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Traditional uses, antimicrobial and acaricidal activities of 20 plants selected among Reunion Island's flora

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1 **Abstract:**

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

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

23

24

25 **1. Introduction**

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

41 Likewise, for several years, resistance to synthetic acaricides has been frequently reported in
42 the literature (Abbas et al., 2014; Jongejan and Uilenberg, 2004). Among Acaria, the cattle
43 tick *Rhipicephalus (Boophilus) microplus* is considered as the most important parasite of
44 livestock in the world (Estrada-Peña et al., 2006). It was introduced to Reunion Island (also
45 called La Réunion) by the importation of “Moka cattle” from Madagascar (Barré and
46 Uilenberg, 2010). This tick causes enormous losses in cattle production, due to blood loss,
47 stress and irritation, which affect the milk and hide. Moreover, this arthropod can transmit
48 diseases to cattle, such as babesiosis caused by *Babesia bovis* and *Babesia bigemina* and
49 anaplasmosis caused by *Anaplasma marginale* (Estrada-Peña et al., 2006). The main

50 treatment to control this arthropod is the use of chemical acaricides, such as organochlorines,
51 carbamates and organophosphates, which have negative consequences in the environment and
52 contribute to the development of resistant populations. There is therefore increasing interest
53 by the scientific community to find new potential sources of compounds with biological
54 activity against pests. Several recent studies have suggested the use of plants to fight cattle
55 tick (Abbas et al., 2014; Adenubi et al., 2016; Borges et al., 2011; Hue et al., 2015).

56 Reunion Island has a great number of assets to find new molecules in the abundant plant
57 biodiversity. Emerging from the Indian Ocean about three million years ago, La Reunion
58 (55°3' E and 21°5'S) is a French volcanic island located about 665 km east of Madagascar in
59 the Mascarene archipelago. The island has a rugged topography and has the highest peak in
60 the Indian Ocean (Piton des Neiges; 3070 m), deep valleys and one of the most active
61 volcanoes in the world (Piton de La Fournaise; 2631 m). Its tropical climate is tempered by
62 the prevailing southeast trade winds and is occasionally unsettled by cyclones. The tropical
63 location and the dramatic landform of the island, combined with the moist trade winds,
64 determine strong asymmetry between the eastern windward coast subjected to daily rainfall
65 (up to 4 to 7 m/year) and the much drier western leeward coast (0.5 to 2.5 m/year). A large
66 variety of microclimates is observed, with radical changes in sun light, precipitation and
67 temperature, allowing a large number of vegetal species to develop and evolve. Consequently,
68 Reunion Island is listed among the world's top biodiversity hotspots with an endemic rate
69 approximately of 40%. Isolated from the mainland and other islands, the biodiversity of La
70 Reunion results from the colonization of species from Madagascar, Africa, Asia, and
71 Australia by marine currents and birds. The evolution of indigenous species, far from their
72 region of origin, led to the emergence of several endemic species. Almost 840 indigenous
73 species constituted the vascular flora of La Reunion, with 236 plants species (28%) being
74 strictly endemic to La Reunion and 153 species endemic (18%) to the archipelago of

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

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

96 **2. Material and methods**

97 **2.1. Plants and extracts**

98 **2.1.1. Selection of plant samples**

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

102 **2.1.2. Preparation of crude extracts**

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

111 **2.2. Small scale survey**

112 **2.2.1. Study area**

113 La Réunion (55°3' E and 21°5'S), with Mauritius and Rodrigues, is one of three islands that
114 composes the Mascarene archipelago located in the Indian Ocean. Discovered in 1507–1512,
115 Reunion Island has been a French overseas department since 1946, and is divided into 24
116 municipalities (average size: 100 km²) (Strasberg et al., 2005). According to the census of
117 2012, the island's population was about 833,944 (403,907 males and 430,037 females). The
118 population is mainly composed of people who originated from France, Africa, Madagascar
119 India (Tamil Nadu and Gujarat) and China ("Insee – Département de La Réunion (974)"
120 2012). Economic activities and around 80% of the population are located in the coastal
121 lowlands due to the rugged topography of the island. Almost 40% of the territory belongs to
122 the National Park, which was established to preserve and conserve the terrestrial biodiversity

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

125

126 **2.2.2. Questionnaire design and data collection**

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

137 **2.3. Biological assays**

138 **2.3.1. Preparation of crude extracts**

139 Collected plant materials (leaves and/or bark) were air-flow dried (40°C), crushed into fine
140 powder and kept dry at room temperature until use. Crude extracts were obtained with an
141 accelerated solvent extractor (ASE® 300, Accelerated Solvent Extractor, France) using ethyl
142 acetate as solvent ($\geq 99.5\%$, Carlo-Erba, France). Conditions were as follows: temperature,
143 40°C; pressure, 100 bars; five cycles with static extraction time of 6 min; and flush volume,
144 100%. The crude extracts were concentrated in a rotary vacuum and were kept at 4°C until
145 further use.

146 **2.3.2. Evaluation of antimicrobial activity**

147 **➤ Microorganisms**

148 Antimicrobial activity was evaluated on bacteria and yeasts with sanitary relevance. Three
149 Gram-positive bacteria, *Listeria monocytogenes* (ATCC 1914), methicillin-resistant
150 *Staphylococcus aureus* (NCTC 12493) and *Streptococcus pyogenes* (ATCC 19615), three
151 Gram-negative bacteria, *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC
152 10145) and *Salmonella enterica* (ATCC 13076), two filamentous fungi, *Aspergillus fumigatus*
153 (ATCC 204305) and *Aspergillus niger* (ATCC 1688), and three yeasts, *Candida albicans*
154 (ATCC 10231), *Candida tropicalis* (ATCC 1369) and *Cryptococcus neoformans* (ATCC
155 76484) (Humeau Laboratory, France) were used. *E. coli*, *S. aureus*, *P. aeruginosa* and *S.*
156 *enterica* were grown on Mueller-Hinton medium (Sigma Aldrich, France), whereas *L.*
157 *monocytogenes* and *S. pyogenes* were grown on Brain Heart medium (bioMérieux, France).
158 Fungi were grown on Sabouraud medium (Sigma Aldrich, France). The media were prepared
159 according to the manufacturer's instructions.

160

161 **➤ Disc diffusion test**

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

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

176 ➤ **Determination of minimum inhibitory concentration by the**
177 **microdilution method**

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

193 **2.3.3. Evaluation of acaricidal activity**

194 ➤ **Preparation of ticks**

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

199 **➤ Larval packet test**

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

210 **➤ Statistical analysis**

211 Mean values of mortality and standard deviation of the mean were calculated for each plant
212 extracts. The LC₅₀ (50% lethal concentration) was calculated using the Probit method
213 (Finney, 1971), generated by the Probit POLOPC program (LeOra Software, 1987, Berkeley,
214 CA, USA).

215 **3. Results and Discussion**

216 **3.1. Traditional use of the selected plants**

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

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

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

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

262 **3.2. Antimicrobial activities of the selected plants**

263 Crude extracts were prepared using ethyl acetate as solvent, and were tested against the
264 different microorganisms. Table 2 shows the results obtained for plants displaying
265 antibacterial activity for at least one bacteria/fungi. The antimicrobial activity varied greatly

266 according to the tested species. The leaves of *Carissa spinarum*, *Indigofera amnoxylum*,
267 *Psiadia amygdalina*, *Sophora denudata*, *Vernonia fimbriifera*, *Zanthoxylum heterophyllum*
268 displayed no antimicrobial activity. The same result was found for the bark of *Psiadia dentata*
269 and *P. retusa* and for the leaves and bark of *P. laurifolia*. Sixteen extracts inhibited at least
270 one microbial species. Among these positive extracts, 12 had a zone of growth inhibition
271 greater than 10 mm: *Antidesma madagascariense* (bark), *Callophylum tacamahaca* (leaves),
272 *Croton mauritianus* (leaves), *Monimia rotundifolia* (leaves), *Psiadia amygdalina* (bark),
273 *Poupartia borbonica* (leaves), *Psiadia boivinii* (leaves), *Psiadia retusa* (leaves), *Secamone*
274 *volubilis* (leaves), *Sophora denudata* (bark), *Stillingia lineata* (leaves) and *Terminalia bentzoe*
275 (leaves). The minimal inhibition concentration (MIC) was determined for the positive extracts
276 following the disk diffusion test (Table 3). It is generally considered that antimicrobial
277 activity is good for extracts with a MIC less than 100 µg/mL; from 100 to 500 µg/mL, the
278 antimicrobial activity is moderate; from 500 to 1000 µg/mL, the antimicrobial activity is
279 weak; and over 1000 µg/mL, the extract is considered inactive (Holetz et al., 2002). Only
280 seven MIC values were >1000 µg/mL. The active extracts displayed a MIC ranged between
281 1000 µg/mL and 15.62 µg/mL. Gram-positive bacteria were more sensitive than Gram-
282 negative bacteria, as was frequently found in the literature (Ríos and Recio, 2005). This is
283 probably due to cell-wall differences, as Gram-negative bacteria have an outer membrane
284 known to act as a barrier to many molecules. The most susceptible bacterium was the
285 methicillin-resistant *Staphylococcus aureus* strain, since 12 extracts were active against it.
286 The best activities against this bacterium were observed with *Callophylum tacamahaca*
287 (leaves) and *Psiadia dentata* (leaves) with a MIC of 62.50 µg/mL. The activity of
288 chloramphenicol was 37.50 µg/mL on this strain. No traditional uses as an antimicrobial were
289 reported for *Callophylum tacamahaca*. According to our survey's results, *Psiadia dentata* is
290 traditionally used to treat dermic problems, such as mycoses in La Réunion, but the ethyl

291 acetate extract of this plant was not active against the tested fungi. Other extraction methods
292 and a wider range of fungi should be used to evaluate the fungicide activity of this plant in the
293 future. In Mauritius, five endemic *Psiadia* species used traditionally to treat pulmonary
294 infections, wounds and burns were evaluated for their antimicrobial activity. They did not
295 show any or showed moderate activity against the studied strains (Govinden-Soulange et al.,
296 2004). The *Terminalia bentzoe* leaf extract showed a weak activity against *Staphylococcus*
297 *aureus* and *Streptococcus pyogenes* with a MIC of 1000.00 µg/mL. Additionally, the current
298 findings showed good antimicrobial activity of the extract of *Sophora denudata* bark against
299 *Listeria monocytogenes* and *Streptococcus pyogenes* with a MIC value of 125.00 µg/mL and
300 15.62 µg/mL respectively. Indeed, the antimicrobial activity of *Sophora* species has often
301 been reported being due to flavonoids (Sohn et al., 2004; Tsuchiya et al., 1996). *Psiadia*
302 *dentata* (leaves) and *Peperomia borbonensis* (leaves) extracts both displayed the broadest
303 spectrum of antibacterial activities. Although MICs were not very low, *P. borbonensis* was
304 active against four bacteria among the six tested, and was especially effective against all the
305 Gram-negative species. Moreover, *P. borbonensis* was the only species active against fungi
306 (one out of five, *Aspergillus fumigatus*). The traditional use of *P. borbonensis* in La Réunion
307 as an antimicrobial is not listed. Likewise, the *Peperomia* species around the world have not
308 been known for this traditional use, except *P. tetraphylla* (G.Forst.) Hook. & Arn., which was
309 used to fight microbial infections in India (Nishanthi et al., 2012). Nevertheless, several
310 studies on the antimicrobial activity of *Peperomia* species were reported in the literature
311 (Ferreira et al., 2014; Langfield et al., 2004; Mbah et al., 2002; Saga Kitamura et al., 2006).
312 The butanolic fraction of *Peperomia pellucida* (L.) Kunth, composed of tannins, flavonoids
313 and saponins, showed good inhibition diameters against *E. coli* and *P. aeruginosa* (Khan and
314 Omoloso, 2002). Patuloside A isolated from *P. pellucida* was tested on four Gram-positive
315 bacteria and six Gram-negative bacteria and a low minimal inhibition concentration (MIC = 8

316 $\mu\text{g/mL}$) was obtained against *Staphylococcus aureus* and *Streptococcus β -haemolyticus* (Khan
317 et al., 2010). Finally, Malquichagua Salazar et al. (2005) showed the activity of two
318 compounds from *Peperomia villipetiola* C.D.C. against the fungus *Cladosporium*
319 *sphaerospermum*.

320 Concerning the three indigenous species of the study, only *Antidesma madagascariense* and
321 *Stillingia lineata* extracts showed antibacterial activity with the disk diffusion method and
322 only against *S. aureus*. The MIC of these extracts were 125.00 $\mu\text{g/mL}$ and >1000.00 $\mu\text{g/mL}$,
323 respectively. Unlike *S. lineata*, the antimicrobial activity of *A. madagascariense* was already
324 evaluated and the methanol leaf extracts showed activity against *Enterococcus faecalis* (MIC
325 = 60.00 $\mu\text{g/mL}$), *S. aureus* (MIC = 500.00 $\mu\text{g/mL}$), methicillin resistant *S. aureus* (MIC =
326 250.00 $\mu\text{g/mL}$) and *Candida albicans* (MIC = 500.00 $\mu\text{g/mL}$) (Mahomoodally et al., 2015). In
327 a study conducted by Rangasamy et al. (2007), the *A. madagascariense* crude methanol
328 extract was active against some of the tested microorganisms of our study. Among them, *S.*
329 *aureus* (MIC = 500.00 $\mu\text{g/mL}$) and *S. enteridis* (MIC = 125.00 $\mu\text{g/mL}$) were the most
330 susceptible strains (Rangasamy et al., 2007). Finally, the species *Carissa spinarum* (leaves)
331 was not active against the tested microorganisms; however, the methanolic extract of the roots
332 of *C. spinarum* from India was active against *E. coli* (MIC = 125 ± 10 $\mu\text{g/mL}$), *S. aureus* (MIC
333 = 110 ± 28 $\mu\text{g/mL}$) and *A. niger* (MIC = 256 ± 30 $\mu\text{g/mL}$) in a study by Sanwal and
334 Chaudhary (2011).

335 That being said, plant extracts with a MIC of 1000 $\mu\text{g/mL}$ should not be neglected, as they
336 could contain interesting antimicrobial molecules. From this point of view, the leaf extracts
337 from *Peperomia borbonensis* should be further explored, since it displayed the widest
338 spectrum of antibacterial activity and also acted as a fungicide. Indeed, bioactive compound
339 concentrations in plant extracts vary depending on the polarity of solvents. Ethyl acetate was
340 used in our work to maximize the collection of a wide range of molecules in order to select

341 interesting plants for further research. Other solvents should now be gradually used to
342 optimize active compound extraction and their isolation by bioguided-fractionation.

343 **3.3. Acaricidal activities of the selected plants**

344 Five extracts showed acaricidal activity on the tick *Rhipicephalus microplus* larvae (Figure 2).

345 At a concentration of 5 %, the most active samples, with a mortality rate of 100%, were

346 extracted from the leaves of *Peperomia borbonensis* (Piperaceae) and the bark of

347 *Zanthoxylum heterophyllum* (Rutaceae). The extracts obtained from *Zanthoxylum*

348 *heterophyllum* leaves showed weaker activity (63.8%) than those obtained from the bark

349 (100%). Medium activity was also observed for *Monimia rotundifolia* (65.7% of mortality).

350 Finally, *Psiadia amygdalina* leaf extract had weak acaricidal activity with 31.8% of mortality.

351 No previous study has reported acaricidal activity of these four plants. However, several

352 studies have reported this property in plants belonging to the Piperaceae and Rutaceae

353 families. Solvent extracts and essential oils from *Piper* (Piperaceae) species have been widely

354 studied for their acaricidal properties (de Souza Chagas et al., 2012; Ferraz et al., 2010; Lima

355 et al., 2014; Silva et al., 2009). Likewise, several species of the genus *Zanthoxylum* were

356 studied for their biocidal activity against arthropods (Moussavi et al., 2015; Prieto et al.,

357 2011). The essential oil of *Zanthoxylum caribaeum* Lam. was assessed for its acaricidal

358 activity against cattle tick. This volatile extract acted on engorged females and inhibited

359 oviposition and egg eclosion (Nogueira et al., 2014). Before this work, no reports about

360 biocidal activity (*in vitro* or traditional) against arthropods was recovered for *Peperomia* spp.,

361 *Psiadia* spp. and *Monimia* spp. As previously said, other solvents should now be gradually

362 used to optimize active compound extraction and their isolation by bioguided-fractionation.

363 Furthermore, these efforts should be complemented by the use of other extraction methods

364 and the implementation of tests on a wide spectrum of biological targets. This is best

365 illustrated by another work conducted in parallel on *Peperomia borbonensis* in our laboratory,
366 which led to demonstrating insecticidal activity of the leaf essential oil of *Peperomia*
367 *borbonensis* and of its isolated major components against the melon fly *Bactrocera*
368 *cucurbitae* (Dorla et al., 2017).

369 **4. Conclusion**

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

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

383 Many potential bioactive plants remain unexplored among the Reunion Island biodiversity.

384 Our multi-criteria approach would also allow the discovery of many other plants with
385 interesting properties. Moreover, several plants selected in this study are endangered and
386 protected in La Réunion. Raising the awareness and knowledge about such plants and
387 improving their valorization by researching biological properties could allow their
388 preservation.

389

390 **Conflict of interest**

391 The authors declare no conflict of interest.

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

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

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

<i>Vernonia fimbrillifera</i> Less. Bois de source Shrub/rainforests	End. R.	nd	blood circulation, cancer, wound healing	<i>V. phosphorea</i> (Valente et al., 2013) <i>V. amygdalina</i> (Mwanauta et al., 2014) <i>V. auriculifera</i> (Gemedo et al., 2014)	<i>V. colorata</i> (Rabe et al., 2002) <i>V. amygdalina</i> (Erasto et al., 2006) <i>V. glabra</i> (Kitonde et al., 2012) <i>V. guineensis</i> (Toyang et al., 2012)
Clusiaceae					
<i>Calophyllum tacamahaca</i> 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	<i>C. inophyllum</i> (Ademola et al., 2014; Agrawal and Mall, 1988; Kadir et al., 2015)	<i>C. moonii</i> , <i>C. thwaitesii</i> (Dharmaratne et al., 1999) <i>C. inophyllum</i> (Yimdjo et al., 2004) <i>C. canum</i> (Alkhamaiseh et al., 2012) <i>C. antillanum</i> (Cuesta-Rubio et al., 2015)
Combretaceae					
<i>Terminalia bentzoe</i> (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	<i>T. catappa</i> (Rani et al., 2011)	<i>T. brachystemma</i> , <i>T. gazensis</i> , <i>T.</i> <i>mollis</i> , <i>T. prunioides</i> , <i>T. sambesiaca</i> , <i>T. sericea</i> , (Masoko et al., 2005)
Euphorbiaceae					
<i>Antidesma madagascariense</i> Lam. Bois de cabri Shrub/medium altitude forests	Ind.	nd	skin disorders, urine secretion	<i>A. bunius</i> (Belmi et al., 2014)	<i>A. thwaitesianum</i> (Dechayont et al., 2012) <i>A. venosum</i> (Mwangomo et al., 2012)
<i>Croton mauritanus</i> Lam. Ti bois de senteur Shrub/coastal areas	End. R. P.	fever (Poullain et al., 2004; Vera et al., 1990)	fever, cold, muscle pains	<i>C. linearis</i> (Alexander et al., 1991) <i>C. argyrophyloides</i> <i>C.</i> <i>nepetaefolius</i> , <i>C. sonderianus</i> <i>zehntneri</i> (Lima et al., 2013)	<i>C. urucurana</i> (Peres et al., 1997) <i>C. megalobotrys</i> (Selowa et al., 2010). <i>C. macrostachyus</i> (Obey et al., 2016)
<i>Stillingia lineata</i> (Lam.) Müll.Arg. Bois de lait Tanguin de pays Tree/lowland dry forests	Ind. P.	nd	chikungunya virus, furuncles	nd	nd
Fabaceae					
<i>Indigofera amnoxylum</i> (DC.) Polhill Bois de sable, Bois de rose Tree/steep gorges	End. R. P.	nd	hypercholesterolemia, diabetes	<i>I. tinctoria</i> (Kamal and Mangla, 1993)	<i>I. oblongifolia</i> (Dahot, 1999) <i>I. suffruticosa</i> (Leite et al., 2006)

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

<i>Sophora denudata</i> 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)	<i>S. flavescens</i> (Mao and Henderson, 2007)	<i>S. alopecuroides</i> (Küçükboyacı et al., 2011) <i>S. oppositifolia</i> (Cota et al., 2011) <i>S. exigua</i> , <i>S. flavescens</i> (Krishna et al., 2012)
Monimiaceae					
<i>Monimia rotundifolia</i> Thouars Mapou Tree/lowland rainforests	End. R.	nd	nd	nd	nd
Piperaceae					
<i>Peperomia borbonensis</i> Miq. Pourpier Epiphyte succulent/highland rainforests	End. R.	nd	ticks	nd	<i>P. villipetiola</i> (Malquichagua Salazar et al., 2005) <i>P. fernandopoina</i> (Mbah et al., 2012; Ngemenya et al., 2006) <i>P. Pellucida</i> (Akinnibosun et al., 2008; Khan and Omoloso, 2002; Oloyede et al., 2011; Wei et al., 2011)
Rutaceae					
<i>Zanthoxylum heterophyllum</i> (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)	<i>Z. caribaeum</i> (Nogueira et al., 2014) <i>Z. dissitum</i> (Wang et al., 2015) <i>Z. heitzii</i> (Moussavi et al., 2015)	<i>Z. budrungea</i> (Islam et al., 2001) <i>Z. chalybeum</i> (Olila et al., 2001) <i>Z. zanthoxyloides</i> , <i>Z. leprieurii</i> (Misra et al., 2013) <i>Z. bungeanum</i> (Zhang et al., 2014)
Stillbaceae					
<i>Nuxia verticillata</i> 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.

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

693

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

694

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

697 ^aL = leaves, B = bark.

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701

702 **Table 3.** Minimal inhibitory concentration (MIC, $\mu\text{g/mL}$) of 16 plants extracts from Reunion Island.
703

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

704

705 The minimum inhibitory concentration (MIC) expressed in $\mu\text{g/mL}$ was determined by broth dilution. Chloramphenicol was used as standard for
706 bacteria, amphotericine B was the standard used for fungi. Three replicates were made for all extracts tested. ‘-’ means not active.

707 ^a L = leaves, B = bark.

708 ^b nt = not tested

709 **Figure captions**

710

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

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

714

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

717 **Table captions**

718

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

720

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

722 The growth inhibition was determined by paper disk diffusion. Inhibition diameters were given in mm ±

723 standard deviation obtained in three replicates, ‘-’ means not active. Chloramphenicol was used as

724 standard for bacteria, amphotericine B was the standard used for fungi.

725 ^aL = leaves, B = bark.

726

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

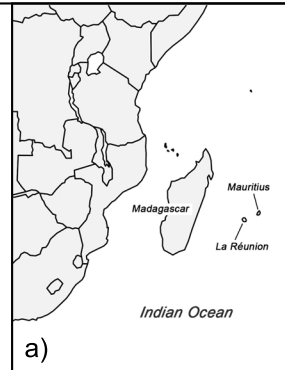
728 The minimum inhibitory concentration (MIC) expressed in µg/mL was determined by broth dilution.

729 Chloramphenicol was used as standard for bacteria, amphotericine B was the standard used for fungi.

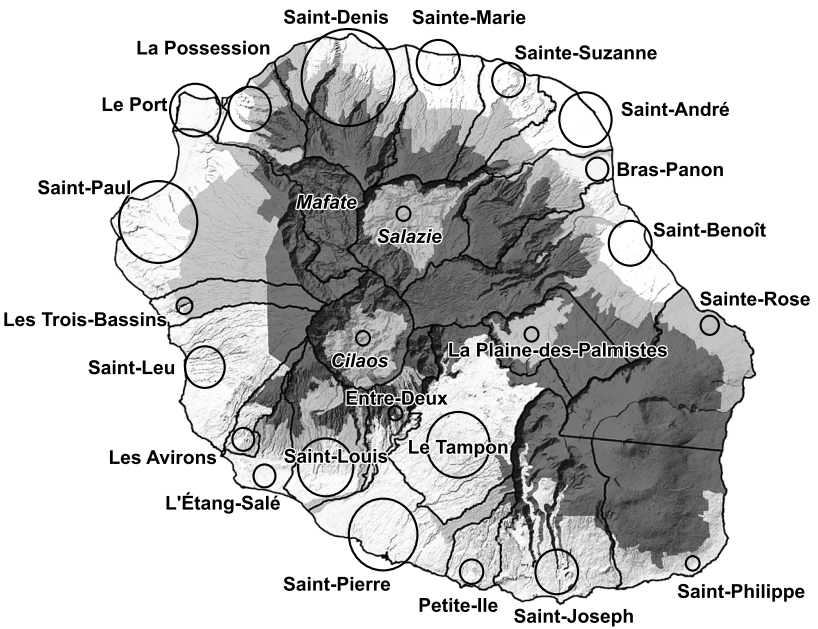
730 Three replicates were made for all extracts tested. ‘-’ means not active.

731 ^aL = leaves, B = bark.

732 ^b nt = not tested



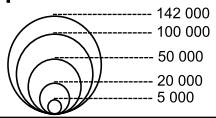
a)



Réunion National Park



Population



b)

