American Journal of Plant Sciences, 2016, 7, 834-840
Published Online April 2016 in SciRes. http://www.scirp.org/journal/ajps
http://dx.doi.org/10.4236/ajps.2016.76079

Leaf Essential Oil from Three Exotic Mytaceae Species Growing in the Botanical Garden of Rio de Janeiro, Brazil

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Received 29 March 2016; accepted 22 April 2016; published 25 April 2016

Abstract

The leaf essential oils of three Myrtaceae species: *Melaleuca leucadendra*, *Lophostemon confertus* and *Ugni molinae*, non-native to Brazil and growing in the Rio de Janeiro Botanical Garden, were obtained by hydrodistillation and analyzed by GC and GC-MS. Oil yields from fresh leaves were 0.76%, 0.08%, and 0.04%, respectively. Sixty-seven percent of *M. leucadendra* oil was made up of monoterpenes, wherein 1,8-cineole was largely predominant (49%), followed by *α*-terpineol (7.6%) and terpinen-4-ol (4.3%). The sesquiterpene profile was characterized by a significant amount of viridiflorol (29%). Oil from *L. confertus* was 42% monoterpenes; the most predominant constituents being *α*-pinene (20.8%) and *α*-thujene (7.1%). Total sesquiterpenes (55.4%) comprised spathulenol (28%), globulol (14%), and aromadendrene derivatives (8.5%) as the main species. *U. Molinae* oil was exclusively composed of sesquiterpenes, with a predominance of *β*-elemene (44%) followed by *β*-caryophyllene (7.1%), and bicyclogermacrene (6.7%); guaiol-type alcohols made up 30% of its profile. To the best of our knowledge, this is the first time *U. Molinae* leaf oil composition has been described. The composition of *M. leucadendra* oil corresponded to a chemotype described for a species growing in Cuba, but was different from that produced by *M. leucadendra* growing in Egypt, Tunisia, and elsewhere in Brazil. The profile of the *L. confertus* leaf oil showed high similarity with those described for Australian native specimens, in particular in relation to the relevant presence of *α*-pinene, aromadendrenes, and the guaiol-type alcohols.

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How to cite this paper: Siani, A.C., Nakamura, M.J., das Neves, G.P., da S. Monteiro, S. and Ramos, M.F.S. (2016) Leaf Essential Oil from Three Exotic Mytaceae Species Growing in the Botanical Garden of Rio de Janeiro, Brazil. *American Journal of Plant Sciences*, **7**, 834-840. http://dx.doi.org/10.4236/ajps.2016.76079

Keywords

***Melaleuca leucadendra*, *Lophostemon confertus*, *Ugni molinae*, Myrtaceae, Essential oil**

1. Introduction

The arboretum section of the Rio de Janeiro's Botanical Garden (BGRJ) comprises about 1500 species and almost 9000 specimens. Although the Brazilian flora is well represented, a large part of the collection constitutes non-native species [1]. For example, the collection includes some exotic species of Myrtaceae, a botanical family that characteristically accumulates volatile compounds in the leaves [2]. The goal of the present study was to assess the composition of leaf essential oil of three exotic species of Myrtaceae species growing in the BGRJ: *Melaleuca leucadendra* (L.), *Lophostemon confertus* Peter G. Wilson & J. T. Waterh. (syn. *Tristania conferta* R.Br.), and *Ugni molinae* Turcz. The former two are woody-fruited species native to Australia, while the latter is native to Chile and is characterized by its production of pleasant tasting berries, as most South American Myrtaceae species are.

The *Melaleuca* genus comprises nearly 300 species, most of which are endemic to Australia [3]. Species of *Melaleuca* thrives in many kinds of environments, including swampy or temporarily inundated lands, poor or degraded soils [4]. Popularly known as tea tree, the leaves of these species produce an abundance of essential oils that possess medicinal properties and are useful as flavor ingredients in the food industries [5]-[7]. *Melaleuca leucadendra* (*white paperbark*; *leucadendron*, Greek for *white tree*) grows up to 40 m in height and has a thick trunk that may reach 1.5 m diameter in its original habitat [8]. This species is conspicuously distinguished by its white, very thin bark that much resembles sheets of paper. Three chemotypes have been identified for *M. leucadendra* based on the essential oil composition. They are characterized by a high content of eugenol-type phenylpropanoids [9] or terpenoids, of which *γ*-terpinene and terpinolene predominate among the monoterpenes [10]. Different chemotypes of the leaf essential oil have also been correlated with the geographical location of plants [5]. Under specific agroforest conditions and depending on the geographic locale, 1,8-cineole [11] or a combination of 1,8-cineole/*α*-terpineol/limonene [12] may be present and characterize the chemotype. Several parts of the plant *M. leucadendra* are traditionally used for their anti-parasitic, antiseptic, and insect repellent activities [13], and the leaf essential oil is used as an antifungal [14]. The 1,8-cineole chemotype (also containing *α*-terpineol) of *M. leucadendra* leaf oil has been found to be active against *Bacilus cereus* and *Staphylococcus aureus* [15] and a series of four other fungi [16] while the methyl eugenol chemotype is an effective virucide against Herpes simplex virus type 1 [6]. Mild antioxidant and anti-hyaluronidase abilities have been observed for the former oil chemotype [16]. The ethanol extract from the branches of *M. leucadendra* is highly selective against *Tripanosoma brucei* [13]. The essential oil of *M. leucadendra*, pure or mixed with other oils, is ineffective in inducing mortality in *Aedes*, *Anopheles*, and *Culex* larvae [17], but significant irritant and repellent properties against *Aedes aegypti* females have been observed in experiments using the excito-repellency test chamber [18] [19]. An anti-feeding assay using gypsy moth larvae and the leaf ethanol extract of *M. leucadendra* led to the isolation of (*E*,*S*)-nerolidol as the active component [20].

Lophostemon confertus (*Queensland brush box*, *Brisbane box*) is a large to very large evergreen tree native to open forest and rainforest ecotones on the east coast of Australia that may reach 25 to 40 m in height [21] [22]. Due to its pest resilience, tolerance to pollution, and excellent shade-providing capacity, it has been used as street tree in the cities of various countries [23]. Leaves of *L. confertus* collected in Queensland have been shown to afford low yields of volatile oil that contained up to 33% *α*-pinene plus 20% limonene; with aromadendrene and spathulenol being relevant among the sesquiterpenes [22].

Ugni molinae is an evergreen shrub that is native to temperate rain forests in Chile (*Chilean cranberry*, *Chilean guava*, *murtilla*, *uñi*). Apart from exceptional cases, this species grows to around 2 m in height. It is cultivated as an ornamental shrub and produces an excellent berry suitable to use in jelly, jam, cakes, liqueurs, etc. [24] [25]. It was eventually introduced in some Pacific British colonies, and thereafter being popularized as the New Zealand cranberry. The leaves of *U. molinae* exhibit astringent and stimulant properties and have been used in folk medicine to treat several kinds of diarrhea [25]. Additionally, the leaves contain triterpenes that exhibit topical anti-inflammatory activity [26]. To the best of our knowledge, no reports exist describing the leaf volatile compounds of this species.

This study aims to describe the composition of the essential oils from the three species non-native to Brazil: *Melaleuca leucadendra*, *Lophostemon confertus* and *Ugni mollinae* and compare them, where possible, with data reported for specimens growing elsewhere in the world.

2. Material and Methods

2.1. Plant Material

Melaleuca leucadendra (tree 23 m high, laminar white bark partially detached from the trunk), *Lophostemon confertus* (tree 24 m high, dark green leafy tree) and *Ugni molinae* (shruby tree, 4 m high) were collected in the Arboretum sections of the Botanical Garden of Rio de Janeiro (BGRJ), Brazil. *L. Confertus* was collected in August 2010; *M. leucadendra* and *U. molinae* were collected between March and May 2011. Voucher of *M. leucadendra* and *L. confertus* were deposited in the herbarium of the BGBJ under registrations RB 345845 and RB 343661, respectively. *U. molinae* was deposited in the plant collection of the Drug and Medicines Institute at Oswaldo Cruz Foundation, Rio de Janeiro, under code FFAR-424.

2.2. Oil Isolation

Fresh leaves (0.7 - 1.0 Kg) of *M. leucadendra*, *L. confertus* and *U. molinae* were individually extracted by placing them with 3 L of water in a 12 L round-bottom flask coupled to a modified Clevenger apparatus and submitted to hydrodistillation for 4 h. After the collection, the oils were centrifuged for 10 minutes at 5000 rpm (Eppendorf model 5810 R, Hamburg, GE) and the residual water was carefully separated out. The oils were placed in amber sealed tubes and stored in the refrigerator. The yields (w/w) were calculated and the refractive indices were determined using a VEE GEE Model C10 Abbe refractometer (Nova-Tech International, Kingwood, USA).

2.3. Gas Chromatographic Analysis

The GC analysis of the oils was performed using an HP 6890N Network GC System gas chromatograph (Hewlett Packard, Palo Alto, CA) with flame ionization detection (GC-FID, injector at 250˚C) equipped with a capillary column HP-5 (30 m × 0.32 mm × 0.25 μm film thickness) with flow at 2.0 ml/min; 1:25 split ratio mode. The oven temperature was programmed from 70˚C (held for 5 minutes) to 290˚C at 4˚C/min and then held for 10 minutes. Helium was the carrier gas at an inlet pressure of 3.14 psi. The injection volume was 1 μL of a 5 mg/ml dichloromethane solution. The percentage compositions were obtained from relative peak areas by electronic integration using GC Station Rev. A.10.02 Agilent Technologies software (Santa Clara, USA). The GC-MS analyses were performed in a similar HP 6890N chromatograph equipped with a HP-5 MS column (30 m × 0.32 mm × 0.25 μm film thickness) at the same conditions as above, excepting the gas flow at 0.5 ml/min and 1:20 split ratio mode. Data were processed using MSD Productivity ChemStation Software operating with ion source at 250˚C and electron impact ionization at 70 eV. Individual components were identified by comparison of both mass spectra and GC retention data with those of authentic compounds stored in the Wiley library software 59943B or in the literature. The retention indices were calculated for all volatile constituents with reference to a homologue n-alkane series injected in the same analytical conditions [27].

3. Results and Discussion

3.1. *Melaleuca leucadendra*

The chemical constitutions of the three oils are displayed in **Table 1**. Earlier studies on the leaf essential oil compositions of *Melaleuca* species have highlighted the predominance of terpinene-type monoterpenes [28], with competitive biosynthetic routes leading to the accumulation of 1,8-cineole, *α*-terpineol and limonene or terpinen-4-ol and *γ*-terpinene [29]. In the present study, the leaf oil yield from *M. leucadendra* was 0.76% (*w*/*w*), presenting 67% and 31% of identified monoterpenes and sesquiterpenes, respectively. The monoterpenes were primarily characterized by 1,8-cineole (49%), *α*-terpineol (7.6%), and terpinen-4-ol (4.3%) as well as lower amounts of limonene, *α*-pinene, and *β*-pinene (<3.4%). The sesquiterpenes were almost totally represented by viridiflorol (29%). This oil profile is quite similar to that obtained by Pino *et al.* for *M. leucadendra* growing in Cuba [30]. On the other hand, the methyl eugenol-chemotype has been described for specimens growing else

Table 1. Essential oil compositions of *Melaleuca leucadendra*, *Lophostemon confertus* and *Ugni molinae*.

RI	Compound	Abundance (%)		
		ML	LC	UM
		Ri 1.4770	Ri 1.6350	Ri 1.5070
847	E-hexanal		4.6 ± 0.11	1.2 ± 0.01
922	*α*-thujene		7.1 ± 0.26	
934	*α*-pinene	3.38 ± 0.06	20.8 ± 0.78	
980	*β*-pinene	1.7 ± 0.03	1.6 ± 0.05	
989	myrcene		3.4 ± 0.09	
1031	limonene	1.4 ± 0.01	2.8 ± 0.1	
1036	1,8-cineole	48.7 ± 0.54		
1130	*α*-campholenal		1.5 ± 0.02	
1184	terpinen-4-ol	4.3 ± 0.03		
1197	*α*-terpineol	7.6 ± 0.03		
1334	*iso*-dihydrocarveol acetate			1.2 ± 0.03
1384	*β*-bourbonene			2.2 ± 0.02
1391	*β*-elemene			44.2 ± 0.19
1423	*β*-caryophyllene			7.1 ± 0.02
1441	aromadendrene		6.1 ± 0.06	0.5 ± 0.01
1459	*α*-humulene			1.5 ± 0.00
1463	allo-aromadendrene		2.4 ± 0.02	0.9 ± 0.00
1490	*β*-selinene			2.2 ± 0.12
1485	germacrene D			1.1 ± 0.01
1499	byclogermacrene			6.7 ± 0.04
1531	*Z*-nerolidol			
1567	maaliol		1.7 ± 0.01	1.0 ± 0.01
1581	spathulenol		27.9 ± 1.24	7 ± 0.04
1587	caryophyllene oxide	1.7 ± 0.12		
1591	globulol		13.8 ± 0.14	8.4 ± 0.04
1599	viridiflorol	27.8 ± 0.75	1.1 ± 0.02	2.4 ± 0.01
1600	ledol	1.8 ± 0.06	2.4 ± 0.03	
1629	10-epi-*γ*-eudesmol			1.1 ± 0.00
1636	muurola-4,10(14)-dien-1-*β*-ol			1.0 ± 1.00
1649	epi-*α*-muurulol			0.6 ± 0.01
1661	selin-11-en-4-*α*-ol			2.6 ± 2.17
1664	*epi*-neointermediol			3.8 ± 2.14
1678	14-hydroxy-9-epi-(*E*)-caryophyllene			0.4 ± 0.00
	hydrocarbon monoterpenes	6.4	35.8	1.2
	oxygenated monoterpenes	60.6	6.1	1.2
	Total monoterpenes	67.0	41.9	2.4
	hydrocarbon sesquiterpenes	0	8.6	67.2
	oxygenated sesquiterpenes	31.3	46.9	30.2
	Total sesquiterpenes		55.5	97.4
	Total identified	98.3	97.4	99.8

RI = Retention index [27]. Ri = Refractive index. ML = *Melaleuca leucadendra*; LC = *Lophostemon confertus*; UM = *Ugni molinae*. Values of relative abundance were determined from the average of three injections in GC.

where in Brazil [31] as well as in Australia [9]. The virtual absence of sesquiterpenes (or just traces at a maximum) characterizes the 1,8-cineole/α-terpineol/limonene chemotype of oils described for the species assigned to *M. leucadendron* cultivated in Egypt [6] and Java (plus <10% β-caryophyllene) [12], where the dry season favored the accumulation of 1,8-cineole [11]. Moreover, acyclic sesquiterpene E-nerolidol was described by Padalia *et al.* to accumulate in the leaf essential oil of *M. leucadendra* growing in India [32]; its accumulation was also reported in specimens assigned to *M. leucadendron* growing in Florida by Doskotch *et al.* [20], who studied the potential anti-feeding characteristics of the extracted compounds. In general, sesquiterpene alcohols (guaiol-, eudesmol- and cadinol-types) occur as minor or trace compounds in profiles reported for this species [9] [12] [16] [32].

3.2. *Lophostemon confertus*

The essential oil from *Lophostemon confertus* (0.08% *w*/*w* for fresh material) yielded hydrocarbon and oxygenated sesquiterpenes in a 2:1 ratio. α-Pinene (20.8%) was predominant in the latter, followed by α-thujene (7.1%), small amounts of myrcene, limonene, and β-pinene. Spathulenol (28%) and globulol (14%) stood out among the 45% of guaiol-type alcohols that constituted the majority of sesquiterpene species. Aromadendrene derivatives (8.5%) represented the second most prevalent group of sesquiterpenes in the oil. The low yield and the oil's main chemical composition showed good agreement with those reported for this species and others belonging to the genus *Lophostemon*, regarding the type of terpenoids present in plants growing in different locales in Australia [22].

3.3. *Ugni molinae*

Among the studied species, the leaves of *Ugni molinae* afforded the lowest yield (0.04% *w*/*w* on fresh material) of volatiles exclusively constituted by sesquiterpenes, in which 67% hydrocarbons and 30% alcohols (mostly of the guaiol-type) were identified. In the former group, there was a large predominance of β-elemene (44%) followed by similar levels of β-caryophyllene (7.1%) and bicyclogermacrene (6.7%). No previously published data seem to be available on the leaf volatile compounds of *U. molinae*; the present study therefore constitutes the first to characterize *Ugni molinae* leaf oil. However, the related species *U. myricoides* Kunth O. Berg has been shown to be rich in α-pinene (52%) and 1,8-cineole (12%), followed by caryophyllenes (9%) and their respective oxides (up to 9%) [33].

4. Conclusion

The present description of the volatile components in the leaves of *L. confertus* and *U. molinae* expands our knowledge of these Myrtaceae species. In the case of *Melaleuca* leaf oil, the chemotypes are especially important, given that they are employed in pharmaceuticals, cosmetics, disinfectants, deodorizers, and many other industrial aromatic products. Thus, as one of the most important *ex-situ* collections in Brazil, the living repository of native and exotic plants of the Botanical Garden of Rio de Janeiro not only contributes to aiding biodiversity conservation, but also enables the conduction of valuable technological studies.

Acknowledgements

The authors are thankful to Ricardo C. C. Reis, curator of the Living Collections Research Institute (BGRJ), for authorizing collections and to Luis C. Giordano and Josias B. da Silva for helping with the collections. We also thank to Arianne C. S. Vaucher for helping with the Refraction Index measurements. The GC analyses were supported by RPTPDTIS-FIOCRUZ.

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