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Research article

Chemical composition, antimicrobial activity, and antioxidant capacity of *Micromeria flagellaris* Baker and *M. madagascariensis* Baker: Two endemic species from Madagascar as sources of essential oils

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ABSTRACT

Background: The aerial parts of *Micromeria madagascariensis* Baker and *M. flagellaris* Baker are used by the population of the Vakinankaratra and Itasy regions (Madagascar) to treat breathing difficulty, fever and/or headache, wounds, and sores.
Purpose: This work aimed to characterise plant materials from *M. madagascariensis* and *M. flagellaris* to report i) chemical composition, ii) antimicrobial properties, and iii) antioxidant capacity of the essential oils extracted from the aerial parts of these species.
Materials and methods: The essential oils from *M. madagascariensis* (MMO) and *M. flagellaris* (MFO) were obtained by hydrodistillation. Their chemical composition was quantified using gas chromatography coupled with mass spectrometry (GC-MS). MMO and MFO were also tested against 7 microbial strains using the disk diffusion method and their antioxidant capacity was assessed using the DPPH scavenging assay.
Results: Hydrodistillation yielded 0.26% MMO and 0.29% MFO (w/w) in relation to the fresh weight. Twenty-seven compounds were identified by GC-MS in MMO extract against 36 in MFO one. The main compounds in MMO were pulegone (24.67%), *trans*-menthone (24.67%), eucalyptol (8.12%), *β*-caryophyllene (4.98%), *α*-guanene (4.47), *iso*-menthone (3.85%), *iso*-pulegone (3.34%), azulene (3.28%) and 2-isopropyl-5-methylcyclohexenone (2.82%). The main compounds in the MFO were eudesma-4,11-dien-2-ol (13.88%), *δ*-guanene (6.62%), pulegone (6.40%), cyperone (5.56%), 4-*epi*-dehydrobietinol acetate (5.39%), eucalyptol (5.12%), *trans*-menthone (4.67%), limonene (3.77%) and sabinene (2.29%). Regarding the chemotaxonomy, *M. flagellaris* was very different from *M. madagascariensis* and both species also differed from the other *Micromeria* species, as confirmed by multivariate statistical analysis. Both MMO and MFO

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exerted activities against a large microbial spectrum; the antimicrobial activity of MMO was higher than MFO one against *S. pneumoniae* and *C. albicans* due to the presence of pulegone as the main component. MFO showed an excellent scavenging capacity with an SC_{50} value of 2.17 ± 0.03 μg/mL.

Conclusion: The biological properties of the essential oils extracted from the selected species may explain their therapeutic value showing that Malagasy *Micromeria* species may be very important as new natural sources of bioactive compounds. This study may promote the effectiveness and quality of Malagasy *Micromeria* species, contributing to sustainable development and commercial valorisation of traditional preparations based on natural local resources.

1. Introduction

Biodiversity conservation, reuse of natural resources, and sustainable rural development are the main challenges for the next years. Important public health issues should also be considered for their influence on productivity, health, and development in some rural areas, such as Madagascar, despite an abundance of often underexploited plant species, grown in seminatural conditions, with high health-promoting properties. The high price of pharmaceuticals makes traditional medicine and medicinal plants more attractive to 80% of the population in some African countries. Moreover, people prefer traditional medicine for several reasons including familiarity, tradition, and safety, but little scientific information is reported on these plant materials. The interest in phytochemicals, medicinal plants, and herbal preparations continues to grow, powered by increasing research into identifying natural substances, consumer demand, and public interest. Studies in different research fields on plants for their medicinal value and good health-promotion properties have considerably increased in recent years [1–3]. Currently, despite some effectiveness limits of specific products used in Allopathic Medicine to treat common, emerging or new diseases, research on natural products derived from plants to be used as drugs, pharmaceuticals, cosmetics or food additives is growing [4–9]. Among these products, the essential oils extracted from aromatic plants have received more attention for their biological properties – i.e. antioxidant, antimicrobial, anthelmintic, insecticidal, cytotoxic, and hemolytic activities [10–12]. Madagascar is an island remarkable for its flora biodiversity and endemicity which concerns 80% of its 14,000 plant species [13]. These endemic plants represent excellent sources of aromatic molecules of medicinal and economic interest for the rural population. 3245 medicinal plants distributed among 230 families and 1050 genera are classified in Madagascar [14]; however, no database has been available for aromatic plants and several plants have still been exploited for their essential oils. Scientific efforts are necessary to evaluate and offer new products to the expanding market for essential oils.

The genus *Micromeria*, belonging to the family of *Lamiaceae*, contains 93 species. Their vegetative forms include perennial herbs, subshrubs or shrubs, and rarely annual herbs that can reach 2–130 cm tall, with simple hairs and glands. They are aromatics and distributed from the Macaronesian-Mediterranean regions to southern Africa, in the Himalayan zone, and the north of America [15]. In Madagascar, this genus is represented by three endemic species including *Micromeria flagellaris* Baker, *M. madagascariensis* Baker, and *M. sphaerophylla* Baker [15]. The aerial parts of the endemic *Micromeria* spp. are used by the population of the Vakinankaratra and Itasy regions in case of breathing difficulty, fever, and/or headache by inhalation as a steam bath and local application of the crushed leaves on wounds and sores. Similar traditional uses of different *Micromeria* species in other countries have been also reported by several researchers. *M. dalmatica* Benth. is used against heart disorders, headaches, infections, and colds in the Mediterranean region [16]; the tea from the leaves of *M. cilicica* Hausskn. ex P.H.Davis is used against heart disorders and colds in Turkey [17]; *M. cristastata* (Hampe) Griseb., *M. myrtifolia* Boiss. & Hohen., and *M. juliana* (L.) Benth. ex Rchb. aerial parts were used in Turkey for bronchitis, common colds, diabetes, headaches, stomachache, kidney diseases, and prostrate treatments [18]. The essential oils from *Micromeria* species have been studied for their biological properties, in particular antioxidant and/or antimicrobial activities [17,19–25]. This work aimed to report for the first time the chemical composition, and the antimicrobial and antioxidant properties of the essential oils extracted from the aerial parts of the *M. flagellaris* and *M. madagascariensis.* The use of the aerial parts from these plants was chosen because only aerial parts are empirically used. Moreover, the plants are herbs, and their root biomass is generally low. The collection of the whole plant may contribute to its eradication reducing the local biodiversity; indeed, the species *M. madagascariensis* is already classified among the plants threatened with extinction according to the IUCN [26]. The population of the other Malagasy *Micromeria* spp. is also of low density.

2. Materials and methods

2.1. Plant materials

Both species were collected in the Itasy Region (Madagascar) during the flowering period in 2021 *M. flagellaris* aerial parts were collected at Tsiafajavona near the Ankaratra summit (19°21′12.4″ S, 047°14′39.4″ E, 1637 m above the sea level), while *M. madagascariensis* aerial parts were collected at Manalalondo (19°21′09.8"S, 047°14′39.2"E, 1720 m above the sea level). The species were identified by Dr Richard Stéphan Rakotonandrasana, the botanist of the Centre National d'Application des Recherches Pharmaceutiques (CNARP). The voucher specimens were deposited at the Herbarium of Medicinal Plants of Madagascar (CNARP) under the references RLL 1549 and RLL 1818 respectively.

2.2. Essential oils extraction

Fresh plant materials (1.5 g, post-harvest) were cut into small pieces (0.5–1.5 cm) and then hydrodistilled for 4 h using a Clevenger-type apparatus for light essential oils. The oils were dried over anhydrous Na_2SO_4. Then, the density was measured using a densimeter (Densito, Mettler Toledo, Italy) and the essential oils were kept in a sealed vial at 4 °C until analysis.

2.3. Gas chromatography-mass spectrometry (GC-MS and GC-FID) analysis

GC-MS analysis of the essential oils was performed using an Agilent 7200 GC (USA) coupled to a mass spectrometer Q-TOF (Agilent, USA). The GC instrument was equipped with an HP5 column (30 m × 0.25 mm, 0.25 μm) (Altmann Analytik, Germany). Helium was used as the carrier gas with a constant flow of 1.2 mL/h. The oven temperature was at 70 °C before the injection (1 μL in split mode), then maintained at this temperature for 2 min after the injection, and finally programmed to increase gradually to reach 310 °C at a rate of 15 °C/min for 10 min and held at this temperature for 28 min. Both injector and detector (FID) temperatures were kept at 220 °C. The MS detection was carried out by a Varian Saturn 2000 (Agilent, USA) ion-trap mass detector in electron-impact ionization mode. The identification of the components was performed based on their retention times and mass spectrum by comparing with the data of REPLIB (Replicate Electronic Impact Mass Spectra Library) and MAINLIB (Main electronic impact mass spectra Library). This approach is the current practice for the identification of peaks in GC chromatograms avoiding some problems and limitations due to the use of the retention index [27].

2.4. Antimicrobial assay

2.4.1. Microorganisms and culture condition

The antimicrobial activity was performed against three Gram-positive strains, including *Bacillus cereus* (ATCC 13061), *Streptococcus pneumoniae* (ATCC 6301), and *Staphylococcus aureus* (ATCC43300); four Gram-negative strains including *Escherichia coli* (ATTC 25922), *Klebsiella oxytoca* (ATTC 700325), *Enterobacter cloacae* (ATTC 700323), and *Salmonella enteridis* (ATTC 13076); and a yeast strain *Candida albicans* provided by the IMRA microbiology department. These microorganisms were selected to show the specificity of the antimicrobial activity of these essential oils. All the microorganism strains were maintained in culture on Mueller Hinton Agar (MHA) for bacterial and Sabouraud Dextrose Agar (SDA) supplemented with chloramphenicol for yeast, according to the manufacturer instructions, and growth at 37 °C for 24 h and 48 h, respectively.

2.4.2. Inoculum preparation

The inoculums were prepared from 1-day and 2-day-old cultures of bacteria and yeast, respectively, by scraping the colonies and putting them into a sterile test tube containing 5 mL of autoclaved 0.85% sodium chloride aqueous solution. The turbidity of the suspension was compared to a 0.5 Mc Farland standard solution corresponding to 1.5 × 108 CFU/mL for bacteria and 2.5 × 106 CFU/mL for yeast [28].

2.4.3. Preparation of oil and antibiotic solutions

The oil solutions were prepared by dissolving 30 μL of the essential oil in 30 μL of ethanol (purity = 99.8%, Merck, Germany). The reference products were streptomycin (Sigma-Aldrich, Germany) for bacteria strains and fluconazole (Sigma-Aldrich, Germany) for yeast strains, both prepared at a concentration of 20 μg/mL in ethanol. All the prepared solutions were stored at 4 °C before use.

2.4.4. Antimicrobial assay

The inoculum was uniformly spread on the surface of the nutrient agar for bacteria and on the surface of Sabouraud agar for yeast. Sterile filter paper discs (Whatman 6 mm in diameter) were impregnated with 20 μL of the oil or reference solutions (used as the positive control). Ethanol was used as the negative control for each test. Each Petri dish was incubated at 37 °C for bacteria and 25 °C for yeast for 24 h. Each experiment was performed in triplicate. The presence of a halo (inhibition zone) around the discs indicates the antimicrobial activity of the tested samples [29]. The diameter of the halo was measured.

2.5. Antioxidant assay

The free radical DPPH (Sigma-Aldrich, Germany) scavenging assay, previously described by Tombozara et al. [30], was slightly modified for the determination of the antioxidant capacity of the oils. The oil was tested at different rates in triplicate. A stock solution of oil in ethanol (50/50, v/v) was prepared. Then, five serial dilutions in ethanol were prepared as tested solutions (25/75; 12.5/87.5; 6.25/93.75; 3.12/96.88, and 1.56/98.44, v/v). Then, 25 μL of the ethanolic solutions of the oils were added to 175 μL of ethanolic solution of DPPH (0.25 mM) in a 96-well microplate and incubated at 25 ± 2 °C for 30 min. Ethanol was used as blank and an ethanol solution of DPPH was used as the negative control. Gallic acid (Sigma-Aldrich, Germany) with concentrations varying from 2.5 to 40 μg/mL was used as the positive control. The results were expressed as means of scavenging concentration (SC), calculated using the following equation: SC (%) = 100 × (ANC – AS)/ANC, where ANC and AS are the absorbance values of the negative control and the sample respectively measured at 517 nm. The scavenging concentration at 50% (SC_{50}) values of the oils and the gallic acid were calculated by linear regression by three replicates.

Table 1
Physical and chemical traits and antioxidant activity of the isolated essential oils from *Micromeria* spp.

	MFO	MMO	Gallic acid
Aspect	Limpid	Limpid	Solid
Colour	Yellow-orange	Yellow	White
Density	0.803	0.838	–
SC_{50} (μg/mL)	2.17 ± 0.03	–	12.85 ± 0.40

SC_{50} values represented the mean ± SD; a: $p < 0.01$ *vs* Gallic acid.
"-": do not calculated.

2.6. Statistical analysis

All the experiments were performed in triplicate and the results were subjected to a Student-*t*-test for mean comparison on SPSS 20.0 Software. The significance was considered for $P < 0.05$. Principal component analysis (PCA) was performed on the essential oils of 20 *Micromeria* species collected during their flowering stage to assess the correlations among the variables (chemical components) and group the individuals (plant species) with similar traits.

3. Results and discussion

3.1. Oil chemical composition and multivariate analysis

The hydro-distillation of aerial parts of *M. flagellaris* and *M. madagascariensis* extracted two oils with respective values of 0.29 and 0.26% (w/w) relative to the fresh materials. The yield of extraction obtained with the similar conditions varied according to the *Micromeria* species. For instance, *M. debilis* Pomel [24] yield was 0.1% (lower yield than *M. flagellaris* and *M. madagascariensis*), while *M. albanica* (Griseb. ex. K. Maly) Silic and *M. barbata* Boiss. & Ky yields were 0.88% and 2.0%, respectively (higher yield than *M. flagellaris* and *M. madagascariensis*) [19,22], showing that it is difficult to classify the extraction yields of Malagasy *Micromeria*. The oil traits are reported in Table 1. Twenty-seven compounds were identified in the *M. madagascariensis* oil (MMO) representing 99.99% of the isolated oil against 36 in the *M. flagellaris* oil (MFO) recovering 87.92% of the total components detected in the oil (Table 2). The main compounds in MMO were pulegone (24.67%), *trans*-menthone (24.67%), eucalyptol (8.12%), *β*-caryophyllene (4.98%), *α*-guanene (4.47), *iso*-menthone (3.85%), *iso*-pulegone (3.34%), azulene (3.28%), and the 2-isopropyl-5-methylcyclohexenone (2.82%). The main compounds in the MFO were eudesma-4,11-dien-2-ol (13.88%), *δ*-guanene (6.62%), pulegone (6.40%), cyperone (5.56%), 4-*epi*-dehydrobietinol acetate (5.39%), eucalyptol (5.12%), *trans*-menthone (4.67%), limonene (3.77%), and sabinene (2.29%). Most of these quantified compounds were oxygenated (ketonic and alcoholic terpenes); moreover, both oils were mainly characterised by oxygenated compounds such as monoterpenones (pulegones and menthones) and terpenols (Table 2). Only 9 of the 54 quantified molecules were found in both oils and 3 of them were considered as main compounds, including eucalyptol, pulegone, and *trans*-menthone. However, their amounts were significantly different in each oil. It may be due to the difference in the collection areas (i.e., the different altitudes among the considered areas), the anthropic conditions, and the edaphic characteristics based on the altitude in the Itasy region [31]. Pulegone inhibits the thermal nociception and increased the reaction latency [32], while *trans*-menthone significantly inhibits the release of pro-inflammatory cytokines, including TNF-α, IL-1β, and IL-6 [33]. These studies showed that these compounds are complementary in the treatment pain and inflammation-associated diseases such as breathing difficulty, fever, and/or headache.

Chemotype was defined by Santesson [34] as "chemically characterized parts of a population of morphologically indistinguishable individuals" [35]. The chemotype of *Micromeria* spp. was evaluated according to the basic structure of the main compounds of the essential oils for each species. Chemotype I included the monocyclic *p*-menthane monoterpenes, mainly piperitenone oxide, *iso*-menthone, pulegone, thymol, and *γ*-terpinene; chemotype II included the bicyclic pinane and camphane monoterpenes, mainly borneol, verbenol, *iso*-borneol and *β*-pinene; the several linear monoterpenes, sesquiterpenes, and other oxygenated compounds were classified in chemotype III. Therefore, the species *M. flagellaris* could be assigned to chemotype III, while the species *M. madagascariensis* could be assigned to chemotype I. The *Micromeria* essential oils could be classified into 3 groups according to their yield values (Table 3): i) the lower yields with a value under 0.5% containing all the chemotypes but dominated by the chemotype II, ii) the intermediate yields with value between 0.5% and 1.5% containing the chemotype I and III but dominated by the chemotype I, and iii) the higher yields with value above 1.5% containing all the chemotypes but also dominated by the chemotype I (Fig. 1). The χ^2 test ($\chi^2 = 12.23$, df = 4, N = 30, $p = 0.41$) of the species frequencies in each yield class did not show a significant difference. Therefore, the yield classification may be not used to assign the chemotype of *Micromeria* spp. It could be observed in the case of the Malagasy *Micromeria* species which were classified in the lower yields class because they belonged to chemotypes I and III.

Few similarities were observed among the chemical profiles of the essential oils in *M. madagascariensis*, *M. flagellaris*, and other *Micromeria* species (*M. cilicica*, *M. frivaldszkyana*, *M. fruticosa*, *M. libanotica*, and *M. thymifolia*). Principal Component Analysis (PCA) on the essential oils of the 18 *Micromeria* species similar to the considered Malagasy species was carried out to clarify the ambiguities among these species by combining data matrix derived from the percentages of 85 components (variables) of their essential oils (Tables S1 and S2). The correlations between the oil components with the two principal components PC1 (34.46% total variance) and PC2 (18.28% total variance) were highlighted in Fig. 2. Among the 85 compounds from the 20 species, 32 compounds (11

Table 2
The chemical component in the *Micromeria* spp. oils.

N°	Compound (abbreviation)	Rt (min)	Relative proportion (%)	
			MFO	MMO
1	**Sabinene**	4218	**2.29**	1.54
2	*α*-Thujene (ATJ)	4276	–	0.66
3	3-Octanone	4315	0.33	–
4	3-Octanol	4409	0.94	–
5	*p*-Cymene	4806	0.34	–
6	**Limonene**	4856	**3.77**	0.77
7	**Eucalyptol (1,8-Cineol)**	4901	**5.12**	**8.12**
8	Linalyl acetate (LA)	5626	0.32	–
9	*Trans-T*-Thujanol (tThujanol)	5628	–	0.79
10	*p*-Menth-2-enol (pMenthenol)	5894	1.15	–
11	Carveol	6053	1.17	–
12	***Iso*-Menthone**	6282	–	**3.85**
13	Menthofurane	6382	2.08	–
15	***Iso*-Pulegone**	6,53	–	**3.34**
16	**Pulegone**	6555	**6.40**	**24.67**
17	*α*-Terpinyl acetate (ATA)	6662	–	1.85
18	1,3,8-*p*-Menthatriene	6765	0.35	–
19	5-Isopropenyl-2-methylcyclopent-1-enecarboxaldehyde (IPMPCA)	6862	–	0.82
20	Carveol acetate (CarveolA)	6941	1.34	–
21	***Trans*-Menthone**	6985	**4.67**	**24.67**
22	Undecanol	7001	–	1.11
23	Carvone	7212	1.68	0
24	**Cyclohexenone, 2-isopropyl-5-methyl (CHIPM)**	7326	–	**2.82**
25	Cumenol	7649	0.44	–
26	Formic acid, decyl ester (FADE)	7960	–	0.69
27	*p*-Menthadienol	8012	0.36	–
28	(+)-4-Carene	8202	0.67	–
29	Eugenol	8280	0.35	–
30	*β*-Elemene	8631	0.47	1.13
31	Formic acid, undecyl ester (FAUE)	8868	–	0.77
32	***β*-Caryophyllene**	8926	0.44	**4.98**
33	***α*-Guanene**	9,05	–	**4.47**
34	***δ*- Guanene**	9052	**6.62**	–
35	Naphtalene	9110	1.38	1.15
36	Valencene	9174	2.69	–
37	Cycloundecatriene	9224	–	1.54
38	Longifolene	9293	3.23	0.71
39	**Azulene**	9431	–	**3.28**
40	γ-gurjunene	9432	3.64	–
41	Aciphyllene	9576	–	1.54
42	Nonyl-2-Methylbutanoate (NMB)	9739	–	0.61
43	Dimethylhexahydronaphthalenone (DMHN)	10,042	1.15	–
44	**Eudesma-4,11-dien-2-ol (EDOH)**	10,167	**13.88**	–
45	*Iso*-caryophyllene	10,259	2.29	–
46	Methylenedecahydronaphtalen-2-en-1-ol (MDHN)	10,318	2.49	1.74
47	10s,11s-Himachala-3(12),4-diene (HMD)	10,388	2.35	–
48	Globulol	10,479	1.48	–
49	Isopropenyl-1,4a,8-dimethyl-4,4a,5,6,7,8-hexahydro-3H-naphthalene-2-one (IPDMHHN)	11,247	–	1.67
50	**Cyperone**	11,577	**5.56**	–
51	Heptanoic acid heptyl ester (HAHE)	11,613	–	0.71
52	Valerenal or Dimethylhexahydroindenmethylacrylaldehyde (DMHIMAA)	12,028	0.45	–
53	**4-*Epi*-Dehydrobietinol acetate (EDHRA)**	13,756	**5.39**	–
54	Retinol	14,973	0.64	–
	Total		**87.92**	**99.99**
	Hydrocarbons		**30.53**	**21.77**
	Monoterpenes		8.80	7.40
	Sesquiterpenes		21.73	12.83
	Oxygenated compounds		**57.39**	**78.23**
	Monoterpenols		8.59	8.91
	Sesquiterpenols		17.85	0.00
	Monoterpenones		14.83	59.35
	Sesquiterpenones		5.56	1.67
	Aldehydes		0.45	0.82
	Esters		7.05	4.63
	Other oxygenated compounds*		3.06	2.85

"-": not detected.
*"Other oxygenated compounds" include alcohols and ketones that have not a terpenic structure.

Table 3
Yields of the main compounds of Micromeria oils obtained by hydrodistillation of wild aerial plant materials collected at the flowering stage.

N°	Species	Main compounds (%)	Oil yields (%)	Chemotype	References
1	*Micromeria albanica* (Griseb. ex. K. Maly) Silic	Piperitenone oxide (38.7), Pulegone (13.4), Piperitenone (9.7)	0.88	I	[19]
2	*M. barbata* Boiss. & Ky	Pulegone (20.2), Limonene (16.6), Neomenthol (12.4)	2	I	[22]
3	*M. biflora* (Buch.-Ham. ex D.Don) Benth.	Geranial (39.0), neral (28.7)	0.26*	III	[37]
4	*M. carminea* P.H.Davis	Borneol (26.0), Camphor (10.6)	0.14	II	[38]
5	*M. cilicica* Hausskn. ex P.H.Davis	Pulegone (66.6), *cis-p*-Menthone (21.7), *trans-p*-menthone (9.59)	0.88	I	[17]
6	*M. congesta* Boiss. et Hausskn. ex Boiss.	Piperitenone oxide (45.0), Pulegone (11.8), Verbenone (9.4)	3	I	[39]
7	*M. cremnophila* Boiss. & Heldr.	Germacrene-D (24.0), *β*-Caryophyllene (22.7), Caryophyllene oxide (9.9)	0.02	III	[40]
8	*M. cristata* (Hampe) Griseb.	*iso*-Borneol (11.3), Borneol (8.5), 10-*epi*-*α*-Cadinol (8.2), Thujan-3-ol (8.0)	0.1	II	[41]
9	*M. croitica* (Pers.) Schott	Caryophyllene oxide (17.1), (*E*)-*β*- Caryophyllene (13.3)	1.63*	III	[42]
10	*M. dalmatica* Benth.	Piperitenone oxide (41.8), Pulegone (15.9), Piperitenone (10.2)	1.11	I	[19]
11	*M. debilis* Pomel	*β*-Pinene (19.3), Germacrene D (11.4), Geranial (8.7), *(E)-β-C*aryophyllene (8.0), Caryophyllene oxide (8.0)	0.1	II	[24]
12	*M. dolichodontha* P.H.Davis	*iso*-Menthone (23.5), Piperitone oxide (16.9), Pulegone (14.9), Piperitone (10.3)	1.3	I	[43]
13	*M. flafellaris* Baker	Eudesma-4,11-dien-2-ol (13.9), *δ*-Guanene (6.6), Pulegone (6.4), Cyperone (5.6)	0.29	III	Present study
14	*M. frivaldszkyana* (Degen) Velen.	Pulegone (51.9), *p*-Menthone (22.5)	0.26*	I	[44,45]
15	*M. fruticosa* Druce	Pulegone (57.2), *iso*-Menthone (20.9)	2.6	I	[46]
16	*M. fruticulosa* (Bertol.) Grande	*γ*-Terpinene (14.5), *β*-Caryophyllene (12.6), *p*-Cymene (8.9), *α*-Pinene (8.2), *β*-Bisabolene (7.2)	1.6	I	[20]
17	*M. gracea* (L.) Benth. ex Rchb.	*α*-Bisabolol (14.7), Caryophyllene oxide (4.7), Linalool oxide (5.4), Spathulenol (4.5)	0.55*	III	[47]
18	*M. hedgei* Rech. F.	Geranial (18.0), Neral (13.8), Geraniol (13.2), Nerol (7.7), Carvacrol (6.2)	1.12	III	[23]
19	*M. herpyllomorpha* Webb & Berthel.	*α*-pinene (9.2), Borneol (6.9)	0.05*	II	[48]
20	*M. hyssopifolia* Webb & Berthel.	Borneol (13.7), *α*-Pinene (8.3)	0.15	II	[48]
21	*M. inodora* (Desf.) Benth.	*α*-Terpinyl acetate (29.1), *cis*-14-*nor*- Muurol-5-en-4-one (13.8)	0.1	III	[49]
22	*M. juliana* (L.) Benth. ex Rchb.	Verbenol (11.8), Thymol (10.8), Caryophyllene oxide (10.5), Borneol (9.3)	0.1	II	[41]
23	*M. lachnophylla* Webb & Berthel.	Borneol (22.0), Bornyl acetate (16.9), Camphene (10.0), Camphor (9.4)	0.14	II	[48]
24	*M. lasiophylla* Webb & Berthel.	Borneol (24.9), Linalool (11.0)	0.14	II	[48]
25	*M. libanotica* Boiss.	*iso*-Menthone (44.5), Pulegone (13.5), *iso*-Pulegone (6.5)	1.1*	I	[50]
26	*M. madagascariensis* Baker	Pulegone (24.7), *Trans*-Menthone (24.7), Eucalyptol (8.1), *β*-Caryophyllene (5.0)	0.26	I	Present study
27	*M. myrtifolia* Boiss. & Hohen.	*β*-Caryophyllene (15.5), Caryophyllene oxide (14.8), Germacrene D (4.9)	0.24	III	[21]
28	*M. persica* Boiss.	Thymol (28.6), Limonene (20.7), *γ*-Terpinene (17.5), *p*-Cymene (17.5)	3.2	II	[51]
29	*M. thymifolia* (Scop.) Fritsch	Pulegone (44.8), Piperitone oxide (14.5), *iso*-Menthone (9.3), Limonene (8.0)	0.49	I	[25]
30	*M. varia* Benth.	Borneol (19.2), *α*-Pinene (13.9), (*E*)-Nerolidol (13.1)	0.45	II	[48]

* mean value of the same species.

sesquiterpenes, 12 monoterpenes, 9 other compounds) showed a positive correlation ($r > 0.5$) with PC1, including sabinene, menthofurane, carveol acetate, and D-carvone as the major contributors; 17 compounds (3 sesquiterpenes, 3 monoterpenes and 11 other compounds) showed a positive correlation ($r > 0.5$) with PC2, including thujanol, undecanol, *iso*-pulegone, and azulene as the main contributors; and 3 compounds (1 sesquiterpene, 1 monoterpene and 1 other unknown) were in intermediate position between PC1 ($r > 0.5$) and PC2 ($r > 0.5$) (Fig. 2, Table S3). Components could be classified into 3 groups; group 1 included the components with a strong positive correlation with PC1 such as 3-octanone and 1,3,8-*p*-menthatriene ($r = 0.993$), carvone and carveol ($r = 0.804$), longifolene and δ-guanene (0.999); group 2 included the components with a strong correlation with the PC2 such as thymol and undecanol ($r = 1$), α-terpinyl acetate (ATA) and 5-isopropenyl-2-methylcyclopent-1-ene carboxaldehyde ($r = 1$), emelene and azulene ($r = 0.973$); and the third group could be attributed to the components with a negative correlation with both PCs (e.g. *trans*-menthone

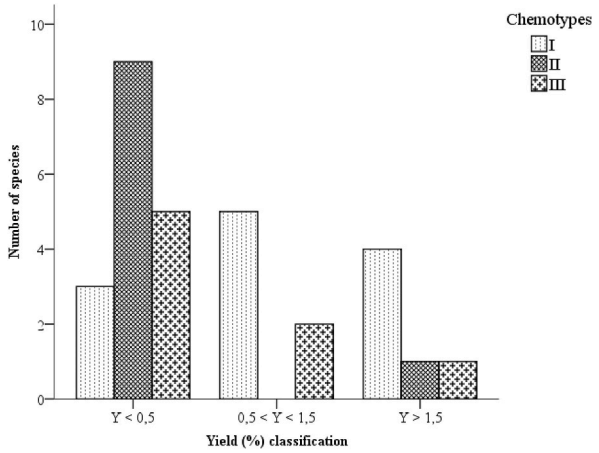

Fig. 1. Yield classification *vs* chemotypes of the 30 *Micromeria* species ($\chi^2 = 12.23$, (df = 4, N = 30), p = 0.41).

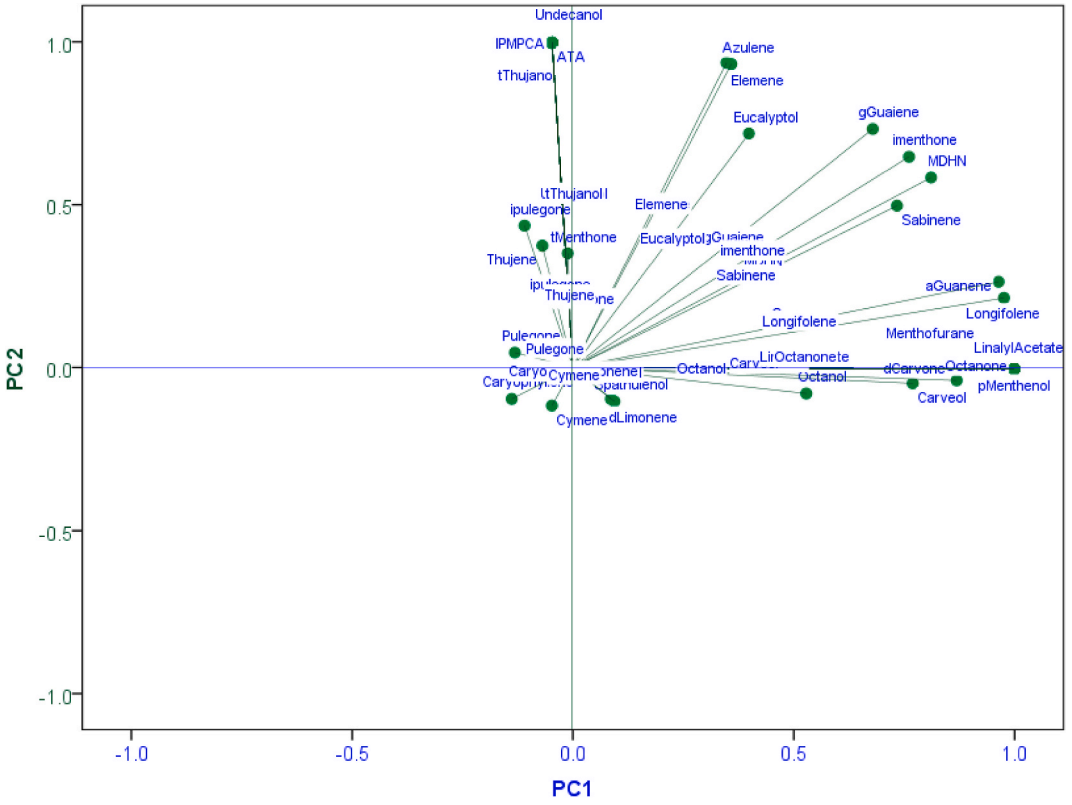

Fig. 2. Loading plot of the essential oils from the selected species similar to Malagasy *Micromeria* species (PC: principal component).

and humulene, r = – 0.305; *trans*-menthone and caryophyllene oxide, r = – 0.302; myrcene and formic acid, undecyl ester r = – 0,824). Similar biosynthesis pathways of these components could be the reason for strong positive correlations, while the negative correlations could be explained by the origin of these molecules (i.e., the first substance may be the precursor of the other ones, for instance, *trans*-menthone and caryophyllene oxide) [36]. The species selected for the PCA may be also classified into three groups; groups 1 and 2 included a single species, *M. flagellaris* and *M. madagascariensis,* respectively, and the other species were included in the third group (Fig. 3). The separation of the Malagasy endemic species compared to other *Micromeria* species may be considered as a distinction of these species according to their distributions (Fig. 3). As the cumulative variance of the PCs represented 52.73% of the information and the two endemic species of Madagascar were oppositely placed compared to the other species, a hypothesis about a difference at the genus level of these Malagasy endemic species (*M. madagascariensis* and *M. flagellaris*) and the other evaluated *Micromeria* species could

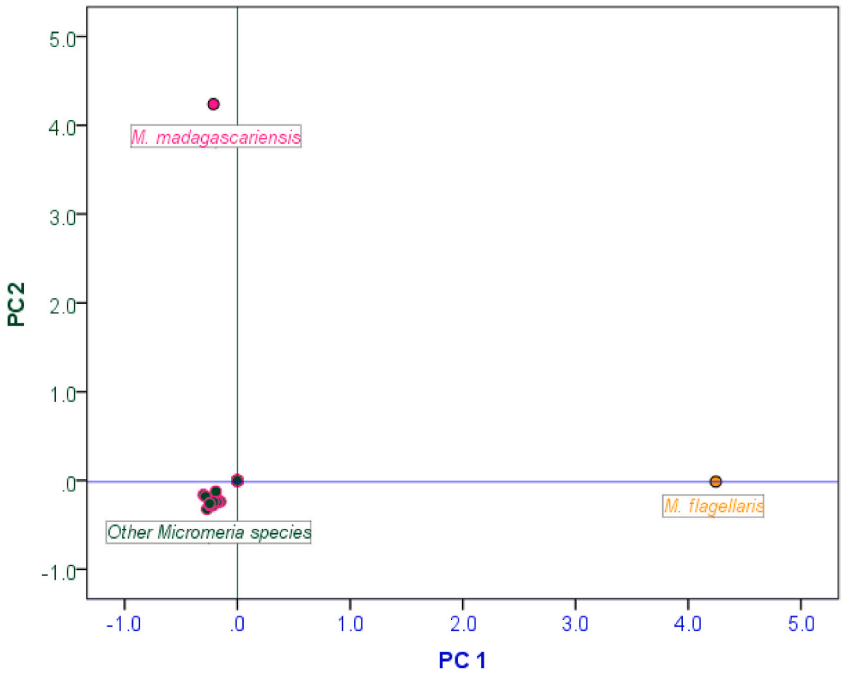

Fig. 3. Score plot of the selected species, similar to the Malagasy *Micromeria* species (PC: principal component).

Table 4
Antimicrobial activities of the *Micromeria* spp. oils.

Microorganism	Zones of inhibition (mm)			
	MFO	MMO	Streptomycin	Fluconazole
Gram (+)				
- *Bacillus cereus* (ATCC 13061)	$7.00 \pm 0.62^{a,b}$	13.00 ± 0.89^{a}	16.97 ± 0.55	NT
- *Streptococcus pneumoniae* (ATCC 6301)	$12.03 \pm 1.15^{a,b}$	18.13 ± 0.96	19.13 ± 071	NT
- *Staphylococcus aureus* (ATCC43300)	9.07 ± 1.37^{a}	8.20 ± 0.90^{a}	23.23 ± 0.75	NT
Gram (−)				
- *Escherichia coli* (ATTC 25922)	11.40 ± 1.28^{a}	9.50 ± 1.41^{a}	16.40 ± 1.35	NT
- *Enterobacter cloacae* (ATTC 700323)	$12.00 \pm 1.00^{a,b}$	9.50 ± 0.80^{a}	14.87 ± 1.40	NT
- *Klebsiella oxytoca* (ATTC 700325)	11.20 ± 1.35^{a}	9.50 ± 1.32^{a}	18.63 ± 1.31	NT
- *Salmonella enteridis* (ATTC 13076)	$10.03 \pm 0.99^{a,b}$	12.07 ± 0.60	13.03 ± 0.61	NT
Yeast				
Candida albicans	$10.00 \pm 0.36^{a,b}$	14.67 ± 0.40	NT	15.43 ± 1.30

Values are expressed as mean ± SD (n = 3).
NC: Negative control; MMO: *M. madagascariensis* oil; MFO: *M. flagellaris* oil; NT: not tested a: $p < 0.05$ *vs* positive control
b: $p < 0.05$ *vs* MMO.

be considered. This hypothesis may be very important for the proposal of Brauchler et al. [15] on the classification of these two Malagasy *Micromeria* species in new or other genera to be determined. Moreover, the separation of these Malagasy species, in relation to PC1, *M. madagascariensis* in positive and *M. flagellaris* in negative (Fig. 3), showed that the two species are largely distinguished by their chemotaxonomy implying that they might belong to two different genera.

3.2. Antimicrobial activities

The antimicrobial activity of MFO and MMO together with the streptomycin and fluconazole, used as positive controls, were reported in Table 4. The highest inhibition zone expressed by MMO and MFO with the respective values of 18.13 ± 0.96 ($p > 0.05$ *vs* streptomycin) and 12.03 ± 1.15 mm were on *S. pneumoniae* against 19.13 ± 0.71 for the streptomycin used as positive control. According to Ponce et al. [52], these values were classified as very sensitive and sensitive respectively. *S. pneumoniae* is one of the bacteria responsible for acute respiratory tract infections causing difficulty breathing [53]. MFO was not sensitive on *B. cereus* with an inhibition zone of 7.00 ± 0.62 mm (< 8 mm) according to Ponce et al. [52]. MMO was slightly more active than MFO on the Gram (+) strains ($p < 0.05$), except for *S. aureus*. MFO was slightly more active against Gram (−) than MMO, except for the *S. enteridis* and the difference among the areas of inhibition was statistically significant ($p < 0.05$). Moreover, the zone of inhibition of MMO and

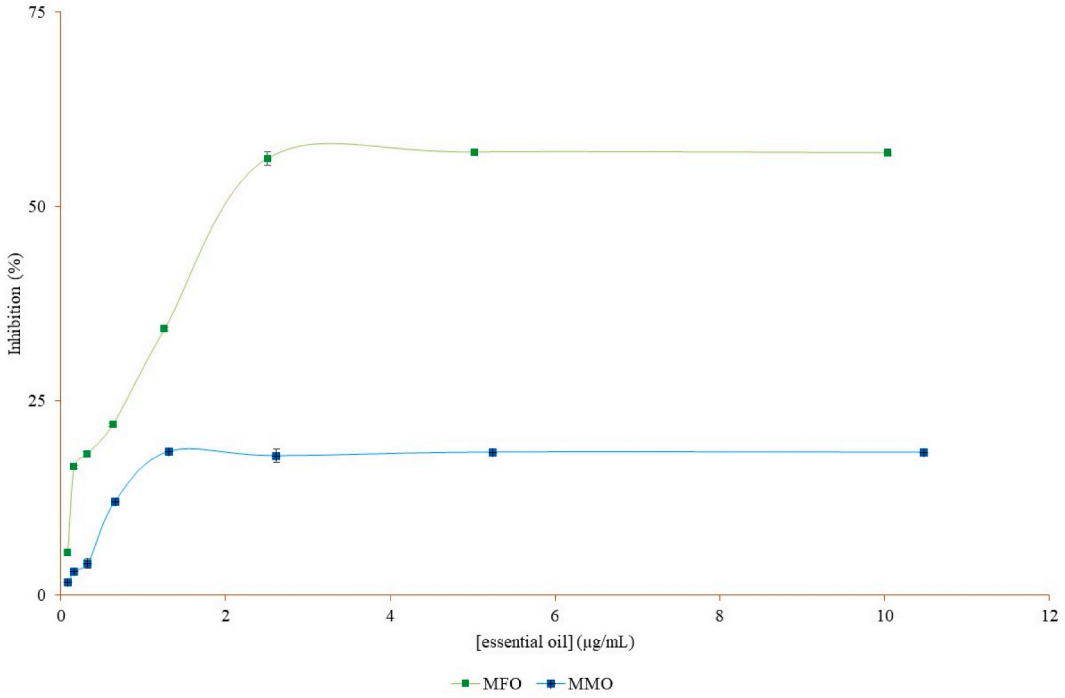

Fig. 4. Antioxidant activity of MFO and MFO.

streptomycin did not show a significant difference in *S. enteridis*. Gram (+) strains were more sensitive to MMO and Gram (−) strains were more sensitive to MFO (Table 4). Compared to the other considered *Micromeria* species, Gram (+) bacteria were more sensitive than Gram (−) for species belonging to chemotype I such as *M. albannica*, *M. dalmatica*, *M. thymifolia*, *M. cilicica*, *M. fruticulosa*, and *M. barbata* [17,19,20,22] and the opposite was observed for *M. hedgei* belonging to the chemotype III [23]. The activity of the essential oils may be due to the ease of low-polarity compounds to penetrate the cell membranes of microbial strains [54]. Regarding yeast inhibition, the activity of MMO was comparable to the values of fluconazole and significantly more active than MFO ($p < 0.05$). It may be due to the presence of Pulegone as one of the main compounds in MMO which shows a high anticandidal effect [17]. Overall, the inhibition exerted by the considered essential oils from Malagasy *Micromeria* spp. on the different microbial strains could explain their traditional applications in the local treatment of wounds and sores, as well as their use by inhalation in the treatment of respiratory difficulty-related diseases, mainly due to the *S. pneumoniae* infections [53].

3.3. Antioxidant capacity

The antioxidant capacity of MFO and MMO was evaluated by their capacity to scavenge the radical DPPH and highlighted in Fig. 4. MFO showed a saturation point at the concentration of 2.51 µg/mL and reached the maximal inhibition of the radical DPPH with the value of 56.95 ± 0.42%, while MMO showed a saturation point at the concentration of 2.62 µg/mL with a maximal inhibition value of 18.36 ± 0.22 %. The SC_{50} of MFO together with the gallic acid (positive control) was reported in Table 1, while those of MMO could not be calculated because the maximal inhibition value was 18.36% (<50%). This method is based on the ability of the oil components to release protons for stable radicals [25]. Both oils showed antioxidant capacity against the radical DPPH, and their activities were better than the other *Micromeria* spp. (*M. myrtifolia* and *M. thymifolia*) essential oils tested against the radical DPPH [21,25]. The antioxidant activity of MFO was significantly higher than that of MMO ($p < 0.01$) regarding the maximal inhibition value. It might be due to the presence of some minor compounds including phenolic terpenes (eugenol and cumenol) and other conjugated alcohols (carveol and retinol). Antioxidants are essential for the cells and tissues to protect them against the radical oxygen species produced by the body during oxidative stress and help in wound and sore recovery.

4. Conclusion

In this study, the essential oils of two Malagasy *Micromeria* species have been isolated and their chemical components were quantified. In the *M. madagascariensis* (MMO), monocyclic monoterpenes, including pulegone (24.67%) and *trans*-menthone (24.67%), were the main detected compounds, while *M. flagellaris* (MFO) was characterised by sesquiterpenes, including eudesma-4,11-dien-2-ol (13.88%), δ-guanene (6.62%). Chemotaxonomy of these species compared to the other ones was reported and the results showed that the variation in the chemical components of the essential oils of *M. madagascariensis* and *M. flagellaris* showed a significant difference

among themselves and also concerning the other essential oils of the other species considered in this study. Moreover, both MMO and MFO showed broad-spectrum antimicrobial activity. In particular, the activity of MMO was very important against *S. pneumoniae* and *C. albicans*, probably due to the presence of pulegone as the main component. MFO showed a good antioxidant capacity compared to MMO. The biological properties of the essential oils extracted by these species may explain their therapeutic values showing that Malagasy *Micromeria* species could be excellent plant materials in the research for new natural sources of bioactive compounds.

These plants may be important in meeting the high demand for natural medicines in Madagascar for cost-effectiveness and safety; natural health-promoting preparations may be produced by industries and used by the local population. This study may promote the effectiveness and quality of Malagasy *Micromeria* species, contributing to sustainable development and commercial valorisation of traditional preparations based on natural local resources.

CRediT authorship contribution statement

Haja Mamison Edouard Rakotofina: Writing – original draft, Methodology, Investigation, Formal analysis. **Dario Donno:** Writing – review & editing, Validation. **Nantenaina Tombozara:** Writing – original draft, Validation, Methodology, Formal analysis. **Zoarilala Rinah Razafindrakoto:** Validation, Methodology. **Stéphan Richard Rakotonandrasana:** Methodology, Investigation. **David Ramanitrahasimbola:** Writing – review & editing, Validation, Supervision. **Solofoherimanana Andrianjaka:** Writing – review & editing. **Valeria Torti:** Writing – review & editing. **Gabriele Loris Beccaro:** Writing – review & editing. **Marcelle Rakotovao:** Writing – review & editing, Validation, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e26865.

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