Research, Society and Development, v. 11, n. 1, e55111121569, 2022
(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v11i1.21569

Chemical composition and toxicity of the essential oil of *Aloysia* Paláu species (Verbenaceae) from South Brazil

Composição química e toxicidade do óleo essencial das espécies de *Aloysia* Paláu (Verbenaceae) do Sul do Brasil

Composición química y toxicidad del aceite esencial de las especies de *Aloysia* Paláu (Verbenaceae) del Sur de Brasil

Received: 10//08/2021 | Reviewed: 10//16/2021 | Accept: 01/13/2022 | Published: 01/15/2022

Eluíza Thomas
ORCID: https://orcid.org/0000-0003-2174-1337
Universidade de Caxias do Sul, Brazil
E-mail: eluizathomas@gmail.com.br
Luciane Corbellini Rufatto
ORCID: https://orcid.org/0000-0002-1691-4566
Universidade de Caxias do Sul, Brazil
E-mail: lurufatto@yahoo.com.br
Luciana Bavaresco Andrade
ORCID: https://orcid.org/0000-0003-0732-8714
Universidade de Caxias do Sul, Brazil
E-mail: lbatougu@ucs.br
Priscila Ceribola Crespam
ORCID: https://orcid.org/0000-0002-0360-442X
Universidade Federal do Rio Grande do Sul, Brazil
E-mail: priscilacrespam@yahoo.com.br
Fabiana Agostini
ORCID: https://orcid.org/0000-0002-7627-1021
Universidade de Caxias do Sul, Brazil
E-mail: mestrefabi@yahoo.com.br
Sidnei Moura
ORCID: https://orcid.org/0000-0003-1903-6735
Universidade de Caxias do Sul, Brazil
E-mail: sidnei.moura@ucs.br
Joséli Schwambach
ORCID: https://orcid.org/0000-0001-6785-4164
Universidade de Caxias do Sul, Brazil
E-mail: jschwambach@ucs.br

Abstract
In Brazil there are nine native species of *Aloysia* which are poorly studied as the chemical compositions and biological activities. Hence, this study describes the chemical composition determinated by GC-MS of essential oil of the five native and one cultivated species of *Aloysia* occurring in Rio Grande do Sul and evaluates the cytotoxic effects of the essential oils from *A. citrodora*, *A. lycioides* and *A. dusenii* through the bioassay with *Artemia salina*. A group of species showed 1,8-cineol as a major compound: *A. dusenii* (16.2 %), *A. citrodora* (32.8 %), *A. lycioides* collected in Guaiba (49.5 %) and *A. lycioides* collected in São Marcos (17.6 %). While species *A. polygalifolia* and *A. virgata* had higher proportions of germacrene-D (11.2 % and 12 %, respectively) and *A. chamaedryfolia* presented spathulenol (15.6 %). The species *A. lycioides* collected in Rosário do Sul had β-Phellandrene (23.7 %) as the major compound. All the essential oils tested presented a high toxicity against *Artemia salina* with LC_{50} values among 48.12 µg mL^{-1} and 55.96 µg mL^{-1}.
Keywords: *Artemia salina*; Hydrodistillation; Rio Grande do Sul.

Resumo
No Brasil existem nove espécies nativas de *Aloysia* que são pouco estudadas quanto às composições químicas e atividades biológicas. Assim, este estudo descreve a composição química determinada por CG-EM do óleo essencial de cinco espécies nativas e uma cultivada de *Aloysia* ocorrentes no Rio Grande do Sul e avalia a toxicidade dos óleos essenciais de *A. citrodora*, *A. lycioides* e *A. dusenii* através do bioensaio com *Artemia salina*. Um grupo de espécies apresentou 1,8-cineol como composto principal: *A. dusenii* (16,2%), *A. citrodora* (32,8%), *A. lycioides* coletada em

Guaíba (49,5%) e *A. lycioides* coletada em São Marcos (17,6%). Enquanto as espécies *A. polygalifolia* e *A. virgata* apresentaram maiores proporções de germacreno-D (11,2% e 12%, respectivamente) e *A. chamaedryfolia* apresentou espatulenol (15,6%). A espécie *A. lycioides* coletada em Rosário do Sul teve β-Felandreno (23,7%) como composto majoritário. Todos os óleos essenciais testados apresentaram alta toxicidade contra *Artemia salina* com valores de CL50 entre 48,12 µg mL^{-1} e 55,96 µg mL^{-1}.
Palavras-chave: *Artemia salina*; Hidrodestilação; Rio Grande do Sul.

Resumen
En Brasil existen nueve especies nativas de *Aloysia* que están poco estudiadas en cuanto a sus composiciones químicas y actividades biológicas. Por lo tanto, este estudio describe la composición química determinada por GC-MS del aceite esencial de cinco especies nativas y una cultivada de *Aloysia* que se encuentran en Rio Grande do Sul y evalúa la toxicidad de los aceites esenciales de *A. citrodora*, *A. lycioides* y *A. dusenii* mediante el bioensayo con *Artemia salina*. Un grupo de especies mostró 1,8-cineol como compuesto mayoritario: *A. dusenii* (16,2%), *A. citrodora* (32,8%), *A. lycioides* recolectada en Guaiba (49,5%) y *A. lycioides* recolectada en São Marcos (17,6%). Mientras que las especies *A. polygalifolia* y *A. virgata* presentaron mayores proporciones de germacreno-D (11,2% y 12%, respectivamente) y *A. chamaedryfolia* presentó espatulenol (15,6%). La especie *A. lycioides* recolectada en Rosário do Sul tenía β-felandreno (23,7%) como compuesto principal. Todos los aceites esenciales probados presentaron alta toxicidad contra *Artemia salina* con valores de LC50 entre 48.12 µg mL^{-1} y 55.96 µg mL^{-1}.
Palabras clave: *Artemia salina*; Hidrodestilación; Rio Grande do Sul.

1. Introduction

Verbenaceae is a family of about 31 genera and approximately 918 species of herbs, shrubs, or small trees, mainly distributed in tropical and subtropical regions (Stevens, 2001). Among the most important genus are *Lippia* and *Aloysia* (Ricco et al, 2010). *Aloysia* comprises 30 species and is distributed from the South of the United States and Mexico until the north of Patagonia (Siedo, 2006), characterized by shrubby form, aromatic inflorescences, and known primarily for its essential oils (Hernandez et al, 2003). Nine species of *Aloysia* are native in Brazil, occurring all in the South region, and *A. citrodora* is found only under cultivation (Reflora, 2016).

Aromatic plants have been used since ancient times for their medicinal properties (Bakkali et al, 2008) and their odors are determined by the presence of essential oils (EOs), a mixture of volatile low-molecular-weight mono- and sesquiterpenes and other isoprenes (Singh et al, 2002). The EOs are involved in various ecological interactions including ones of medicinal properties such as bactericidal, fungicidal and antiviral properties (Chao et al, 2000), besides they may also exhibit cytotoxic activity (Sacchetti et al, 2005).

The brine shrimp lethality bioassay is rapid, simple, easily mastered, inexpensive, and requires small amounts of test material (Ghisalberti, 1993) to predict toxicity (McLaughlin, 1991). Since its introduction, this test has been successively employed to provide a frontline screen backed up by more specific and more sophisticated bioassays (Apu et al, 2010).

The present study reports the chemical composition by GC-MS of the essential oils of five native species of the genus *Aloysia* distributed in the Rio Grande do Sul - South Brazil and one cultivated species. Moreover, the cytotoxic activity of essential oil of the species *A. citrodora*, *A. lycioides* and *A. dusenii* were evaluated by brine shrimp bioassay.

2. Methodology

2.1 Plant material

Aerial parts from six species of *Aloysia* (leaves, flowers, and stem) were collected at seven locations in the Rio Grande do Sul State, Brazil (Table 1). The samples were identified, and voucher specimens were deposited in the Herbarium of the Universidade de Caxias do Sul (HUCS) and Herbarium of the Universidade Federal do Rio Grande do Sul (ICN). The detailed data referent to each species can be found in Table 1.

Table 1. Location, Herbarium Number, and Collect Date of each specimens of *Aloysia* species used for chemical characterization.

Plant Name	Location	Herbarium Number	Collect Date
(I) *Aloysia chamaedryfolia* Cham.	Rosário do Sul – 106m S 30º25'35.7" – W 55º16'30.8"	HUCS 40702	April 2013
(II) *Aloysia citrodora* Paláu	Carlos Barbosa – 666m S 29º16'55.62" – W 51º29'34.58"	HUCS 41907	December 2013
(III) *Aloysia dusenii* Moldenke	Garibaldi – 48 m S 29º13'58.51" – W 51º39'48.02"	HUCS 39692	February 2014
(IV) *Aloysia lycioides* Cham.	Guaíba – 23 m S 30º10'47" – W 51º23'33"	HUCS 40700	December 2012
(V) *Aloysia lycioides* Cham.	São Marcos – 746 m S 28º56'07.20" – W 51º07'23.81"	HUCS 40699	February 2013
(VI) *Aloysia lycioides* Cham.	Rosário do Sul – 106 m S 30º25'35.7" – W 55º16'30.8"	HUCS 40703	April 2013
(VII) *Aloysia polygalifolia* Cham.	Guaíba – 23 m S 30º10'47" – W 51º23'33"	HUCS 40701	December 2012
(VIII) *Aloysia virgata* (Ruiz & Pav.) Juss.	Três Passos – 451 m S 27º28'14" – W 23º59'52"	ICN 161927	April 2014

Source: Authors (2021).

2.2 Essential oil extraction

After collection, aerial parts of *Aloysia* species were dried at room temperature, fragmented, and subjected to extraction. Essential oils were obtained by hydrodistillation in a Clevenger apparatus for 1 hour (Agostini et al, 2009). Anhydrous sodium sulfate was employed to eliminate essential oil humidity. The essential oils were stored in airtight tubes, wrapped in aluminum foil, and stored in the freezer (-20 °C) prior to use.

2.3 Essential oil chemical characterization

Chromatographic analysis was performed using a gas chromatograph coupled to a mass spectrometer detector (GC-MS) and a gas chromatograph with a flame ionization detector (GC-FID) (Hewlett Packard 6890) (Tomazoni et al, 2016). The analyses used two capillary columns HP-Innowax (GC-FID: 30 m × 320 mm × 0.50 mm; GC-MS: 30 m × 250 mm × 0.50 mm), (Hewlett Packard, Palo Alto, USA). GC-MS analysis were carried out on the conditions: column temperature, 40 ºC (8 min) to 180 ºC at 3 ºC/min, 180-230 ºC at 20 ºC/min, 230 ºC (20 min); interface 280 ºC; split ratio 1:100; carrier gas He (56 KPa); flow rate: 1.0 mL/min; ionization energy 70 eV; mass range 40-350; volume injected 0.4 µL diluted in hexane (1:10). GC-FID analyses were carried out on the conditions: column temperature, 40 ºC (8 min) to 180 ºC at 3 ºC/min, 180-230 ºC at 20 ºC/min, 230 ºC (20 min); injector temperature 250 ºC, detector temperature 250 ºC; split ratio 1:50; carrier gas H_2 (34 KPa). The volume injected was 1 µL diluted in hexane (1:10). Identification of the individual components was based on comparing their GC retention times (R.T.) on polar columns and comparison with mass spectra of components by GC-MS. The components were identified by a combination of the mass spectrum of Wiley library and by comparison with data from literature (Adams, 2007). The relative percentage of each component was obtained from chromatographic peak areas, assuming the sum of all eluted peaks was 100 %.

2.4 Brine-shrimp bioassay

The cytotoxicity bioassay was done according to Meyer's procedure (Meyer et al, 1982) with modifications on the preparation of the samples. The essential oils tested were obtained from *A. citrodora*, *A. lycioides* (V) and *A. dusenii*.

Approximately 1 g brine shrimp eggs (*Artemia salina* - Flagner Soares de Souza Ind.) was hatched in a rectangular aquarium (10 × 20 cm) filled with artificial seawater, which was prepared with 1 L beaker of distilled water containing 30 g of commercial salt mixture (Azevedo Bento S.A. Comércio e Indústria). After 48 hours of incubation, the active shrimp (10-15) were collected by pipette. The nauplii were transferred to culture plates with diluted solutions of the essential oils in 1000 µg mL^{-1}, 500 µg mL^{-1}, 100 µg mL^{-1}, 50 µg mL^{-1} e 20 µg mL^{-1} with dimethyl sulfoxide (DMSO) 1%. The total volume was adjusted to 1 mL with artificial seawater. Three replications were done for each dose level and control with artificial seawater and DMSO 1%. After 24 hours, the survivors were counted. The absence of movement of nauplii for 5 minutes was regarded as dead. The bioassay was done three times independently, and the LC_{50} (Lethal Concentration 50) and 95% CI (Confidence Intervals) were calculated using the Probit Analysis with the software IBM SPSS 21.0.

3. Results and Discussion

3.1 Chemical composition of the essential oils

Essential oils of six species of *Aloysia* (Table 1) were analyzed for chemical composition by GC-MS. The essential oils yields ranged from 0.2% (mL 100 g^{-1} of dried leaves) for *A. chamaedryfolia* to 2.5% for *A. citrodora*, depending on the species (Table 2). But it also varied according to provenance from 0.8% for *A. lycioides* from Guaiba to 2.0% for *A. lycioides* from São Marcos (Table 2). A total of 25 (I), 36 (II), 23 (III), 16 (IV), 23 (V), 21 (VI), 13 (VII), and 26 (VIII) compounds were identified for each species or provenance, representing 56.1% to 88.4% of the composition of the essential oils (Table 2).

Table 2. Chemical composition, chemical groups, and yield of essential oils from aerial parts of *Aloysia* species: (I) *A. chamaedryfolia*; (II) *A. citrodora*; (III) *A. dusenii*; (IV) *A. lycioides* collected in Guaíba; (V) *A. lycioides* collected in São Marcos; (VI) *A. lycioides* collected in Rosário do Sul, (VII) *A. polygalifolia*, (VIII) *A. virgata*.

Compound	Group	I	II	III	IV	V	VI	VII	VIII
α-Pinene	HM	2.4	3.4	3.7	2.2	3.5	1.6	-	1.1
α-Thujene	HM	0.2	0.2	0.6	-	0.7	-	-	-
Camphene	HM	-	0.3	-	-	-	-	-	-
β-Pinene	HM	3.3	0.2	13.0	-	11.6	1.2	-	-
β-Phellandrene	HM	0.8	9.3	1.7	-	1.4	23.7	-	-
Myrcene	HM	0.5	1.0	1.8	-	1.6	-	-	-
Limonene	HM	1.2	6.0	9.9	2.9	8.6	0.4	-	-
1,8-Cineol	OM	3.1	32.8	16.2	49.5	17.6	-	2.1	-
ɣ-Terpinene	HM	-	-	1.8	-	0.6	-	-	-
p-Cymene	HM	4,0	0.5	5.4	1.4	7.0	0.5	-	-
1-Octadien-3-ol	OM	0.1	0.5	0.2	-	0.1	-	-	0.4
Δ-Elemene	HS	-	-	-	-	-	-	-	1.5
Terpineol	OM	0.2	2.2	0.3	2.4	0.5	1.5	-	-
α-Terpinene	OM	-	-	-	-	-	0.4	-	-
α-Cedrene	HS	-	-	-	-	-	-	-	0.6
β-Bourbonene	HS	0.6	0.3	-	-	-	-	1.3	4.0
Linalool	OM	7.7	0.7	1.8	1.5	2.1	1.8	0.8	0.5
Copaene	HS	-	-	-	-	-	0.4	-	-
β-Cubebene	HS	-	-	-	-	-	-	-	1.5
p-Caryophyllene	HS	-	-	-	-	-	-	-	-
β-Caryophyllene	HS	1.6	1.0	6.5	0.3	4.2	1.8	10.8	7.2
Terpinene-4-ol	OM	0.5	0.5	1.4	1.4	1.3	1.8	-	-
Myrtenal	OM	0.4	-	-	-	-	-	-	-
Alloaromadendrene	HS	-	-	-	-	-	-	-	0.8
α-Caryophyllene	HS	1.4	0.4	0.9	-	0.7	1.8	8.9	0.8
γ-Murolene	HS	-	-	-	-	-	-	-	1.1
cis-Verbenol	OM	-	-	-	0.8	-	-	-	-
Myrtenyl Acetate	HS	3.2	-	-	-	-	-	-	-
Germacrene-D	HS	4.1	1.9	6.1	-	2.7	1.3	11.2	12.0
Bicyclogermacrene	HS	3.3	3.1	4.3	-	2.0	0.6	1.7	-
Carvone	OM	-	-	-	-	-	-	6.8	-
8-isopropenyl-1,1,5-dimethyl-5 iclocadiene	HS	-	-	-	0.5	-	-	-	-
Δ-3-Carene	HM	-	0.3	-	-	-	-	-	-
β-Elemene	HS	-	-	-	-	-	-	-	1.1
α-Carene	HM	-	3.2	-	-	-	-	-	-
Myrtenol	OM	0.5	-	-	-	-	-	-	-
γ-Elemene	HS	-	-	0.2	0.6	0.2	0.4	1.4	7.1
p-Elemene	HS	0.5	-	-	-	-	-	-	-
Menthadienol	OM	-	-	-	0.4	-	-	-	-
α-Cubebene	HS	-	-	-	-	-	-	-	2.6
Δ-Cadinene	HS	0.7	0.8	0.2	0.7	0.4	0.8	-	-
Caryophyllene Oxide	OM	4.6	1.8	5.4	3.6	8.3	8.7	7.8	3.2
Epibiciclo-sesquiphelandrene	HS	-	-	-	-	-	-	-	2.3
Nerolidol	OS	-	0.6	-	-	-	4.6	1.1	1.6
Elemol	OS	0.9	-	4.4	-	4.7	1.0	0.6	-
α-Elemol	OS	-	-	-	-	-	15.3	-	-
α-Selinene	HS	-	0.3	-	-	-	-	-	-
Guaiol	OS	-	-	-	-	-	-	-	1.6
Cedrol	OS	-	10.3	0.1	2.3	0.2	-	1.5	1.7
(-) Spathulenol	OS	15.7	3.1	2.6	6.3	4.9	0.6	-	4.9
Calarene	HS	-	-	-	-	-	-	-	1.0
β-Patchoulene	HS	-	-	-	-	-	-	-	1.5
Bulnesol	OS	-	-	-	-	-	-	-	0.9
(+) Spathulenol	OS	-	-	-	-	-	-	-	10.6
Caryophylladienol	OS	-	-	-	-	-	-	-	1.1
Total identified		**61.5**	**84.7**	**88.5**	**76.8**	**84.9**	**70.2**	**56.0**	**72.7**
Oil yield v/p (%)		**0.2**	**2.5**	**1.1**	**0.8**	**2.0**	**1.0**	**0.4**	**0.8**

* (R.T.) Retention Time, (-) absence or no detected, (OM) oxygenated monoterpene, (HM) hydrocarbons monoterpene, (OS) oxygenated sesquiterpene, (HS) hydrocarbons sesquiterpene.

Source: Authors (2021).

The main compounds of each EO showed that each species presented a different composition. However, some constituents are conserved in several species, as linalool and ß-caryophyllene, for example (Table 2). Possibly, the conservation of these constituents may have chemotaxonomic significance to maintain similar morphologic and biochemical characteristics, which will determine the biosynthesis of their secondary metabolism (Sousa et al, 2012). For *A. lycioides*, collections were performed in three different locations and resulted in different chemical compositions. Thereby, these species were separated into two groups: major compound 1,8-cineol (IV and V) and β-Phellandrene (VI). These differences in major compounds found among the *A.lycioides* from Guaíba and São Marcos and from Rosário do Sul could be associated with the geographical origin of the material, but could also suggest different chemotypes. The species *A. citrodora* is known for the predominant presence of citral in their chemical composition (Zigadlo et al, 1994). However, our results did not show the presence of this component. These data may suggest a new chemotype for the species. These results are based on a local collection and do not analyze the intraspecific variation. The different chemistry can occur through the influence of environmental conditions and seasonal variations (Ricciardi et al, 2011). On the other hand, the composition of the essential oil of a plant is also genetically determined and usually specific to a particular organ and characteristic for their stage of development, giving rise to chemotypes in plants rich in essential oils. Tavares et al (2005) showed that differences in the composition of different chemotypes of *Lippia alba* are not only a product of the influence of environmental factors but mainly reflect the genotypic variation of these plants.

There were predominant monoterpenes (Table 3) in *A. citrodora* (59.6%), *A. dusenii* (63.1%), and *A. lycioides* (collected in Guaíba 66.7%, collected in São Marcos 65.1%, and collected in Rosário do Sul 41.3%). The species *A. virgata* and *A. polygalifolia* showed higher levels of sesquiterpenes (45.4% and 66.1%, respectively), while *A. chamaedryfolia* presented a similar quantity of mono- and sesquiterpenes (29.4% and 28,9%, respectively). These classes of terpenes are related to different biological activities (Singh & Sharma 2015) what can explain the popular use of some *Aloysia* species for medicinal purposes (Santos et al, 2015).

Table 3. Percentage of chemical groups (mono- and sesquiterpenes) in essential oils from *Aloysia* species: (I) *A. chamaedryfolia*; (II) *A. citrodora*; (III) *A. dusenii*; (IV) *A. lycioides* collected in Guaíba; (V) *A. lycioides* collected in São Marcos; (VI) *A. lycioides* collected in Rosário do Sul, (VII) *A. polygalifolia*, (VIII) *A. virgata*.

Chemical groups	I	II	III	IV	V	VI	VII	VIII
Hydrocarbon monoterpenes	12.4	21.2	37.8	6.5	35.1	27.5	0.0	2.2
Oxygenated monoterpenes	17.1	38.4	25.3	60.2	30.0	13.8	10.6	4.7
Total Monoterpenes	**29.4**	**59.6**	**63.1**	**66.7**	**65.1**	**41.3**	**10.6**	**6.9**
Hydrocarbon sesquiterpenes	13.3	10.7	22.6	1.5	15.0	8.5	42.8	43.5
Oxygenated sesquiterpenes	15.6	14.4	2.7	8.6	5.1	20.5	2.6	22.6
Total sesquiterpenes	**28.9**	**25.1**	**25.3**	**10.1**	**20.1**	**29.0**	**45.4**	**66.1**

Source: Authors (2021).

3.2 Cytotoxicity activities

As expected, the degree of lethality was directly proportional to the essential oil concentration (Table 4). The mortality rate of brine shrimp nauplii was drastically increased as the dose level was increased from 20 µg mL^{-1} to 100 µg mL^{-1}. Moreover, a 100 % mortality was observed at 500 µg mL^{-1} and 1000 µg mL^{-1} dose levels for all essential oil evaluated. More than 80% of the nauplii remained active in control, with DMSO 1%.

Table 4. Toxicity of essential oils from *Aloysia* species on *Artemia salina* in different doses level with their LC_{50} and 95% confidence intervals determined by Probit Analysis.

Species	Percentage of death at 24 hours/dose (μg mL^{-1})						LC50 (μg mL^{-1})	95% CI (μg mL^{-1})
	1000	500	100	50	20	Control		
A. citrodora	100.00	100.00	75.33	32.41	25.20	15.26	55.96	45.97 – 67.49
A. dusenii	100.00	99.62	74.89	37.45	29.88	13.72	48.98	43.74 – 54.58
A. lycioides	100.00	99.82	73.73	35.25	33.60	14.35	48.12	40.10 – 57.14

Source: Authors (2021).

The value of LC_{50}, calculated from the 24-hour counts, was not different among the essential oils of the species tested. Therefore, the toxicity was considered high, and LC_{50} values were from 48.12 μg mL^{-1} in *A. lycioides* (V) to 55.96 μg mL^{-1} in *A. citrodora*. The similar toxicity of these essential oils can probably be explained by the main constituent, 1.8-cineol, present in all of them. According to Meyer et al (1982) the LC_{50} value under 1000 μg mL^{-1} is pharmacologically active and toxic, classifying these as potentially of pharmacological interest as some authors have already related the brine shrimp lethality with the detection of antitumoral compounds in terrestrial plants (Carballo et al, 2002, Mackeen et al, 2000). However, there is no correlation between the degree of toxicity found for brine shrimp and the toxicity to mammalian cells and the brine shrimp test is used as a tool for approaching the real toxicity (Oliva et al, 2007). Oliva et al (2007) showed in their study the low toxicity of the essential oil from *Aloysia tomentosa* (LC_{50} 968 μg mL^{-1}), and nontoxicity of essential oils from *Aloysia polystachia* (LC_{50} 6459 μg mL^{-1}) and *Aloysia triphylla* (synonym for *A. citrodora*) (LC_{50} 1279 μg mL^{-1}), differently of the high toxicity observed in this work for all species, especially *A. citrodora*. Nevertheless, the essential oil composition determined by them for *A. triphylla* (*A. citrodora*) was different and rich in limonene, citral, spathulenol, and thujone, showing the importance of chemical characterization to study biological activities.

4. Conclusion

This work is the first study evaluating the chemical composition of the essential oil of six species of *Aloysia* from the South of Brazil. The species showed different chemical compositions, but some constituents are conserved in several species. This chemical information can assist in the taxonomy of the genus. Also, the results demonstrated the cytotoxic activity of some *Aloysia* essential oils, showing that studies like this are essential in the screening for new substances with potential biological activities. Therefore, the following steps should be a more detailed evaluation of the toxicity presented by this essential oil to secure the safety of using these natural products.

Acknowledgments

This study was supported by Coordenação de Aperfeiçoamento de Pessoal de Ensino Superior (1162577). We want to thank Nelson Ivo Matzenbacher for sharing knowledge and helping with the collection of *A. polyfgalifolia*. Also, we would like to thank Diego Maciel and Felipe Gonzatti for helping collect *A. virgata* and *A. dusenii*, respectively.

References

Adams, R. P. (2007). *Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy*. (4th ed). Allure Publishing Corporation.

Agotini, F., Santos, A. C. A., Rossato, M., Pansera, M. R., Santos, P. L., Serafini, L. A., Molon, R. & Moyna, P. (2009). Essential oil yield and composition of Lamiaceae species growing in Southern Brazil. *Brazilian Archives of Biology and Technology*, 52(2), 473-478. https://doi.org/10.1590/S1516-89132009000200026

Apu, A., Muhit, M., Tareq, S., Pathan, A., Jamaluddin, A. & Ahmed, M. (2010). Antimicrobial activity and brine shrimp lethality bioassay of the leaves extract of *Dillenia indica* Linn. *Journal of Young Pharmacists*, 2(1), 50-53. https://doi.org/10.4103/0975-1483.62213

Bakkali, F., Averbeck, S., Averbeck, D. & Idaomar, M. (2008). Biological effects of essential oils - a review. *Food and Chemical Toxicology*, 46(2), 446-475. https://doi.org/10.1016/j.fct.2007.09.106

Carballo, J.L., Hernandez-Inda, Z. L., Pilar, P. & Garcia-Gravalos, M. D. (2002). A comparison between two brine shrimp assays to detect in vitro cytotoxicity in marine natural products. *BMC Biotechnology*, 2,17. https://bmcbiotechnol.biomedcentral.com/articles/10.1186/1472-6750-2-17

Chao, S. C., Young D. G. & Oberg, C. J. (2000). Screening for inhibitory activity of essential oils on selected bacteria, fungi and viruses. *Journal of Essential Oil Research*, 12(5), 639-649. https://doi.org/10.1080/10412905.2000.9712177

Ghisalberti, E. L. (1993). Detection and isolation of bioactive natural products. In S.M, Colegate. & R.J, Molyneux (Ed.). *Bioactive Natural products: Detection, Isolation and Structure Elucidation* (2nd ed.), 15-18. Boca Raton: CRC Press.

Hernandez, T., Canales, M., Avila, J. G., Duran, A., Caballero J., Romo de Vivar, A. & Lira, R. (2003). Ethnobotany and antibacterial activity of some plants used in traditional medicine of Zapotitlán de las Salinas, Puebla (Mexico). *Journal of Ethnopharmacology*, 88(2-3), 181-188. https://doi.org/10.1016/S0378-8741(03)00213-7

Khafagi, I., Dewedar, A. & Farouk, S. (2000). *In vitro* cytotoxicity and antimicrobial activities of some common essential oils. *Egyptian Journal of Biology*, 2, 20-27. https://www.ajol.info/index.php/ejb/article/view/29900

Mackeen, M. M., Ali, A. M., Lajis, N. H., Kawazu, K., Hassan, Z., Amran, M., Habsah, M., Mooi, L.Y. & Mohamed, S. M. (2000). Antimicrobial, antioxidant, antitumour-promoting and cytotoxic activities of different plant part extracts of *Garcinia atroviridis* Griff. Ex T. Anders. *Journal of Ethnopharmacology*, 72,395-402. https://doi.org/10.1016/S0378-8741(00)00245-2

McLaughlin, J. L. (1991). Assays for bioactivity. In K, Hostettmann (Ed.). *Methods in Plant Biochemistry* (pp. 1-33). Academic Press.

Meyer, B. N., Ferrigni, N. R., Putnam, J. E., Jacobsen, L. B., Nichols, D. E. & McLaughlin, J. L. (1982). Brine shrimp: a convenient general bioassay for active plant constituents. *Planta Medica*, 45(5), 31-34. 10.1055/s-2007-971236

Oliva, M. M., Gallucci, N., Zygadlo, J. A. & Demo, M. S. (2007). Cytotoxic Activity of Argentinean Essential Oils on *Artemia salina*., *Pharmaceutical Biology*, 45(4), 259-262. 10.1080/13880200701214557

Reflora - Herbário Virtual. (2016). http://reflora.jbrj.gov.br/reflora/herbarioVirtual/

Ricciardi, G., Torres, A. M., Bubenik, A. L., Ricciardi, A., Lorenzo, D. & Dellacassa, E. (2011). Environmental effect on essential oil composition of *Aloysia citriodora* from Corrientes (Argentina). *Natural Product Communications*, 6(11), 1711-1714. 10.1177/1934578X1100601135

Ricco, R. A., Wagner, M. L., Portmann, E., Reides, C., Llesuy, S., Gurni, A. A. & Carballo, M. A. (2010). Survey on polyphenols, antioxidant activity and genotoxicity on Argentinean species of *Lippia* and *Aloysia (Verbenaceae). Boletim Latinoamericano e do Caribe de Plantas Medicinais e Aromáticas*, 9(5), 388-396. https://www.redalyc.org/pdf/856/85615225010.pdf

Sacchetti, G., Maietti, S., Muzzoli, M., Scaglianti, M., Manfredini, S., Radice, M. & Bruni, R. (2005). Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobial in food. *Food Chemistry*, 91(4), 621-632. https://doi.org/10.1016/j.foodchem.2004.06.031

Santos, A. C. B., Nunes, T. S., Coutinho, T. S. & Silva, M. A. P. (2015). Uso popular de espécies medicinais da família Verbenaceae no Brasil. *Revista Brasileira de Plantas Medicinais*. 17(4-II), 980-991. https://doi.org/10.1590/1983-084X/14_083

Siedo, S. J. (2006). Systematics of *Aloysia* (Verbenaceae). [dissertation]. Texas (TX): University of Texas at Austin. https://repositories.lib.utexas.edu/handle/2152/3492

Singh, B. & Sharma, R. A. (2015). Plant terpenes: defense responses, phylogenetic analysis, regulation and clinical applications. 3 Biotech 5, 129–151. https://doi.org/10.1007/s13205-014-0220-2

Singh, H. P., Batish, D. R., Kaur, S. & Kohli, R. K. (2002). Allelopathic effect of two volatile monoterpenes against bill goat weed (*Ageratum conyzoides L.*). *Crop Protection*, 21(4), 347-350. https://doi.org/10.1016/S0261-2194(01)00096-5

Sousa, O. V., Del-Vechio-Vieira, G., Alves, M. S., Aílson, A. L. A., Pinto, M. A. O., Amaral, M. P. H., Rodarte, M. P. & Kaplan, M. A. C. (2012). Chemical composition and biological activities of the essential oils from *Dauguetia lanceolata* St. Hil. Barks. *Molecules*, 17(9), 11056-11066. 10.3390/molecules170911056

Stevens, P. F. (2001). Angiosperm Phylogeny Website. Version 14, July 2017. http://www.mobot.org/MOBOT/research/APweb/

Tavares, E. S., Julião, L. S., Lopes, D., Bizzo, H. R., Lage, C. L. S. & Leitão, S. G. (2005). Analysis of the essential oil from leaves of three *Lippia alba* (Mill.) N. E. Br. (Verbenaceae) chemotypes cultivated on the same conditions. *Brazilian Journal of Pharmacognosy*, 15(1), 1-5. https://doi.org/10.1590/S0102-695X2005000100002

Tomazoni, E. Z., Pansera, M. R., Pauletti, G. F., Moura, S., Ribeiro, R. S. T. & Schwambach, J. (2016). *In vitro* antifungal activity of four chemotypes of *Lippia alba* (Verbenaceae) essential oils against *Alternaria solani* (Pleosporeaceae) isolates. *Anais da Academia Brasileira de Ciências*, 88(2), 999-1010. http://dx.doi.org/10.1590/0001-3765201620150019

Zigadlo, J. A., Lamarque, L. A., Maestri, D. M., Guzmán, C. A., Lucini, E. I., Grosso, N. R. & Ariza-Espinar, L. (1994). Volatile Constituents of *A. triphylla (L'Herit.)* Britton. *Journal of Essential Oil Research*, 6(4), 407-409. https://doi.org/10.1080/10412905.1994.9698409