Anais da Academia Brasileira de Ciências (2016) 88(3 Suppl.): 1899-1907
(Annals of the Brazilian Academy of Sciences)
Printed version ISSN 0001-3765 / Online version ISSN 1678-2690
http://dx.doi.org/10.1590/0001-376520162015-0732
www.scielo.br/aabc

Seasonal variation of the essential oil from two Brazilian native *Aldama* La Llave (Asteraceae) species

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Manuscript received on October 1, 2015; accepted for publication on March 1, 2016

ABSTRACT

Aldama arenaria and *A. robusta* are morphologically similar aromatic species that have seasonal development. The yield and chemical composition of essential oils from aerial and underground vegetative organs of these species were compared to verify the production of volatile metabolites in flowering and dormant phases of development and to identify if there are unique compounds for either species. The major compound in the essential oils from *A. arenaria* leaves was palustrol (16.22%) and for aerial stems was limonene (15.3%), whereas limonene (11.16%) and α-pinene (19.64%) were the major compounds for leaves and aerial stems from *A. robusta*, respectively. The major compound for the underground organs was α-pinene, in both species and phenological stages. High amounts of diterpenes were found especially for *A. arenaria* essential oils. Each analyzed species presented unique compounds, which can provide a characteristic chemical profile for both species helping to solve their taxonomic problems. This study characterized for the first time the yield and essential oil composition of *A. arenaria* and *A. robusta*, which have medicinal potential, and some of the compounds in their essential oils are unique to each one and may be useful in helping the correct identification of them.

Key words: *Aldama arenaria*, *Aldama robusta*, *Viguiera*, phenological phases, terpenes, vegetative organs.

INTRODUCTION

Aldama arenaria (Baker) E.E.Schill. & Panero (=*Viguiera arenaria* Baker in Martius) and *A. robusta* (Gardner) E.E.Schill. & Panero (=*Viguiera robusta* Gardner in Hook) are herbs and subshrubs with a thickened underground stem and seasonal development (Magenta et al. 2010). During favorable periods of development, these species emit shoots from the underground system and produce flowers. In cold and dry periods, these species lose their shoots and survive only via underground organs, which characterize the dormant period. Both species are strongly aromatic due to the production of essential oils (EOs).

Essential oils are generally odoriferous, liquid (Bakkali et al. 2008, Simões and Spitzer 2004), complex mixtures of lipophilic substances that

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primarily consist of low molecular weight volatile terpenes (Fahn 1979, 2000). In general, these compounds play important ecological roles in plants (Harborne and Turner 1984), such as protecting against microorganisms and herbivores, attracting pollinators, and acting as allelochemicals (Ryan 2001, Wink 2009, Arimura et al. 2010, Chauveau et al. 2011). Thus, the biosynthesis, composition, and yield of EOs may be influenced by biotic or abiotic environmental factors (Marotti et al. 1996, Sangwan et al. 2001, Deschamps et al. 2008). In this context, the chemical composition and yield of EOs may differ between organs from the same plant and between different phenological phases of a plant's development (Deschamps et al. 2008, Murari et al. 2008).

Although environmental factors may influence the chemical composition and yield of EOs, some compounds are characteristic of certain plant groups and may thus be used in chemotaxonomy (Gören et al. 2002, Croteau et al. 2005). For example, some monoterpenes, such as α-pinene and β-pinene, are common among angiosperms, and quantitative variations in these compounds may be a diagnostic feature for distinguishing between species (Harborne and Turner 1984). The species *Aldama arenaria* and *A. robusta* belong to Asteraceae family (Magenta and Pirani 2014) and are easily confused in herbalized materials, being only distinguished based on pollen features (Magenta 2006). Data on the chemical composition of EOs from these species could help distinguish between both of them. In a study comparing the chemical composition of secretions from glandular trichomes of *Aldama robusta*, Da Costa et al. (2001) rated it as a "chemo consistent" taxon, with a similar chemical pattern between different populations.

Additionally, phytochemical studies have demonstrated the biological activity of root compounds from *Aldama arenaria* (Tirapelli et al. 2002, Ambrosio et al. 2008, Hipolito et al. 2009, Porto et al. 2009) and *A. robusta* (Tirapelli et al. 2002, Valerio et al. 2007).

For these reasons, the purpose of the present study was to characterize and compare the yield and chemical composition of EOs from the vegetative organs of *Aldama arenaria* and *A. robusta*, to verify if there is any difference in the production of volatile metabolites during flowering and dormant periods, and to determine whether either species produces unique compounds that may be useful as distinguishing features.

MATERIALS AND METHODS

Botanical Material

Samples were collected from vegetative organs of *Aldama arenaria* (Baker) E.E.Schill. & Panero and *A. robusta* (Gardner) E.E.Schill. & Panero in the flowering stage (leaf, aerial stem, xylopodium, and root) and dormant stage (xylopodium and root). At least four individuals for each species and developmental stage were sampled. Samples from *A. arenaria* were collected at the Itirapina Ecological Station (22°14'S and 47°51'W), and samples from *A. robusta* were collected at the Mogi Guaçu Biological Reserve (Campininha Farm) (22°42'S and 47°37'W), both located in São Paulo, Brazil. The species were identified by Mara Angelina Galvão Magenta and the material was registered and incorporated into the herbarium collection (ESA) of the Escola Superior de Agricultura "Luiz de Queiroz", at the University of São Paulo, under the numbers 111847 and 114255, respectively.

Obtaining the Essential Oils

Samples of fresh leaves, aerial stems, xylopodia, and roots from different individuals were pooled and submitted separately to the essential oils extraction by hydrodistillation method in a Clevenger system, for three hours. The essential oils were separated and stored at low temperatures, and the yields were calculated based on the fresh plant material.

Analysis of Essential Oils

Samples of essential oils were analysed by gas chromatography coupled with flame ionization detector (GC-FID) and gas chromatography coupled with mass spectrometry (GC–MS). All the samples were prepared in ethyl acetate at a concentration of 20 $mg.mL^{-1}$.

The GC-FID analyses were done using a Thermo Mod. Serie Trace CG-ULTRA, which was equipped with a flame ionization detector (FID); AS 3000 auto-sampler, a split/splitless injector, and an HP-5 (25 m x 0.20 mm x 0.33 μm) capillary column. The temperatures were used: injector = 220 °C; detector = 280 °C; and column = 60 °C, raised at 3°C.min^{-1} to 240 °C (held for 7 min at this temperature); the flow rate of carrier gas (super-dry He) = 1.0 $mL.min^{-1}$.

The GC/MS analysis were carried out in a HP 5890 series II chromatograph (Hewlett-Packard, Palo Alto, CA, USA) with Hewlett-Packard 5971 mass-selective detector, split/splitless injector, and HP-5 capillary column (25 m x 0.20 mm x 0.33 mm). The temperatures were: injector = 220 °C; detector = 280 °C; and column = 60 °C, raised at 3°C.min^{-1} to 240 °C (held for 7 min at this temperature); the flow rate of carrier gas (super-dry He) = 1.0 $mL.min^{-1}$.

The chemical composition was determined based on retention time data obtained from GC-FID, and based on mass spectra obtained from GC-MS, and comparison of the mass spectra with data from the NIST 11 Mass Spectral Library (Fabrication Varian Inc.). Arithmetic indexes (IA's) were calculated using Van den Dool Kratz equation and co-injection patterns for hydrocarbons and the data described by Adams (2007).

RESULTS

Yield of Essential Oils

There were differences in the yield of EOs obtained from the vegetative organs of *Aldama arenaria* and *A. robusta* (Tables I, II and Fig. 1). The highest yield of EOs in *Aldama arenaria* in the flowering stage was obtained from the leaves (0.23%), followed by roots (0.19%) and xylopodium (0.07%) (Table I). In the dormant stage, the level of EOs from the xylopodium increased by 100% relative to the flowering stage, whereas EOs from the roots increased by 73%.

In *A. robusta*, in the flowering phase, the highest yield of EOs was obtained from the roots (0.32%), followed by the leaves (0.21%), and xylopodium (0.08%) (Table II). In the dormant stage, the level of EOs from the xylopodium increased by 62% relative to the flowering stage, while the level of EOs from the roots did not change.

Chemical Composition of Essential Oils

In addition to changes in yield, there were also differences in chemical composition for EOs from the vegetative organs in the flowering (leaf, stem, root, and xylopodium) and in the dormant stage (root and xylopodium) in both species (Table I and II). Monoterpenes represent the major compounds identified of EOs obtained from roots and xylopodia, in both flowering and dormant stages, with predominance of α-pinene. The sesquiterpenes content was similar for both species and stages. Cyperene was the predominant compound in the roots of both species. The majority identified compounds of xylopodia was carotol in *A. arenaria* and germacrene D in *A. robusta*. Diterpene content was higher to the flowering stage of *A. arenaria*, in both subterranean organs. In dormant stage, the monoterpene content in the EO from xylopodia and roots increased, while the sesquiterpene and diterpene contents decreased. The identification of diterpenes was performed by comparative analysis of their mass spectra with literature data (Adams 2007) (Mass Spectra of Diterpenes - Supplementary Material).

Concerning the essential oils from the aerial organs, the EOs from leaves were primarily made

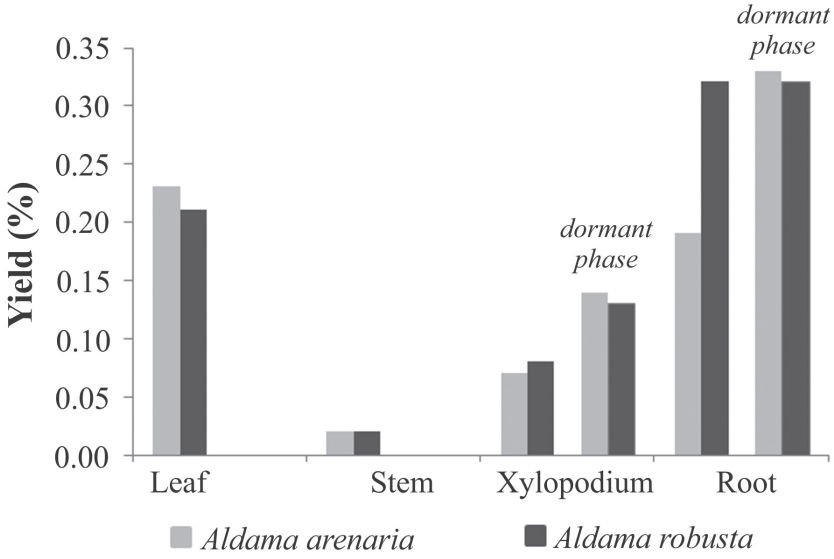

Figure 1 - Comparison of the essential oils yield (% w/w) from vegetative organs of *Aldama arenaria* and *A. robusta*, obtained from leaves, stems, xylopodia, and roots.

up of sesquiterpenes (52.31%), whereas the EOs from stems presented similar proportion of mono and sesquiterpenes (24.42% and 18.93%) followed lower diterpene content (10.33%). For *A. robusta* the EOs from leaves showed higher monoterpenes content (47.83%), followed by sesquiterpenes (32.31%) and low concentrations of diterpenes (1.49%), whereas the OEs from stems showed higher content monoterpenes (67.82%), followed by sesquiterpenes (16.84%) and diterpenes (3.53%).

There were identified 14 exclusive compounds for *Aldama arenaria* and 10 exclusive compounds for *A. robusta*. For the first species, these compounds were sesquiterpenes and diterpenes and for *A. robusta*, they included mono, sesqui and diterpenes. The exclusive compounds are highlighted in Tables I and II.

TABLE I

Relative percentage of identified compounds of EOs from the vegetative organs of *Aldama arenaria (*Baker) E.E.Schill. & Panero in the flowering (leaf, stem, root, and xylopodium) and dormant (root and xylopodium) stages. AI = Arithmetic Index, nc = not calculated, t = traces, nd = not determined. Exclusive compounds are highlighted in the table.

Phenological phase		Flowering				Dormant	
		Leaf	Stem	Xylopodium	Root	Xylopodium	Root
Yield (% m/m)		0.23	0.02	0.07	0.19	0.14	0.33
Compound	$AI_{(calculated)}$	Relative area (%)					
α-pinene	933	1.14	4.05	31.44	40.72	45.47	40.35
Camphene	948	0.20	0.15	0.45	1.39	1.56	1.74
β-pinene	977	1.26	2.21	4.15	5.16	7.85	8.05
β-myrcene	991	1.41	2.43	0.33	0.59	0.64	2.29
δ-3-carene	1011	t	0.28	0.86	5.17	3.82	13.58
Limonene	1028	5.18	15.30	2.45	2.46	2.94	5.19
MONOTERPENES		9.20	24.42	39.68	55.49	62.28	71.2
Cyperene	1395	t	0.37	3.22	9.50	2.10	7.45
E-caryophyllene	1415	3.02	0.53	t	t	**nd**	**nd**

TABLE I (continuation)

Phenological phase				Flowering		Dormant	
α-santalene	**1417**	**2.10**	**0.94**	**nd**	**nd**	**nd**	**nd**
α-guaiene	**1435**	**nd**	**nd**	**nd**	**nd**	1.34	nd
α-humulene	**1450**	**2.19**	**t**	nd	nd	nd	nd
γ-muurolene	**1477**	**4.38**	**0.88**	**0.95**	**0.53**	**4.53**	**2.56**
Bicyclogermacrene	1495	2.38	t	t	t	nd	nd
γ-cadinene	**1513**	**2.32**	**t**	**t**	**t**	**t**	**t**
δ-cadinene	**1520**	**2.91**	**2.52**	**2.81**	**1.78**	**2.04**	**1.30**
Palustrol	**1564**	**16.22**	**3.32**	**t**	**t**	**t**	**t**
Spathulenol	1573	4.52	2.26	t	t	t	t
Caryophyllene oxide	**1579**	**2.39**	**0.70**	**nd**	**nd**	**nd**	**nd**
Carotol	**1593**	**2.80**	**5.49**	**11.79**	**11.74**	**5.14**	**7.46**
Ledol	**1598**	**3.79**	**1.01**	**nd**	**nd**	**nd**	**nd**
Muurola-4,10-(14)-dien-1-1β-ol	**1626**	**3.29**	**0.91**	**0.84**	**t**	**nd**	**nd**
SESQUITERPENES		52.31	18.93	19.91	23.55	15.15	18.77
Pimara-8(14),15-diene	**1940**	**t**	**1.76**	**6.55**	**5.08**	**4.0**	**1.08**
8β-podocarpan-8-ol	**nc**	**nd**	**1.11**	**3.37**	**1.51**	**nd**	**nd**
Pimaral	**nc**	**nd**	**2.01**	**5.08**	**2.08**	**1.90**	**0.55**
Ent-8(14),15-pimaradien-3β-ol	nc	nd	5.45	12.80	4.46	4.40	1.39
DITERPENES		0.0	10.33	27.8	13.13	10.30	3.02
TOTAL		61.51	53.68	87.39	92.17	87.73	93.0

TABLE II

Relative percentage of identified compounds of EOs from the vegetative organs of *Aldama robusta* E.E.Schill. & Panero in the flowering (leaf, stem, root, and xylopodium) and dormant (root and xylopodium) stages. AI = Arithmetic Index, nc = not calculated, t = traces, nd = not determined. Exclusive compounds are highlighted in the table.

Phenological phase				Flowering		Dormant	
		Leaf	**Stem**	**Xylopodim**	**Root**	**Xylopodium**	**Root**
Yield (% m/m)		0.21	0.02	0.08	0.32	0.13	0.32
Compound	**$AI_{(calculated)}$**	**Relative area (%)**					
α-pinene	936	9.02	19.64	39.03	31.57	65.90	69.00
Camphene	947	2.03	1.12	1.28	2.24	2.10	2.80
Sabinene	**972**	**1.95**	**3.01**	**0.70**	**0.60**	**1.00**	**1.00**
β-pinene	976	9.82	11.59	0.83	0.87	1.00	1.10
β-myrcene	991	4.05	11.26	0.64	1.09	nd	nd
α-phellandrene	**1007**	**t**	**0.38**	**10.11**	**23.10**	**3.10**	**4.80**
δ-3-carene	1012	nd	nd	1.16	3.91	3.50	7.60
p-cymene	**1025**	**t**	**0.51**	**3.72**	**5.31**	**nd**	**nd**
Limonene	1028	11.16	15.84	0.71	0.78	nd	nd
Trans-β-ocimene	**1047**	**7.92**	**2.96**	**nd**	**nd**	**nd**	**nd**
Bornyl acetate	**1284**	**1.88**	**1.51**	**1.50**	**3.62**	**2.00**	**2.89**
MONOTERPENES		47.83	67.82	59.68	73.09	78.6	89.19
α-copaene	**1373**	**t**	**0.44**	**2.81**	**1.92**	**1.60**	**t**
Cyperene	1397	t	t	3.19	11.66	3.10	7.79
E-caryophyllene	1415	4.52	0.79	t	t	t	t

TABLE II (continuation)

Phenological phase				Flowering		Dormant	
γ-gurjunene	**1471**	**t**	**t**	**1.13**	**2.72**	**nd**	**nd**
Germacrene D	**1480**	**11.98**	**7.56**	**10.41**	**3.54**	**11.30**	**3.0**
Bicyclogermacrene	1495	12.52	4.97	**t**	**t**	nd	nd
Spathulenol	1575	3.29	3.08	0.89	t	nd	nd
SESQUITERPENES		32.31	16.84	18.43	19.84	16.00	16.00
Manoyl oxide	**nc**	**1.49**	**2.86**	**2.26**	**0.59**	**nd**	**nd**
Manool	**nc**	**t**	**t**	**2.67**	**0.56**	**2.1**	**nd**
Ent-8(14),15-pimaradien-3β-ol	nc	t	0.67	2.26	nd	nd	nd
DITERPENES		1.49	3.53	7.19	1.15	2.1	0.0
TOTAL		81.63	88.19	85.30	94.08	96.7	99.98

DISCUSSION

There were differences in both the yield and chemical composition of EOs from different organs of *Aldama arenaria* and *A. robusta*. In *A. arenaria*, the leaves had the highest levels of EOs, whereas the highest levels of EOs in *A. robusta* were found in the roots. When compared to other *Aldama* species (Bombo et al. 2012, 2014) the EOs yield from leaves and stems were lower, while the yield from xylopodia and roots were similar in values. Furthermore, in *A. arenaria*, the roots and xylopodia had higher levels of EOs during the dormant stage, whereas in *A. robusta,* only the xylopodia had higher levels of EOs during the dormant stage. Differences in the yield and chemical composition were also observed for EOs from leaves and aerial and underground stems of *Senecio crassiflorus* var. *crassiflorus* (Asteraceae) (Murari et al. 2008). Such differences are common and several aspects can influence the production and composition of secondary metabolites in plants, such as physiological variations, environmental conditions, geographic variation, genetic factors (Gershenzon et al. 2000, Figueiredo et al. 2008).

Differences between flowering and dormant stages in chemical composition of EOs were also observed for underground organs of both species. The monoterpenes levels in the roots and xylopodia increased during the dormant stage, whereas the levels of sesqui and diterpenes decreased. Monoterpenes have been shown to protect against herbivores and pathogens (Bakkali et al. 2008). However, even in the absence of herbivores, some plants show seasonal increases in the concentrations of defence compounds, which must provide a selective advantage over those species that only increase the concentration of monoterpenes after an attack (Lerdau et al. 1994). Thus, the increase in monoterpenes in EOs during the dormant stage of the species studied herein may be interpreted as a chemical defence mechanism in a plant that not only survives in conditions unfavorable to growth but also has only an underground system to maintain itself until the next growth stage (Bombo et al. 2014).

Essential oils from the underground organs of *Aldama arenaria* and *A. robusta* were rich in monoterpenes during the flowering stage. The same was observed in *A. linearifolia*, *A. filifolia* and *A. trichophylla*, which presented more than 80% of the EO composition constituted by monoterpenes in the underground organs (Bombo et al. 2014).

High amounts of sesquiterpenes were also found in *A. arenaria* and *A. robusta*, mainly in the EOs from the leaves. According to Loomis and Croteau (1973), sesquiterpenes are metabolically active during plant growth. Thus, individuals from *Aldama arenaria* and *A. robusta* would be expected to invest more in the biosynthesis of sesquiterpenes during the flowering stage, when the aerial structures are sprouting.

Several biological activities were attributed to some of the compounds described here for both studied species. Canales et al. (2008) attributed the antimicrobial activity of the EO from the aerial organs of *Viguiera dentata* to the presence of compounds such as limonene, bornyl acetate, and spathulenol. These three compounds were also present in the EOs from *Aldama robusta*, while just bornyl acetate was not detected in *A. arenaria*; moreover limonene was one of the major compounds in the EOs from the aerial organs in both species. Studies with *Varronia curassavica* (Jacq.) (=*Cordia verbenacea* DC) (Boraginaceae) have demonstrated anti-inflammatory properties of (-)-*trans*-caryophyllene and α-humulene (Fernandes et al. 2007, Passos et al. 2007). The α-humulene may also possess cytotoxic and insecticide activities (Murari et al. 2008). Furthermore, α- pinene, β-pinene, and δ-3-carene, which were present in flowering and dormant stages for both species, have been indicated in phytochemical studies as possessing antimicrobial activity (Lorenzetti et al. 1991, Leite et al. 2007).

Some diterpenes were identified for both species through comparative analysis of their mass spectra with literature data (Adams 2007), mainly for *Aldama arenaria*. In this species, these compounds belong to the pimaranes class, as previously reported for dichloromethane extracts from the tuberous roots of *A. arenaria* (=*Viguiera arenaria*) (Ambrosio et al. 2006). An antiproliferative activity has been also associated to the presence of these compounds in chloroform extracts (Oliveira 2013). In *A. robusta*, the diterpenes manoyl oxide and manool, which belong to the labdanes class, were identified in the present study. Previous studies have identified heliangolides (Da Costa et al. 2001) and diterpenes derived from kauranes (Da Costa et al. 1996) in *Aldama robusta* (=*Viguiera robusta*).

There are unique compounds in *Aldama arenaria* and *A. robusta* that may help to distinguish these two species. Fourteen compounds were unique to *A. arenaria* and among them, the sesquiterpene carotol, which exhibits antifungal activity (Jasicka-Misiaka et al. 2004). For *A. robusta*, ten compounds were exclusive, and germacrene D, one of the major sesquiterpenes in the EOs from this species, exhibits antimicrobial activity (Murari et al. 2008). Exclusive compounds, as presented here for *A. arenaria* and *A. robusta*, along with the common compounds to each species, provide a characteristic chemical profile that can help in solving taxonomic problems among *Aldama* species (Bombo et al. 2012, 2014).

This study characterized the yield and essential oil composition of two native Brazilian *Aldama* species that have been pointed out as having medicinal potential (Arakawa et al. 2008, Nicolete et al. 2009, Carvalho et al. 2011). It was possible to identify some compounds of interest in the pharmaceutical context. Moreover, the comparison between flowering and dormant stages can indicate the best phenological phase to obtain the essential oils considering the target compound in future studies.

ACKNOWLEDGMENTS

We thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for grants (Proc. nº 303715/2014-6) and the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) for providing financial support and grants for the first author (Thematic Project Proc. nº 2010/51454-3 and Proc. nº 2010/02005-1). We would furthermore like to thank the coordinators of the Estação Ecológica de Itirapina and Mogi Guaçu Biological Reserve (Campininha Farm), SP, Brazil for granting permission to collect plant material for this study.

RESUMO

Aldama arenaria e *A. robusta* são espécies aromáticas morfologicamente semelhantes e que apresentam

desenvolvimento sazonal. O rendimento e a composição química dos óleos essenciais dos órgãos vegetativos aéreos e subterrâneos dessas espécies foram caracterizados e comparados, a fim de verificar a produção dos metabólitos voláteis nas fases de floração e dormência e, ainda, para tentar identificar compostos exclusivos para cada espécie. O composto majoritário no óleo essencial das folhas de *A. arenaria* foi o palustrol (16,22%) e para os caules aéreos o limoneno (15,3%), enquanto que limoneno (11,16%) e α-pineno (19,64%) foram os majoritários para o óleo das folhas e caules aéreos de *A. robusta*, respectivamente. O composto majoritário nos óleos essenciais do sistema subterrâneos foi α-pineno em ambas as espécies e fases fenológicas. Grandes quantidades de diterpenos foram encontradas especialmente nos óleos essenciais de *A. arenaria*. Diversos compostos foram únicos para cada uma das espécies analisadas. Este estudo caracterizou o rendimento e a composição química do óleo essencial de *A. arenaria* e *A. robusta*, espécies que possuem potencial medicinal. A comparação entre as fases de floração e dormência pode indicar a melhor época para obter os óleos essenciais, considerando o composto alvo em futuros estudos farmacológicos. Além disso, alguns dos compostos identificados foram exclusivos para cada uma delas, fornecendo um perfil químico único para cada espécie, o que pode ajudar na solução de problemas taxonômicos.

Palavras-chave: *Aldama arenaria*, *Aldama robusta*, *Viguiera*, fases fenológicas, terpenos, órgãos vegetativos.

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SUPPLEMENTARY MATERIAL

Mass Spectra of Diterpenes