guinaudeau H, Angerhofer CK, Wongpanich V, Soejarto
87. Chokchaisiri R, Chaichompoo W, Chalermglin R, Suksamrarn A. Potent
88. Mbah JA, Tane P, Ngadjui BT, Connolly JD, Okunji CC, Iwu MM, et al. Anti-
89. Langat MK, Crouch NR, Pohjala L, Tammela P, Smith PJ, Mulholland
90. Melo GF, Machado IC, Luceno M. Reprodução de três espécies de
91. Abdellaoui SE, Destandau E, Krolikiewicz-Renimel I, Cancellieri P, Toribio
92. Deharo E, Bourdy G, Quenevo C, Muñoz V, Ruiz G, Sauvain M. A search
93. Mors WB, Rizzini CT, Pereira NA. Medicinal plants of Brazil. In: DeFilipps
94. Souza MP, Matos MEO, Matos FJA, Machado MIL, Craveiro AA. Constitu-
95. Rukunga GM, Gathirwa AW, Omar SA, Muregi FW, Muthaura CN, Kirina PG, et al. Anti-plasmodial activity of the extracts of some Kenyan medicinal plants. J Ethnopharmacol. 2009;121:282–5.
96. Tchinda AT, Fuendjiep V, Sajjad A, Matchawe C, Wafo P, Khan S, et al. Bio-
97. Kirira PG, Rukunga GM, Wanyonyi AW, Muregi FM, Gathirwa JW, Muth-
98. Nguta JM, Mbaria JM, Gakuya DW, Gathumbi PK, Kiama SG. Traditional
99. Tchinda AT, Fuendjiep V, Sajjad A, Matchawe C, Wafo P, Khan S, et al. Bio-
100. Ginsburg H, Deharrow A. E. a call for using natural compounds in the development of new antimalarial treatments—an introduction. Malar J. 2011;10(Suppl 1):S1.
101. Gansane A, Sanon S, Ouattara LP, Traore A, Hutter S, Livelier E, et al. Antiplasmodial activity and toxicity of crude extracts from alternatives plants of plants widely used for the treatment of malaria in Burkina Faso: contribution for their preservation. Parasitol Res. 2010;106:335–40.
102. Gessler MC, Nkunya MH, Mwasumbi LB, Heinrich M, Tanner M. Screening of Tanzanian medicinal plants for antimalarial activity. Acta Trop. 1994;56:65–77.
103. Kirina PG, Rukunga GM, Wanyonyi AW, Muregi FM, Gathirwa JW, Muth-
104. Nguita JM, Mbara JM, Gakuya DW, Gathumbi PK, Kiama SG. Traditional antimalarial phytotherapy remedies used by the South Coast community, Kenya. J Ethnopharmacol. 2010;131:256–67.
105. Penalí L, Mulholland DA, Tano KD, Cheplogó PK, Randrianarivelosia M. Low antiplasmodial activity of alkaloids and amides from the stem bark of Zanthoxylum rubescens (Rutaceae). Parasite. 2007;14:161–4.
106. Rukunga GM, Gathirwa AW, Omar SA, Muregi FW, Muthaura CN, Kirina PG, et al. Antiplasmodial activity and toxicity of extracts of plants used in traditional malaria therapy in Menu and Kóf Districts of Kenya. J Ethnopharmacol. 2006;106:403–7.
107. Tchinda AT, Fuendjiep V, Sajjad A, Matchawe C, Wafo P, Khan S, et al. Bio-
108. Tchinda AT, Fuendjiep V, Sajjad A, Matchawe C, Wafo P, Khan S, et al. Bio-
109. Traore MS, Baldé MA, Diaa M, Diassane D, Diallo MB, Diallo MB, Samba MA, Diallo A, et al. Ethnobotanical survey on medicinal plants used by Guinean traditional healers in the treatment of malaria. J Ethnopharmacol. 2013;150:114–53.
110. Weenen H, Nkunya MH, Bray DH, Mwasumbi LB, Kinabo LS, Kilimali VAEB, et al. Antimalarial activity of Tanzanian plants. Part 2. Antimalarial compounds containing an α,β-unsaturated carbonyl moiety from Tanzanian medicinal plants. Planta Med. 1990;56:371–3.
111. Kato A, Moriyasu M, Ichimaru M, Nishiyama Y, Juma F, Nganga J, et al. Isolation of alkaloidal constituents of Zanthoxylum usambarensis and Zanthoxylum chalybeum using ion-pair HPLC. J Nat Prod. 1996;59:316–9.
112. Gakunju DMN, Mberu ED, Dossaj SF, Gray AL, Waigb RD, Waterman PG, et al. Potent antimalarial activity of the alkaloid nitidine, isolated from a Kenyan herbal remedy. Antimicrob Agents Chemother. 1995;39:2606–9.
113. Aviwo GO, Iyola S, Enoghayin EI. Effects of some Nigerian antimalarial medicinal plants on glucose levels in Wistar rats. Arch Appl Sci Res. 2010;2:112–6.
114. Hemeshkher M, Santosh MS, Kemparaju K, Girish KS. Emerging roles of anacardic acid and its derivatives: a pharmacological overview. Basic Clin Pharmacol Toxicol. 2012;110:222–32.
115. Cui L, Miao J, Furuya T, Fan Q, Li X, Rathod PK, et al. Histone acetyltrans-
116. Yenesew ABIY, Twinomuhwezi H, Kabaru JM, Akala HM, Kiremire BT,
117. Falade MO, Akinboye DO, Gbotosho GO, Abiodun OO, Ajaiyeoba
118. Margaret MO, Akinboye DO, Gbotosho GO, Abiodun OO, Ajaiyeoba
119. Banks (Ochnaceae), Blume (Moraceae) and Lophira alata (Melastomataceae) in Cameroon. Rev Biol Trop. 1999;47:359–63.
120. Heydenreich M, et al. Antiplasmodial and larvicidal flavonoids from
121. Cui L, Miao J, Furuya T, Fan Q, Li X, Rathod PK, et al. Histone acetyltrans-
122. Croton-kauren-19-oic acid derivatives from the stem bark of Croton pseudopulchellus (Euphorbiaceae) in Nigeria. J Ethnopharmacol. 2012;139:184–90.
123. Wos M, Roth PK, et al. Histone acetyltransferase inhibitor anacardic acid causes changes in global gene expression during in vitro Plasmodium falciparum development. Eukaryot Cell. 2008;7:1200–10.
124. Yenesew ABY, Twinomuhwezi H, Kabaru JM, Akala HM, Kiremire BT, Heydenreich M, et al. Antiplasmodial and larvicidal flavonoids from Croton pseudopulchellus (Euphorbiaceae) in Nigeria. J Ethnopharmacol. 2012;139:184–90.
125. Meefin A, Giday M, Animun A, Tekeleyanmotan T. Ethnobotanical study of antimalarial plants in Shirel District, Somali Region, Ethiopia, and in vivo evaluation of selected ones against Plasmodium berghei. J Ethnopharmacol. 2012;139:221–7.
126. Sangian H, Faramarz H, Yazdinezhad A, Mousavi SJ, Zamari Z, Nou-
127. Falade MO, Akinboye DO, Gbotosho GO, Abiodun OO, Ajaiyeoba EO, Happe TC, et al. In vitro and in vivo antimalarial activity of Ficus thornii Blume (Moraceae) and Lopha dota Banks (Dichocereae), identified from the ethnomedicine of the Nigerian Middle Belt. J Parasitiol. 2014;100:2853.
128. Upadhyay HC, Sisodia BS, Agrawal J, Pal A, Darokar MP, Srivastava SK. Antimalarial potential of extracts and isolated compounds from four species of genus Ammannia. Med Chem Res. 2014;23:870–6.
121. Musila MF, Dossaji SF, Nguta JM, Lukhoba CW, Munyao JM. In vivo antimalarial activity, toxicity and phytochemical screening of selected antimalarial plants. J Ethnopharmacol. 2013;146(2):557–61.

122. Nguta JM, Mbaria JM. Brine shrimp toxicity and antimalarial activity of some plants traditionally used in treatment of malaria in Msambweni district of Kenya. J Ethnopharmacol. 2013;148:986–92.

123. Gathirwa W, Rukunga GM, Mwitari PG, Mwikwabe NM, Kimani CW, Muthaura CN, et al. Traditional herbal antimalarial therapy in Kilifi district, Kenya. J Ethnopharmacol. 2011;134:434–42.

124. Jiménez-Díaz MB, Viera S, Ibáñez J, Mulet T, Magán-Marchal N, Garuti H, et al. A new in vivo screening paradigm to accelerate antimalarial drug discovery. PLoS One. 2013;8:e66967.

125. Blair S, Correa A, Madrigal B, Zuluaga CB, Franco HD. Plantas antimaláricas, una revisión bibliográfica. Medellin: Universidad de Antioquia; 1991. p. 214.

126. Osorio E, Arango GJ, Jimenez N, Alzate F, Ruiz G, Gutierrez D. Antiprotozoal and cytotoxic activities in vitro of Colombian Annonaceae. J Ethnopharmacol. 2007;11:630–5.

127. Steele JCP, Phelps RJ, Simmonds MSJ, Warhurst DC, Meyer DJ. Two novel assays for the detection of hemin-binding properties of antimalarials evaluated with compounds isolated from medicinal plants. J Antimicrob Chemother. 2002;50:25–31.

128. Adia MM, Anywar NG, Byamukama R, Kamatenesi-Mugisha M, Sekagya Y, Kakudidi EK, et al. Medicinal plants used in malaria treatment by Prometra herbalists in Uganda. J Ethnopharmacol. 2014;155:580–8.

129. Mujtaba Shah G, Abbasi AM, Khan N, Guo X, Khan MA, Hussain M, et al. Traditional uses of medicinal plants against malarial disease by the tribal communities of Lesser Himalayas-Pakistan. J Ethnopharmacol. 2014;155:450–62.

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