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Antiplasmodial, anti-inflammatory and cytotoxic activities of various plant extracts from the Mascarene Archipelago

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ABSTRACT

Aim of the study: Antiplasmodial activity, inhibition of nitric oxide (NO) overproduction, and anti-proliferative activity were investigated *in vitro* to evaluate the bioactive potential of the traditional pharmacopoeia of the Mascarene Archipelago, which is known for its biodiversity and for the richness of its endemic flora.

Materials and methods: A total of 45 methanol (MeOH) and dichloromethane (DCM) extracts were prepared from 19 plant species collected on Réunion and Mauritius Islands. Ninety-six-well microplate assays were performed on chloroquine sensitive *Plasmodium falciparum* 3D7 strain, on LPS-stimulated Raw 264.7 murine macrophages and on A-549, DLD-1 and WS1 human cells. Activity was evaluated through spectrophotometric methods.

Results: Activity was attributed to plant extracts expressing $IC_{50} < 50$ μg/ml for antiplasmodial response, $IC_{50} < 100$ μg/ml for cytotoxicity, and $IC_{50} < 130$ μg/ml for anti-inflammatory reaction. The majority of the extracts tested (69%) exhibited potency in at least one of these three types of activity. This is the first report describing promising antiplasmodial activity ($IC_{50} < 15$ μg/ml) for *Psiadia dentata* DCM extract and *Terminalia bentzoe* MeOH bark extract. NO inhibition assay revealed seven interesting plants, described for the first time as anti-inflammatory: *Aphloia theiformis*, *Buddleja salviifolia*, *Eupatorium riparium*, *Hiptage benghalensis*, *Psiadia arguta*, *Psiadia dentata*, and *Scutia commersonii*. Finally, anti-proliferative activity was observed for two endemic species, *Geniostoma borbonicum* and *Nuxia verticillata*.

Conclusion: Using the criterion of endemism as part of the criteria for traditional medicinal use raises the chances of finding original active principles. In our case, 86% of the endemic plants tested displayed pharmacological interest.

1. Introduction

The Mascarene Archipelago, consisting of the Réunion, Mauritius and Rodrigues islands, is located in the Indian Ocean, East of Madagascar. The Archipelago is described as a biodiversity hotspot, mainly due to the remarkable flora and particularly to its high rate of endemic species. As the islands are located far from a continent, the flora has evolved separately, creating endemic plants. This evolution is an adaptation to the special geomorphology and microclimate that is particularly present in Réunion Island, where most of the plants were collected for this study. Thus, it is well worth conserving this biodiversity and promoting the vegetation there for healthcare, especially when we know that 80% of the world's population still uses medicinal plants, and that about 30% of drugs on the pharmaceutical market come from nature (Newman and Cragg, 2007).

To our knowledge, few investigations have previously been carried out using pharmacological assays to endorse the flora of these islands. Therefore, the screening of these plants is urgently needed. Indeed, it is important to act before some endemic or endangered species disappear. Various ethnobotanical surveys have already been conducted in Réunion Island; the most comprehensive reference is Roger Lavergne's study (Lavergne, 2001).

In our continuous search for new antiplasmodial agents, further plants were selected on the Mascarene Archipelago based on the ethnopharmacological data compiled in Réunion Island, particularly according to their medicinal use in treating fever or malaria. Two points should be kept in mind when analysing an ethnopharmacological survey. Firstly, a plant could have various traditional medicinal applications, and a disease usually causes a range of

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symptoms. Secondly, symptoms/diseases described by traditional medicine might not always be transposable to modern medicine. For instance, the symptom of fever could be attributed to various diseases including parasitic or microbiologic infections, but it could also be caused by an inflammatory reaction. Therefore numerous biologic assays would need to be performed to confirm these indications.

In this study, a total of nineteen plants were collected and screened for antiplasmodial, anti-inflammatory and cytotoxic activities. Using *in vitro* assays, we report preliminary results regarding inhibition of *Plasmodium falciparum* growth, regarding inhibition of nitric oxide (NO) overproduction on LPS-stimulated Raw 264.7 murine macrophages and regarding cytotoxicity against human lung cancer cell lines (A-549), colon cancer cell lines (DLD-1) and the normal cell line, WS1.

2. Materials and methods

2.1. Selection and collection of plant material

Nineteen plant species were selected in the Mascarene Archipelago (Table 1). Six plants were collected on Mauritius Island and were identified by Claudia Flores (Mauritius Sugar Industry and Research Institute). Fourteen plants were collected on Réunion Island and were identified by Dr. D. Strasberg (Herbier Universitaire de la Réunion). Voucher specimens were deposited in the Herbarium of Réunion University. Some specimens were quick-frozen, pounded with liquid nitrogen and freeze-dried (*cryo* in Table 1). Others were air-dried at room temperature, with no direct sunlight, and then pulverized using an electrical grinder.

2.2. Preparation of extracts

For each plant part, 5 g of powdered dried material was macerated three times in 50 ml solvent (MeOH or DCM), for 30 min with constant shaking, at room temperature. The filtrates were pooled and evaporated to dryness under reduced pressure at 40 °C.

2.3. Cell culture

The murine macrophage Raw 264.7 (ATCC #TIB-71), human lung carcinoma A-549 (ATCC #CCL-185), human colorectal adenocarcinoma DLD-1 (ATCC #CCL-221), and human skin fibroblast WS1 (ATCC #CRL-1502) cell lines obtained from the American Type Culture Collection (ATCC, Manassas, USA) were grown in Dubelco's Minimum Essential Medium supplemented with 10% foetal calf serum (Hyclone, Logan, USA), and a solution of vitamins (1X), sodium pyruvate (1X), non-essential amino acids (1X), penicillin (100 IU) and streptomycin (100 μg/ml) (Mediatech Cellgro®). Cells were cultured in a humidified atmosphere at 37 °C in 5% CO_2.

2.4. Cytotoxic assay

Exponentially growing cells were plated at a density of 5×10^3 carcinoma cells and 7.5×10^3 WS1 cells per well in 96-well microplates (Costar, Corning Inc.) in 100 μl of culture medium and were allowed to adhere for 24 h at 37 °C and 5% CO_2 before treatment. Each extract sample was prepared in a series of eight twofold dilutions (final concentrations ranging from 0 to 200 μg/ml) in DMSO and medium culture. Following this, 100 μl of increasing concentrations of extracts were added to three rows. The final concentration of DMSO in the culture medium was maintained at 0.5% (v/v) to avoid solvent toxicity. The cells were incubated for 48 h in the presence or absence of the extracts. Etoposide (≥98%, Sigma–Aldrich) was used as a positive control. Cell viability was measured using the resazurin reduction test, as described by O'Brien et al. (2000). Fluorescence was read on an automated 96-well Fluoroskan Ascent FL Thermo plate reader (Labsystem) using an excitation wavelength of 530 nm and an emission wavelength of 590 nm. Cytotoxic activity was expressed as the concentration of extract inhibiting cell growth by 50% (IC_{50}).

2.5. Measurement of anti-inflammatory activity by nitrite quantification

Exponentially growing cells were plated in 96-well microplates (Costar, Corning Inc.) at a density of 7.5×10^4 cells per well in 100 μl of culture medium and were allowed to adhere overnight. Each extract sample was prepared in a series of eight twofold dilutions (final concentrations ranging from 0 to 160 μg/ml) in DMSO and medium culture. Cells were treated or not with positive control N(ω)-nitro-L-arginine methyl ester hydrochloride (L-NAME, ≥98%, Sigma–Aldrich), or increasing concentrations of plant extracts on three rows. Cells were then stimulated with 100 μg/ml of lipopolysaccharide (LPS) and incubated at 37 °C, 5% CO_2 for 24 h. After 24 h, cell-free supernatants were collected and NO concentration was immediately determined using the Griess reaction with minor modifications (Green et al., 1990). Briefly, 100 μl aliquots of cell supernatants were incubated with 100 μl of a mix of 1% sulfanilamide in 2.5% H_3PO_4 and of 0.1% N-1-naphtylethylenediamine dihydrochloride in water at room temperature for 20 min. Absorbance at 550 nm was then measured using an automated 96-well Varioskan Ascent plate reader (Thermo Electron) and the presence of nitrite was quantified by comparison with an $NaNO_2$ standard curve. Macrophage survival was assessed using the resazurin reduction test.

2.6. Antiplasmodial assay

Continuous cultures of *Plasmodium falciparum*, chloroquine sensitive (3D7) strain, were assessed following the procedure already described in Frederich et al. (2002). The strain was obtained from Prof. Grellier (Museum d'Histoire Naturelle in Paris, France). Each extract sample was applied in a series of eight threefold dilutions (final concentrations ranging from 0.09 to 200 μg/ml) on two rows of a 96-well microplate and was tested in triplicate. Parasite growth was estimated by determination of lactate dehydrogenase activity as described previously in Kenmogne et al. (2006). Artemisinin (98%, Sigma–Aldrich) was used as a positive control.

3. Results

In the present study, 19 species were selected based on the work of Lavergne (Lavergne, 2001) and on chemotaxonomy. Antiplasmodial, anti-inflammatory and cytotoxic activities occurred *in vitro* on methanolic (MeOH) and dichloromethane (DCM) extracts of each species. Some species had various parts collected, as described in Table 1.

Following the results obtained, the 45 plant extracts tested were split into three groups: the first group consists of the extracts that did not provide any significant effect among those tested. This includes *Ageratum conyzoïdes* MeOH extract, *Buddleja salvifolia* MeOH bark extract, *Cassia fistula* MeOH extract, *Erythoxylon laurifolium* DCM and MeOH extracts, *Eupatorium triplinervis* DCM extract, *Geniostoma borbonicum* MeOH bark and leaf extracts, *Hiptage benghalensis* MeOH extract, *Justicia gendarusa* MeOH extract, *Morinda citrifolia* MeOH fruit and leaf extracts, *Nuxia verticillata* MeOH extract, *Rubus roseafolius* MeOH extract, *Scutia commersonii*

Table 1
Plants collected from Mauritius or Réunion Islands.

Species (family)	Local name	Locality	Date	Plant part used	Traditional use
Ageratum conyzoides L. (Asteraceae)	Herbe à bouc	Mauritius: Gaulettes serrées	February 2008	AP	Fever, anti-inflammatory (Lavergne, 2001), anti-diarrhoeal (Adjanohoun et al., 1983)
Aphloia theiformis (Vahl) Benn. (Aphloiaceae)	Change écorce, Goyave marron	Réunion: Plaines des Fougères	September 2006	B	Paludism (Lavergne, 2001), fever, rheumatism, pain (Adjanohoun et al., 1983; Lavergne and Véra, 1989)
Buddleja salvii folia (L.) Lam (Loganiaceae)	n.d.	Réunion: Plaine des Fougères	September 2006	B L (cryo)	n.d.
Cassia fistula L. (Fabaceae)	Cytise indien	Mauritius: Terre rouge	February 2008	L (cryo)	Wormer, laxative (Adjanohoun et al., 1983)
***Erythoxylum laurifolium* Lam. (Erythroxylaceae)**	Bois de rongue	Réunion: La montagne	February 2008	L (cryo)	Paludism, fever (Lavergne, 2001), high blood pressure, nephritic colic (Adjanohoun et al., 1983)
Eupatorium riparium Regel (Asteraceae)	Ortichifon, herbe la tension	Réunion: La montagne Réunion: La montagne	March 2009 February 2008	L (cryo) AP	High blood pressure (Adjanohoun et al., 1983; Lavergne and Véra, 1989; Lavergne, 2001)
Eupatorium triplinerve Vahl (Asteraceae)	Ayapana	Réunion: Saint André	January 2007	AP	Fever, high blood pressure, flu, vomiting, nausea (Adjanohoun et al., 1983; Lavergne and Véra, 1989; Lavergne, 2001)
***Geniostoma borbonicum* Spreng. (Loganiaceae)**	Bois de piment	Réunion: Plaine des Palmistes	June 2006	B L	n.d.
Hiptage benghalensis Kurz (Malpighiaceae)	Liane papillon	Réunion: La montagne	February 2008	L (cryo)	Epilepsy (Lavergne, 2001)
Justicia gendarussa Burm f. (Acanthaceae)	Ayapana marron	Réunion: Saint-André	January 2007	AP (cryo)	High blood pressure, pain, rheumatism, myorelaxant (Adjanohoun et al., 1983; Lavergne and Véra, 1989; Lavergne, 2001)
Morinda citrifolia L. (Rubiaceae)	Noni	Réunion: La Providence	February 2008	F L (cryo)	Sprains, rheumatism (Adjanohoun et al., 1983; Lavergne, 2001)
***Nuxia verticillata* Lam. (Loganiaceae)**	Bois maigre	Mauritius: Gaulettes serrées	February 2008	L	Albuminurie, venereal disease, purgative (Lavergne and Véra, 1989; Lavergne, 2001)
***Ochrosia borbonica* J.F.Gmel. (Apocynaceae)**	Bois jaune	Réunion: La montagne	February 2008	L	Paludism (Lavergne, 2001), fever (Lavergne and Véra, 1989)
***Psiadia arguta* Voigt (Asteraceae)**	n.d.	Réunion: Fac de Sciences	November 2006	L (cryo)	n.d.
***Psiadia dentata* DC. (Asteraceae)**	Bois collant, Ti-mangue	Réunion: La montagne	February 2008	AP (cryo)	n.d.
Rubus rosifolius Sm. (Rosaceae)	Framboisier	Mauritius: Gaulettes serrées	February 2008	AP (cryo)	Paludism, fever, anti-inflammatory, astringent, anti-diarrhoeal (Lavergne, 2001)
Scutia commersonii Brongn. (Rhamnaceae)	Bois de sinte	Mauritius: Gaulettes serrées	February 2008	L	Paludism, fever (Lavergne, 2001)
***Terminalia bentzoe* L. (Combretaceae)**	Benjoin	Réunion: Fac des Sciences	February 2008	B	Paludism, fever (Lavergne, 2001), flu, cough (Lavergne and Véra, 1989)
Toddalia asiatica Lam. (Rutaceae)	Liane patte poule	Mauritius: Gaulettes serrées	February 2008	L (cryo)	Paludism, fever (Lavergne, 2001), purgative, cramp, muscle tears (Lavergne and Véra, 1989)

n.d. = not determined; AP = aerial part; B = bark; F = fruit; L = leaf; cryo = freeze-dried.
Plant names in bold: endemic to Réunion and/or Mauritius Island.

MeOH extract. The second group, summarized in Table 2, includes extracts displaying more than one type of activity. The third group, described in Table 3, represents the extracts displaying a single activity.

3.1. Antiplasmodial activity

In vitro screening against the chloroquine sensitive *Plasmodium falciparum* strain, 3D7, supplied eight extracts showing moderate activity (IC_{50} = 15–50 μg/ml), and six displaying promising activity ($IC_{50} \leq 15$ μg/ml). The results are detailed in Tables 2 and 3. The most interesting of the extracts was the *Psidia dentata* DCM extract followed by the *Terminalia bentzoe* MeOH bark extract.

3.2. Anti-inflammatory activity

Inflammation is a complex mechanism developed as an immunological response to a viral, bacterial or even parasitic infection. During the ultimate phase of a series of signalling events, macrophages induce the expression of pro-inflammatory genes such as inducible nitric oxide synthase (iNOS). This enzyme is up-regulated by secretion of pro-inflammatory cytokines, and produces NO from L-arginine. Large amounts of such a biological

Table 2
In vitro antiplasmodial, anti-inflammatory and cytotoxic activities of the collected plant extracts (part 1).

Plant species	Extract	3D7, IC_{50} (μg/ml)	NO inhibition, IC_{50} (μg/ml)	WS1, IC_{50} (μg/ml)	DLD-1, IC_{50} (μg/ml)	A-549, IC_{50} (μg/ml)
Aphloia theiformis	MeOH	**13**	**19**	61	65	40
Buddleja salviifolia leaves	DCM	22	**42**	>200	>200	>200
Eupatorium riparium (cryo)	DCM	30	**43**	98	131	84
	MeOH f.p.	**5**	n.d.	n.d.	n.d.	n.d.
	MeOH	66	66	>200	>200	>200
Eupatorium riparium	DCM	26	**21**	50	68	64
Geniostoma borbonicum leaves	DCM	>50	85	**41**	**42**	57
Geniostoma borbonicum bark	DCM	>50	81	68	65	62
Nuxia verticillata	DCM	23	Toxic	125	**42**	**45**
Ochrosia borbonica	DCM	23	Toxic	149	95	>200
	MeOH	43	62	194	53	145
Psiadia arguta (2007)	DCM	**10**	**13**	**24**	**23**	**25**
	MeOH	22	**20**	**43**	**32**	**37**
Psiadia dentata	DCM	**7**	87	55	56	**35**
	MeOH	**15**	**21**	125	188	136
Artemisinin		0.006	n.d.	n.d.	n.d.	n.d.
L-NAME		n.d.	129	n.d.	n.d.	n.d.
Etoposide		n.d.	n.d.	18.5	2.5	0.7

f.p.: freshly prepared; n.d.: not determined.
Results in bold showed promising activity.

Table 3
In vitro antiplasmodial, anti-inflammatory and cytotoxic activity of the collected plant extracts (part 2).

Plant species	Extract	3D7, IC_{50} (μg/ml)	NO inhibition, IC_{50} (μg/ml)	WS1, IC_{50} (μg/ml)	DLD-1, IC_{50} (μg/ml)	A-549, IC_{50} (μg/ml)
Ageratum conyzoides	DCM	>50	**40**	99	155	102
	MeOH	>50	102	>200	>200	>200
Buddleja salviifolia leaves	MeOH	>50	81	>200	>200	>200
Buddleja salviifolia bark	DCM	>50	**24**	162	88	103
Cassia fistula	DCM	>50	83	>200	>200	>200
Eupatorium riparium	MeOH	>50	**42**	>200	>200	>200
Eupatorium triplinerve	MeOH	36	>130	>200	>200	>200
Hiptage benghalensis	DCM	>50	**38**	90	>200	102
Justicia gendarussa	DCM	>50	84	>200	>200	>200
Morinda citrifolia fruit	DCM	>50	67	>200	>200	>200
Morinda citrifolia leaves	DCM	>50	**13**	>200	>200	>200
Rubus rosifolius	DCM	>50	56	119	109	70
Scutia commersonii	DCM	>50	**50**	116	112	96
Terminalia bentzoe bark	DCM	>50	>130	102	134	80
	MeOH	**8**	>130	>200	>200	>200
Toddalia asiatica	DCM	>50	**15**	194	125	142
	MeOH	>50	78	>200	>200	>200

Results in bold showed promising activity.

mediator can cause severe or collateral tissue injury because NO can form peroxynitrite ($ONOO^-$), a cytotoxic molecule. The regulation of NO production is therefore an important target for inflammatory diseases (Guzik et al., 2003).

Anti-inflammatory activity was assessed using LPS-stimulated Raw 264.7 macrophages and NO production quantification using the Griess reaction as described in Section 2.5. The cytotoxic effect of the extract was evaluated on macrophages using resazurin to ensure that the anti-inflammatory activity was not due to cytotoxicity. Below 90% macrophage survival, the NO concentration was not considered to be significant. *Nuxia verticillata* DCM leaf extract and *Ochrosia borbonica* DCM extract were too toxic even at low concentration (5 μg/ml); thus an IC_{50} could not be estimated. In this study, extracts displaying $IC_{50} < 130$ μg/ml were regarded as interesting according to the reference used (L-NAME, $IC_{50} = 129$ μg/ml). Fourteen extracts displayed a promising effect ($IC_{50} < 50$ μg/ml, according to the WHO) and 12 extracts showed moderate activity ($50 < IC_{50} < 130$ μg/ml) (Tables 2 and 3).

3.3. Cytotoxic activity

Inhibition of cell proliferation was assessed using the resazurin test as the vital strain. A plant extract is usually regarded as interesting for *in vitro* cytotoxic activity when $IC_{50} < 100$ μg/ml (Boyd, 1997). In our study, extracts were considered to show moderate cytotoxicity when IC_{50} values were between 50 and 100 μg/ml. The definition of promising activity was reserved for extracts with IC_{50} values of below 50 μg/ml; this was the case for 6 extracts (Table 2). Our attention was focused on *Nuxia verticillata* DCM leaf extract for its selectivity toward cancer cell lines and on *Geniostoma borbonicum* DCM leaf extract for its single promising cytotoxic activity.

4. Discussion

The purposes of the study were to evaluate the *in vitro* efficacy of medicinal plants used by the local population and to select plant candidates that might provide new compounds against malaria.

Consequently, some plant extracts could be used *in totum* as a medicine, while other more toxic extracts would need to be fractionated in order to find the active principle responsible for the various activities. For this reason, two tables of results have been drawn up. Table 3 shows extracts demonstrating a specific activity among the three evaluated. Due to low or absent *in vitro* cytotoxicity, these extracts could be firstly tested *in vivo* on mice for their safety and efficacy, leading to the later development of improved traditional drugs (ITDs), which are more affordable to tropical population than pure compounds.

In practice, *Terminalia bentzoe* (*endemic*) MeOH *bark* extract (IC_{50} = 8 μg/ml) is a good candidate for use as an ITD for the treatment of malaria. Moreover, the result obtained here *in vitro* is a primary confirmation of the traditional effect reported. We found the bark to be more active and less toxic than the leaf extract. Indeed, as already noted, *Terminalia* sp. bark is often used to treat malaria in Africa (Jonville et al., 2008).

Several extracts were found to have a specific anti-inflammatory potency. The highest activity was provided by *Morinda citrifolia* DCM leaf extract (IC_{50} = 13 μg/ml, without any cytotoxicity). This result tallies with the plant's traditional use and with numerous scientific studies on *Morinda citrifolia* (Rasal et al., 2008; Basar et al., 2010). Safety tests have also been performed on polar leaf extract. The extract was found to lack oral toxicity effect and allergenicity (West et al., 2007). These auspicious results could be integrated into an ITD program for *Morinda citrifolia* (noni).

Toddalia asiatica DCM leaf extract was also found to be interesting in the present study, with an IC_{50} level of 15 μg/ml. A previous study reported that oil from the leaves exhibited *in vivo* anti-inflammatory properties (Kavimani et al., 1996). Several compounds found in the oil could also be present in the DCM extract and could in part be responsible for the activity found, although, according to Hao et al. (2004), alkaloids also display anti-inflammatory and analgesic effects. Benzo[c]phenanthridine alkaloids have been isolated from the *stem* of the plant: nitidine and dihydronitidine revealed specific cytotoxicity against A-549 cells *in vitro* (Iwasaki et al., 2006). Moreover, nitidine has also been shown to inhibit tumour growth in lung adenocarcinoma bearing mice (xenograft model) (Iwasaki et al., 2006, 2010). As the MeOH *leaf* extract did not show any cytotoxicity against either of the cancer cell lines tested here, this suggests that the extract does not contain these alkaloids. This hypothesis needs to be confirmed.

To our knowledge, little phytochemical or pharmacological research has been conducted on *Hiptage benghalensis*. Only a phosphodiesterase inhibiting activity has been described for the EtOH stem extract (Temkitthawon et al., 2008). In the present study, the DCM leaf extract showed promising NO inhibitory activity (IC_{50} = 38 μg/ml). This is the first report concerning the anti-inflammatory potency of this plant.

The DCM leaf extract of *Scutia commersonii*, traditionally used to treat fever and paludism, showed anti-inflammatory activity with IC_{50} of 50 μg/ml. *Scutia commersonii* has never been studied. However, non-polar fractions of stems and leaves of *Scutia buxifolia* have been shown to display a free radical-scavenging and antioxidant activity (Boligon et al., 2009).

Rubus rosifolius, a common red raspberry, has been studied several times. Bowen-Forbes et al. (2008) reported moderate inhibition of Cox 1 and 2 enzymes by the EtOAc fruit extract. Kanegusuku et al. (2007) isolated the main active compound (28-methoxytormentic acid), which exhibit *in vivo* analgesic activity from the extract of the polar aerial part. This analgesic activity and our result (DCM aerial part extract IC_{50} = 56 μg/ml) support the traditional anti-inflammatory use, which could in part explain the use of *Rubus rosifolius* against malaria (for its painkilling effect).

Some attention needs to be focused on *Justicia gendarussa*. Several studies have been carried out on the antioxidant activity of its polar leaf extract. Only one team (Wahi et al., 1974) has investigated the non-polar extract, which was found to contain alkaloids and β-sitosterol, also known to be anti-inflammatory and analgesic (Delporte et al., 2005). In the present study, the DCM aerial part extract tested showed moderate NO inhibition effect, indicating that it probably contains sterols and alkaloids. Taken together, these results encourage the traditional use of *Justicia gendarussa* (against high blood pressure, pain, rheumatism). Moreover, absence of *in vitro* cytotoxicity provides a reason for carrying out more assays, possibly leading to a later development of an ITD.

Various pharmacological properties of *Ageratum conyzoides* have been commonly tested. Polar (Moura et al., 2005) and non-polar leaf extracts of this plant have already been described as anti-inflammatory agents. In 1976, stigmasterol, and α-spinasterol were isolated from the plant (Horng et al., 1976). These phytosterols are known to have an anti-inflammatory effect (Garcia et al., 1999). Essential oil from the leaf possesses *in vivo* anti-inflammatory, analgesic and anti-pyretic dose-dependent activity without producing gastric lesions (Abena et al., 1996). These lipophilic compounds could explain anti-inflammatory activity regarding the DCM aerial part extract (IC_{50} = 40 μg/ml), and this supports the traditional use of the plant.

Plant extracts exhibiting two or three types of activity are presented in Table 2. Plants displaying more cytotoxicity than antiplasmodial or anti-inflammatory activity are potentially effective but are hazardous to use *in totum*. Thus an ITD could not be developed from those extracts without very great care. However, the extracts could be used to later isolate pure active compounds, as more than one compound could be responsible for the effects noticed. In contrast, a compound could act on various symptoms, as shown in the literature: ordinary natural anti-malarial drugs have been tested for effects other than antiplasmodial activity: quinine has been shown to display *in vivo* anti-inflammatory and anti-pyretic potential (Santos and Rao, 1998). Artemisinin, which acts using a different mode of action from quinine in the treatment against *Plasmodium falciparum* infection, has been shown to demonstrate selective immunosuppressive activity (Tawfik et al., 1990). This compound is now being evaluated in cancer therapy (Firestone and Sundar, 2009).

The antiplasmodial activity of *Aphloia theiformis* MeOH bark extract was described for the first time by Jonville et al. (2008). As previously shown against WI38 fibroblasts (IC_{50} = 58 μg/ml), cytotoxic activity was found here to be moderate toward WS1 fibroblasts. But the activity was promising against A-549 cells (IC_{50} = 40 μg/ml) and for its anti-inflammatory effect (IC50 = 19 μg/ml). This bark seems to have a lot of potential, and besides, no phytochemical studies have been conducted on it.

It seems that *Psiadia* is a genus with a high pharmacological potency; the non-polar and polar extracts of the two *endemic* species tested in the present study responded moderately or actively to the assays. This confirms our previous cytotoxic results on *Psiadia arguta*. The non-specificity of action toward plasmodium, macrophages, fibroblasts and carcinoma cells could uncover compounds that also have non-specificity of action, and thus they would not be able to be used for treatments. This plant will not have a high priority for isolation work. *Psiadia dentata* MeOH extract was found here to be less cytotoxic than DCM extract and to display a high level of activity toward the inhibition of NO release. Methoxyflavones and coumarins have been isolated from *Psiadia dentata* (Fortin et al., 2001; Jakobsen et al., 2001). Robin et al. (2001) revealed that 3-methylkaempferol inhibited the genomic RNA synthesis of poliovirus. Aside from this aspect, *Psiadia dentata* needs to be investigated further.

Concerning *Eupatorium riparium* (also named *Ageratina riparia* (Regel) R.M. King & H. Rob), DCM extracts from both batches (freeze-dried and air-dried) in the present study displayed an

interesting anti-inflammatory effect and moderate antiplasmodial activity. A difference was noticed between the two batches concerning the MeOH extract. The freeze-dried batch (*cryo*) showed a high level of activity toward *Plasmodium falciparum* only when the extract was freshly prepared. However, activity was lost when the extract was tested a few weeks later. On the other hand, the air-dried batch never exhibited significant antiplasmodial activity. The antiplasmodial effect is probably due to unstable compounds. Concerning anti-inflammatory activity, both batches were revealed to be promising. Therefore, the active compound is probably different from the one that is responsible for the antiplasmodial effect. Interestingly, the air-dried batch was more specific than the freeze-dried batch and no cytotoxic effect was found. Only a few phytochemical and pharmacological investigations have been conducted on this species. However, stigmasterol, epi-friedelinol, taraxasteryl acetate and palmitate have been isolated from the aerial parts (Patra et al., 1981). Some sterols, including stigmatsterol and taraxasteryl acetate, have been shown to display an anti-inflammatory effect (Perez-Garcia et al., 2005).

Buddleja salviifolia DCM bark extract was one of the most interesting extracts tested in the present study for NO inhibition. The DCM leaf extract also displayed a promising anti-inflammatory activity, but it also exhibited moderate antiplasmodial activity. Neither pharmacological nor phytochemical studies have been carried out on this species. Other *Buddleja* species contain triterpenoids, and flavonoids showing anti-inflammatory, analgesic, anti-pyretic and antioxidant properties (Liao et al., 1999; Houghton et al., 2003; Backhouse et al., 2008). These studies provide encouragement for continuing a careful exploration of *Buddleja salviifolia*.

Little research has been carried out regarding the endangered *endemic* species *Ochrosia borbonica*. Anti-cancer activity, due to a 9-methoxyellipticine and neurosedative effect induced by reserpine, has been described by Svoboda et al. (1968). Alkaloids are usually significantly active from a pharmacologic point of view. The toxicity of this plant toward macrophages (IC_{50} = 49 μg/ml) or the moderate antiplasmodial activity found in this study could therefore be due to alkaloids.

Another extract tested in the present study, which was found to be toxic toward macrophages was *Nuxia verticillata* DCM leaf extract (IC_{50} = 44 μg/ml). However, the antiplasmodial effect was moderate (because of the drying method used, as already discussed in Jonville et al., 2008). Interestingly, this extract inhibited selectively cancer cell growth in comparison with normal cells. This is the first report describing the potential anti-proliferative activity of an endemic plant from the Mascarene Archipelago.

Finally, in this study, we reported a moderate NO inhibition effect and an anti-proliferative activity of DCM bark and leaf extracts from *Geniostoma borbonicum*. To the best of our knowledge, this *endemic* shrub had never been studied before.

Lastly, we should keep in mind that *in vitro* assays are typically focused on one feature; they cannot express the connections between the different effects that could be perceived during *in vivo* experiments. Therefore, negative results that were registered here during *in vitro* tests cannot be construed as an indicator of inefficiency of the plant extract.

5. Conclusion

The high level of endemism in Mascarene flora provides a wealth of new healing compounds. In the present study, of the seven collected endemic plants, six displayed therapeutic interest. Regarding the traditional use of these plants, three of the seven plants employed to treat malaria inhibited the intraerythrocytic stage of *Plasmodium falciparum*. Concerning traditional use of the plants for their anti-inflammatory effects (including against fever, pain and rheumatism symptoms), eight out of a total of eleven plants showed a positive result in NO inhibition assays. This score is very high, since NO inhibition is one of the numerous *in vitro* targets that may be used to signal anti-inflammatory activity. These findings suggest that the traditional use of these plants is mostly justified.

The preliminary results presented in this paper have highlighted four plants that might be used in the development of an ITD: polar extract of *Terminalia bentzoe* bark for the treatment of malaria; lipophilic extract of *Scutia commersonii* leaf, *Morinda citrifolia* fruit or leaf, and the aerial part of *Justicia gendarussa* for the treatment of anti-inflammatory diseases. Furthermore, this study has highlighted plant extracts exhibiting various pharmacological applications. We focused on the active extract, for which no anti-inflammatory, antiplasmodial, or cytotoxic data has been described in the literature to date. The plants concerned were: *Terminalia bentzoe* bark hydrophilic extract (antiplasmodial), *Aphloia theiformis* bark polar extract (antiplasmodial, anti-inflammatory and cytotoxic), *Psiadia dentata* (antiplasmodial and anti-inflammatory) and *Psiadia arguta* (antiplasmodial, cytotoxic and anti-inflammatory) polar and non-polar extracts, *Nuxia verticillata* leaf lipophilic extract (cytotoxic), *Eupatorium riparium* polar and non-polar extracts (anti-inflammatory), *Geniostoma borbonicum* leaf lipophilic extract (cytotoxic), *Buddleja salviifolia* bark and leaf lipophilic extracts (anti-inflammatory), and *Hiptage benghalensis* lipophilic extract (anti-inflammatory). Further investigations, such as bioguided fractionation, need to be carried out firstly to isolate and then to elucidate the structure of the active compounds in these plants.

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