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Antibacterial activities of fourteen medicinal plants from the endemic plant diversity of Madagascar

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ABSTRACT

Methanolic extracts of 14 plant species used in traditional medicine in Madagascar were investigated for their antibacterial activities. Several extracts showed high inhibitory activities (*Maytenus polyacantha*, *Mystroxylon aethiopicum*, *Psychotria bridsoniae, Dombeya tsaratananensis, Psychotria oreotrephes*, *Razafimandimbisonia sambiranensis*) against *Listeria monocytogenes*, *Staphylococcus aureus* and *Streptococcus pyogenes* strains, some of which reached a MIC value lower than 0.1 mg/ml. The extract of *P. oreotrephes* was the most active against *Clostridium perfringens, Proteus mirabilis* and *Yersinia enterocolitica*. *Escherichia coli* and *Salmonella* Enteritidis were resistant to all tested extracts. This study demonstrates that plant extracts from species belonging to the Lauraceae, Proteaceae, Celastraceae, Malvaceae and Rubiaceae families have high antibacterial activity, some of which are bactericidal, against Gram-positive pathogenic bacteria that are known to cause infectious diseases.

1. Introduction

Plant use in traditional medicine remains a major practice of the health care system in Madagascar based on a rich know-how from the population and that the country represents a hot spot for biodiversity in the world. Some studies have already been reported on the activities of some plants used in the traditional medicine in Madagascar. A methanol extract of *Tetradenia riparia* was active against *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus*, *Shigella flexneri*, and *Klebsiella pneumoniae* with MIC values ranging between 1 and 10 mg/ml (Ndamane et al., 2013). Active molecules have been elucidated from the ethyl acetate leaf extract of *Dilobeia thouarsii* Roem and Schult, which is used as decoctions for diarrhea and skin infections (Rabesa, 1986; Razafintsalama et al., 2013a). Fourteen species endemic to Madagascar and which have been reported in the literature for their use in traditional medicine for treatment of gastroenteritis, diarrhea, skin infections and fatigue were selected for this study (Bost, 1961; Debray et al., 1971; Schmitt, 1971; Boiteau, 1975; Boiteau, 1986; Rabesa, 1986; Rakotobe et al., 1993; Boiteau et al., 1999). These include *Beilschmiedia microphylla* Kosterm (Lauraceae); *Cryptocarya dealbata* Baker (Lauraceae); *Cryptocarya floribunda* Baill. (Lauraceae); *Ravensara affinis* (Kosterm.) Kosterm (Lauraceae); *D. thouarsii* Roem. & Schult. (Proteaceae); *Maytenus polyacantha* (Sond.) Marais (Celastraceae); *Mystroxylon aethiopicum* (Thunb.) Loes. (Celastraceae); *Dombeya tsaratananensis* (Hochr.) Arènes (Malvaceae); *Hyperacanthus poivrei* (Drake) Rakotonas. & A. P. Davis (Rubiaceae); *Hyperacanthus* sp.1 (Rubiaceae); *Hyperacanthus* sp.2 (Rubiaceae); *Psychotria bridsoniae* A. Davis & Govaerts (Rubiaceae); *Psychotria oreotrephes* (Bremek) A. Davis & Govaerts (Rubiaceae) and *Razafimandimbisonia sambiranensis* (Homolle ex Cavaco) Kainul. & B. Bremer (Rubiaceae). The aim of this study was to assess the antibacterial activities of their methanolic extracts in order to provide a better characterization of the biological activity of the plants endemic to Madagascar and scientific validation for their traditional use.

2. Material and methods

2.1. Preparation of extracts

Voucher specimens of collected plant samples from different regions of Madagascar (around Tsaratanana Mountain, Mandraka, Arivonimamo) (Fig. 1) were deposited at the Botany Department of the "Centre National d'Application des Recherches Pharmaceutiques" (CNARP, Antananarivo, Madagascar). Once shade-dried, plant samples were ground into

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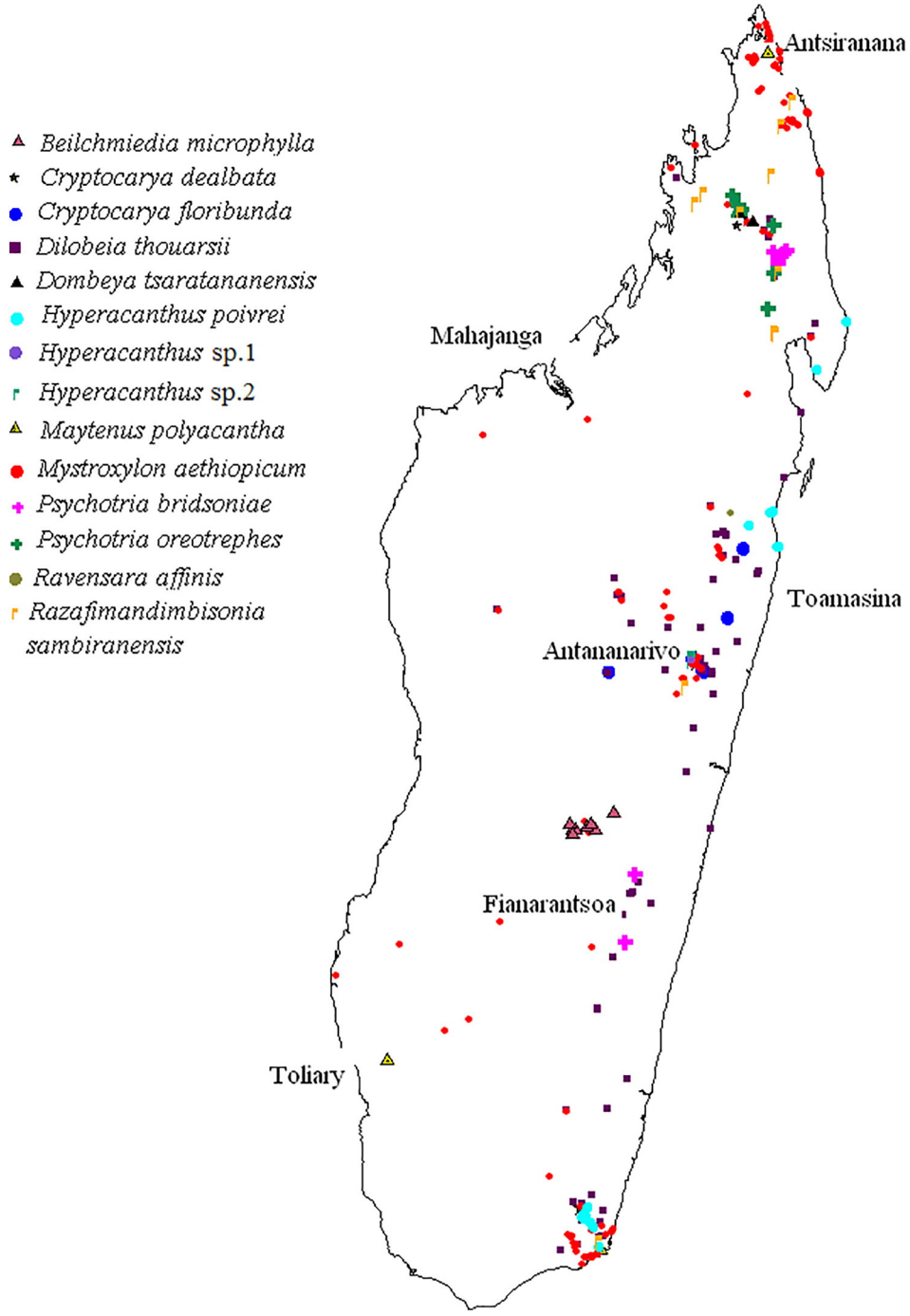

Fig. 1. Geographic repartition of the 14 medicinal plants collected in Madagascar.

powder and stored at room temperature before performing individual methanol extraction procedure. The powder (500 mg) was extracted successively through maceration using 500 ml × 4 of methanol. Methanol is among the best solvents used for extraction of antimicrobial substances when compared to other solvents, such as water, ethanol and hexane (Eloff, 1998). The solvent was evaporated under reduced pressure to yield methanol extracts. Extracts were stored in glass vials at room temperature until use.

2.2. Antibacterial activity

Four Gram-positive and five Gram-negative strains (Table 1) were used for susceptibility-screening tests using the disc diffusion method (Kil et al., 2009). Sterilized filter paper discs of 6 mm (Biomérieux, Marcy l'Etoile, France) were saturated with 10 μl of the methanol extract (1 mg/disc). Kanamycin 30 μg and Streptomycin 10 μg (Bio-Rad, Marnes-la-Coquette, France) were used as positive controls. Tests were performed in triplicate. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were evaluated using the microdilution method (Razafintsalama et al., 2013b). Each extract concentration (100 μl), ranging from 0.001 to 5 mg/ml, was distributed in a well (96-well microplate) containing 95 μl of Mueller–Hinton broth and 5 μl of the inoculum (10^8 CFU/ml by adjusting the optical density to 0.125 at 600 nm corresponding to 0.5 McFarland) and incubated for 24 h at 37 °C. The MIC of each extract was the lowest concentration that inhibited the bacterial growth which was visually