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Emmanuelle Dorla [a)], Isabelle Grondin [a)], Thomas Hue [b)], Patricia Clerc [a)], Séverine Dumas [a)], Anne Gauvin-Bialecki [a)], Philippe Laurent [a)*]

[a] Laboratoire de Chimie des Substances Naturelles et des Sciences des Aliments (LCSNSA), Université de La Réunion, avenue René Cassin–CS 92003–97744 Saint-Denis Cedex 9, France.

[b] Laboratoire de Parasitologie, Institut Agronomique néo-Calédonien, BP 73, 98890 Païta, New Caledonia

[*] Corresponding author: philippe.laurent@univ-reunion.fr
Tel: +262 96 28 50

Abstract:

The aim of this study was to screen the antimicrobial and acaricidal activity of 20 endemic or indigenous plants from Reunion Island (Indian Ocean). Plants were chosen on the basis of their traditional uses and their biocidal activities found in the literature. A survey was conducted in the local population to assess and supplement knowledge about the selected plants. The collected information confirmed and/or supplemented the data obtained for nine plants. Seven plants were described for the first time for their traditional uses in medicine and ethnoveterinary practices. To evaluate their biocidal activities, leaves or bark were treated with ethyl acetate using an accelerated solvent extraction method. Six bacteria and five fungi, frequently implicated in infectious diseases, were used to assess the antimicrobial activity of these extracts. A preliminary screening using the paper disk diffusion assay showed an effective antibacterial activity of 16 extracts. The minimum inhibitory concentration (MIC) of active plant extracts was then determined using a microdilution method. The leaf extract from *Peperomia borbonensis* displayed the widest spectrum of antibacterial activity and was the only one to act as a fungicide. In parallel, acaricidal bioassays were performed on the larvae of the tick *Rhipicephalus microplus* (Ixodidae), and plant extracts from *Peperomia borbonensis* and *Zanthoxylum heterophyllum* were the most effective. The preliminary studies of these plant extracts exhibited biocidal activities that were not described in the literature and that are congruent with traditional uses for some of them. Investigations are currently being conducted to isolate the active compound(s) and evaluate their potential for future developments and applications.

Keywords: acaricidal, antimicrobial, La Réunion, plant extracts, traditional use, survey

1. Introduction

The resistance of microorganisms and arthropods to chemicals is becoming a major concern for agriculture and public health issues. According to several reports on antimicrobial resistance, edited by the World Health Organization, new resistance mechanisms have emerged in the last few years and have spread globally, threatening our ability to treat common infectious diseases. The non-availability and the high costs of a new generation of antibiotics have led to an increase in mortality and morbidity. In the European Union, strains of resistant bacteria are responsible for 25,000 deaths and an extra health care cost of €1.5 billion each year (World Health Organization, 2015a, 2015b, 2014). Worldwide, drug-resistant infections cause 700,000 deaths every year, and a recent report has suggested that without policies to stop the spread of antimicrobial resistance, the number of deaths in 2050 would rise to 10 million every year (O'Neil, 2016). Hence, there is an urgent need to react, and one option is to find effective novel molecules to circumvent this resistance phenomenon. In this context, several studies have highlighted the antimicrobial activities of plants from different regions of the world (Agyare et al., 2016; Aumeeruddy-Elalfi et al., 2015; Mgbeahuruike et al., 2017; Mickymaray et al., 2016).

Likewise, for several years, resistance to synthetic acaricides has been frequently reported in the literature (Abbas et al., 2014; Jongejan and Uilenberg, 2004). Among Acaria, the cattle tick *Rhipicephalus (Boophilus) microplus* is considered as the most important parasite of livestock in the world (Estrada-Peña et al., 2006). It was introduced to Reunion Island (also called La Réunion) by the importation of "Moka cattle" from Madagascar (Barré and Uilenberg, 2010). This tick causes enormous losses in cattle production, due to blood loss, stress and irritation, which affect the milk and hide. Moreover, this arthropod can transmit diseases to cattle, such as babesiosis caused by *Babesia bovis* and *Babesia bigemina* and anaplasmosis caused by *Anaplasma marginale* (Estrada-Peña et al., 2006). The main treatment to control this arthropod is the use of chemical acaricides, such as organochlorines, carbamates and organophosphates, which have negative consequences in the environment and contribute to the development of resistant populations. There is therefore increasing interest by the scientific community to find new potential sources of compounds with biological activity against pests. Several recent studies have suggested the use of plants to fight cattle tick (Abbas et al., 2014; Adenubi et al., 2016; Borges et al., 2011; Hüe et al., 2015).

Reunion Island has a great number of assets to find new molecules in the abundant plant biodiversity. Emerging from the Indian Ocean about three million years ago, La Réunion (55°3' E and 21°5'S) is a French volcanic island located about 665 km east of Madagascar in the Mascarene archipelago. The island has a rugged topography and has the highest peak in the Indian Ocean (Piton des Neiges; 3070 m), deep valleys and one of the most active volcanoes in the world (Piton de La Fournaise; 2631 m). Its tropical climate is tempered by the prevailing southeast trade winds and is occasionally unsettled by cyclones. The tropical location and the dramatic landform of the island, combined with the moist trade winds, determine strong asymmetry between the eastern windward coast subjected to daily rainfall (up to 4 to 7 m/year) and the much drier western leeward coast (0.5 to 2.5 m/year). A large variety of microclimates is observed, with radical changes in sun light, precipitation and temperature, allowing a large number of vegetal species to develop and evolve. Consequently, Reunion Island is listed among the world's top biodiversity hotspots with an endemic rate approximately of 40%. Isolated from the mainland and other islands, the biodiversity of La Réunion results from the colonization of species from Madagascar, Africa, Asia, and Australia by marine currents and birds. The evolution of indigenous species, far from their region of origin, led to the emergence of several endemic species. Almost 840 indigenous species constituted the vascular flora of La Réunion, with 236 plants species (28%) being strictly endemic to La Réunion and 153 species endemic (18%) to the archipelago of

Mascarene. Since the arrival of the Europeans in the 16^{th} century, Reunion Island flora has experienced major perturbations, such as forest clearance and exotic plant introduction. Nevertheless, 25% of the original forest cover remains, and this allows unique plant communities to develop (Lavergne, 2001; Parc National de La Réunion, 2008; Strasberg et al., 2005; Thébaud et al., 2009). The human inhabitants are composed of people who originated from Europe (mainly France), Africa, Madagascar, India and China. The local traditional medicine was the result of the mixed knowledge of this cosmopolitan population (Pourchez, 2011). For instance *Ayapana triplinervis* (Vahl) R.M. King et H. Rob was introduced from India at the end of 18^{th} century and was used by the local population for digestive problems. Additionally, numerous endemic plants from La Réunion are used in traditional medicine; for instance, Ambaville (*Hubertia ambavilla* Bory) for dermic problems, blood circulation, gastric anti-ulcer and diabetes ("Aplamedom"). The exploration of this empirical knowledge could lead to the discovery of new active molecules.

The screening of 20 plants from La Réunion for biocidal activities constituted the core of this study. Plants were first chosen because of their endemic or indigenous status. Then, we considered their traditional uses in medicine and ethnoveterinary practices, and if no data were available, we selected the plants on the basis of existing biocidal activities in species of the same genus. To complement the fragmentary and limited knowledge of the selected plants, a small-scale survey of the population was conducted. Plant extracts were tested for antimicrobial and acaricidal activities. To our knowledge, this is the first study to report such properties of plants from La Réunion.

2. Material and methods

2.1. Plants and extracts

2.1.1. Selection of plant samples

Samples were chosen from our laboratory's plant collection formed during the extensive research program BIOMOL-TCN, which aimed to find new therapeutic, cosmetic and nutraceutical molecules in the marine, terrestrial and microbial biodiversity from La Réunion.

2.1.2. Preparation of crude extracts

The 20 plants chosen in this study were collected between 2009 and 2013 in different forests of Reunion Island. As described above, the selection criteria of the plants were (1) their biological status, (2) the availability of information concerning traditional practices and/or (3) data for biocidal activity of each species found in the literature or for the genus if no data were available concerning the species. This information is summarized in Table 1. The plant materials were identified by the botanists Jacques Fournel and Professor Dominique Strasberg (Faculty of Science and Technology, University of La Réunion). Voucher specimens are kept and were deposited in the Herbarium of the University.

2.2. Small scale survey

2.2.1. Study area

La Réunion (55°3' E and 21°5'S), with Mauritius and Rodrigues, is one of three islands that composes the Mascarene archipelago located in the Indian Ocean. Discovered in 1507–1512, Reunion Island has been a French overseas department since 1946, and is divided into 24 municipalities (average size: 100 km^2) (Strasberg et al., 2005). According to the census of 2012, the island's population was about 833,944 (403,907 males and 430,037 females). The population is mainly composed of people who originated from France, Africa, Madagascar India (Tamil Nadu and Gujarat) and China ("Insee – Département de La Réunion (974)" 2012). Economic activities and around 80% of the population are located in the coastal lowlands due to the rugged topography of the island. Almost 40% of the territory belongs to the National Park, which was established to preserve and conserve the terrestrial biodiversity

(Figure 1). (Strasberg et al., 2005). The core zone of La Réunion National Park coincides with the area of "Pitons, cirques and remparts" in the World Heritage List of the UNESCO.

2.2.2. Questionnaire design and data collection

A small-scale survey of the general population was carried out in Reunion Island from October 2015 to February 2016. The questionnaire was adapted from previous studies (Grønhaug et al., 2008; Samoisy and Mahomoodally, 2015). Data were recorded via face-to-face interviews in the local language (Créole) or French, depending on the interviewee. The first section concerned general information about gender, age, and place of residence. The second section concerned the knowledge of the selected plants and their traditional uses. Non-specialist people (n = 55) were randomly chosen and interviewed in the street, at their home or in their garden. Nineteen municipalities out of 24 were covered in this study. In addition, four traditional healers, one pharmacist, two ethnobotanists and one farmer were interviewed about the medicinal usages of the 20 studied plants.

2.3. Biological assays

2.3.1. Preparation of crude extracts

Collected plant materials (leaves and/or bark) were air-flow dried (40°C), crushed into fine powder and kept dry at room temperature until use. Crude extracts were obtained with an accelerated solvent extractor (ASE® 300, Accelerated Solvent Extractor, France) using ethyl acetate as solvent (≥ 99.5 %, Carlo-Erba, France). Conditions were as follows: temperature, 40°C; pressure, 100 bars; five cycles with static extraction time of 6 min; and flush volume, 100%. The crude extracts were concentrated in a rotary vacuum and were kept at 4°C until further use.

2.3.2. Evaluation of antimicrobial activity

- **Microorganisms**

Antimicrobial activity was evaluated on bacteria and yeasts with sanitary relevance. Three Gram-positive bacteria, *Listeria monocytogenes* (ATCC 1914), methicillin-resistant *Staphylococcus aureus* (NCTC 12493) and *Streptococcus pyogenes* (ATCC 19615), three Gram-negative bacteria, *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 10145) and *Salmonella enterica* (ATCC 13076), two filamentous fungi, *Aspergillus fumigatus* (ATCC 204305) and *Aspergillus niger* (ATCC 1688), and three yeasts, *Candida albicans* (ATCC 10231), *Candida tropicalis* (ATCC 1369) and *Cryptococcus neoformans* (ATCC 76484) (Humeau Laboratory, France) were used. *E. coli, S. aureus, P. aeruginosa and S. enterica* were grown on Mueller-Hinton medium (Sigma Aldrich, France), whereas *L. monocytogenes and S. pyogenes* were grown on Brain Heart medium (bioMérieux, France). Fungi were grown on Sabouraud medium (Sigma Aldrich, France). The media were prepared according to the manufacturer's instructions.

- **Disc diffusion test**

The assay was conducted by a modified disk diffusion method of the Clinical and Laboratory Standard Institute (Cavalieri et al., 2009). Suspensions of microorganisms were made in sterile medium and adjusted spectrophotometrically between 1×10^5 and 1×10^6 CFU/mL for bacteria and between 1×10^3 and 1×10^4 spores/mL for fungi. A 150 mL volume of sterile medium was added to Petri dishes (245 × 245 × 25 mm) to a thickness of 4 mm. Once the surface of media was inoculated with 1 mL of the microbial suspension, the disks (6 mm diameter) were placed on the surface of the medium. Each extract was dissolved in dimethylsulphoxide (DMSO ≥ 99.5 %, Sigma-Aldrich, France) to have a final concentration of 10 mg/mL and was tested at 20 µL/disk. Positive controls were chloramphenicol (BDH

Chemicals, England) for bacteria and amphotericine B (Sigma-Aldrich, France) for fungi. The plates were left for 30 min at room temperature to allow diffusion of the extract into the agar. They were then incubated at 37°C for 24 h and 48 h for bacteria and fungi, respectively. Antimicrobial activity was determined by measuring the inhibition zone with a caliper in millimeters. The experiment was repeated three times.

- **Determination of minimum inhibitory concentration by the microdilution method**

The minimal inhibition concentration (MIC) determination method was applied to extracts that had demonstrated their efficiency against microorganisms by the disk diffusion method (Kuete et al., 2009). The extracts and antibiotics were dissolved in DMSO (max 6.2% of DMSO per well). The initial concentration of the extracts was 1000.00 µg/mL and they were serially diluted two-fold in order to obtain a concentration range from 1000.00 to 0.98 µg/mL in sterile nutrient broth. A negative control was run in parallel to study the impact of the solvent on the microorganism growth. Each well was inoculated with 50 µL of suspension containing between 1×10^5 and 1×10^6 CFU/mL for the bacteria and between 1×10^3 and 1×10^4 spores/mL for the fungi. After incubation (37°C, 24 h and 48 h for bacteria and fungi, respectively), 20 µL (0.2 mg/mL) of *p*-iodonitrotetrazolium violet (INT, Sigma-Aldrich, France) was added to each well and the plates were incubated for an additional hour. MIC was determined as the lowest concentration of plant extract inhibiting microbial growth, indicated by a decrease in the intensity of the red colour of the formazan product. Positive controls were chloramphenicol (BDH Chemicals, England) for bacteria and amphotericine B (Sigma-Aldrich, France) for fungi. Three replicates were made for each extract.

2.3.3. Evaluation of acaricidal activity

- **Preparation of ticks**

R. microplus engorged females were collected after detachment from cattle. The females were incubated in the laboratory of the Agronomic Institute of New Caledonia (IAC) at a temperature of 27°C and a relative humidity (RH) of 85% for one week. Eggs were then collected and placed in the same conditions until larvae were 2–3 weeks old.

- **Larval packet test**

The acaricidal activity of plant extracts was evaluated using the modified larval packet test (LPT) (Stone and Haydock, 1962) on 14–21-day-old larvae. Extracts were diluted in ethanol to a 5% solution. A 7.5 × 8.5-cm nylon paper (Anowo Ltd, Switzerland) was impregnated with the different extracts and placed in a fume hood for 1 h to allow ethanol evaporation, before being folded into packets using bulldog clips. Approximately 100 *R. microplus* larvae were placed into each treated nylon paper packet, which were then sealed with additional bulldog clips and placed in an incubator (27°C, 85% Relative Humidity) for 24 h. Two replicates for each plant extracts, a negative control (nylon paper with ethanol) and a positive control (amitraz 1 g/L) were used. After 24 h, the numbers of live and dead larvae were counted to calculate the percentage of larval mortality.

- **Statistical analysis**

Mean values of mortality and standard deviation of the mean were calculated for each plant extracts. The LC_{50} (50% lethal concentration) was calculated using the Probit method (Finney, 1971), generated by the Probit POLOPC program (LeOra Software, 1987, Berkeley, CA, USA).

3. Results and Discussion

3.1. Traditional use of the selected plants

Despite traditional plants uses being an important cultural component of Reunion Island, surprisingly, no survey had been made in the population to collect information about these plants and their uses until our work.

Of the people interviewed, 58.2% were male and 41.8% were female; 32.7% were aged 18–35 years old, 49.1% were 36–60 years old and 18.2% were >60 years old. Finally, 65.5% of them lived in a rural area. A substantial proportion of the interviewed non-specialists (78.2%) cultivated and used plants mainly from their gardens as medicinal remedies. Among them, the proportion of women (81.8%) who used traditional remedies was slightly higher than the proportion of men (75.7%). This result is aligned with other studies around the world (Samoisy and Mahomoodally, 2015). In La Réunion, as in Mauritius and Rodrigues, people are particularly attached to traditional practices, and women are often the principal source of knowledge transmission (Pourchez, 2011). More than 85% of herbal medicine traditional uses were indeed transmitted through generations within the families. The knowledge of traditional uses of plants is indeed held by older people, and it is likely that the transfer of this information is now greatly affected by lifestyle modernization, as in many regions of the world. The preliminary literature analysis showed that only a few traditional uses were reported concerning the 20 selected plants (Table 1). During the survey, no traditional use in relation to antimicrobial or acaricidal properties was cited by the non-specialist population even though one or more of the 20 studied plants were recognized by the interviewees. For example, *Terminalia bentzoe* (L.) Pers. (benjoin) was recognized by 89% of interviewed persons, *Zanthoxylum heterophyllum* (Lam.) Sm. (bois de poivre) 50% and *Calophyllum tacamahaca* Willd. (takamaka) was recognized by 40%. This knowledge was observed only for some representative plants from La Réunion because the general population did not know the selected plants of this study well. For instance, the *Psiadia* species were the least known probably because of their low distribution and scarcity in the island. *Psiadia amygdalina*

Cordem. and *Psiadia boivinii* B.L.Rob. were recognized by 6 and 4% of interviewed people, respectively.

We also questioned eight specialized people (four traditional healers, one pharmacist, two ethnobotanists and one farmer) about the traditional uses of the 20 selected plants. These people acted daily for the valorization, conservation and preservation of the biodiversity of Reunion Island and the associated traditional knowledge, which is endangered by the lack of verbal transfer to new generations. As shown in Table 1, this small scale-survey allowed the collection of helpful information about the traditional use of little-known plants. This is the first time that seven plants were described for their traditional uses in medicine and ethnoveterinary practices: *Psiadia dentata* DC., *Psiadia retusa* DC., *Vernonia fimbrillifera* Less., *Antidesma madagascariense* Lam., *Stillingia lineata* (Lam.) Müll.Arg., *Indigofera ammoxylum* (DC.) Polhill and *Peperomia borbonensis* Miq. Furthermore, the collected information confirmed and/or supplemented the data obtained from the literature for nine plants (*Poupartia borbonica* J.F.Gmel, *Carissa spinarum* L., *Secamone volubilis* (Lam.) Marais., *Calophyllum tacamahaca* Willd, *Terminalia bentzoe* (L.) Pers., *Croton mauritianus* Lam., *Sophora denudata* Bory, *Zanthoxylum heterophyllum* (Lam.) Sm., *Nuxia verticillata* Lam.). Finally, no traditional use was identified for *Psiadia amygdalina* Cordem, *Psiadia boivinii* B.L.Rob., *Psiadia laurifolia* Cordem and *Monimia rotundifolia* Thouars. These results reinforce bioprospecting of plants and herbal traditional uses to discover new substances. They should help us to direct our bioactivity tests and phytochemical studies.

3.2. Antimicrobial activities of the selected plants

Crude extracts were prepared using ethyl acetate as solvent, and were tested against the different microorganisms. Table 2 shows the results obtained for plants displaying antibacterial activity for at least one bacteria/fungi. The antimicrobial activity varied greatly according to the tested species. The leaves of *Carissa spinarum*, *Indigofera ammoxylum*, *Psiadia amygdalina*, *Sophora denudata*, *Vernonia fimbrillifera*, *Zanthoxylum heterophyllum* displayed no antimicrobial activity. The same result was found for the bark of *Psiadia dentata* and *P. retusa* and for the leaves and bark of *P. laurifolia*. Sixteen extracts inhibited at least one microbial species. Among these positive extracts, 12 had a zone of growth inhibition greater than 10 mm: *Antidesma madagascariense* (bark), *Callophylum tacamahaca* (leaves), *Croton mauritianus* (leaves), *Monimia rotundifolia* (leaves), *Psiadia amygdalina* (bark), *Poupartia borbonica* (leaves), *Psiadia boivinii* (leaves), *Psiadia retusa* (leaves*), Secamone volubilis* (leaves), *Sophora denudata* (bark), *Stillingia lineata* (leaves) and *Terminalia bentzoe* (leaves). The minimal inhibition concentration (MIC) was determined for the positive extracts following the disk diffusion test (Table 3). It is generally considered that antimicrobial activity is good for extracts with a MIC less than 100 µg/mL; from 100 to 500 µg/mL, the antimicrobial activity is moderate; from 500 to 1000 µg/mL, the antimicrobial activity is weak; and over 1000 µg/mL, the extract is considered inactive (Holetz et al., 2002). Only seven MIC values were >1000 µg/mL. The active extracts displayed a MIC ranged between 1000 µg/mL and 15.62 µg/mL. Gram-positive bacteria were more sensitive than Gram-negative bacteria, as was frequently found in the literature (Ríos and Recio, 2005). This is probably due to cell-wall differences, as Gram-negative bacteria have an outer membrane known to act as a barrier to many molecules. The most susceptible bacterium was the methicillin-resistant *Staphylococcus aureus* strain, since 12 extracts were active against it. The best activities against this bacterium were observed with *Callophylum tacamahaca* (leaves) and *Psiadia dentata* (leaves) with a MIC of 62.50 µg/mL. The activity of chloramphenicol was 37.50 µg/mL on this strain. No traditional uses as an antimicrobial were reported for *Callophylum tacamahaca*. According to our survey's results, *Psiadia dentata* is traditionally used to treat dermic problems, such as mycoses in La Réunion, but the ethyl acetate extract of this plant was not active against the tested fungi. Other extraction methods and a wider range of fungi should be used to evaluate the fungicide activity of this plant in the future. In Mauritius, five endemic *Psiadia* species used traditionally to treat pulmonary infections, wounds and burns were evaluated for their antimicrobial activity. They did not show any or showed moderate activity against the studied strains (Govinden-Soulange et al., 2004). The *Terminalia bentzoe* leaf extract showed a weak activity against *Staphylococcus aureus* and *Streptococcus pyogenes* with a MIC of 1000.00 µg/mL. Additionally, the current findings showed good antimicrobial activity of the extract of *Sophora denudata* bark against *Listeria monocytogenes* and *Streptococcus pyogenes* with a MIC value of 125.00 µg/mL and 15.62 µg/mL respectively. Indeed, the antimicrobial activity of *Sophora* species has often been reported being due to flavonoids (Sohn et al., 2004; Tsuchiya et al., 1996). *Psiadia dentata* (leaves) and *Peperomia borbonensis* (leaves) extracts both displayed the broadest spectrum of antibacterial activities. Although MICs were not very low, *P. borbonensis* was active against four bacteria among the six tested, and was especially effective against all the Gram-negative species. Moreover, *P. borbonensis* was the only species active against fungi (one out of five, *Aspergillus fumigatus*). The traditional use of *P. borbonensis* in La Réunion as an antimicrobial is not listed. Likewise, the *Peperomia* species around the world have not been known for this traditional use, except *P. tetraphylla* (G.Forst.) Hook. &Arn., which was used to fight microbial infections in India (Nishanthi et al., 2012). Nevertheless, several studies on the antimicrobial activity of *Peperomia* species were reported in the literature (Ferreira et al., 2014; Langfield et al., 2004; Mbah et al., 2002; Saga Kitamura et al., 2006). The butanolic fraction of *Peperomia pellucida* (L.) Kunth, composed of tannins, flavonoids and saponins, showed good inhibition diameters against *E. coli* and *P. aeruginosa* (Khan and Omoloso, 2002). Patuloside A isolated from *P. pellucida* was tested on four Gram-positive bacteria and six Gram-negative bacteria and a low minimal inhibition concentration (MIC = 8 µg/mL) was obtained against *Staphylococcus aureus* and *Streptococcus β-haemolyticus* (Khan et al., 2010). Finally, Malquichagua Salazar et al. (2005) showed the activity of two compounds from *Peperomia villipetiola* C.D.C. against the fungus *Cladosporium sphaerospermum*.

Concerning the three indigenous species of the study, only *Antidesma madagascariense* and *Stillingia lineata* extracts showed antibacterial activity with the disk diffusion method and only against *S. aureus.* The MIC of these extracts were 125.00 µg/mL and >1000.00 µg/mL, respectively. Unlike *S. lineata*, the antimicrobial activity of *A. madagascariense* was already evaluated and the methanol leaf extracts showed activity against *Enterococcus faecalis* (MIC = 60.00 µg/mL), *S. aureus (*MIC = 500.00 µg/mL), methicillin resistant *S. aureus* (MIC = 250.00 µg/mL) and *Candida albicans* (MIC = 500.00 µg/mL) (Mahomoodally et al., 2015). In a study conducted by Rangasamy et al. (2007), the *A. madagascariense* crude methanol extract was active against some of the tested microorganisms of our study. Among them, *S. aureus* (MIC = 500.00 µg/mL) and *S. enteridis* (MIC = 125.00 µg/mL) were the most susceptible strains (Rangasamy et al., 2007). Finally, the species *Carissa spinarum* (leaves) was not active against the tested microorganisms; however, the methanolic extract of the roots of *C. spinarum* from India was active against *E. coli* (MIC = 125 ±10 µg/mL), *S. aureus* (MIC = 110 ± 28 µg/mL) and *A. niger* (MIC = 256 ±30 µg/mL) in a study by Sanwal and Chaudhary (2011).

That being said, plant extracts with a MIC of 1000 µg/mL should not be neglected, as they could contain interesting antimicrobial molecules. From this point of view, the leaf extracts from *Peperomia borbonensis* should be further explored, since it displayed the widest spectrum of antibacterial activity and also acted as a fungicide. Indeed, bioactive compound concentrations in plant extracts vary depending on the polarity of solvents. Ethyl acetate was used in our work to maximize the collection of a wide range of molecules in order to select interesting plants for further research. Other solvents should now be gradually used to optimize active compound extraction and their isolation by bioguided-fractionation.

3.3. Acaricidal activities of the selected plants

Five extracts showed acaricidal activity on the tick *Rhipicephalus microplus* larvae (Figure 2). At a concentration of 5 %, the most active samples, with a mortality rate of 100%, were extracted from the leaves of *Peperomia borbonensis* (Piperaceae) and the bark of *Zanthoxylum heterophyllum* (Rutaceae). The extracts obtained from *Zanthoxylum heterophyllum* leaves showed weaker activity (63.8%) than those obtained from the bark (100%). Medium activity was also observed for *Monimia rotundifolia* (65.7% of mortality). Finally, *Psiadia amygdalina* leaf extract had weak acaricidal activity with 31.8% of mortality.

No previous study has reported acaricidal activity of these four plants. However, several studies have reported this property in plants belonging to the Piperaceae and Rutaceae families. Solvent extracts and essential oils from *Piper* (Piperaceae) species have been widely studied for their acaricidal properties (de Souza Chagas et al., 2012; Ferraz et al., 2010; Lima et al., 2014; Silva et al., 2009). Likewise, several species of the genus *Zanthoxylum* were studied for their biocidal activity against arthropods (Moussavi et al., 2015; Prieto et al., 2011). The essential oil of *Zanthoxylum caribaeum* Lam. was assessed for its acaricidal activity against cattle tick. This volatile extract acted on engorged females and inhibited oviposition and egg eclosion (Nogueira et al., 2014). Before this work, no reports about biocidal activity (*in vitro* or traditional) against arthropods was recovered for *Peperomia* spp., *Psiadia* spp. and *Monimia* spp. As previously said, other solvents should now be gradually used to optimize active compound extraction and their isolation by bioguided-fractionation. Furthermore, these efforts should be complemented by the use of other extraction methods and the implementation of tests on a wide spectrum of biological targets. This is best illustrated by another work conducted in parallel on *Peperomia borbonensis* in our laboratory, which led to demonstrating insecticidal activity of the leaf essential oil of *Peperomia borbonensis* and of its isolated major components against the melon fly *Bactrocera cucurbitae* (Dorla et al., 2017).

4. Conclusion

To conclude, this *in vitro* study corroborated the acaricidal activity of *Peperomia borbonensis* traditionally used by a few farmers on the island to protect their cattle from ticks. The crude extract could possess one or several bioactive molecules acting in combination. Further research is currently being conducted to isolate the active component(s) by bioguided-fractionation. Furthermore, this study also demonstrated, for the first time, the acaricidal activity of *Zanthoxylum heteropyllum* and the antimicrobial activity of *Psiadia dentata* (MIC of 62.50 µg/mL against *S. aureus*) and *Sophora denudata* (MIC of 15.62 µg/mL against *S. pyogenes*). These results will further be used in bioguided phytochemical studies.

Lastly, a large-scale survey should be carried out to collect more information throughout the territory from older people. It could be especially interesting to dedicate a part of this future survey to the three mountain cirques Mafate, Cilaos and Salazie (Figure 1), and more particularly Mafate, which is accessible only by a pedestrian path network. In this landlocked area, the traditional lifestyle is more preserved than in coastal areas.

Many potential bioactive plants remain unexplored among the Reunion Island biodiversity. Our multi-criteria approach would also allow the discovery of many other plants with interesting properties. Moreover, several plants selected in this study are endangered and protected in La Réunion. Raising the awareness and knowledge about such plants and improving their valorization by researching biological properties could allow their preservation.

Conflict of interest

The authors declare no conflict of interest.

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Table 1. The studied plants: type, habitat, traditional uses and biocidal activity for the genus.

Family *Botanical name* Vernacular Créole name Plant type/habitat	Status	**Traditional uses in medicine and ethnoveterinary practices** Found in literature	Collected in this study	Biocidal activities known in the genus Against arthropods	Against microbes
Anacardiaceae					
Poupartia borbonica J.F.Gmel. Bois de poupart, Zévi marron Shrub/semi-dry forests	End. R. M. P.	contraceptive, furuncle, nephritis (Lavergne, 2001; Smadja and Vera, 1991)	menopause, blood circulation disorders, fungi, insects	nd	nd
Apocynaceae					
Carissa spinarum L. Bois amer Shrub/semi-dry forests	Ind. P.	skin disorders, wounds, gonorrhoea, stomach disorders, tonic, nephritis. (Poullain et al., 2004; Vera et al., 1990)	typhoid fever, fever	*C. edulis* (Nyahangare et al., 2015)	*C. lanceolata* (Lindsay et al., 2000) *C. spinarum* (Sanwal and Chaudhary, 2011).
Secamone volubilis (Lam.) Marais Liane bois d'olive Liana/semi-dry forests	End. R. M.	hernia, diarrhoea, fever, diabetes, hypertension. (Poullain et al., 2004; Smadja and Vera, 1991)	cancer	*S. afzelli* (Adesina et al., 2012)	nd
Asteraceae					
Psiadia amygdalina Cordem. Bois collant, Ti mangue Shrub/cloud forests	End. R	nd	nd	nd	
Psiadia boivinii B.L.Rob. Bouillon blanc Shrub/dense cloud forests	End. R	nd	nd	nd	
Psiadia dentata DC. Bois collant, Ti mangue Shrub/dense cloud forests	End. R	nd	skin disorders (mycoses), insects	nd	*P. trinervia* (Wang et al., 1989) *P. arguta, P. lithospermifolia, P. penninervia, P. terebinthina, P. viscosa* (Govinden-Soulange et al., 2004)
Psiadia laurifolia Cordem. Bois de tabac, Bois de chenille Tree/dense cloud forests	End. R.	nd	nd	nd	nd
Psiadia retusa DC. Saliette Shrub/rocky coastal areas	End. R. P.	nd	source of mineral salts	nd	

End: endemic, Ind.: Indigenous, R: Reunion Island, M: Mauritius Island, Mas: Mascarenes, P: Protected species, nd: no data

Vernonia fimbrillifera Less. Bois de source Shrub/rainforests	End. R.	nd	blood circulation, cancer, wound healing	*V. phosphorea* (Valente et al., 2013) *V. amygdalyna* (Mwanauta et al., 2014) *V. auriculifera* (Gemeda et al., 2014)	*V. colorata* (Rabe et al., 2002) *V. amygdalina* (Erasto et al., 2006) *V. glabra* (Kitonde et al., 2012) *V. guineesis* (Toyang et al., 2012)
Clusiaceae					
Calophyllum tacamahaca Willd. Takamaka Tree/lowland rainforests	End. R. M.	eye diseases, rheumatism, headache, gout, arthritis, dermic problems (Lavergne, 2001)	skin disorders, memory troubles, rheumatism, blood circulation	*C. inophyllum* (Ademola et al., 2014; Agrawal and Mall, 1988; Kadir et al., 2015)	*C. moonii, C. thwaitesii* (Dharmaratne et al., 1999) *C. inophyllum* (Yimdjo et al., 2004) *C. canum* (Alkhamaiseh et al., 2012) *C. antillanum* (Cuesta-Rubio et al., 2015)
Combretaceae					
Terminalia bentzoe (L.) Pers. Faux benjoin Tree/dry lowland forests	End. Mas.	fever, cold, cough, influenza, asthma, dysmenorrhoea, pleuritis paludism. (Lavergne, 2001; Poullain et al., 2004; Smadja and Vera, 1991)	reproductive disorders (spermatozoids), flu, bronchitis, cold	*T. catappa* (Rani et al., 2011)	*T. brachystemma, T. gazensis, T. mollis, T. prunioides*, *T. sambesiaca, T. sericea*, (Masoko et al., 2005)
Euphorbiaceae					
Antidesma madagascariense Lam. Bois de cabri Shrub/medium altitude forests	Ind.	nd	skin disorders, urine secretion	*A. bunius* (Belmi et al., 2014)	*A. thwaitesianum* (Dechayont et al., 2012) *A. venosum* (Mwangomo et al., 2012)
Croton mauritianus Lam. Ti bois de senteur Shrub/coastal areas	End. R. P.	fever (Poullain et al., 2004; Vera et al., 1990)	fever, cold, muscle pains	*C. linearis* (Alexander et al., 1991) *C argyrophylloides C. nepetaefolius, C. sonderianus zehntneri* (Lima et al., 2013)	*C. urucurana* (Peres et al., 1997) *C. megalobotrys* (Selowa et al., 2010). *C. macrostachyus* (Obey et al., 2016)
Stillingia lineata (Lam.) Müll.Arg. Bois de lait Tanguin de pays Tree/lowland dry forests	Ind. P.	nd	chikungunya virus, furuncles	nd	nd
Fabaceae					
Indigofera ammoxylum (DC.) Polhill Bois de sable, Bois de rose Tree/steep gorges	End. R. P.	nd	hypercholesterolemia, diabetes	*I. tinctoria* (Kamal and Mangla, 1993)	*I. oblongifolia* (Dahot, 1999) *I. suffruticosa* (Leite et al., 2006)

End: endemic, Ind.: Indigenous, R: Reunion Island, M: Mauritius Island, Mas: Mascarenes, P: Protected species, nd: no data.

Sophora denudata Bory Petit tamarin des hauts Tree/high-altitude forests	End. R. M.	skin cancer (Poullain et al., 2004; Vera et al., 1990)	skin disorders (psoriasis, eczema)	*S. flavescens* (Mao and Henderson, 2007)	*S. alopecuroides* (Küçükboyaci et al., 2011) *S. oppositifolia* (Cota et al., 2011) *S. exigua, S. flavescens* (Krishna et al., 2012)
Monimiaceae					
Monimia rotundifolia Thouars Mapou Tree/lowland rainforests	End. R.	nd	nd	nd	nd
Piperaceae					
Peperomia borbonensis Miq. Pourpier Epiphyte succulent/highland rainforests	End. R.	nd	ticks	nd	*P. villipetiola* (Malquichagua Salazar et al., 2005) *P. fernandopoina* (Mbah et al., 2012; Ngemenya et al., 2006) *P Pellucida* (Akinnibosun et al., 2008; Khan and Omoloso, 2002; Oloyede et al., 2011; Wei et al., 2011)
Rutaceae					
Zanthoxylum heterophyllum (Lam.) Sm. Poivrier des hauts Tree/rain/semi-dry forests	End. Mas. P.	back pain, toxins, fever (Lavergne, 2001; Poullain et al., 2004)	tooth lesion, local anaesthetic (mooth)	*Z. caribaeum* (Nogueira et al., 2014) Z. dissitum (Wang et al., 2015) *Z. heitzii* (Moussavi et al., 2015)	*Z. budrunga* (Islam et al., 2001) *Z. chalybeum* (Olila et al., 2001) *Z. zanthoxyloides, Z. leprieurii* (Misra et al., 2013) *Z. bungeanum* (Zhang et al., 2014)
Stillbaceae					
Nuxia verticillata Lam. Bois maigre Tree/medium altitude forests	End. R. M.	toxins, albuminuria, venereal diseases, intestinal transit disorders. (Jonville et al., 2011; Lavergne, 2001; Poullain et al., 2004; Smadja and Vera, 1991)	hypercholesterolemia, stomach problems, malaria, urine secretion, albuminuria	nd	nd

End: endemic, Ind.: Indigenous, R: Reunion Island, M: Mauritius Island, Mas: Mascarenes, P: Protected species, nd: no data.

Table 2. Antimicrobial activity of ethyl acetate extracts of 16 plants from Reunion Island.

Plants	Parts used[a]	**Gram-negative bacteria** *Salmonella enterica*	*Pseudomonas aeruginosa*	*Escherichia coli*	**Gram-positive bacteria** *Streptococcus pyogenes*	*Listeria monocytogenes*	*Staphylococcus aureus*	**Fungi** *Aspergillus fumigatus*
Antidesma madagascariense	**B**	-	-	-	-	-	13.15 ± 0.21	-
Callophylum tacamahaca	**L**	-	-	-	-	-	10.20 ± 0.28	-
Croton mauritianus	**L**	-	-	-	-	-	10.25 ± 1.18	-
Monimia rotundifolia	**L**	-	-	-	-	-	10.70 ± 0.23	-
Nuxia verticillata	**L**	-	-	-	-	9.67 ± 0.58	-	-
Peperomia borbonensis	**L**	8.77 ± 0.49	8.77 ± 0.40	9.87 ± 0.81	-	9.20 ± 1.11	-	7.97 ± 0.67
Poupartia borbonica	**L**	-	-	-	-	-	15.00 ± 0.60	-
Psiadia amygdalina	**B**	-	-	10.33 ± 0.58	-	-	-	-
Psiadia boivinii	**L**	-	-	-	-	-	10.67 ± 0.83	-
Psiadia dentata	**L**	-	9.87 ± 0.23	9.60 ± 0.40	-	9.70 ± 0.52	9.15 ± 1.20	-
Psiadia retusa	**L**	-	-	-	-	9.77 ± 0.38	10.50 ± 0.71	-
Secamone volubilis	**L**	-	-	-	-	-	11.70 ± 2.01	-
Sophora denudata	**B**	-	-	-	8.00 ± 0.00	10.27 ± 0.46	-	-
Stillingia lineata	**L**	-	-	-	-	-	10.15 ± 0.21	-
Terminalia bentzoe	**L**	-	-	-	11.70 ± 0.42	9.23 ± 0.06	8.80 ± 0.28	-
Zanthoxylum heterophyllum	**B**	-	-	-	-	-	9.70 ± 0.42	-
Chloramphenicol		31.30 ± 2.70	8.40 ± 0.53	21.60 ± 2.27	29.20 ± 0.81	29.57 ± 3.19	25.50 ± 0.81	-
Amphotericine B		-	-	-				10.67 ± 0.61

The growth inhibition was determined by paper disk diffusion. Inhibition diameters were given in mm ± standard deviation obtained in three replicates, '-' means not active. Chloramphenicol was used as standard for bacteria, amphotericine B was the standard used for fungi.

[a] L = leaves, B = bark.

Table 3. Minimal inhibitory concentration (MIC, µg/mL) of 16 plants extracts from Reunion Island.

Plants	Parts used[a]	**Gram-negative bacteria**			**Gram-positive bacteria**			**Fungi**
		Salmonella enterica	***Pseudomonas aeruginosa***	***Escherichia coli***	***Streptococcus pyogenes***	***Listeria monocytogenes***	***Staphylococcus aureus***	***Aspergillus fumigatus***
Antidesma madagascariense	**B**	-	-	-	-	-	125.00	-
Callophylum tacamahaca	**L**	-	-	-	-	-	62.50	-
Croton mauritianus	**L**	-	-	-	-	-	>1000.00	-
Monimia rotundifolia	**L**	-	-	-	-	-	1000.00	-
Nuxia verticillata	**L**	-	-	-	-	1000.00	-	-
Peperomia borbonensis	**L**	1000.00	1000.00	1000.00	-	1000.00	-	500.00
Poupartia borbonica	**L**	-	-	-	-	-	125	-
Psiadia amygdalina	**B**	-	-	>1000.00	-	-	-	-
Psiadia boivinii	**L**	-	-	-	-	-	>1000.00	-
Psiadia dentata	**L**	-	1000.00	1000.00	-	500.00	62.50	-
Psiadia retusa	**L**	-	-	-	-	500.00	125.00	-
Secamone volubilis	**L**	-	-	-	-	-	>1000.00	-
Sophora denudata	**B**	-	-	-	15.62	125.00	-	-
Stillingia lineata	**L**	-	-	-	-	-	>1000.00	-
Terminalia bentzoe	**L**	-	-	-	1000.00	>1000	1000.00	-
Zanthoxylum heterophyllum	**B**	-	-	-	-	-	>1000.00	-
Chloramphenicol		18.75	150.00	37.50	4.68	nt[b]	37.50	-
Amphotericine B		-	-	-	-	-	-	0.50

The minimum inhibitory concentration (MIC) expressed in µg/mL was determined by broth dilution. Chloramphenicol was used as standard for bacteria, amphotericine B was the standard used for fungi. Three replicates were made for all extracts tested. '-' means not active.

[a] L = leaves, B = bark.

[b] nt = not tested

Figure captions

Figure 1. a) Map of the south-west Indian Ocean. b) The National Park and the repartition of the Reunion Island population.

The 24 municipalities are represented as circles. The three mountains cirques appear in italic font.

Figure 2. Percentage mortality for *Rhipicephalus microplus* larvae exposed to plant extracts (dilution 5%). The Standard Deviation of the mean values are represented by error bars.

Table captions

Table 1. The studied plants: type, habitat, traditional uses and biocidal activity for the genus.

Table 2. Antimicrobial activity of ethyl acetate extracts of 16 plants from Reunion Island.

The growth inhibition was determined by paper disk diffusion. Inhibition diameters were given in mm ± standard deviation obtained in three replicates, '-' means not active. Chloramphenicol was used as standard for bacteria, amphotericine B was the standard used for fungi.

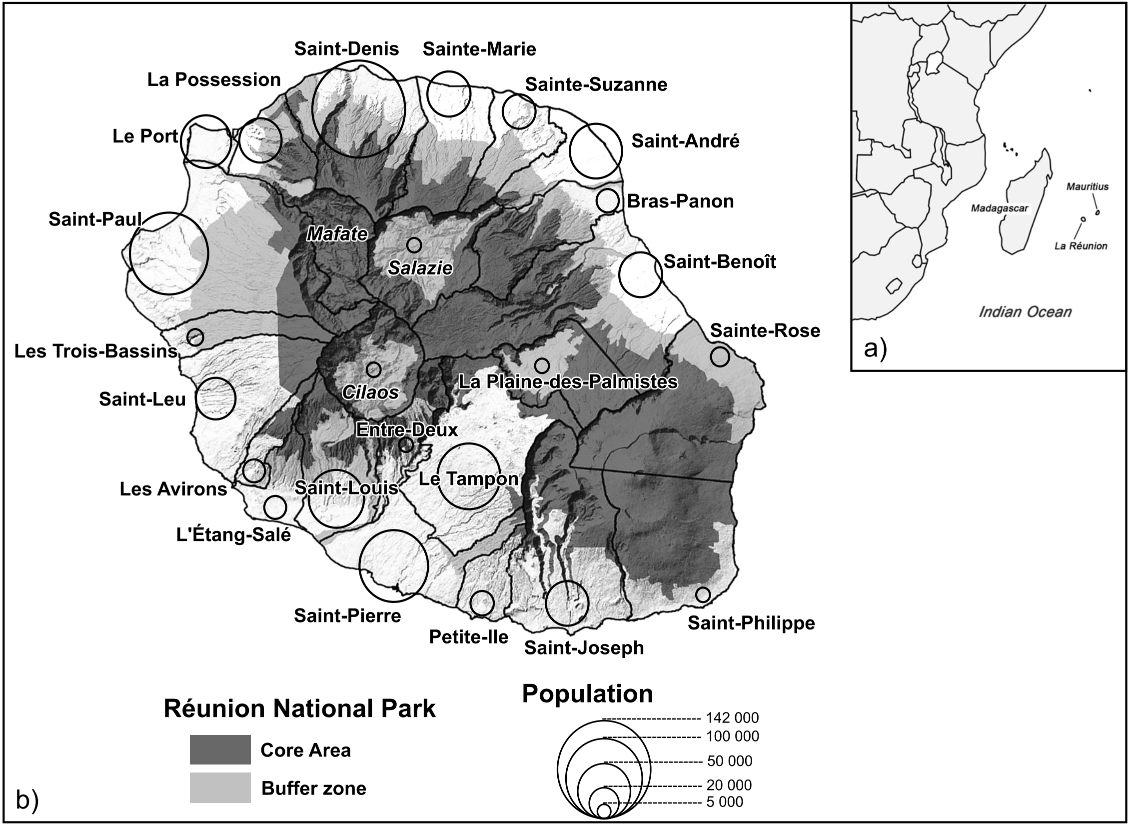
[a] L = leaves, B = bark.

Table 3. Minimal inhibitory concentration (MIC, µg/mL) of 16 plant extracts from Reunion Island.

The minimum inhibitory concentration (MIC) expressed in µg/mL was determined by broth dilution. Chloramphenicol was used as standard for bacteria, amphotericine B was the standard used for fungi. Three replicates were made for all extracts tested. '-' means not active.

[a] L = leaves, B = bark.

[b] nt = not tested


Saint-Denis
Sainte-Marie
La Possession
Sainte-Suzanne
Le Port
Saint-André
Bras-Panon
Saint-Paul
Mafate
Salazie
Saint-Benoît
Sainte-Rose
Les Trois-Bassins
Cilaos
La Plaine-des-Palmistes
Saint-Leu
Entre-Deux
Les Avirons
Saint-Louis
Le Tampon
L'Étang-Salé
Saint-Pierre
Petite-Ile
Saint-Joseph
Saint-Philippe
Réunion National Park
Core Area
Buffer zone
Population
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20 000
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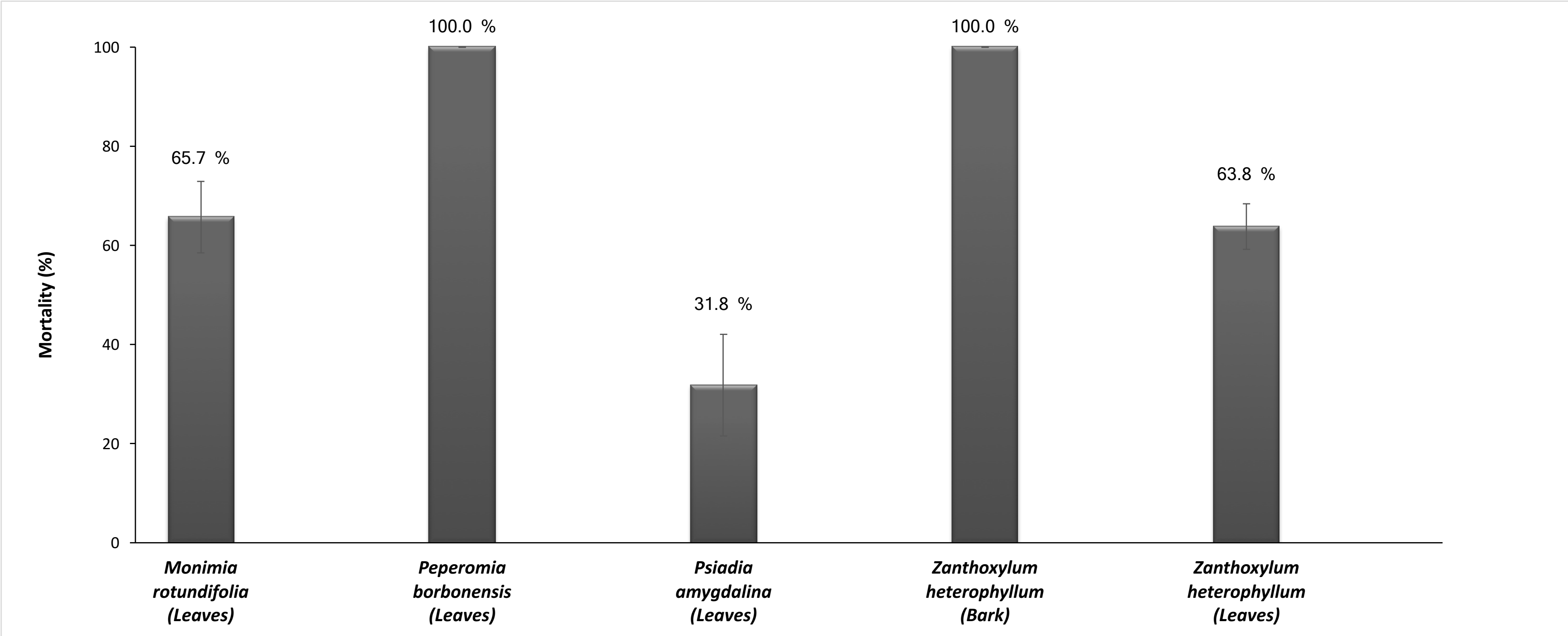
b)
Mauritius
Madagascar
La Réunion
Indian Ocean
a)



100.0 %
100.0 %
65.7 %
63.8 %
31.8 %
100
80
60
40
20
0
Mortality (%)
Monimia rotundifolia (Leaves)
Peperomia borbonensis (Leaves)
Psiadia amygdalina (Leaves)
Zanthoxylum heterophyllum (Bark)
Zanthoxylum heterophyllum (Leaves)