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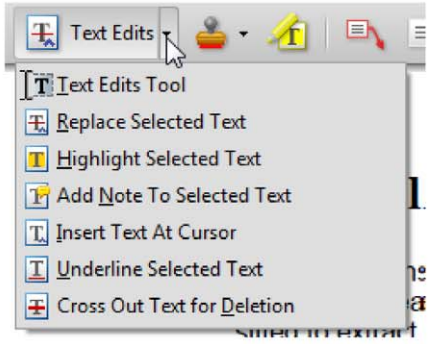

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Phytochemical investigation of the essential oil from the 'resurrection plant' *Myrothamnus moschatus* (Baillon) Niedenzu endemic to Madagascar

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The composition of the essential oil hydrodistilled from the aerial parts of the 'resurrection plant' *Myrothamnus moschatus* (Baillon) Niedenzu endemic to Madagascar was investigated for the first time by gas chromatography (GC/flame ionization detector and GC/mass spectrometry). Forty components were identified in the oil, representing 98.2–98.6% of the total composition. The oil composition was dominated by oxygenated monoterpenes (73.5–75.0%), with *trans*-pinocarveol (35.6–36.3%) and pinocarvone (19.8–20.0%) as the most representative. Other components occurring in significant amounts were β-selinene (8.5%) and perillyl acetate (6.0–12.7%). No significant differences were detected in the major volatiles between the two different biological forms (flowering period and dry season) in which the plant naturally occurs.

Keywords: *Myrothamnus moschatus*; Myrothamnaceae; essential oil composition; GC/FID and GC/MS; *trans*-pinocarveol; pinocarvone; α-pinene

Introduction

Of the approximately 260,000 species of angiosperms, about 100 are called 'resurrection plants', i.e. those that, by various means, are able to recover from complete desiccation (1). These plants are termed 'desiccation tolerant' and have the remarkable ability of being able to survive in an air-dried state for months.

Beside angiosperms, the majority of resurrection plants are mosses, ferns and other spore-bearing plants, but there are a couple of flowering plants that are poikilohydric, e.g. those in genus *Myrothamnus* ('myron' meaning aromatic and 'thamnos' meaning bush), which is apparently the only woody shrub that has the ability to resurrect. These plants are characterized by roots and leaves that possess the rare capacity of surviving virtually to a complete desiccation by loss of most of their tissue water content until a quiescent stage is achieved. In severely drought-stressed areas, the plants can remain quiescent for considerable periods of time, but at the first substantial fall of rain they can resurrect, and grow and reproduce long before other species have the opportunity to do so (1).

The Myrothamnaceae family comprises one genus, *Myrothamnus*, including only two species: the most studied *Myrothamnus flabellifolia* Welw. from arid mountains in Sub-Kalaharian Africa (2–5), and the rare *Myrothamnus moschatus* (Baillon) Niedenzu.

Myrothamnus moschatus (synonym *Myosurandra moschata* Baillon) is a small shrub about 0.5 m in height native to Madagascar, where it grows on shallow granitic soils and is subjected to drought during the dry winter months (6).

It is known under several Malagasy vernacular names according to ethnic groups (between brackets):

- '*Maimbeloma'* (Merina): *maina*=dry, *belona*∼*velona*=alive; this name reflects the resurrection property of the species;
- '*Maharoaka', maroaky, maharoaky*, (Antandroy): *maha*=capable of, *roaka*∼*mandroaka*=expelling; this name refers to the magical ethnobotanical use of the plant capable of expelling devil; another meaning is to facilitate divorce;
- '*Somorombato*' (Bara): *somorona* is the common name of plants that are smoked in Madagascar for their narcotic properties, mbato∼*vato*=stones; this name reflects both the narcotic properties of the species and its ecology;
- '*Radiatra'* (Antanosy) from *riatra*, that means 'making noise if burned';
- '*Fanalalahy', 'fanalahy*' (Betsileo): *fanala*=action of removing, *lahy*=male, this name designates the use of the plant as a magical philtre to create problems and confusions among married persons, leading to divorce.

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When fresh, the plant emits a pleasant and characteristic balsamic odor. Once desiccated, the plant is collected for different purposes. The dried leaves are smoked like a cigar for the treatment of asthma; they are also used in infusion against cough (6). They can also be employed as a psychoactive plant in the South region of Madagascar, near Betroka, Ivohibe and around Beraketa. In the central highlands, the decoction is used against vomiting and the leaves are burned for magical rituals (7).

While most of phytochemical investigations focused on *M. flabellifolia*, little is known of this species, as no extensive scientific investigation has been performed so far, although its potential biological activity was reported (8).

Therefore, in the present work, we performed the first phytochemical investigation of the essential oil, using gas chromatography [GC/flame ionization detector (FID) and GC/mass spectrometry (MS)], obtained from the aerial parts of the plant collected in the two main phases of the plant biological cycle.

Experimental

Plant material

Aerial parts of *M. moschatus* were collected in the flowering period in January 2010, and in the dry season in May 2011, in the Isalo Region (southwestern Madagascar). The plant was identified by botanists at the Parc Botanique et Zoologique de Tsimbazaza, Antananarivo. A voucher specimen was deposited in the Herbarium of the Institut Malgache de Recherches Appliquées (IMRA), under the accession code MAD-0013/RECs.

Extraction of the essential oil

The essential oil of *M. moschatus* was obtained by using a portable alembic. Freshly collected aerial parts (6 kg) were extracted by steam distillation for 3 hours yielding 0.73% of yellowish oil for the batch collected in January, and 0.67% for the batch collected in May. Before analysis, the oil was dried with anhydrous Na_2SO_4, transferred into an amber glass flask and kept at a temperature of −20°C.

Chemicals

Standard compounds used for identification (Table 2) of essential oil constituents were purchased from Sigma-Aldrich (Milan, Italy). For retention index determination, a mix of hydrocarbons ranging from *n*-octane (C_8) to *n*-triacontane (C_{30}) (Supelco, USA) was used and run at the experimental conditions reported below. For the determination of GC-FID response factors (RFs), the following standards purchased from Sigma-Aldrich were also used: isobornyl acetate for monoterpenoid acetates; octane and octadecane for alkanes; *p*-cymene for aromatic monoterpenes; β-pinene, γ-terpinene and limonene for monoterpene hydrocarbons; *trans*-pinocarveol, 1,8-cineole, terpinen-4-ol and carvone for oxygenated monoterpenes; (*E*)-caryophyllene and α-humulene for sesquiterpene hydrocarbons. All compounds were of analytical standard grade. Analytical grade *n*-hexane solvent was purchased from Carlo Erba (Milan, Italy); it was successively distilled by a Vigreux column before use.

GC/FID and GC/MS conditions

For GC separations, an Agilent 4890D instrument coupled to an FID was used. Volatile components were separated on a HP-5 capillary column (5% phenylmethylpolysiloxane, 25 m, 0.32 mm inner diameter, i.d.; 0.17 μm film thickness) (J and W Scientific, Folsom, CA), with the following temperature program: 5 minutes at 60°C, subsequently 4°C/minute up to 220°C, then 11°C/minute up to 280°C, held for 15 minutes, for a total run of 65 minutes. Injector and transfer line temperatures were 280°C. Helium was used as the carrier gas, at a flow rate of 1.4 mL/minute; injection volume: 1 L; split ratio, 1:34. A mixture of aliphatic hydrocarbons (C_8–C_{30}) (Sigma) in hexane, was directly injected into the GC injector under the above temperature program, in order to calculate the linear retention index of each compound. Oil samples were diluted 1:100 in acetone and injected at a volume of 1 μL. The analysis was repeated three times. Data were collected by using HP3398A GC Chemstation software (Hewlett Packard, Rev. A.01.01). The GC/FID analyses were run in triplicate. The reliability of the analytical method was demonstrated by the values of CV%, which were in the range 0.2–11.7%. The highest values of CV% were observed only for some peaks present at low levels (<0.1% peak area). GC-MS analysis was performed on an Agilent 6890N gas chromatograph coupled to a 5973N mass spectrometer using a HP-5MS (5% phenylmethylpolysiloxane, 30 m, 0.25 mm i.d., 0.1 μm film thickness) (J and W Scientific), using the same temperature programme reported above. Injector and transfer line temperatures were 280°C. Helium was used as the carrier gas, at a flow rate of 1 mL/minute. Injection volume: 2 L; split ratio: 1:50; acquisition mass range: 29–400 *m/z*. All mass spectra were acquired in electron impact (EI) mode with a ionization voltage of 70 eV. Oil samples were diluted 1:100 in acetone and the volume injected was 2 μL. The analysis was repeated three times. Data were analyzed by using MSD ChemStation software (Agilent, Version G1701DA D.01.00).

Qualitative and quantitative analysis

For 15 of 40 compounds, corresponding to 48.6–55.7% of the total oils, the identification was carried out by

co-injection with authentic standards (Table 2). Otherwise, the peak assignment was based in accordance with the standard of the International Organization of the Flavor Industry (IOFI, http://www.iofi.org/) (9) statement, i.e. by combining the computer matching with the WILEY275, NIST 08 and ADAMS libraries, with the correspondence of the calculated retention indices with respect to those reported by Adams (10) and NIST 08 (11). Quantification of essential oil components was obtained by FID peak-area internal normalization for the calculation of the FID RFs, which were calculated by using different available standard compounds representative of the main chemical groups of volatiles detected in the essential oil of *M. moschatus* (Table 1). Each standard, diluted in *n*-hexane, was injected at four different concentrations (0.04, 0.08, 0.16 and 0.4 mg/mL) using *n*-octane and *n*-octadecane as internal standards at a concentration of 0.04 mg/mL. For each standard, a regression line was obtained by plotting the ratio of internal standard (mean value of *n*-octane and *n*-octadecane) to reference standard peak area versus the ratio of internal standard to reference standard concentration. Each dilution was analyzed five times. The correlation coefficients (R^2) obtained for calibration curves of representative standards were all higher than 0.997.

Results and discussion

The essential oil compositions of *M. moschatus*, obtained during the two different biological phases, are reported in Table 2, whereas the GC/FID chromatograms of the same oils are depicted in Figure 1.

A total of 40 volatile components were identified in the essential oils obtained from the two different biological forms of the plant, corresponding to 98.2–98.6% of the total compositions. The major components, accounting together for 80.6–83.1% of the oils, were the bicyclic monoterpenoids *trans*-pinocarveol (35.6–36.3%) and its oxidation product pinocarvone (19.8–20.0%). These volatile constituents will also be released and absorbed during rituals in which the smoke from the burnt leaves is inhaled (4). Other constituents detected in significant levels were α-pinene (5.8–10.5%), β-selinene (8.5%) and perillyl acetate (6.0–12.7%). Most of the oil was constituted by oxygenated monoterpenes (73.5–75.0%), with alcohols and ketones the most abundant. The remaining was given mainly by monoterpene hydrocarbons (13.5–15.9%) and sesquiterpene hydrocarbons (8.7–8.8%).

No qualitative differences were noticed comparing the compositions obtained for the two different biological forms of the plant, as clearly evident in the comparison of the chromatograms reported in Figure. 1. From a quantitative point of view, in the desiccation period, there was only a decrease in the α-pinene content (from 10.5% to 5.8%) and a corresponding increase in that of perillyl acetate (from 6.0% to 12.7%), whereas the other major compounds remained constant. Thus, we can assume that environmental conditions did not qualitatively influence the biosynthesis of the major volatiles of the plant within the different biological forms in which the plant occurs; however, the increase of perillyl acetate can be indicative of a presence of more active constituents. In fact, this molecule is an

Table 1. Determinations of gas chromatography/flame ionization detector (GC/FID) response factor (RF), expressed as slope of the regression line, for different classes of volatiles occurring in *Myrothamnus moschatus*.

Grouped compounds	R^{2a}	Slope[b]	RF±SD[c]
Monoterpenes hydrocarbons			
Β-Pinene	0.999	1.00	1.10±0.08
Limonene	0.999	1.16	
γ-Terpinene	0.999	1.09	
Aromatic monoterpenes			
P-Cymene	0.998	1.09	1.09±0.00
Monoterpenoid acetates			
Isobornyl acetate	0.998	1.45	1.45±0.00
Oxygenated monoterpenes			
trans-Pinocarveol	0.998	1.45	1.44±0.10
1,8-Cineole	0.999	1.34	
Terpinen-4-ol	0.998	1.39	
Carvone	0.997	1.58	
Sesquiterpene hydrocarbons			
(*E*)-Caryophyllene	1.000	0.95	1.05±0.14
α-Humulene	0.999	1.15	

[a]R^2, linear regression coefficient.
[b]Slope obtained of the straight regression line in equation of the curve for each reference standard compounds.
[c]Response factor (RF) for a volatile class as the mean of the slopes in the regression lines of representative compounds belonging to that class.

Table 2. Composition of the essential oils from the aerial parts of *Myrothamnus moschatus* obtained during desiccation and re-hydration period.

No.	Component[a]	RF[b]	RI[c]	Literature RIs[d]		Biological form				ID[e]
						Desiccation		Re-hydration		
				ADAMS	NIST 08	% (n=3) ±SD	RSD%[f]	% (n=3)	RSD%[f]	
1	α-Thujene	1.10	926	930	929			tr	5.8	Std
2	**α-Pinene**	**1.10**	**932**	**939**	**937**	**5.8±0.20**	**3.4**	**10.5±0.05**	**0.5**	**Std**
3	Camphene	1.10	946	954	949	0.2±0.01	4.6	0.3±0.00	0.3	Std
4	Thuja-2,4(10)-diene	1.10	952	960		0.1±0.00	4.4	tr[g]	2.7	RI,MS
5	β-Pinene	1.10	974	979	976	0.4±0.02	3.5	0.7±0.00	0.6	Std
6	Myrcene	1.10	992	990	991	tr	4.6	0.1±0.00	2.6	Std
7	*p*-Cymene	1.09	1026	1024	1024	0.9±0.01	0.8	1.6±0.01	0.6	Std
8	Limonene	1.10	1030	1029	1026	0.4±0.01	1.8	2.3±0.00	0.2	Std
9	1,8-Cineole	1.44	1032	1031	1032	0.4±0.00	0.4	0.7±0.03	3.9	Std
10	*p*-Cymenene	1.10	1089	1091		0.1±0.00	4.4	0.2±0.00	2.8	RI,MS
11	6-Camphenone	1.44	1095	1096		0.1±0.00	1.6	0.2±0.02	6.3	RI,MS
12	*trans*-*p*-Mentha-2,8-dien-1-ol	1.44	1121	1122	1113	0.7±0.00	0.2	0.5±0.01	1.1	RI,MS
13	α-Campholenal	1.44	1126	1126		0.3±0.01	3.1	0.3±0.00	0.8	RI,MS
14	***trans*-Pinocarveol**	**1.44**	**1139**	**1139**	**1139**	**36.3±0.14**	**0.4**	**35.6±0.07**	**0.2**	**Std**
15	*trans*-Pinocamphone	1.44	1159	1162	1163	0.5±0.11	8.7	tr	4.8	RI,MS
16	**Pinocarvone**	**1.44**	**1162**	**1164**	**1163**	**19.8±0.08**	**0.4**	**20.0±0.04**	**0.2**	**RI,MS**
17	*cis*-Pinocamphone	1.44	1172	1175		tr	8.9	0.1±0.01	8.1	RI,MS
18	Terpinen-4-ol	1.44	1177	1177	1177	tr	4.3	tr	3.5	Std
19	*trans*-*p*-Mentha-1(7),8-dien-2-ol	1.44	1186	1189		2.8±0.04	1.4	3.3±0.05	1.4	RI,MS
20	Myrtenal	1.44	1191	1195	1191	1.1±0.01	1.1	1.2±0.01	1.1	Std
21	Myrtenol	1.44	1193	1195	1196	1.8±0.01	0.3	1.4±0.03	0.3	Std
22	*trans*-Carveol	1.44	1218	1216	1218	0.9±0.00	0.4	0.9±0.03	1.0	Std
23	*cis*-*p*-Mentha-1(7),8-dien-2-ol	1.44	1227	1227		2.3±0.02	0.7	2.1±0.05	1.2	RI,MS
24	*cis*-Carveol	1.44	1231	1229	1229	tr	11.7	tr	9.9	RI,MS
25	(*Z*)-Ocimenone	1.44	1231	1229		0.1±0.01	4.1	tr	1.4	RI,MS
26	Cumin aldehyde	1.44	1239	1241	1240	tr	1.7	tr	4.0	RI,MS
27	Carvone	1.44	1243	1243	1242	0.4±0.02	4.6	0.4±0.01	2.3	Std
28	Perilla aldehyde	1.44	1272	1271	1274	0.2±0.01	6.0	0.2±0.00	2.5	RI,MS
29	Isobornyl acetate	1.45	1284	1285		0.2±0.04	4.9	0.2±0.01	3.1	RI,MS
30	*trans*-Sabinyl acetate	1.45	1289	1290		tr	6.6	tr	5.7	RI,MS
31	Perilla alcohol	1.44	1294	1295	1295	tr	2.5	tr	8.7	RI,MS
32	*trans*-Pinocarvyl acetate	1.45	1297	1298		0.3±0.02	8.6	0.2±0.01	6.7	RI,MS
33	Myrtenyl acetate	1.45	1324	1326	1328	0.3±0.01	2.3	0.3±0.02	5.7	RI,MS
34	β-Elemene	1.05	1387	1390	1385	tr	10.5	tr	9.4	RI,MS
35	(*E*)-Caryophyllene	1.05	1410	1408	1416			tr	5.9	Std
36	*p*-Cymen-7-ol acetate	1.45	1420	1422		tr	11.4	tr	8.9	RI,MS
37	**Perillyl acetate**	**1.45**	**1436**	**1436**		**12.7±0.26**	**2.1**	**6.0±0.09**	**1.5**	**RI, MS[h]**
38	**β-Selinene**	**1.05**	**1479**	**1490**	**1485**	**8.5±0.09**	**1.1**	**8.5±0.03**	**0.4**	**RI,MS**
39	δ-Selinene	1.05	1484	1492	1491	tr	1.0	0.2±0.00	2.9	RI,MS
40	Fukinanolide	1.00	1800	1798	1798			0.5±0.01	2.1	RI,MS
	Total identified (%)					98.2		98.6		
	Grouped compounds (%)									
	Monoterpene hydrocarbons					13.5		15.9		
	Oxygenated monoterpenes					75.0		73.5		
	Sesquiterpene hydrocarbons					8.7		8.8		
	Others					1.0		0.5		

[a]Compounds belonging to each class are listed in order of their elution from a HP-5 column.
[b]Relative response factor of FID detector.
[c]Retention index on HP-5 column, experimentally determined using homologous series of C_8–C_{30} alkanes.
[d]Relative retention index taken from Adams (10) and/or NIST 08 (11) for DB-5 and HP-5 capillary column, respectively.
[e]Identification methods: std, based on co-injection with authentic standards; MS, based on comparison with WILEY, ADAMS and NIST 08 MS database; RI, based on comparison of RI with those reported in ADAMS and NIST 08.
[f]Relative standard deviation.
[g]tr, traces (mean value below 0.1%).
[h]Retention index and MS taken from NIST 08, Brophy et al. (12) and Silva et al. (16).

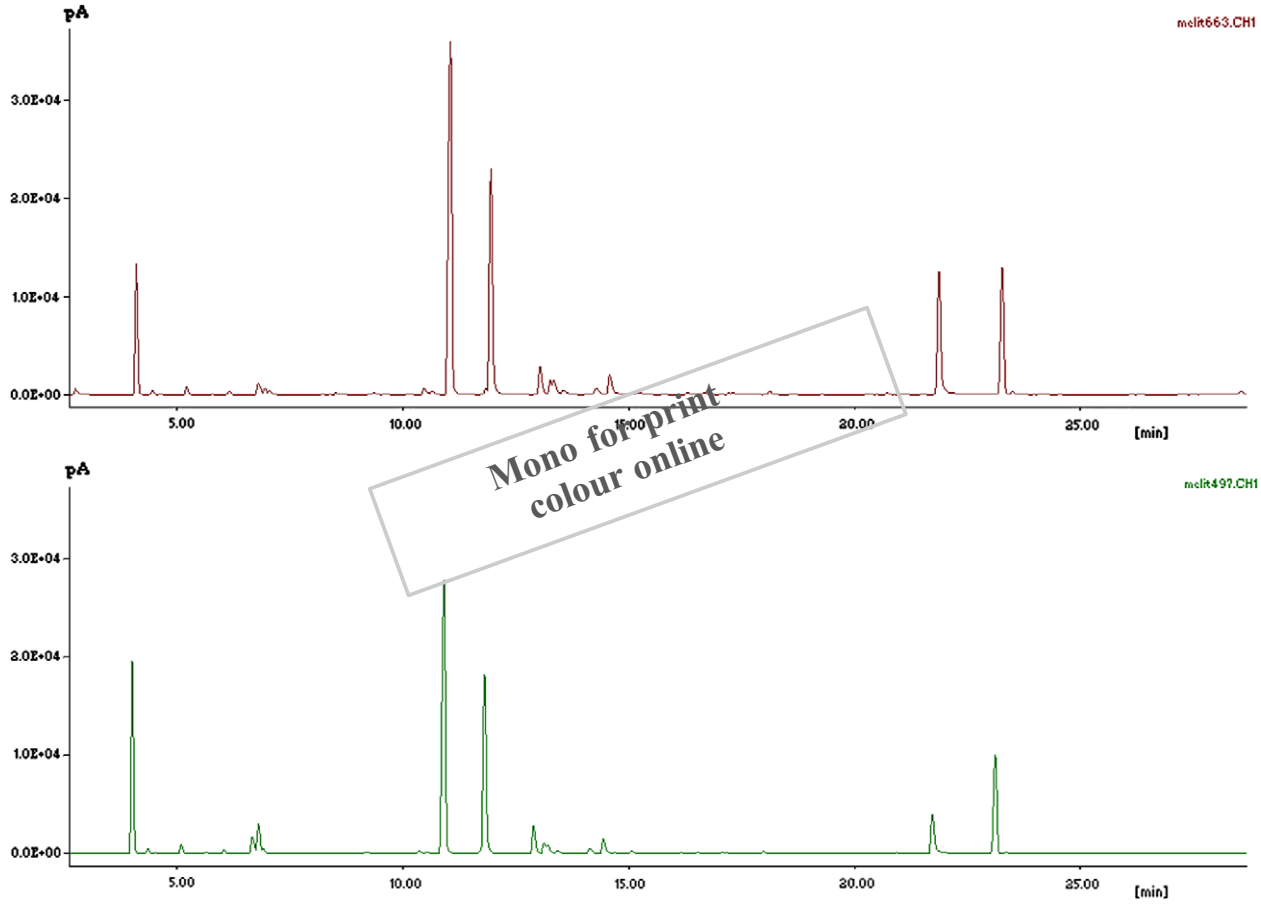

Figure 1. Gas chromatography/flame ionization detector (GC/FID) chromatograms of the essential oils hydrodistilled from the resurrection plant *Myrothamnus moschatus* during (a) desiccation and (b) re-hydration.

uncommon essential oil constituent, having some application in perfumery (12). It is a metabolite of the monoterpene perillyl alcohol and it was found to possess significant antineoplastic activity in treating and preventing carcinoma of the breast in rats (13).

It is interesting to note that *trans*-pinocarveol, the major compound of the oil, is used in pharmaceutical preparations such as Ozopulmin™ to treat respiratory tract disorders including asthma (4), thus confirming one traditional use of the plant.

The present study represents the first investigation of the essential oil of *M. moschatus*, which is endemic to Madagascar. Other works were previously carried out by some authors on the other resurrection plant, *M. flabellifolia*, growing in South Africa (4) and Zimbabwe (3). In the former study, the major components of the oil resulted *trans*-pinocarveol (19.6%), pinocarvone (11.1%), limonene (6.1%), *trans-p*-menth-1-(7)-8-diene-2-ol (7.4%) and *cis-p*-menth-1-(7)-8-diene-2-ol (6.8%), whereas in the latter, the major volatiles were *trans*-pinocarveol (28.7–28.9), pinocarvone (13.4–21.3%), α-pinene (trace–23.0%) and β-selinene (5.0–9.9%). On the other hand, other authors previously reported different compositions, related to different geographic areas from which plants were sampled (14,15). They reported as major essential oils constituents carvone and perillic acid, and 1,8-cineole and diosphenol, respectively.

In conclusion, we can assume that geographical isolation, in addition to genetic factors, did not influence significantly the composition of the essential oil of *M. moschatus* with respect to that of *M. flabellifolia* from Southern Africa resulting in a similar chemical profile, notably with respect to the Zimbabwe population (3).

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