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ETHNO-BOTANICAL SURVEY, ANTIMICROBIAL ACTIVITY AND GC/SM ANALYSES OF ESSENTIAL OIL FROM THE LEAVES OF BROCHOUNERA MADAGASCARIENSIS (MYRISTICACEAE)

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ABSTRACT

Brochounera madagascariensis (Myristicaceae) is an endemic plant species, distributed in the Eastern and the South-Eastern regions of Madagascar. During an ethno-botanical survey conducted in the South-Eastern of Madagascar, it was reported that *Brochounera madagascariensis* is traditionally used by the local communities to treat asthma, hypertension, bacterial infections and for wound-healing. The Aim of the study was to evaluate the chemical composition and antimicrobial activity of essential oil from the leaves of *Brochounera madagascariensis.* The plant species *B. madagascariensis* was selected based on its relative citation frequency (use value= 0.65) and the informant consensus factor value (0.38) and collected in Alanirano village, district of Fort-Dauphin (Southeast of Madagascar) on December 2023 and identified at the Department of Botany, Tsimbazaza Zoological and Botanical Park, Antananarivo, Madagascar. Essential oil was isolated from the root bark of this plant species by hydro-distillation. Antimicrobial activities were carried out using agar disc diffusion and micro-dilution broth methods with Gram-positive and Gram-negative bacteria as models. The chemical compositions of the essential oils were determined by Gas chromatography-Flame Ionization Detector (GC-FID) and Gas chromatography-Mass spectrometry (GC/MS). Analysis of the leaves essential oil of *B. madagascariensis* by GC-FID and GC/MS enabled the identification of 41 compounds corresponding to 96.02 % of the total essential oil composition. Based on functional groups classification, the essential oil from the leaves essential oil of *B. madagascariensis* contained 80.16% of terpenic hydrocarbons, and 15.86% of oxygenated terpenic functions. The major compounds were α-thuyène (16.37%), α-pinène (15.46%), Terpinolène (11.33%), β-myrcène (8.31%), β-pinène (7.44%), α-terpineol (6.47%), Limonene (5.94%), ϒ-terpinène (5.61%), Sabinene (2.80%), α-terpinène (2.68%), Linalol (2.24%), Trans-p-menth-2-ène-1-ol (1.36%), 8-hydroxy-4-ène-3-one (1.34%), Cis-cis-dihydro-p-menthenolide (1.27%), 1, 8-cinéol (1.21%), p-cymène (1.11%) and piperitenone (1.07%).The essential oil from the root bark of H. voyronii originated from Madagascar showed bactericidal activity (MBC/MIC ≤ 4).

KEYWORD: Medicinal plant, *Brochounera madagascariensis*, essential oil, antimicrobial activity, Madagascar.

INTRODUCTION

Infectious diseases remain the major public health problem, and are currently the world's leading cause of death.[1-2] The control of these diseases constitutes new challenges because of the emergence of multidrug resistance among several pathogens to some of the drugs commonly used in the treatment of infectious diseases.[3-5] This explains the need to intensify the search for more efficient drugs to combat these diseases.

In addition, some conventional drugs are sometimes associated with adverse effects on the human cell host including hypersensitivity, immune-suppression, allergic reactions and even loss of hearing.[6-8] Despite the efforts of bio-scientists, few new antimicrobial drugs have emerged. For efficient drug discovery, it's important to identify new biologically active chemical complex that will lead to effective antimicrobial drugs. The plant kingdom constitutes a good target for this purpose

because of its enormous chemical and structural diversity.[9-11]

Madagascar as constitute each one a rich reservoir of medicinal plants (hotspot of biodiversity) and the first line of treatment for poor people in such countries is the use of herbal medicines at home.[12-14]

In order to preserve the ethno-medical cultural heritage of these countries, a multidisciplinary research program aiming at the identification, the validation and the sustainable use and conservation of medicinal plant species with pharmacological properties established a database for public purpose.[2,8,15-17]

Several essential oils have been reported to possess interesting antimicrobial activity suggesting the possibility of using them as alternative of synthetic antimicrobials in order to overcome the increasing resistance of some pathogens to the conventional antibiotics drugs.[18-21] During an ethno-botanical survey conducted in the South-Eastern of Madagascar, it was reported that *Brochounera madagascariensis* is traditionally used by the local communities to treat asthma, hypertension, bacterial infections and for wound-healing. Since wounds are generally infected by microbial pathogenic agents, it can therefore, be hypothesized that *B. madagascariensis* essential oil could be effective in the management of diseases caused by microbial agents. This plant species belongs to the family of Myristicaceae is widely distributed in the Eastern and the South-Eastern regions, and the *Brochounera madagascariensis* specie is originated from Madagascar.[22-29] The chemical composition and biological activity of essential oils from the selected plant species has not been fully studied yet. They could be promising sources of secondary metabolites with pharmacological properties, including antimicrobial activity.

The aim of this study was to determine the chemical composition of essential oils extracted from the leaves of *Brochounera madagascariensis* and to investigate the in vitro antimicrobial activity of these essential oils against Gram-positive bacteria [Bacillus subtilis (B. subtilis), Staphylococcus aureus (S. aureus) and Bacillus cereus (B. cereus)] strains and Gram-negative bacteria [Escherichia coli (E. coli), Salmonella typhii (S. typhii), Pseudomonas aeruginosa (P. aeruginosa), and Enterobacter cloacae (E. cloacae)] strains respectively, in order to validate scientifically the traditional use of these aromatic plant species.

The search for natural antibacterial products is the new challenges for the control and prevention of bacterial diseases in human health. This is the first report involving the complete chemical composition and antimicrobial activity of essential oils of the selected plant originated from the south-eastern of Madagascar.

2. MATERIALS AND METHODS

Ethno-botanical survey

Ethno-botanical information about the plant species selected for this study was obtained by interviewing traditional healers during field work which was conducted in the Southeast and the East part of Madagascar. Surveys were conducted from October to December 2023 in six villages of the Southeast and East of Madagascar. These villages are Fort-dauphin, Alanirano, Manakara, Vohipeno, Mananjary and Vangaindrano. There are six different ethnic groups (tribes) inhabiting the South of Madagascar: Antevondro, Antanosy, Antaisaka, Antefasy, Antaimora and Antagnala. They all share a common language Malagasy, which is the unique characteristic of this island. A total of 35 traditional healers were interviewed. Informants were selected for their authentic knowledge on the utilization of medicinal plants. Malagasy, the national language of Madagascar was used during anthropological interviews. Traditional healers were interviewed on a voluntary basis. The study followed principles laid out in the Declaration of Helsinki as previously reported.[30]

The questionnaires were divided into three sections: (i) personal information such as name, age, sex, marital status and studies level; (ii) traditional medicine practice (including knowledge of diseases and symptoms); (iii) plant vernacular names, plant part used, preparation methods, and administration route of remedies. Informed consent was obtained from both the Government of Madagascar to collect plant samples and to conduct non-commercial research on Malagasy medicinal plants and the respondents to divulge information. A benefit-sharing agreement on mutually agreed terms were also established between Madagascar (Malagasy Institute of Applied Research and University of Toliara) and France (Pharmacognosy Laboratory, Paul Sabatier University) according to the principles laid out in the Nagoya protocol.[30-31]

2.1. Selection and collection of plant materials: The plant species *Brochounera madagascariensis* (*B. madagascariensis*) was selected based on its relative citation frequency (use value= 0.65) and the informant consensus factor value (0.38). Plant leaves samples were collected in Alanirano village, district of Fort-Dauphin (Southeast of Madagascar) on December 2023. The plant sample was identified by comparison with reference specimens available at the Department of Botany, Tsimbazaza Zoological and Botanical Park, Antananarivo. Voucher specimens with assigned sample number FA-01was deposited at the Herbarium of the Laboratory of Applied Chemistry, Layflaylle Street, University of Toliara.

2.2. Essential oil extraction: Fresh leaves of each plant species were separately cut and about 400 grams of each sample were extracted for four hours by hydro-distillation using a Clevenger-type apparatus. Briefly, the plant leaves were completely immersed in water and

heated to boiling after which the essential oil was evaporated together with water vapor and finally collected after decantation. The oil obtained was dried over anhydrous sodium sulphate and then stored in a dark glass bottle, and kept at 0 °C until the time of further analysis. The percent yield was calculated relative to the dried mass of the initial sample.

2.3. Determination of physico-chemical indices of extracted essential oils: Physico-chemical characterization of essential oil was carried out according to the International Organization of Normalization and the French Association of Normalization Standard in order to determine relative density, refractive index, optical rotation, acidity index and ester index.[32] Specific gravity was measured with an Aton Paar densimeter (DMA 23N model). Optical rotation was measured with a CETI Polaris polarimeter instrument. Essential oil samples were previously dissolved in analytical grade chloroform (Merk). Refractive index was measured at 20 °C with a CETI Quartz refractometer. The acid value (mg/KOH/g) of essential oils was evaluated by neutralizing the free acids of those by KOH. The saponification value (mg/KOH/g) was calculated as the quantity of KOH necessary to saponify esters of acids and to neutralize the free acids in one gram of essential oil. The ester index is the difference between the saponification value and the acid value.

2.4. Chemical analysis of essential oils and identification of the constituents: The quantification and analysis of the essential oil sample of the leave of *B. madagascariensis* was carried out using gas chromatography (GC) and gas chromatography coupled to a mass spectroscopy (GC/MS).

The quantification of oils was performed in a Varian model 3400 gas chromatograph/flame ionization detector (GC-FID), under the following conditions: one-column injector, a DB-Wax (Université Blaise Pascal de Clermont, France) fused silica capillary column (60 mx0.32 mm inner diameter inner 0.25 µm film). The temperature of the oven increased from 50 °C to 200 °C in a rate of 5 °C/min; up there it was held for 30 min. The injector and detector temperature was 260 °C. The carrier gas was helium maintained at 2.0 mL/min, dual FID; split ratio 1:70. The response factors were taken as 1.0 for all compounds, with reference of n-hexanol as internal standard. The linear retention indices were calculated with reference of C5-C22. Concentrations are given as the average of triplicate analysis. GC-MS analysis of oil was performed under the same conditions with GC using an AGILENT 5973 gas chromatography equipped with an AGILENT 6890 series mass selective detector. Analytic conditions were: injector and transfer line temperature, 220 °C and 240 °C, respectively; oven temperature programmed from 50 °C to 200 °C at 5 °C/ min; carried gas used was helium at a flow rate of 1 mL/min; and the injection volume of 0.1 µL (10% hexane solution); and the split ratio was 1:50. The electron impact energy was set at 70 eV. The identifications of the constituents were based on the comparison of their mass spectra with those of Wiley and NIST (National Institute of Standards and Technology) libraries and literature data.[33-43]

2.5. Biological evaluation

2.5.1. Microbial strains: The activity of the essential oil sample was tested toward 9 different microorganisms: Gram positive bacteria represented by Bacillus subtilis (B. subtilis ATCC 6633), Staphylococcus aureus (S. aureus ATCC 25923), Bacillus cereus (B. cereus ATCC 10876), and Gram negative bacteria: Escherichia coli (E. coli ATCC 25922), Pseudomonas aeruginosa (P. aeruginosa ATCC 27853), and Enterobacter cloacae (E. cloacae ATCC 13047) and the yeast Candida albicans (C. albicans ATCC 10231). The tested strains were obtained from the American Type Culture Collection (ATCC, Rockville MD, USA).

2.5.2. Antimicrobial activity

a. Disc diffusion: The agar disc diffusion method was used to determine the antibacterial activity of essential oil as follow: A 1 mL of suspension of 18 hours culture bacteria containing about 106 UFC/mL were spread on Mueller Hinton agar medium using sterile swabs. Filter paper discs (6 mm in diameter) were soaked in 5 or 10 µL of pure essential oil and placed on the inoculated plates and allowed to dry for 30 min, then incubated at 37 °C for 24 hours. The diameters of the inhibition zones were measured in millimeters.

Two controls were included in the test: the first was a control involving the presence of microorganisms but without the test oils sample and the last was a standard antibiotic penicillin G which was used in order to control the sensitivity of the tested micro-organisms. Studies were performed in triplicate, and the developing inhibition zones were compared with those of reference discs.[44]

b. Minimum inhibitory and minimum bactericidal concentration: An aliquot (10 µL) of a 106 CFU/mL overnight culture was added to wells of a sterile 96-well micro-plate titer. Essential oil (EO) was diluted in Mueller Hinton broth (MHB) containing 0.1% (v/v) Tween 80 and added to wells to give final concentrations ranging from 0.03 to 10 µL/mL. The positive control wells contained MHB+ bacteria suspension without essential oil while negative control wells contained MHB only. Optical density (OD) was measured at 630 nm using a microplate reader (Titertek Twin-reader, Finland) and again after incubation for 24 hours at 37 °C.

The minimum inhibitory concentration (MIC) was determined as the lowest EO concentration at which the OD after 24 h of incubation of the inoculum remained the same or reduced compared with the initial reading. MTT (30 µL) in aqueous solution (0.01%) was used to evaluate the micro-organism viability. For minimum

bactericidal (MBC) determination, 10 µL was taken from each well after incubation and spot inoculated on to MHB and incubated for 24, 48 and 72 hours at 37 °C. The concentration at which no growth observed on subculture was determined as the MBC [45]. The mean MBC/MIC ratio was evaluated for each sample.

2.6. Statistical analysis: All statistical calculations were carried out with Graph Pad Prism 4. The results are expressed as the mean±standard error of mean (SEM) of an independent experiments with individual values. Unpaired Student's t-test was used for statistical comparison; P values less than 0.01 were considered as significantly different against the control.

3. RESULTATS

3.1. Ethno-botanical survey: During ethno-botanical survey, twenty-five traditional healers were interviewed about medicinal plants used in folk medicine to treat asthma and bacterial infections. The most cited plant was *B. madagascariensis* with the use value and informant consensus factor of 0.59 and 0.35 respectively.

3.2. Physico-chemical indices: A Physico-chemical index of the extracted essential oil is given in the Table 1. From this table, it can be noticed that the relative density values of the essential oil is less than 1. All extracted essential oil was found to be dextrogyre but the optical rotation of essential oil.

Table 1: Physico-chemical indices of essential oil from the leaves of B. madagascariensis.

Physico-chemical indices	Essential oil of B. madagascariensis
Relative density	0.934±0.155
Refractive index	1.4853±0.2340
Optical rotation (°)	50.640±0.194
Acidity index	17.21±0.00123
Ester index	53. 150±1.866

3.3. Chemical analyses

3.3.1 Extraction yields and Chemical composition: The yield of the leaves essential oil of *B. madagascariensis* (*Myristicaceae*) obtained by hydro-distillation was 4.16%. From GC-FID and GC/SM analyses, a total of 41 volatiles compounds were identified corresponding to 96.02% of the total essential oil composition. The essential oil from the leave essential oil of *B. madagascariensis* contained 80.16% of terpenic hydrocarbons, and 15.86% of oxygenated terpenic functions. The major compounds were α-thuyène (16.37%), α-pinène (15.46%), Terpinolène (11.33%), β-myrcène (8.31%), β-pinène (7.44%), α-terpineol (6.47%), Limonene (5.94%) and ϒ-terpinène (5.61%). The minor compounds where Sabinene (2.80%), α-terpinène (2.68%), Linalol (2.24%), Trans-p-menth-2-ène-1-ol (1.36%), 8-hydroxy-4-ène-3-one (1.34%), Cis-cis-dihydro-p-menthenolide (1.27%), 1, 8-cinéol (1.21%), p-cymène (1.11%), piperitenone (1.07%). The other compounds are in trace state i.e. less than 1% (Table 2).

Table 2: Composition of the essential oil of *B. madagascariensis.*

N°	Component name	Percentage	R.I	Identification method
1	Tricyclene	0.05	515.1	a ,b ,c
2	α-pinene	15.46	528.3	a ,b
3	α-thuyene	16.37	532.3	a ,b
4	Camphene	0.11	571.1	a ,b
5	β-pinène	7.44	613.4	a ,b
6	Sabinene	2.80	625.1	a ,b
7	δ-3-carène	0.27	651.0	a ,b
8	β-myrcène	8.31	668.1	a ,b
9	α-terpinène	2.68	683.9	a ,b ,c
10	2,3-déhydrociéole	0.03	697.0	b, c
11	Limonene	5.94	703.9	a ,b ,c
12	1,8-cinéol	1.21	712.6	a ,b ,c
13	(Z)- β-ocimène	0.07	739.0	a ,b ,c
14	ϒ-terpinène	5.61	747.5	a ,b ,c
15	(E)- β-ocimène	0.41	755.2	a ,b ,c
16	p-cymène	1.11	771.0	a ,b ,c
17	Terpinolène	11.33	785.3	a ,b ,c
18	α-cubébène	0.12	958.3	a ,b ,c

19	Oct-1-ène-3-ol	0.23	959.8	b, c
20	δ-élémène	0.05	982.7	a ,b
21	α-copaène	0.05	993.9	a ,b ,c
22	Camphor	0.42	999.6	a ,b ,c
23	Linalol	2.24	1045.6	a ,b ,c
24	Trans-p-menth-2-ène-1-ol	1.36	1053.9	b ,c
25	α-humulène	0.26	1152.1	a ,b ,c
26	Nèral	0.11	1163.2	c
27	Néoiso-isopulégyle	0.84	1168.4	b ,c
28	α-terpineol	6.47	1180.5	a ,b ,c
29	β-sélinène	0.04	1202.5	a ,b ,c
30	β-bisabolène	0.11	1207.5	a ,b ,c
31	δ-cadinène	0.05	1239.6	a ,b ,c
32	β-citronellol	0.04	1253.3	a ,b ,c
33	8-hydroxy-menth-4-ène-3-one	1.34	1256.4	c
34	β-sesquiphellandrène	0.04	1258.6	a ,b ,c
35	Nérol	0.16	1283.7	b ,c
36	piperiténone	1.07	1309.1	b ,c
37	Calaménène	0.06	1322.3	a ,b
38	isopiperiténone	0.42	1333.5	b ,c
39	Cis-cis-dihydro-p-menthenolide	1.27	1409.2	c
40	Oxyde de caryophyllène	0.04	1438.7	a ,b ,c
41	Eugenol	0.03	1614.1	a ,b ,c

(a): GC-FID; (b): GC/SM and (c): RMN ^{13}C

3.4. Antimicrobial activity: The antimicrobial activity of essential oils from *B. madagascariensis* against some pathogenic microorganisms was determined. The results are shown in Tables 2. Compared to the standard antibiotic, all microbial strains displayed sensitivity to the tested essential oils with the inhibition zones varying from 7.43±1.032 (or12.43±1.32) to 22.73±2.12 (25.80±3.02) mm (Table 3). The most sensitive microbes were B. subtilis followed respectively by B. cereus; S. aureus, E. cloacae, E. coli and P. aeruginosa. The minimum inhibitory concentration (MIC) and the Minimum Bactericidal Concentration (MBC) values ranged from 0.312 to 2.5 µL/mL (Table 4).

Table 3: Inhibitory effect of essential oils against bacteria (expressed as the inhibition zones of bacterial growth).

Bacterial strains	Diameter of inhibition zones (mm)		
	5 µL/disc	10 µL/disc	Penicillin G (10 units)
B. cereus ATCC 6633	22.73±2.12	25.80±3.02	17±0.7
S. aureus ATCC 25923	17.91 ±2.80	20.76 ±2.80	15±0.5
B. subtilis ATCC 10876	18.09±4.36	21.00±3.77	16±0.2
E. coli ATCC 25922	13.50±1.27	17.66±1.32	14±0.3
E. cloacae ATCC 13047	11.35±2.52	15.50±2.72	12±0.8
P. aeruginosa ATCC 27853	7.43±1.032	12.43±1.32	11.±0.2

Table 4: Inhibitory effect of *B. madagascariensis* essential oils against bacteria (expressed as the minimum inhibitory concentration MIC and the minimum bactericidal concentration MBC).

Bacterial strains	MIC (µL/mL) (24 hours)	MBC (µL/mL) (72 hours)
B. cereus ATCC 6633	0.312	0.312
S. aureus ATCC 25923	0.625	0.625
B. subtilis ATCC 10876	0.312	0.312
E. coli ATCC 25922	0.625	0.625
E. cloacae ATCC 13047	1.25	1.25
P. aeruginosa ATCC 27853	2.50	2.50

4. DISCUSSION

During the extraction, it was noticed that the essential oils of the leaves of the *B. madagascariensis* was white color. This character was demonstrated by the relative density measured at 20 °C. The refractive index and optical rotation (dextrogyre) values of each essential oil was almost similar to the values found by others authors [2]. For the other physico-chemical indices such as the ester index, we found different values.

The ester index of *B. madagascariensis* oil is 53.150±1.866. This value was very high because it's richness in terpenic hydrocarbon components.

The results of the present study revealed a variation in chemical composition of essential oils. The leaves essential oil of *B. madagascariensis* was rich in hydrocarbon terpenic with a high proportion of α-thuyène (16.37%), α-pinène (15.46%), Terpinolène (11.33%), β-myrcène (8.31%), β-pinène (7.44%), α-terpineol (6.47%), Limonene (5.94%) and ϒ-terpinène (5.61%). The minor compounds where Sabinene (2.80%), α-terpinène (2.68%), Linalol (2.24%), Trans-p-menth-2-ène-1-ol (1.36%), 8-hydroxy-4-ène-3-one (1.34%), Cis-cis-dihydro-p-menthenolide (1.27%), 1, 8-cinéol (1.21%), p-cymène (1.11%), piperitenone (1.07%). The difference in the chemical composition of essential oils could explain the difference in antimicrobial activities of tested oils. This chemical composition is controlled by the environmental factors such as the climate, the geological nature of the site of harvest and the period of the harvest of the aromatic plants samples.[12-13]

Recent findings have indicated that essential oil extracts with MIC values below 100 µg/mL are considered promising as potential antimicrobial agents. In this study, the essential oils of the leaves of *B. madagascariensis* exhibited strong antibacterial effect against tested microorganisms showing MIC values lower than 15 µg/mL and lower than other studies in the literature.[46] Results of antimicrobial assay showed that the essential oil of B. madagascariensis was more active than the positive control (Penicillin G)). The values of MIC and MBC were identical for all tested bacterial strains. So, the Malagasy medicinal plants essential oils could act both by inhibiting bacterial growth and/or by killing them. This activity was found to be bacterial strains dependent. The present study indicates that medicinal plants species should be a powerful source of antimicrobial compounds due to their environment. Indeed, the plant kingdom offers a way of hope because of the enormous structural and chemical diversity of its secondary metabolites.[11] Historically, plants always have been confronted with microorganisms. They have evolved numerous chemical strategies for deterring pathogen attack, including the production of bactericidal and anti-infective compounds, leading to their use as medicines.[47]

However, despite the fact that plant pathogenic microorganisms have played a key role in the early evolution of the secondary metabolites diversity, there is little chance for a microbe to gain resistance from a plant as it is known for antibiotic producing microbes which possess genes protecting them from the toxic effects of these compounds. Plants, on the other hand, are genetically dissimilar from the microorganisms they are trying to eradicate. Like microbial antibiotics, plant antimicrobial compounds could kill pathogen via a non-species specific mechanism such as disrupting microbial cell membranes.[48]

However, it was recently reported that, plant secondary metabolites could also act against microbes by targeting cell's communication system (quorum sensing). The breakdown of this system causes an attenuation of microbial pathogenicity.[49]

The effect of essential oils on bacterial growth and quorum sensing is well known in the literature.[50-53] It was reported that essential oils act by destabilizing bacterial communities in the host. The anti-quorum sensing effect of essential oils may reduce pathogenicity, antibiotic resistance and biofilm formation.

It has been suggested that targeting pathogenesis instead of killing the microbial organism may provide less selective pressure and therefore decreased emergence of resistant strains.[54-55]

This study focused on the evaluation of chemical composition and the antimicrobial activity of the essential oils from C. greveanus, C. borarium and C. geayi which have been selected through ethno-botanical survey conducted in the south of Madagascar. At the end of this study, we have demonstrated that the essential oils from Croton species possess promising antimicrobial activity in vitro against tested bacteria.

Interactions between the major and minor constituents within each of oil could be responsible for the displayed inhibitory effects. The ability of the oils to display antibacterial activity may represent a rational explanation for the use of these aromatic plant species by the traditional healers to treat infections caused by pathogenic bacteria in Madagascar. This study makes them particularly interesting for further studies including cytotoxicity bioassay in order to evaluate the selectivity/therapeutic index of these essential oils before developing them as novel antimicrobial agents.

CONCLUSION

The present research work evaluated the chemical composition and the in vitro antimicrobial activity of the essential oil from *Brochounera madagascariensis*. This essential oil displayed promising antimicrobial activity in vitro. The ability of the oil to kill bacteria may represent a rational explanation for the use of this plant species by the traditional healers to treat incurable wounds and bacterial infections in Madagascar.

Further studies involving the evaluation of both cytotoxicity of this oil toward human cells and the role of the lead molecules in the antimicrobial activity of the essential oil and combinational studies with conventional drugs are in progress.

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