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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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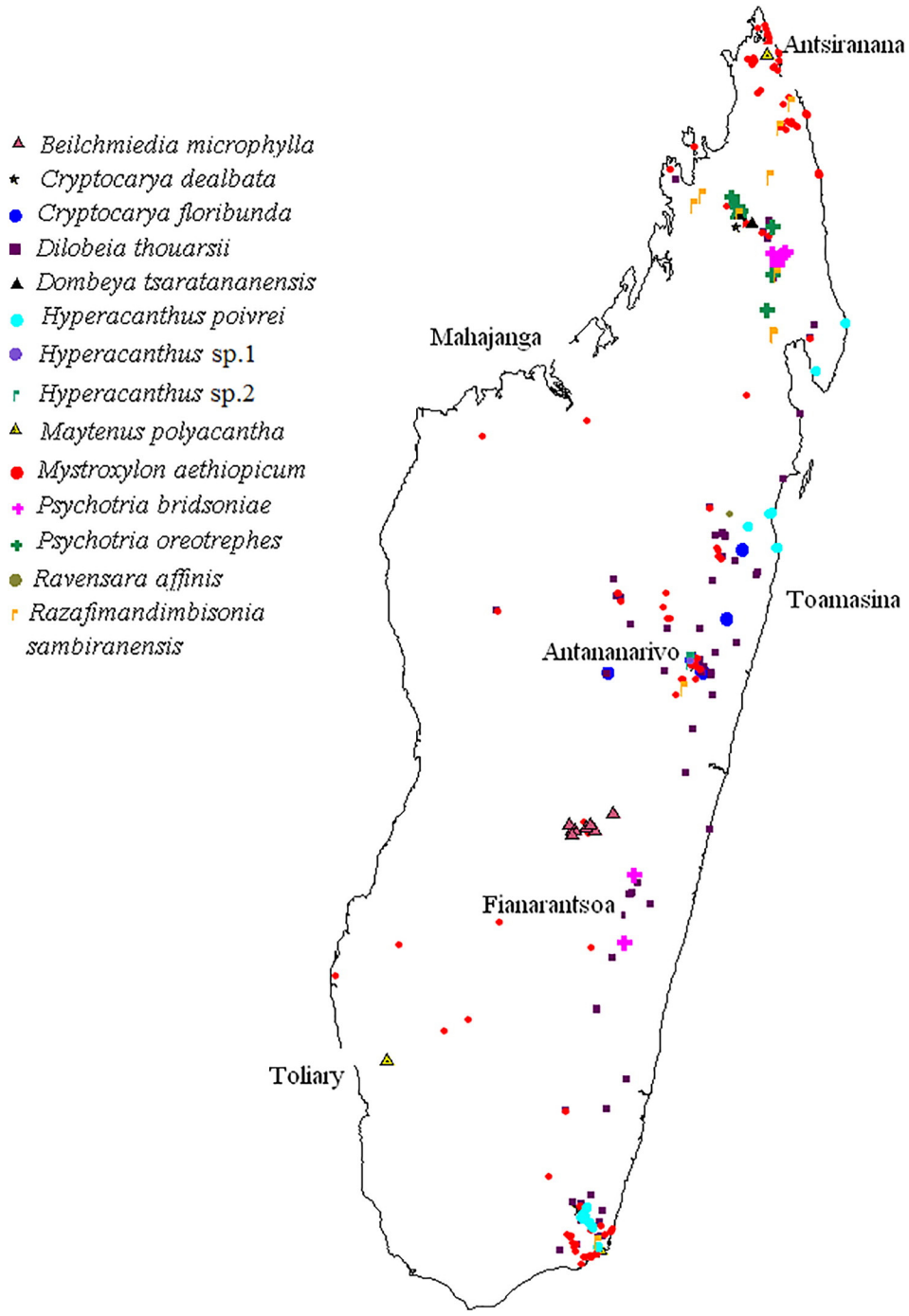

Fig. 1. Geographic repartition of the 14 medicinal plants collected in Madagascar.

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

Table 1
Antibacterial activities of the methanolic extracts (n = 16) from different endemic plants species of Madagascar against Gram-negative and Gram-positive bacteria.

Plant extracts (part) and antibiotics	Gram-negative bacteria												Gram-positive bacteria														
	Proteus mirabilis			*Escherichia coli*			*Shigella flexneri*			*Salmonella* Enteritidis			*Yersinia enterocolitica*			*Listeria monocytogenes*			*Clostridium perfringens*			*Staphylococcus aureus*			*Streptococcus pyogenes*		
	ATCC35659			ATCC 8739			ATCC12022			ATCC13076			ATCC23715			ATCC19114			ATCC13124			ATCC11632			ATCC19615		
	DD	MIC	MBC	DD	MIC	MBC	DD	MIC	MBC	DD	MIC	MBC	DD	MIC	MBC	DD	MIC	MBC	DD	MIC	MBC	DD	MIC	MBC	DD	MIC	MBC
Beilschmiedia microphylla (fruit)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	7	3.52^{h}	3.52^{g}	10	3.500^{d}	3.500^{c}	8	0.310^{ab}	2.250^{c}	12	3.500^{e}	3.500^{d}
Cryptocarya dealbata (fruit)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	8	0.783^{b}	1.583^{a}	10	2.257^{c}	–	–	–	–
Cryptocarya floribunda (fruit)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	7	2.257^{c}	–	13	$0,177^{a}$	–
Ravensara affinis (bark)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	7	0.620^{b}	2.510^{c}	11	$0,177^{a}$	–
Dilobeia thouarsii (bark)	10	0.437^{b}	2.0^{c}	–	–	–	–	–	–	–	–	–	–	–	–	14	0.833^{f}	1.75^{e}	7	0.870^{b}	1.750^{a}	12	0.310^{ab}	5.0^{d}	10	$0,197^{a}$	1.750^{b}
Dombeya tsaratananensis (bark)	10	0.870^{d}	1.750^{bc}	–	–	–	–	–	–	–	–	–	–	–	–	12	0.537^{d}	3.5^{g}	11	3.167^{d}	3.166^{c}	11	0.027^{a}	0.026^{a}	18	$3,167^{d}$	3.167^{d}
Hyperacanthus poivrei (stem with leaves)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	7	2.5^{g}	2.5^{f}	–	–	–	7	2.503^{c}	–	10	2.500^{c}	2.500^{c}
Hyperacanthus sp.1 (leaves)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	7	3.167^{d}	–	11	$0,156^{a}$	–
Hyperacanthus sp.2 (leaves)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	7	3.167^{d}	–	11	$0,157^{a}$	–
Maytenus polyacantha (wood)	10	2.500^{e}	–	–	–	–	–	–	–	–	–	–	–	–	–	19	0.002^{a}	0.008^{a}	8	2.500^{c}	2.500^{b}	10	0.310^{ab}	1.583^{b}	15	0.003^{a}	0.008^{a}
Mystroxylon aethiopicum 1 (bark)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	12	0.001^{a}	0.75^{d}	11	0.708^{ab}	–	11	0.040^{a}	2.256^{c}	10	0.750^{b}	2.500^{c}
Mystroxylon aethiopicum 2 (roots)	14	0.667^{c}	2.500^{c}	–	–	–	–	–	–	–	–	–	7	0.667^{a}	0.667^{a}	12	0.004^{a}	2.5^{f}	8	0.667^{ab}	–	9	0.040^{a}	2.256^{c}	15	0.901^{b}	2.500^{c}
Psychotria bridsoniae (inflorescence)	12	0.667^{c}	1.417^{ab}	–	–	–	–	–		–	–	–	10	0.667^{a}	1.417^{bc}	12	0.281^{bc}	0.284^{b}	8	0.667^{ab}	1.416^{a}	11	0.040^{a}	5.0^{d}	16	0.004^{a}	0.008^{a}
P. oreotrephes (stem with leaves and fruits)	8	0.240^{a}	0.875^{a}	–	–	–	7	3.167^{b}	3.167^{a}	–	–	–	10	0.249^{b}	1.583^{c}	10	0.249^{b}	0.249^{b}	8	0.437^{a}	1.750^{a}	11	0.310^{ab}	5.0^{d}	12	$0,003^{a}$	0.240^{a}
R. sambiranensis 1 (bark)	14	0.281^{a}	2.500^{c}	–	–	–	7	0.310^{a}	2.833^{a}	–	–	–	10	0.31^{c}	2.833^{d}	13	0.667^{c}	–	8	2.500^{c}	2.500^{b}	11	0.167^{a}	2.250^{c}	25	$0,002^{a}$	2.500^{c}
R. sambiranensis 2 (leaves)	14	0.875^{d}	3.167^{d}	–	–	–	7	0.437^{a}	2.233^{a}	–	–	–	10	0.437^{c}	0.875^{ab}	13	0.395^{c}	0.395^{c}	8	0.875^{b}	3.166^{c}	10	0.15^{a}	2.51^{c}	11	$0,173^{a}$	3.167^{d}
Kanamycin (30 μg/disc)	27	NT	NT	25	NT	NT	21	NT	NT	19	NT	NT	20	NT	NT	30	NT	NT	20	NT	NT	20	NT	NT	35	0.008	NT
Streptomycin (10 μg/disc)	20	NT	NT	15	NT	NT	20	NT	NT	24	NT	NT	17	NT	NT	22	NT	NT	15	NT	NT	13	NT	NT	35	0.009	NT

Values are means of triplicate.
DD: Disc Diffusion (diameter of inhibition zone including disc diameter of 6 mm).
MIC: Minimum Inhibitory Concentration (mg/ml).
MBC: Minimum Bactericidal Concentration (mg/ml).
NT: not tested.
–: no growth inhibition.
Values followed by different letters within a column are significantly different according to the Fisher LSD test ($P < 0.05$).

evaluated based on the degree of turbidity and the measure of absorbance at 600 nm. Thereafter, 5 µl from each well was deposited on to Mueller–Hinton agar. The MBC was determined as the lowest concentration at which no colony growth occurred on the plates after incubation for 24 h at 37 °C.

2.3. Statistical analysis

For comparison of MIC and MBC values, analysis of variance was performed using ANOVA. Significant differences between mean values were determined by Fisher LSD test at the threshold of $P < 0.05$.

3. Results and discussion

Inhibition zones of extracts were lower than those of the antibiotics for all strains (Table 1). Gram-positive strains were more sensitive than Gram-negative strains with MIC values lower than 0.1 mg/ml, which can be considered as significant activity according to Kuete (2010) and reaching in some cases values lower than 0.01 mg/ml which are noteworthy (*Maytenus* spp., *Mystroxylon* spp., *Psychotria* spp., *R. sambiranensis* bark). Plants displaying moderate activity (0.1 mg/ml < MIC <0.625 mg/ml) were more frequent for Gram-positive bacteria as well. *Streptococcus pyogenes* was the most sensitive (MIC range of 0.002–0.197 mg/ml) for the most active group (*R. sambiranensis* 1–2, *R. affinis*, *D. thouarsii*, *P. oreotrephes*, *P. bridsoniae*, *Hyperacanthus* sp.1–sp.2, *C. floribunda*) ($P < 0.001$). *R. sambiranensis* bark, *M. aethiopicum* 1–2, *P. bridsoniae* and *D. tsaratananensis* showed the highest activity ($P < 0.001$) against *S. aureus* (MICs from 0.027 mg/ml to 0.167 mg/ml). Both *M. aethiopicum* extracts were the most active against *Listeria* spp. ($P < 0.001$), showing the lowest MIC values of 0.001–0.004 mg/ml, which are noteworthy. *E. coli* and *Salmonella* spp. were resistant to all extracts, as was observed by Khan et al. (2009) for the crude ethanolic extracts of *Terminalia arjuna* and *Eucalyptus globulus*. Higher resistance of Gram-negative bacteria has been attributed to the greater complexity of their double membrane-containing cell wall in contrast with the single membrane-glycoprotein/teichoic acid of Gram-positive bacteria (Ndamane et al., 2013). Considering the ratio of MBC/MIC < 4 (Oussou et al., 2008), several extracts (*D. thouarsii*, *P. bridsoniae*, *P. oreotrephes*, *R. sambiranensis*) were bactericidal against *Listeria* spp., *Clostridium* spp. and *Streptococcus* spp. Different parts of the plant may possess varying antimicrobial properties including the flowers, bark, stems, leaves, twigs or roots (Okigbo et al., 2009). For instance, the MICs of the bark extract from *R. sambiranensis* (0.002 mg/ml) or *Mystroxylon* spp. (0.75 mg/ml) were, respectively, lower than those from the leaf extracts (0.173 mg/ml) or the roots (0.9 mg/ml) of both plants against *Streptococcus* spp.

To the best of our knowledge, this is the first study that demonstrates the antibacterial activity of the selected parts of the plants belonging to the Lauraceae, Proteaceae, Celastraceae, Malvaceae and Rubiaceae families, some of which are noteworthy, and may contribute to support the traditional use against bacterial infections.

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References

Boiteau, P., 1975. Dictionnaire des noms malgaches de végétaux. Fitoterapia 5, 201–237.
Boiteau, P., 1986. Médecine traditionnelle et pharmacopée: Précis de matière médicale malgache. Agence de Coopération Culturelle et Technique, Antananarivo.
Boiteau, P., Boiteau, M., Allorge-Boiteau, L., 1999. Dictionnaire des noms malgaches de végétaux (Éditions Alzieu).
Bost, R., 1961. Pharmacopée malgache. Institut Scientifique de Madagascar X 2, 159–234.
Debray, M., Jacquemin, H., Razafindrambao, R., 1971. Contribution à l'inventaire des plantes médicinales de Madagascar. ORSTOM, Paris.
Eloff, J., 1998. Which extractant should be used for the screening and isolation of antimicrobial components from plants? Journal of Ethnopharmacology 60, 1–8.
Khan, R., Islam, B., Akram, M., Shakil, S., Ahmad, A.A., Ali, S.M., Siddiqui, M., Khan, A.U., 2009. Antimicrobial activity of five herbal extracts against multi drug resistant (MDR) strains of bacteria and fungus of clinical origin. Molecules 14, 586–597.
Kil, H.Y., Seong, E.S., Ghimire, B.K., Chung, I.-M., Kwon, S.S., Goh, E.J., Heo, K., Kim, M.J., Lim, J.D., Lee, D., 2009. Antioxidant and antimicrobial activities of crude sorghum extract. Food Chemistry 115, 1234–1239.
Kuete, V., 2010. Potential of Cameroonian plants and derived products against microbial infections: a review. Planta Medica 76, 1479–1491.
Ndamane, Y., Kambizi, L., Songca, S.P., Oluwafemi, O.S., 2013. Antibacterial effectiveness of *Tetradenia riparia* extract, a plant traditionally used in the Eastern Cape Province to treat diseases of the respiratory system. Journal of Medicinal Plant Research 7, 2755–2760.
Okigbo, R., Anuagasi, C., Amadi, J., 2009. Advances in selected medicinal and aromatic plants indigenous to Africa. Journal of Medicinal Plant Research 3, 086–095.
Oussou, K.R., Yolou, S., Boti, J.B., Guessennd, K.N., 2008. Etude chimique et activité anti diarrhéique des huiles essentielles de deux plantes aromatiques de la pharmacopée ivoirienne. European Journal of Scientific Research 24, 94–103.
Rabesa, Z.A., 1986. Pharmacopée de l'Alaotra. Edition Fanantenana, Antananarivo.
Rakotobe, E.A., Rasolomanana, C.J.C., Randrianasolo, S.S., 1993. Pharmacopées de l'Ambongo et du Boina. Centre d' Informartion et de Documentation Scientifique et Technique, Antananarivo.
Razafintsalama, V., Girardot, M., Randrianarivo, R., Rakoto, D., Sarter, S., Petit, T., Ralambonirina, S., Deville, A., Grellier, P., Jeannoda, V., 2013a. Dilobenol A–G, diprenylated dihydroflavonols from the leaves of *Dilobeia thouarsii*. European Journal of Organic Chemistry 2013, 1929–1936.
Razafintsalama, V., Sarter, S., Mambu, L., Randrianarivo, R., Petit, T., Rajaonarison, J.F., Mertz, C., Rakoto, D., Jeannoda, V., 2013b. Antimicrobial activities of *Dilobeia thouarsii* Roemer and Schulte, a traditional medicinal plant from Madagascar. South African Journal of Botany 87, 1–3.
Schmitt, J.P., 1971. Contribution à l'inventaire des plantes médicinales de Madagascar. ORSTOM, Antananarivo.