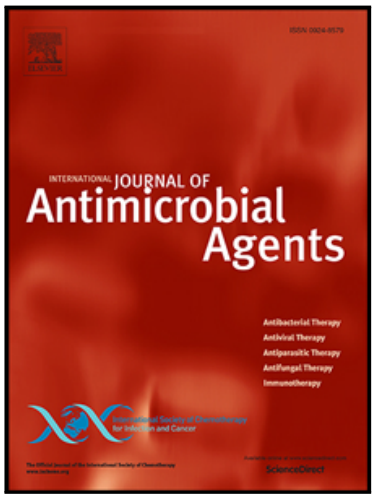

Antiplasmodial, Anti-chikungunya virus and Antioxidant Activities of 64 endemic Plants from the Mascarene Islands

Allison Ledoux , Martine Cao , Olivia Jansen , Mamede Lucia , Pierre-Eric Campos , Bertrand Payet , Patricia Clerc , Isabelle Grondin , Emmanuelle Girard-Valenciennes , Thomas Hermann , Marc Litaudon , Charlotte Vanderheydt , Leen Delang , Johan Neyts , Pieter Leyssen , Michel Frédérich , Jacqueline Smadja

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Highlights

- **Natural products are an immeasurable therapeutic potential resource for drug discovery**
- **64 endemic plants has been selected to evaluate their antiplasmodial and anti-chikungunya activities**
- **Casearia coriaceae, Monimia rotundifolia, Poupartia borbonica, Psiadia retusa, Vernonia fimbrillifera, and Zanthoxylum heterophyllum demonstrated high antiplasmodial activity**
- **Aphloia theiformis, Stillingia lineata, Croton mauritianus, Indigofera ammoxylum, and Securinega durissima showed high anti-chikungunya activity**
- **These plants are good candidates to further investigations to obtain new antimalarial and anti-chikungunya agents**

Antiplasmodial, Anti-chikungunya virus and Antioxidant Activities of 64 endemic Plants from the Mascarene Islands

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Abstract

Vector-borne diseases cause more than 1 million deaths annually. The research of new medicines is urgent, especially since there is currently no specific treatment. In this study, the authors have selected 64 endemic plants from the Mascarene Islands based on their endemism, their medicinal use and their registration in the French Pharmacopeia to evaluate the antiplasmodial, anti-chikungunya and antioxidant activities. The list of these 64 plants including their local name, population, data of collection and voucher number are available in the Supporting Information section. 40 active extracts have been identified from the 38 species: 22 responded positively to the antiplasmodial activity, 8 to the anti-chikungunya activity and 8 to the antioxidant activity.

Six plants demonstrated high antiplasmodial activity (IC_{50} < 15 µg/mL): *Casearia coriaceae*, *Monimia rotundifolia*, *Poupartia borbonica*, *Psiadia retusa*, *Vernonia fimbrillifera*, and *Zanthoxylum heterophyllum*; and five showed high anti-chikungunya activity (IC_{50} < 20 µg/mL): *Aphloia theiformis*, *Stillingia lineata*, *Croton mauritianus*, *Indigofera ammoxylum*, and *Securinega durissima*. Eight plants displayed an important antioxidant activity, with values of ABTS, FRAP or ORAC > 2000 µM of Trolox equivalent per mg/mL of extract: *Bertiera borbonica*, *Erythroxylon laurifolium*, *Erythroxylon sideroxyloides*, *Indigofera ammoxylum*, *Poupartia borbonica*, *Scolopia heterophylla*, *Sophora denudata*, and *Terminalia*

bentzoe. Some data obtained tend to corroborate with the reported traditional use of the plant, such as *Zanthoxylum heterophyllum* (antiplasmodial), *Aphloia theiformis* (anti-chikungunya), and *Erythroxylon laurifolium* (antioxidant).

Keywords

Chikungunya virus; Malaria; Antiplasmodial; Antioxidant

1. Introduction

Vector-borne diseases are human pathologies caused by parasites, virus or bacteria. According to WHO, they are responsible for more than 1 million deaths annually [1]. Mosquitoes are the most studied insects and also the deadliest due to their ability to carry and propagate pathogens to humans. Malaria is one of the most serious vector-borne diseases, accountable for 429.000 deaths worldwide in 2016 [2]. The resistance of parasites to medicines has become a widespread problem. Malaria is the consequence of a protozoan parasite, *Plasmodium sp*., transmitted by the bite of anopheles mosquitoes. The *Plasmodium* uses a lot of energy for survival and reproduction increasing the production of reactive oxygen species in hosts, which can impact the general condition of patients [3]. Chikungunya is caused by a single-stranded RNA virus, chikungunya virus (CHIKV) and is transmitted to humans by the bite of *Aedes aegypti* and *Aedes albopictus* mosquitoes. Chikungunya is characterized by an important fever and arthralgia, which can frequently become chronic [4]. Outside the known infection areas (Africa, Islands

in the Indian and Pacific Oceans, Southern Europe, and Southeast Africa), the chikungunya epidemic in 2005 that occurred in Reunion Island and its neighbors involved about one-third of the population and led to more than 250 deaths [5]. Unfortunately, there is currently no specific treatment. The use of antipyretic and analgesic helps to alleviate symptoms [6]. Antioxidant treatments can be used as adjuvant to decrease articular inflammatory pain. Vector-borne diseases are a major public health problem that makes the search for new treatment essential. Natural products are an immeasurable therapeutic potential resource for drug discovery. Recently, a review showed that more than 60% of all anti-infective (antibacterial, fungal, parasitic and viral) drugs commercialized from January 1981 to December 2014 are unaltered natural products (5.5%), natural product derivatives (35.2%), synthetic drugs with a natural product pharmacophore (18.6%) or synthetic drugs with natural product mimic (3.3%) (vaccines not included) [7]. Reunion Island is described as a biodiversity hotspot containing a high rate of endemic plants, due to its particular specific geomorphology and micro-climate. In this work, all the plants were collected in Reunion Island based on their medicinal use, endemism and registration in the French Pharmacopoeia(Smadja, 2016; S. Giraud-Techer, 2016). Some of the studied plants are endangered species, implying the importance of their screening to valorize their potential. We evaluated the antiplasmodial, anti-chikungunya virus and antioxidant activities. Only the plants which demonstrated activities in our assays are presented for the sake of clarity. The complete list of 64 plants including their local name, population, data of collection and voucher number are available in the Supporting Information.

2. Materials and methods

2.1 Plant materials and extraction

64 plants were collected in Reunion Island for the BIOMOL TCN project through a cooperative network of botanist collaborators. The harvests were made with best regard to the biological patrimony represented by the exceptional biodiversity of the island (Pitons, Cirques and Remparts of Reunion Island are listed in UNESCO World Heritage). The species were identified by Prof. Dominique STRASBERG and Jacques FOURNEL (University of Reunion) and by Hermann THOMAS (Parc National de La Réunion). A voucher specimen of each species was deposited in the University Herbarium. The plants were air-dried at room temperature with no direct sunlight and then pulverized using an electrical grinder. Extracts were prepared with 5 g of powdered dried material in 50 mL of solvent (Ethyl acetate - EtOAc) using an ASE 300 system (Accelerated Solvent Extraction). The EtOAc extracts were then submitted on a small-cartridge polyamide filtration to remove tannins and then weighed and stored in appropriate vials. The filtrates were pooled and evaporated to dryness under reduced pressure at 40°C. These extracts were evaluated for their antiplasmodial and anti-chikungunya activities.

For the antioxidant activity assays, the plants have been dried in an oven at 45°C and crushed. All extractions were also performed using the ASE 300 system. Approximately 50 g of plant powder was extracted using 100 mL-stainless steel cells. Five static cycles of 6 min followed by 2

min of purge with pressurized nitrogen (10 bars) were performed at 40°C, 100 bars leading to approximately 180 mL of extract. The solvent of extraction was ethyl acetate for all the extracts. These extracts have been concentrated and dried on rotary evaporator (Büchi).

2.2 Antiplasmodial assays

Continuous *in vitro* cultures of asexual erythrocyte stages of *P. falciparum*, chloroquine-sensitive strain 3D7 were maintained following the procedure of Trager and Jensen [10]. The strain was obtained from the Malaria Research and Reference Reagent Resource Center, MR4. The host cells were human red blood cells (A+). The culture medium composed of RPMI 1640 (Gibco, Fisher) containing $NaHCO_3$ (32 mM), HEPES (25 mM) and L-glutamine, was supplemented with 1.76 g/L of glucose (Sigma-Aldrich), 44 mg/mL of hypoxanthin (Sigma-Aldrich), 100 mg/L of gentamicin (Gibco) and 10% human pooled serum (A+), as previously described [11].

Each crude extract was dissolved in DMSO or in EtOH/DMSO [1:1], according to their solubility, at a concentration of 10 mg/mL. The solutions of tested extracts were diluted in the medium; each test sample was applied in a series of eight 2-fold dilutions in a 96-well plate and tested in triplicate. The parasitemia was 2%, and the hematocrit was 1%, as described by Murebwayire et al [12]. The highest concentration of solvent to which the parasites were exposed was 1% (which is devoid of toxicity). The *P. falciparum* culture was placed in contact for 48h. Parasite growth was estimated by colorimetric revelation with the measurement of absorbance at 630 nm by the determination of lactate dehydrogenase

(pLDH) activity, according to the methods described by Makler et al [13]. Artemisinin (Sigma-Aldrich) at an initial concentration of 100 ng/mL was used as positive control in all experiments. IC_{50} (concentration inhibiting 50% of parasitic growth) values were calculated from graphs.

2.3 Anti-chikungunya assay

CHIKV causes a pronounced cytopathic effect in cell, such as Vero cells, which served as a starting point for the optimization of a virus-cell-based screening assay employing the MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt) method[14]. Following the microtiter plate readout, assay conditions can be quickly and automatically sorted as inactive/toxic (no increase in optical density (OD) at 493 nm) or with potential antiviral activity (an increase greater than 50% in OD compared with the control conditions). However, and this was expected based on prior experience with the screening of complex samples, a high initial positives rate was obtained (up to 10%). To discriminate true hits from false positives, a microscopic quality-control step was introduced: only samples that completely inhibited virus-induced cell death without causing an adverse effect on the host cell and monolayer morphology were retained for further study.

The same assay was used to guide the fractionation process, in the end yielding selective inhibitors of CHIKV replication. The eight plant extracts from Reunion presenting selective antiviral activity were submitted to bioassay-guided fractionation in order to identify some active

molecules.

Serial dilutions of extracts, fractions or compounds were prepared in assay medium [MEM Rega3 (Cat. N19993013; Invitrogen), 2% FCS (Integro), 5 mL 200 mM L-glutamine, and 5 mL 7.5% sodium bicarbonate] that was added to empty wells of a 96-well microtiter plate (Falcon, BD). Subsequently, 50 µL of virus dilution in assay medium was added, followed by 50 µL of a cell suspension. This suspension, with a cell density of 25,000 cells/50 µL, was prepared from a Vero cell line sub-cultured in cell growth medium (MEM Rega3 supplemented with 10% FCS, 5 mL L-glutamine, and 5 mL sodium bicarbonate) at a ratio of 1:4 and grown for 7 days in 150 cm^2 tissue culture flasks (Techno Plastic Products). The assay plates were placed in the incubator for 6–7 days (37 °C, 5% CO_2, 95–99% relative humidity), a time at which maximal virus-induced cell death or cytopathic effect (CPE) is observed in untreated, infected controls. The assay medium was then aspirated, replaced with 75 µL of a 5% MTS (Promega) solution in phenol red-free medium and incubated for 1.5 h. The absorbance was measured at a wavelength of 498 nm (Safire2, Tecan); and optical densities reached values between 0.6–0.8 for the untreated, uninfected controls. Raw data was converted to percentage of controls and the EC_{50} (50% effective concentration or concentration which is calculated to inhibit virus induced cell death by 50%) and CC_{50} (50% anti-metabolic concentration or concentration calculated to inhibit the overall cell metabolism by 50%) were derived from the dose–response curves. The selectivity index (SI) is calculated as CC_{50}/EC_{50} and is used as an indication for the antiviral potential of a compound: the larger this value is, the larger is the therapeutic window or the distance between the concentration of compound

having a pronounced antiviral effect and causing an adverse effect on the host cell. All assay conditions producing an antiviral effect exceeding 50% were checked microscopically for minor signs of CPE or adverse effects on the host cell (i.e. altered cell morphology). A compound is considered to elicit a selective antiviral effect on virus replication only if, following microscopic quality control, and at least at one concentration of compound, no CPE or any adverse effect is observed.

2.4 Antioxidant assays

Extracts were solubilized at a concentration of 1 mg/mL in ethyl acetate for the FRAP and the ABTS assays and in methanol for ORAC assay. For these three tests, if the results obtained were out of the Trolox range, the extracts were two-fold diluted and the test repeated.

Ferric reducing antioxidant power (FRAP) assay

The method described by Benzie and Strain[15] was used. It is based on the reduction of a ferric (III) complex to its colored ferrous (II) form in the presence of antioxidants. The FRAP reagent was prepared with three solutions as follows: acetate buffer at pH 3.6 (3.1g of $C_2H_3NaO_2 \cdot 3H_2O$ and 16 mL $C_2H_4O_2$ per liter of buffer solution); 10 mmol/L solution of TPTZ in 40 mmol/L HCl; and 20 mmol/L solution of $FeCl_3 \cdot 6H_2O$. The working solution was prepared by mixing 10 mL of acetate buffer, 1 mL of TPTZ solution and 1mL of $FeCl_3 \cdot 6H_2O$ solution. For the preparation of Trolox standards, 0.250 g of Trolox was dissolved in 50 mL of methanol to give a 0.02 M solution. This solution was diluted with methanol to prepare a standard range between 800 and 25 µM. For the assay, 10 µL of the extract at a concentration of 1mg/mL was introduced into a 96-

well microplate with 290 µL of FRAP reagent. After 1h of incubation at 37°C, the absorbance was measured at 593 nm. Trolox equivalents of samples were calculated with a calibration curve of pure Trolox in acetate buffer.

ABTS radical scavenging activity

We used the method described by Re et al.[16]. ABTS radical cation (ABTS•+) was produced by the reaction between ABTS and potassium persulfate. ABTS was dissolved in water to a 7 mM concentration (19.2 mg of ABTS in 5 mL of water) and added to 5 mL of a 2.45 mM solution of potassium persulfate. The mixture was left in the dark at room temperature for 12–16 h before use in order to obtain the maximal absorbance of the solution. The ABTS•+ solution was diluted with methanol to obtain an absorbance of 0.70 (± 0.2) at 734 nm and equilibrated at 30°C. Trolox standards were prepared as explained above. The solution was diluted with methanol to prepare a standard range between 400 and 25 µM. For the assay, 20 µL of the extract at a concentration of 1 mg/mL were introduced into a 96-well microplate with 280 µL of the ABTS•+ solution. The absorbance was measured at 734 nm after 5 min of incubation at 30°C. Trolox equivalents of samples were calculated with a calibration curve of pure Trolox in methanol.

Oxygen Radical Absorbance Capacity (ORAC) assay

As described in the method developed by Ou et al.(Ou et al, 2001), the AAPH reagent solution was prepared just before use and kept on ice due to the instability of the radical. AAPH (0.414 g) was dissolved in 10 mL of 75 mM phosphate buffer (pH 7.4) to a final concentration of 153

mM. Fluorescein stock solution (4.19 x 10^{-3} mM) was prepared in 75 mM phosphate buffer (pH 7.4) and kept at 4 °C in dark condition. The 8.16 x 10^{-5} mM fresh fluorescein working solution was made daily from the stock solution in 75 mM phosphate buffer (pH 7.4). Trolox standards were prepared as described hereinabove and the solution was diluted with methanol to prepare a standard range between 75 and 6.25 µM. For the assay, 25 µL of sample at a concentration of 1 mg/mL were introduced in each well into a 96-well microplate with 150 µL of the fluorescein solution. 25 µL of AAPH (153 mM) were added in each well with the injector of the spectrophotometer and the fluorescence was recorded every minute for 100 min. The area under the curve (AUC) was determined automatically for each sample and each Trolox concentration. Standard curve was established by plotting AUC versus Trolox concentrations. Antioxidant activity was expressed as micromoles of Trolox equivalent per mg/mL of extract.

2.5 Statistical Analysis

The experimental results were expressed as an average of three replicates in each case. For all samples, the Trolox equivalent was calculated for 1 mg/mL of extract with a calibration curve of Trolox according to the concentration. For each point of the standard range, 4 replications have been performed.

3. Results and Discussion

Concerning the screening of the crude extracts for antiplasmodial activity, 17 plant extracts showed moderate activity with an IC_{50} comprised between 15 and 50 µg/mL. The bark extract of *Casearia coriaceae* and 6 leaves extracts displayed a promising activity with an IC_{50} < 15 µg/mL: *Casearia coriaceae*, *Monimia rontundifolia*, *Poupartia borbonica*, *Psiadia retusa*, *Vernonia fimbrilifera* and *Zanthoxylum heterophyllum*. These results are detailed in Table 1. The most interesting extracts were obtained from *Casearia coriaceae* (IC_{50} < 5µg/mL), *Poupartia borbonica* (IC_{50} < 5 µg/mL) and *Vernonia fimbrilifera* (IC_{50}= 5.2 µg/mL ± 1.3 for the leaves extracts). As far as we know, these 3 plants have never been either phytochemically nor pharmacology studied. Our team further investigated these plants and discovered that hydroxy-γ-isosanshool was the main active compound of the extract of *Z. heterophyllum*, with IC_{50} corresponding to 11.3 ± 1.5 µg/mL (Ledoux *et al.*, 2015) and Poupartones A, B and C (alkylphenol cyclohexenones derivatives, with IC_{50} < 1 µg/mL) in *P. borbonic*a, the main antiplasmodial compounds in our *in vitro* and *in vivo* assays (Ledoux et al, 2017). The use of *Zanthoxylum heterophyllum* in decoction to treat fever in traditional medicine[20] may be correlated with its antiplasmodial properties. The *Vernonia* genus is known to contain vernolides, a class of sesquiterpene lactones which showed interesting antiplasmodial bioactivity [21]. Three sesquiterpene lactones namely 8-(4'-hydroxymethacrylate)-dehydromelitensin, onopordopicrin and 8α-[4'-hydroxymethacryloyloxy]-4-epi-sonchucarpolide have been isolated and identified as the main active compounds with IC_{50} < 5µg/mL [22]. Leaves of *Terminalia bentzoe* and *Psiadia dentata* showed moderate activity (IC_{50} of 18.7 µg/mL ± 10.6 and 23.0 µg/mL ± 3.0, respectively), challenging a previous study [23] which demonstrated the promising activity of these plants (IC_{50} < 10 µg/mL). This suggests

that the leaves of *Psiadia dentata* are less active than the aerial parts previously tested and that the leaves extract of *Terminalia bentzoe* are less active than the bark extract. These differences could be due to different harvest time or location. In this study, EtOAc extracts of *Aphloia theiformis* were not active, although our team previously showed the activity of the MeOH bark extract and the CH_2Cl_2 leaves extract. The discrepancy observed might be explained by the location or time of harvest. Regarding the plants used by local population, 5 species were in use to treat fever or malaria [20] ; *Croton mauritianus* (decoction), *Eugenia uniflora* (infusion), *Terminalia bentzoe* (decoction and sometimes macerated in alcohol), *Toddalia asiatica* and *Zanthoxylum heterophyllum* (decoction or infusion), demonstrated antiplasmodial activities, which validate their traditional application. Even if aqueous extracts are closer to the traditional preparation method, they contain a lot of polar molecules such as glycosides. Glycosides can be inactive *in vitro* but active *in vivo* after metabolization, and the EtOAc extract contains the active corresponding aglycone. Since the aqueous extracts hold many compounds, their presence can mask minor interesting extracted molecules and thus appear to be less active than it is in reality. Another reason that could explain the *in vivo* activity of traditional aqueous preparations is linked to the remedies consumption. Indeed, when the herbal tea is prepared, filtration is not complete and some plants fragments can be swallowed (including active compounds which have not been extracted in water). It could be the case for example, of *Croton mauritianus*. The traditional remedies actually involve a decoction of chopped leaves [20] which are difficult to fully filter.

From *Toddalia asiatica*, already known for its antiplasmodial properties, two active molecules were isolated: toddayanis (terpenalkaloid) and 8-methoxynorchelerythrine (IC_{50} < 10 µg/mL) [24]. Although *Terminalia bentzoe* and *Zanthoxylum heterophyllum* have already been studied by our team, it is the first time that the antiplasmodial activity of *Croton mauritianus* and *Eugenia uniflora* (IC_{50} < 25 µg/mL) is demonstrated.

Regarding the anti-chikungunya activity, *Stillingia lineata* was one of the most interesting plants, with IC_{50} of 1.9 ± 0.4 µg/mL (the results are shown in Table 1). In this study, the crude EtOAc extract obtained from the stem bark of *Stillingia lineata* ssp. *lineata* exhibited a selective antiviral activity against the chikungunya virus with an EC_{50} < 2 µg/mL whereas only a weak cytotoxic effect was observed on the host cells. A phytochemical investigation of this extract led to the isolation of tonantzitlolone A, tonantzitlolone B, of an original 4'-hydroxytonantzitlolone, named tonantzitlolone C which has an uncommon C_{15}-flexibilane skeleton, and of a new ent-12-hydroxy-3,7- dioxoisopimara-8,15-diene. Subsequent evaluation of the inhibition of chikungunya virus replication in cell demonstrated that the 4'-acetoxytonantzitlolone was endowed with antiviral activity against CHIKV [14]. Moreover, the LC/MS and the bioassay-guided purification of the EtOAc leaves extract using a chikungunya virus-cell-based assay led to the isolation of six new and three known tonantzitlolones possessing the rare C_{20}-flexibilane skeleton, along with tonantzitloic acid, a new linear diterpenoid, and three new and two known tigliane-type diterpenoids[25]. A bioassay-guided purification of an EtOAc extract of the leaves of *Croton mauritianus* using a chikungunya virus-cell-based assay led to the isolation of 12-O-decanoylphorbol-13-acetate and the new 12-O-decanoyl-7-hydroperoxy-phorbol-5-ene-13-acetate, along with loliolide, vomifoliol, dehydrovomifoliol, annuionone D and bluemol C. 12-O-decanoylphorbol-13-acetate and 12-O-decanoyl-7-hydroperoxy-phorbol-5-ene-13-

acetate inhibited chikungunya virus-induced cell death in cell culture with EC_{50} of 2.4 ± 0.3 and 4.0 ± 0.8 µM, respectively [26]. *Aphloia theiformis* has already been phytochemically and pharmacologically investigated and is known to contain saponins[27] and xanthone glucosides[28] and is known to act on multiple pharmacological targets [29]. The bark of *Aphloia theiformis* was used in decoction against Chikungunya virus during the epidemic in 2006 in Reunion Island [30]. It was traditionally prepared to treat fever and articular pain [20]. Here, we confirmed for the first time the high anti-chikungunya activity of this plant, with IC_{50} of 9.7 ± 1.2 µg/mL and a selectivity index of 5.5. The barks of *Indigofera ammoxylum* and *Securinega durissima* showed an interesting antiviral activity, with IC_{50} of 15.6 ± 2.9 µg/mL and 15.9 ± 3.9 µg/mL, respectively. Even if the activities of these two plants are similar, *Indigofera ammoxylum* has more potentiality for further investigations, as its selectivity index is 3.5 vs.1.6 for *Securinega durissima* . As far as it could be established, these endemic plants have never been studied.

Some species showed an interesting antioxidant activity of their bark extracts according to the three assays: *Bertiera borbonica*, *Erythroxylon laurifolium* and *Erythroxylon sideroxyloides*, *Indigofera ammoxylum*, *Poupartia borbonica*, *Scolopia heterophylla*, *Sophora denudata* and *Terminalia bentzoe* (see results in Table 1). The leaves extract of *Bertiera borbonica* and the bark extract of *Erythroxylon laurifolium* displayed the highest response to the ORAC assay, respectively 6510.8 and 6310.3 µM of Trolox equivalent per mg/mL of extract. *Bertiera borbonica* also exhibited the highest Trolox equivalent response for the ABTS assay (3612.0 ± 42.1 Trolox equivalent per mg/mL of extract). *Erythroxylon laurifolium* is known to contain oligomeric and polymeric proanthocyanidins and showed moderate activity against Herpes simplex virus type

1.[31] The decoction of *Erythroxylon laurifolium* is used in traditional medicine as anti-inflammatory (Supporting information). This use can be validated by its high antioxidant activity observed in this study. *Scolopia heterophylla* and *Terminalia Bentzoe* were the only two leaves extracts that have also exhibited a relatively high activity, respectively 2195.5 and 1005.7 µM of Trolox equivalent per mg/mL of extract. *Terminalia bentzoe* seemed particularly interesting, as both its leaves and bark extracts showed an antioxidant activity and their values were really high for the ABTS (2758.4 and 3063.3 µM of Trolox equivalent per mg/mL of extract) and FRAP (332.6 and 1954.5 µM of Trolox equivalent per mg/mL of extract) assays. Up to now, *Bertiera borbonica* and *Scolopia heterophylla* have never been either phytochemically nor pharmacology studied.

4. Conclusion

In this study, elements were put in evidence to justify several traditional uses of the flora of Mascarene Islands. Some uses were not confirmed, but it does not mean the plants do not have any activity. Indeed, they may act on other pathways than the erythrocytic stage tested in this study (on the liver stage of *P. falciparum* for example) or they may act on the symptoms of the pathologies (fever, cephalgias) and impact the patient's recovery. In this study, EtOAc extracts were performed, because some compounds could be more active *in vivo* after metabolization, and not *in vitro*, which is frequently the case for plants containing prodrugs. This can explain why EtOAc or dichloromethane extracts are generally more active than *in vitro* aqueous extracts [32]. These results demonstrated the biological activities of plants which were highlighted for the first time, as C*asearia coriaceae*, *Monimia rotundifolia, Indigofera ammoxylum, Scolopia durissima, Bertiera borbonica, and Scolopia heterophylla*. Further investigations, such as bioassay guided fractionation should be carried out to isolate and elucidate the structure of the active compounds from these plants.

5. Supporting Information

The list of the 64 plants including their local name, population, data of collection and voucher number are available in the Supporting Information.

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Declarations

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Competing Interests: None

Ethical Approval: Not required

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Table 1: *In vitro* IC_{50} values against *Plasmodium falciparum* 3D7 strain; IC_{50}, IC_{90}, CC_{50} and Selectivity Index (SI) values against Chikungunya virus (CHKV); ABTS ($C_{18}H_{16}N_4O_6S_4$-$(NH_4)_2$) Radical Scavenging Activity (ABTS), Ferric Reducing Antioxidant Power (FRAP) and Oxygen Radical Absorbance Capacity (ORAC) antioxidant activities values. NA = not active at higher concentration tested.

Species	Family	Part	3D7, IC_{50} (µg/mL)	CHKV, IC_{50} (µg/mL)	CHKV, IC_{90} (µg/mL)	CHKV, CC_{50} (µg/mL)	CHKV, SI	ABTS (µM/mg/mL)	FRAP (µM/mg/mL)	ORAC (µM/mg/mL)
***Aphloia theiformis* (Vahl) Benn.**[1, 2]	Aphloiaceae	L.	>50	**9.7 ± 1.18**	**18.4 ± 1.88**	**53.5 ± 0.18**	**5.5**	NA	NA	NA
		B.		NA	NA	NA	-			
***Bertiera borbonica* A. Rich. ex DC**	Rubiaceae	L.	>50	NA	NA	NA	-	**161.2 ± 4.4**	**5.2 ± 0.6**	**159.5 ± 33.9**
		B.						**3612.0 ± 42.1**	**625.4 ± 25.0**	**6510.8 ± 617.6**
***Callophylum tacamahaca* Willd.**[2]	Clusiaceae	L.	**30.78 ± 8.8**	NA	NA	NA	-	NA	NA	NA
		B.	>50							
***Casearia coriacea* Vent.**	Flacourtiaceae	L.	**4.24 ± 2.2**	NA	NA	NA	-	NA	NA	NA
		B.	**3.62 ± 1.02**							
***Coffea mauritiana* Lam.**	Rubiaceae	L.	>50	NA	NA	NA	-	NA	NA	NA
		B.	**37,63 ± 0.7**							
***Croton mauritianus* Lam.**[1]	Euphorbiaceae	L.	**16.4 ± 5.58**	**18.41 ± 0.3**	**> 33.33**	**>100**	**>5.4**	NA	NA	NA
***Doratoxylon apetalum* (Poir.) Radlk. *var. diphyllum* (Cordem.) F. Friedmann**	Sapindaceae	L.	>50	**28.4 ± 3.92**	**44.7 ± 11.8**	**>100**	**> 3.5**	NA	NA	NA
		B.		NA	NA	NA				
***Erica arborescens* (Willd.) E.G.H. Oliv.**	Ericaceae	L.	>50	NA	NA	NA	-	NA	NA	NA
		B.	**37.15 ± 1.33**							
***Erica reunionensis* E.G.H. Oliv.**	Ericaceae	L.	>50	NA	NA	NA	-	NA	NA	NA

		B.	**32.77 ± 0.59**							
***Erythroxylum laurifolium* Lam.**[1, 2]	Erythroxylaceae	L.	>50	NA	NA	NA	-	**134.5 ± 2.5**	**18.1 ± 0.8**	**72.2 ± 7.1**
		B.						**1927.0 ± 217.9**	**391.9 ± 38.1**	**6310.3 ± 1404.2**
***Erythroxylum sideroxyloides* Lam.**	Erythroxylaceae	L.	>50	NA	NA	NA	-	**157.4 ± 3.0**	**63.9 ± 4.7**	**229.1 ± 22.2**
		B.		**22 ± 6.37**	**32.9 ± 2.96**	**>100**	**> 5.4**	**1493.7 ± 2.9**	**88.8 ± 2.6**	**4441.1 ± 901.6**
***Eugenia uniflora* L.**[1, 2]	Myrtaceae	L.	**24.18 ± 11.13**	NA	NA	NA	-	NA	NA	NA
		B.	**21.03 ± 6.03**							
***Indigofera ammoxylum* (DC.) Polhill**	Fabaceae	L.	>50	NA	NA	NA	-	**150.9 ± 1.9**	**18.9 ± 5.2**	**252.9 ± 30.0**
		B.		**15.6 ± 2.88**	ND	**53.9 ± 2.47**	**3.5**	**1343.5 ± 23.2**	**953.1 ± 79.6**	**5882.7 ± 1295.9**
***Maillardia borbonica* Duch.**[2]	Moraceae	L.	**36.56 ± 17.96**	NA	NA	NA	-	NA	NA	NA
		B.	>50							
***Monimia rotundifolia* Thouars**	Monimiaceae	L.	**11.26 ± 2.88**	NA	NA	NA	-	NA	NA	NA
		B.	>50							
***Nuxia verticillata* Lam.**	Stilbaceae	L.	**18.33 ± 5.61**	NA	NA	NA	-	NA	NA	NA
		B.	>50							
***Phyllanthus phillyreifolius* Poir.**	Phyllanthaceae	L.	>50	**24.5 ± 4.12**	**47.7 ± 10**	**47.4 ± 22.5**	**1.9**	NA	NA	NA
		B.	**44.81 ± 7.32**	NA	NA	NA	-			
***Poupartia borbonica* J. F. Gmel.**	Anacardiaceae	L.	**4.92 ± 2.35**	NA	NA	NA	-	**244.0 ± 9.6**	**55.8 ± 1.3**	**121.8 ± 17.6**
		B.	>50					**2086.8 ± 30.6**	**543.5 ± 26.5**	**1823.7 ± 166.6**
***Psiadia amygdalina* (Lam.) Cordem.**	Asteraceae	L.	>50	NA	NA	NA	-	NA	NA	NA
		B.	**16.61 ± 0.57**							
Psiadia boivini	Asteraceae	L.	**23.69 ± 4.91**	NA	NA	NA	-	NA	NA	NA

(Klatt) Rob.										
		B.	>50							
***Psiadia dentata* (Cass.) DC.**	Asteraceae	L.	**22.99 ± 2.96**	NA	NA	NA	-	NA	NA	NA
		B.	>50							
***Psiadia retusa* (Lam.) DC.**	Asteraceae	L.	**12.09 ± 0.8**	NA	NA	NA	-	NA	NA	NA
		B.	>50							
***Scolopia heterophylla* (Lam.) Sleumer**[1]	Salicaceae	L.	>50	NA	NA	NA	-	**441.0 ± 33.2**	**732.0 ± 34.1**	**2195.5 ± 592.4**
		B.						**613.6 ± 6.7**	**409.0 ± 15.3**	**1959.6 ± 122.1**
***Securinega durissima* J.F. Gmel.**	Phyllanthacaceae	L.	>50	NA	NA	NA	-	NA	NA	NA
		B.		**15.9 ± 3.88**	**30 ± 7.62**	**24.9 ± 1.57**	**1.57**			
***Sophora denudata* Bory**	Fabaceae	L.	>50	NA	NA	NA	-	**357.9 ± 4.7**	**160.0 ± 8.2**	**940.0 ± 92.4**
		B.	**17.88 ± 3.39**					**1361.1 ± 1.9**	**130.0 ± 4.2**	**3621.1 ± 509.7**
***Stillingia lineata* (Lam.) Müll. Arg.**	Euphorbiaceae	L.	>50	**1.94 ± 0.36**	**3.71 ± 0.88**	**21.1 ± 3.51**	**10.9**	NA	NA	NA
		B.		NA	NA	NA	-			
Terminalia bentzoë* (L.) L. f. subsp. *Bentzoë[1, 2]	Combretaceae	L.	**18.65 ± 10.6**	NA	NA	NA	-	**2758.4 ± 80.7**	**332.6 ± 38.1**	**1005.7 ± 258.1**
		B.	>50					**3063.3 ± 302.9**	**1954.5 ± 81.6**	**2122.3 ± 126.0**
***Toddalia asiatica* (L.) Lam.**[1, 2]	Rutaceae	L.	**43.12 ± 12.4**	NA	NA	NA	-	NA	NA	NA
		B.	>50							
***Turraea thouarsiana* (Baill.) Cavaco et Keraudren**[2]	Meliaceae	L.	>50	NA	NA	NA	-	NA	NA	NA
		B.	**33.43 ± 6.49**							
***Vernonia fimbrillifera* (Cass.) Less.**	Asteraceae	L.	**5.2 ± 1.3**	NA	NA	NA	-	NA	NA	NA
		B.	>50							
Zanthoxylum heterophyllum	Rutaceae	L.	**12.46 ± 4.14**	NA	NA	NA	-	NA	NA	NA

(Lam.) Sm.[1]		B.	>50							
Artemisinine	-	-	0.006 ± 0.002	-	-	-	-	-	-	-

[1]Species that are used to treat fever or malaria. [2]Species that are used as anti-inflammatory, rheumatisms, arthrosis, or against CHKV during the epidemic of 2006