ABES

Revista Brasileira de Ciências Ambientais
Brazilian Journal of Environmental Sciences

Essential oil composition and biological activities of *Aloysia gratissima* (Gillies & Hook.) Tronc. (Verbenaceae): a systematic review

Composição do óleo essencial e atividades biológicas de *Aloysia gratissima* (Gillies & Hook.) Tronc. (Verbenaceae): uma revisão sistemática

Laura Montero de Avila[1], *Adriana Carla Dias Trevisan*[2], *Antonio Batista Pereira*[1]

ABSTRACT

Essential oils are secondary metabolites stored in different organs of aromatic plants. Among the plants found in the Pampa biome with potential for essential oil extraction, *Aloysia gratissima*, the garupá, stands out. The sustainable use of native plants is a strategy for conserving biodiversity. Therefore, by connecting research and the strategic requirement to value sociobiodiversity in South America, this review aimed to systematically analyze scientific studies about the biological effects, yield, and chemical composition of the essential oil of garupá. The review considered articles published between 2012 and 2022 based on the following inclusion criteria: a) publications in English, Spanish, or Portuguese, b) related to essential oils, and c) on chemical compounds and biological activities. Of the 99 articles obtained, 13 were included in the analysis based on the construction of the textual corpus. Similarity and descending hierarchical analyses were carried out using Iramuteq. The 13 articles presented data on the composition of *A. gratissima* oils, of which ten evaluated the biological properties and eight presented yield data. Thus, the yield varied from 0.25 to 1.14% and the main compounds found in the studies were: 1,8-cineole, β-pinene, guaiol, sabinene, ß-caryophyllene, α-pinene, bicyclogermacrene, spathulenol, trans-pinocamphone, and trans-pinocarveol acetate. Furthermore, the results show that essential oils have potential for use as phytomedicines and agricultural bio-inputs due to their antifungal, antibacterial, analgesic, and repellent activities.

Keywords: biological effects; chemical composition; native plants; natural products; Pampa biome.

RESUMO

Os óleos essenciais são metabólitos secundários armazenados em diferentes órgãos de plantas aromáticas. Dentre as plantas encontradas no bioma Pampa com potencial para extração de óleo essencial, destaca-se a *Aloysia gratissima,* o garupá. O uso sustentável de plantas nativas é uma estratégia de conservação da biodiversidade. Assim, ao conectar as pesquisas e a estratégica necessidade de valorização da sociobiodiversidade na América do Sul, esta revisão busca analisar sistematicamente estudos científicos sobre os efeitos biológicos, rendimento e composição química do óleo essencial do garupá. A revisão considerou artigos publicados entre 2012 e 2022 a partir dos seguintes critérios de inclusão: a. publicações nos idiomas inglês, espanhol ou português, b. relacionadas a óleos essenciais, e c. sobre compostos químicos e atividade biológica. Dentre 99 artigos obtidos, 13 fizeram parte da análise a partir da construção do corpus textual. Foram realizadas as análises de similitude e hierárquica descendente com o uso do Iramuteq. Os 13 artigos apresentaram dados sobre a composição dos óleos de *A. gratissima* e destes, dez avaliaram as propriedades biológicas e oito apresentaram dados de rendimento. Assim, o rendimento variou de 0,25 a 1,14% e os principais compostos encontrados nos estudos foram: 1,8-cineol, β-pineno, guaiol, sabineno, ß-cariofileno, α-pineno, biciclogermacreno, espatulenol, trans-pinocanfona e acetato de trans-pinocarveol. Ainda, os resultados mostram que os óleos essenciais possuem potencial de uso para insumos em fitomedicamentos e bioinsumos agropecuários, uma vez que possuem atividades antifúngica, antibacteriana, analgésica e repelente.

Palavras-chave: efeitos biológicos; composição química; plantas nativas; produtos naturais; bioma Pampa.

[1]Universidade Federal do Pampa – São Gabriel (RS), Brasil.

[2]Universidade Estadual do Rio Grande do Sul – Sant'Ana do Livramento (RS), Brasil.

Correspondence address: Laura Montero de Avila – Universidade Federal do Pampa – Rua Aloízio Barros Macedo, s/n, BR 290, km 423 – CEP: 97300-970 – São Gabriel (RS), Brazil. E-mail: laura_monteiro_t@hotmail.com

Conflicts of interest: the authors declare no conflicts of interest.

Funding: Coordination for the Improvement of Higher Education Personnel (CAPES) – funding code 001.

Received on: 04/17/2023. Accepted on: 09/26/2023.

https://doi.org/10.5327/Z2176-94781603

Introduction

Brazil is one of the countries with the greatest prospects for defining conservation strategies for the use of megabiodiversity. This is due to its appropriate climatic, soil and water resources, in combination with the wisdom of sociobiodiversity (Zuanazzi and Mayorga, 2010; IPBES, 2019). However, due to the lack of policies and appreciation of its natural and cultural heritage, this reality is strongly threatened (Fernandes et al., 2017). In this reality, the Pampa biome, located in the southern part of South America, with 193,000 km² under Brazilian rule, exclusive in Rio Grande do Sul, is currently the second most threatened biome in Brazil, mainly for the predominance of open ecosystems (MapBiomas 2022; Menezes et al., 2022; Hasenack et al., 2023).

According to Andrade et al. (2023), more than 3,642 species belonging to 191 families and 1,108 genera are known to occur in the Pampas. It is a biome with enormous biodiversity and distinctive landscapes, with endemic flora and fauna, and a valuable genetic mosaic, but little known in Brazil (König et al., 2014). This heritage is highly threatened and ecological dynamics have resulted in floristic communities dominated by pioneer and invasive exotic species (Torchelsen et al., 2020).

Given the extent of this native plant diversity, the use of medicinal plants by local communities to treat and prevent diseases is considered one of the oldest human medicinal practices, becoming the first therapeutic resource used by primitive people (Tomazzoni et al., 2006). In addition to the typical uses of sociobiodiversity, such as medicinal and aromatic plants, there is currently a great demand for the consumption and use of products from natural sources, and one of the emerging products is the essential oil (EO), due to the large presence of bioactive compounds that give them pharmacological action (Da Silva et al., 2017). EOs are produced by more than 17,500 aromatic plants and are stored in different organs of the plant, such as flowers, leaves, cortex, roots, rhizomes, fruits, and seeds. They are secondary metabolites with a strong aroma consisting of a system of several components, mainly terpenes and volatile hydrocarbons (Baptista-Silva et al., 2020).

Among the plants found in the Pampa biome with potential for EO extraction are representatives of the genus *Aloysia* (Verbenaceae), which originated in South America and contains 34 cataloged species, 12 of which are found in Brazil (Benovit et al., 2015). Among this genus that occur in the Pampa and are typical of sociobiodiversity, *A. gratissima* stands out, an aromatic and medicinal species popularly known as Brazilian lavender, holy herb, or garupá (O'Leary and Moroni, 2020). Widely distributed in North America, *A. gratissima* is found in the southern United States and northern and central Mexico, and in South America in Bolivia, Paraguay, southern Brazil, Uruguay, northern and central Argentina, and central Chile (Risso et al., 2021).

It is a shrubby perennial plant that can reach up to three meters in height, grows in a disorderly manner, and has white flowers arranged in clusters along the branches that are very fragrant (Franco et al., 2007); its leaves are simple and opposite, with smooth edges up to the lower half of the leaf and serrated edges from the middle to the tip (Garlet, 2019). It has a lanceolate shape and may have a subcoriaceous or even soft texture, with a dark green color on the ventral side and a grayish color on the dorsal side; stems are usually thin and long, with a rough, grayish or olive-green appearance, and even shoots are herbaceous and resistant (Schreiner, 2019).

Some studies with *A. gratissima* EO have identified the potential antibiotic, anti-inflammatory, antibacterial, antifungal, antidepressant, and anesthetic effects (Benovit et al., 2015; Santos et al., 2015; Galvez et al., 2018; Paulo et al., 2021; Santos et al., 2021a; Santos et al., 2021b). Despite the interest of researchers in the species, native plants have been neglected in the context of incentive policies and innovation processes. Therefore, given the importance of the popular use of native plants, the interest in research on croup, and the need for strategies to promote the use of sociobiodiversity in South America, this review aimed to systematically analyze scientific studies on the biological effects and chemical composition of EO from *A. gratissima*.

Methodology

This review conformed to the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guidelines statement (Urrútia and Bonfill, 2010). The research was carried out in 2021 and 2022 and included a search of scientific articles published in the last ten years using the subsequent search engines: *A. gratissima, A. gratissima* AND essential oil, and *A. gratissima* AND chemical compounds, as well as replacing *A. gratissima* in the search algorithms with the synonyms *Lippia gratissima* and *Verbena gratissima*. The articles were selected according to the specific inclusion criteria: 1. original articles published in English, Spanish, or Portuguese, 2. related to EO, and 3. on chemical compounds and biological activities. The databases used were eduCAPES, SciELO, ResearchGate, and ScienceDirect. Repeated studies, bibliographic reviews, and studies that did not discuss the compounds present in *A. gratissima* or EO were excluded. The data were organized in Excel spreadsheets with the following information: 1. article data (authors, year of publication); 2. chemical composition of the oils; and 3. identified biological activities.

Firstly, the articles were selected, and after being studied and organized into a textual body, called corpus, the analysis was performed using the statistical software Iramuteq (version 0.7 alpha 2). The textual content of the corpus was subjected to a descending hierarchical analysis, aimed at finding similarities and differences between the words used in the researchers' discourses and classifying them into classes, as well as to comparative analysis, in order to examine the link between the discourses, i.e., concepts, methodological scope, and results.

Results and Discussion

We found 99 articles published between 2011 and 2022, of which 10 were available in the ResearchGate database, 21 in eduCAPES, 10 in SciELO, and 58 in ScienceDirect. Articles that were duplicates on the different search platforms were excluded. The content evaluation of the articles resulted in the elimination of 73 papers that did not meet the inclusion criteria. According to the search protocols, only 13 articles were included in this review. The flowchart (Figure 1) illustrates the methodological scope of this systematic review, with the number of articles selected at each stage indicated in parentheses.

All 13 papers studied provided primary data on the chemical composition of oils extracted from the aerial parts of *A. gratissima*, while ten of them investigated their biological properties. In the qualitative framework, the hierarchical classification analysis, which represents the relationship of similarity and divergence of the text segments analyzed in the textual corpus, identified seven classes (Figure 2).

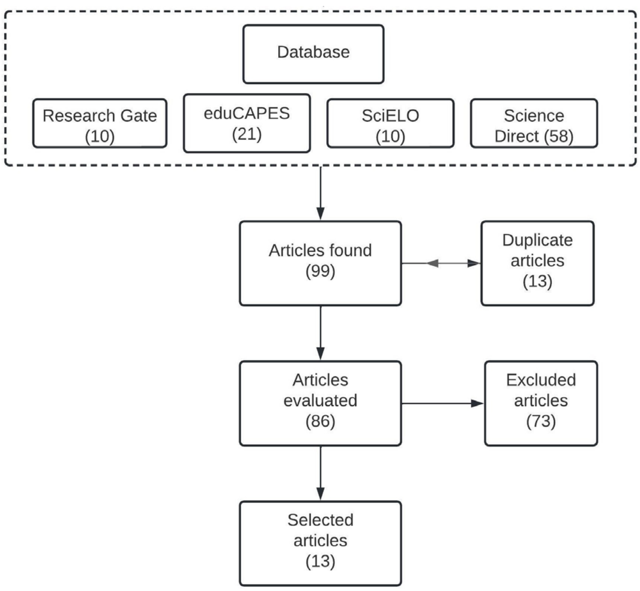

Figure 1 – Flowchart for the selection of articles used in the review on *A. gratissima*.

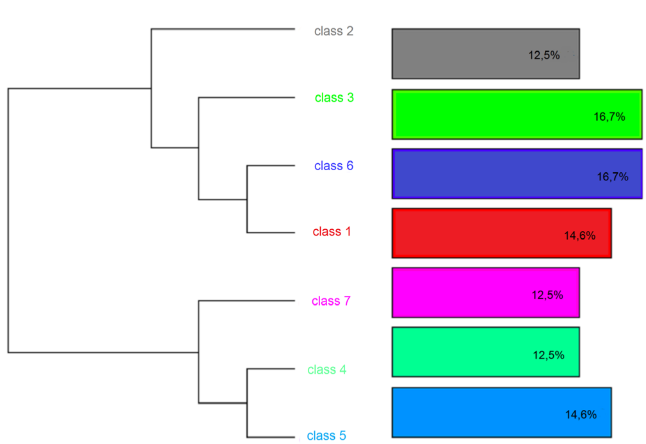

Figure 2 – Hierarchical analysis dendrogram of the text segments.

In the dendrogram of hierarchical analysis (Figure 2) it is shown that there were two groups of studies. The first group included studies related to chemical analysis and biological activity, and the second one, in addition to providing data on the chemistry and/or biological activity of EOs, also emphasized the importance of using natural products from native species in various sectors of the economy.

Paul and Sharma (2006) reported that some active principles found in extracts of certain plants can have a toxic effect on pathogens and can attack them. Silva et al. (2023) related the use of EOs as a toxic effect on phytopathogens, with positive antibacterial and antifungal activity, which are able to inhibit the growth of bacteria and fungi, respectively, or even reduce their resistance. For example, 1,8-cineol is a monoterpene known for its antimicrobial activity and ability to inhibit microorganisms such as *Staphylococcus aureus* Rosenbach (Gachkar et al., 2007). Da Silva et al. (2014) were able to show that natural products derived from EO can be more effective than a commercial fungicide in controlling diseases such as *Oidium eucalypti* Rostr. The use of natural products to gradually replace the use of synthetic fungicides is a current topic of interest in the fields of alternative control, medicine, and organic agriculture (Pansera et al., 2021). These natural products, especially EO from native species, can be a vector for innovation in areas under pressure from agribusiness (Elguy et al., 2021).

The volatile oil of *A. gratissima* is characterized by a diverse composition derived mainly from two groups of compounds, the terpenoids (monoterpenes and sesquiterpenes) and the phenylpropanoids (Calsamiglia et al., 2007). These compounds originate from a wide range of primary metabolic precursors and are synthesized through different metabolic pathways, thus conferring a diverse spectrum of biological activity (Khan et al., 2011).

The EO extraction method adopted in the 13 studies reviewed was hydrodistillation, of which 12 used a laboratory bench extractor called a Clevenger and one used an industrial scale extractor (Elguy et al., 2021). For the identification of the compounds present in EO, gas chromatography coupled with mass spectrometry was the method used in all studies. The 29 compounds identified in the studies analyzed are described in Table 1.

Of the 29 compounds referred to above (Table 1), 24 had quantitative data recorded in the studies evaluated. Thus, from this perspective of quantitative analysis, the ten compounds found in the highest percentage concentration were: 1. β-thujone (36.10%); 2. α-thujone (32.20%); 3. 6α-hydroxygermacra-1 (16.30%); 4. spathulenol (16.23%); 5. e-pinocamphone (16.07%); 6. 1,8-cineole (14.82%); 7. trans-pinocamphone (14.12%); 8. β-caryophyllene (13.88%); 9. trans-pinocarveol acetate (13.27%); and 10. β-pinene (12.93%). However, it should be observed that the first five compounds highlighted above were identified in only one of the studies assessed, the sixth compound was identified in five studies, and the last four compounds were detected in two, four, two, and five studies, respectively.

Table 1 – Compounds found in essential oil of *Aloysia gratissima*.

Compounds	1	2	3	4	5	6	7	8	9	10	11	12	13	Part of the plant	n	Study location
1,8-cineole					x	x	x	x					x	LE, TW	5	RS, SC, RJ (BR), (ARG)
Guaiol		x	x	x	x			x						LE, FL, TW	5	MG, SP, RS, RJ (BR)
β-pinene			x	x		x			x	x				LE, FL, TW	5	MG, SP, SC, PR (BR)
Sabinene					x		x				x		x	LE, TW	4	RS (BR), (ARG)
Bicyclogermacrene					x	x			x	x				LE, FL, TW	4	RS, SC, PR (BR)
α-pinene									x	x		x	x	LE, FL, TW	4	SC, PR (BR)
β-caryophyllene	x					x			x	x				LE, FL, TW	4	SC, PR (BR), (BO),
Trans-pinocarveol acetate		x	x	x										LE, FL	3	MG, SP (BR)
Germacrene B		x			x									LE, FL	3	MG, RS (BR)
Germacrene D						x						x		LE, TW	2	SC (BR)
Spathulenol												x	x	LE, TW	2	RS (BR), (ARG)
Trans-pinocamphone		x	x											LE, FL	2	MG (BR)
(E)-nerolidol									x	x				LE, FL, TW	2	SC, PR (BR)
E-pinocamphone				x										LE	1	SP (BR)
E-pinocamphone acetate			x											LE	1	MG (BR)
3-carene											x			LE, TW	1	RS (BR)
Pinene											x			LE, TW	1	RS (BR)
γ-terpinene											x			LE, TW	1	RS (BR)
Limonene											x			LE, TW	1	RS (BR)
Nerolidol												x		LE, TW	1	RS (BR)
β-thujone							x							LE	1	(ARG)
α-thujone							x							LE	1	(ARG)
6α-Hydroxygermacra-1	x													LE	1	(BO)
Cubenol	x													LE	1	(BO)
δ-Cadinene	x													LE	1	(BO)
Germacrene D-4-ol	x													LE	1	(BO)
Myrcene			x											LE	1	MG (BR)
α-caryophyllene				x										LE	1	SP (BR)
Verbenol													x	LE	1	(ARG)

Arze et al. (2013)[1]; Santos et al. (2013)[2]; Da Silva et al. (2014)[3]; Bersan et al. (2014)[4]; Benovit et al. (2015)[5]; Santos et al. (2015)[6]; Galvez et al. (2018)[7]; Garcia et al. (2018)[8]; Santos et al. (2021a)[9]; Santos et al. (2021b)[10]; Elguy et al. (2021)[11]; Pansera et al. (2021)[12]; Risso et al. (2022)[13]. LE: leaves; FL: flowers; TW: twigs. RS: Rio Grande do Sul; SC: Santa Catarina; RJ: Rio de Janeiro; MG: Minas Gerais; SP: São Paulo; PR: Paraná; BR: Brazil; ARG: Argentina; BO: Bolivia.

It also should be noted that β-thujone and α-thujone are the compounds that stand out as they showed concentration peaks well above the highest peak of the compound recorded in more than one study. In addition, both compounds were identified in a study conducted in Argentina. This study did not record the time and season in which the parts of *A. gratissima* used to obtain the oils were harvested, so it is believed that these may be the factors that influenced their composition.

Figure 3 shows the concentration spread of the 24 compounds with quantitative data identified in the evaluated studies.

Among these 24 compounds, some were reported in more than one study during the course of the review; 17 compounds were common among the reviewed papers and 12 had quantitative values, as shown in the descriptive statistical analysis of concentrations below (Figure 4). The highest standard deviation was observed for β-pinene at 8.2% and the lowest at 0.4% for germacrene D.

Chemical composition of *A. gratissima*

Some researchers such as Benovit et al. (2015), when extracting EO from *A. gratissima* using a Clevenger, identified the presence of four major components, as shown in Table 1. However, when the oil was fractionated, the following compounds were observed: E- (-)-pinocamphone, (-)-caryophyllene oxide, (-)-guaiol, and (+)-spathulenol.

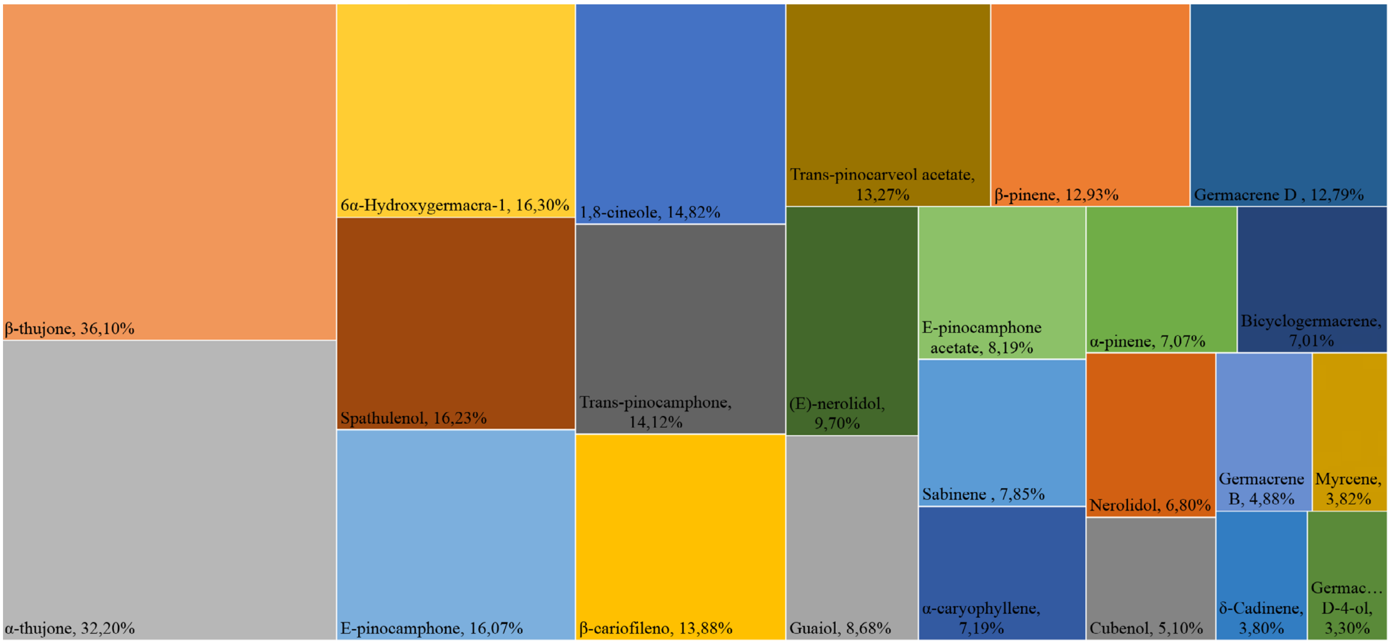

Figure 3 – Compounds identified in the essential oils of *Aloysia gratissima* in the studies analyzed.

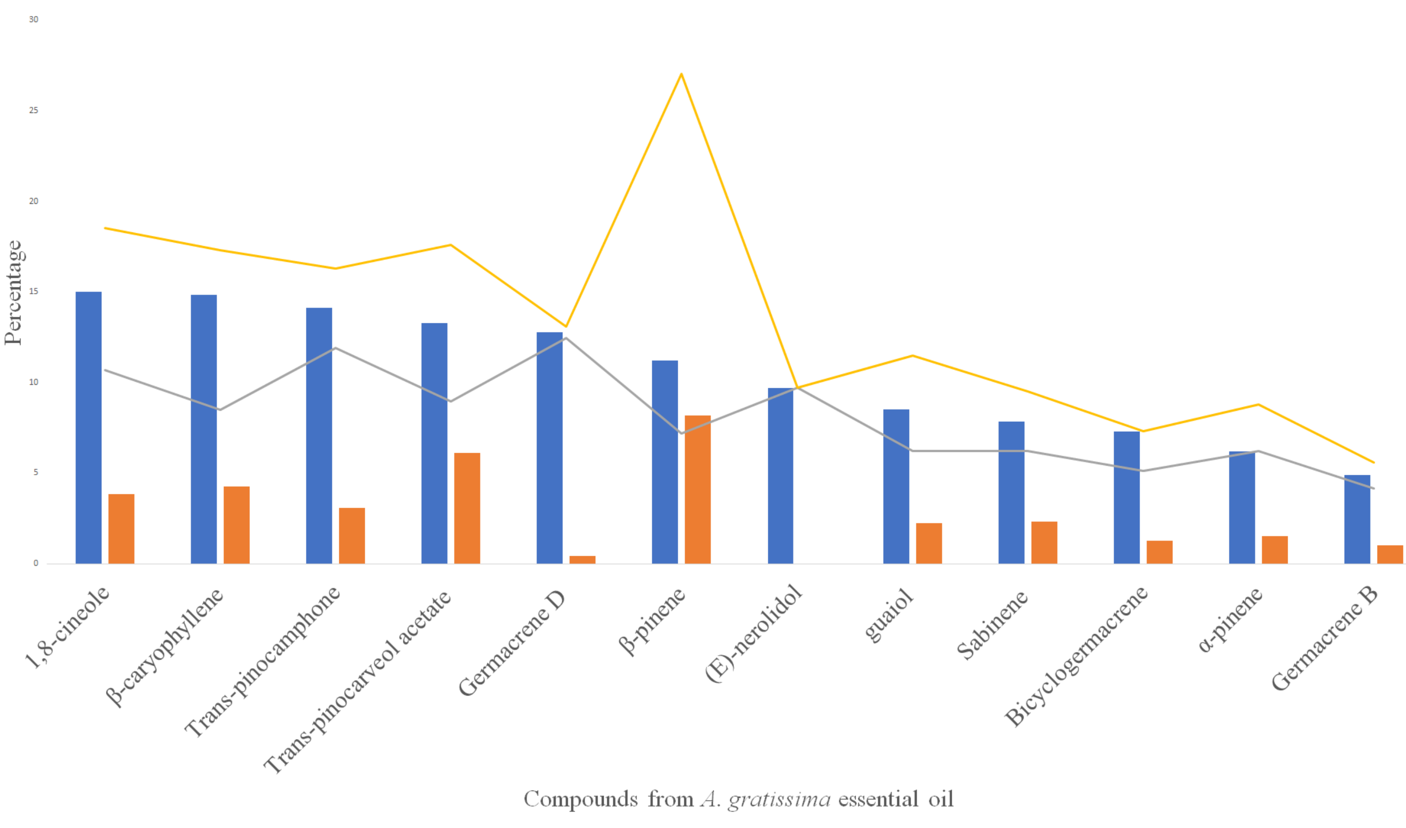

Figure 4 – Compounds identified in *A. gratissima* essential oil in more than one study.

Elguy et al. (2021) extracted the oil of *A. gratissima* using an industrial steam extractor, which resulted in the identification of ten major components, including seven monoterpenes, representing 72.6% of the composition, and three sesquiterpenes, with a total of 24.3%. Both studies mentioned above were carried out in the state of Rio Grande do Sul, Brazil.

Arze et al. (2013) collected *A. gratissima* in the province of Mizque, in Cochabamba (Bolivia) and identified the presence of five major compounds in the EO, also shown in Table 1. In the Province of San Luis (Argentina), Risso et al. (2022) identified the presence of the monoterpenes α-pinene, sabinene, and verbenol, and the sesquiterpenes, 1,8-cineole, spathulenol, cubenol, and β-caryophyllene in the oils.

Santos et al. (2013) extracted the EO from the leaves and flowers of *A. gratissima* using Clevenger hydrodistillation and analysis by gas chromatography coupled with mass spectrometry. The three major compounds identified from the EO of *A. gratissima* leaves were trans-pinocarveol acetate, trans-pinocamphone, and guaiol, which together accounted for 45.4% of the identified compounds. The same study highlighted that the main compounds in the flowers were e-caryophyllene, germacrene B, and guaiol.

When extracting EO from croup in the fall, Risso et al. (2021) identified the presence of monoterpenes and sesquiterpenes in the composition of the oil, with the majority compound of each group being 1,8-cineole and spathulenol, respectively. Pansera et al. (2021), when collecting and extracting the EO of *A. gratissima* in autumn, identified the majority presence of sesquiterpene spathulenol (16.23%), followed by germacrene D (12.48%).

In summer, Santos et al. (2015) collected and extracted the EO from *A. gratissima*, in the states of Santa Catarina and Paraná in Brazil, and found that 50.6% of the compounds identified in the oils were monoterpenes and 43.1% were sesquiterpenes, with the majority being germacrene D (13.1%), β-caryophyllene (12.4%), and 1,8-cineole (12.4%). Santos et al. (2021a) also collected samples in the state of Santa Catarina, but unlike the above authors, the samples were collected in spring.

Yield of essential oils

When extracting EO from *A. gratissima* leaves (120 g) and flowers (60 g), Santos et al. (2013) found that the flowers had an EO yield of 0.56% and the leaves 0.35%. Arze et al. (2013) reported that after extracting 76 kg of *A. gratissima* leaves by Clevenger, they obtained 110 mL of EO, corresponding to 1.14%. From 100 g of fresh *A. gratissima* leaves, Bersan et al. (2014) got 1.10% of the mass of the material used. Benovit et al. (2015) extracted the EO of *A. gratissima* from the leaves and produced five oil fractions, with a yield of 1.94±0.03% (w/w). Garcia et al. (2018) reached a yield of 0.52% EO when extracting 1,020 g of *A. gratissima* leaves and twigs. Pansera et al. (2021) obtained 0.50 % v/m oil when extracting leaves and twigs of *A. gratissima*.

Elguy et al. (2021) extracted EO from *A. gratissima* at two different times of the year, fall and spring. In the fall, they extracted 20 kg of fresh matter from the leaves of the plant and reached a yield of 0.25%. In the spring, the leaves and branches of the plant were extracted and a yield of 0.75% was achieved from 20 kg of fresh matter. Santos et al. (2021a) obtained a yield of 0.33% EO after extracting 50 g of dry matter (flowers, twigs, and leaves) from *A. gratissima* using the Clevenger method.

It can be seen that there is no standard unit to measure the EO yield in the reviewed articles since of the eight studies that reported the EO yield, six used the percentage extracted over the weight of the mass used, one used the percentage over the weight, and another presented the volume over the mass. Among the six studies that used the same unit of measurement, the maximum reported value was 1.14% and the minimum was 0.25%.

Biological activities

The biological properties of the EO extracted from the species found in the works are anesthetic (Benovit et al., 2015), virucidal (Garcia et al., 2018), antifungal (Santos et al., 2013; Da Silva et al., 2014; Santos et al., 2015; Galvez et al., 2018; Pansera et al., 2021), antibacterial (Santos et al., 2013, Santos et al., 2015; Santos et al., 2021a), and antibiotic (Santos et al., 2021b), as shown in Figure 5.

The antimicrobial activity of *A. gratissima* EO was evaluated by Bersan et al. (2014) using the microdilution against the fungus *Candida albicans* (Robin) Berkhout (CBS 562) and the bacteria *Streptococcus sanguis* White & Niven (ATCC 10556), *Streptococcus* mitis Andrewes and Horder (ATCC 903), *Porphyromonas gingivalis* Straint (ATCC 33277), and *Fusobacterium nucleatum* (ATCC 25586). They found that *A. gratissima* EO significantly inhibited the biofilm growth of *P. gingivalis, S. sanguis*, and *S. mitis*. Santos et al. (2013) assessed the chemical composition of the oil from the leaves and flowers of *A. gratissima* and its antimicrobial activity against the Gram-positive bacteria *Bacillus subtilis* (Cohn 1872) (CCT 2576), *Staphylococcus aureus* Rosenbach (CCT 2740), and *Streptococcus pneumoniae* Chester (ATCC 11733), the Gram-negative bacteria *Salmonella choleraesuis* Le Minor (CCT 4296) and *Pseudomonas aeruginosa* Schroeter (ATCC 13388), and the fungus *C. albicans* (ATCC 10231). The antimicrobial activity varied according to the microorganisms evaluated, such that the EO of *A. gratissima* leaves showed activity against *P. aeruginosa* (CIM 0.8 mg mL^{-1}) and *S. pneumoniae* (CIM 0.8 mg mL^{-1}) and the EO of the inflorescence showed activity against *P. aeruginosa* (CIM 0.15 mg mL^{-1}), *S. pneumoniae* (CIM 0.025 mg mL^{-1}), and *C. albicans* (0.02 mg mL^{-1}). According to the authors, the EO activity of the flower was more effective than that of the leaf and was particularly pronounced against the Gram-negative bacterium *P. aeruginosa*, the Gram-positive bacterium *S. pneumoniae*, and the yeast *C. albicans*.

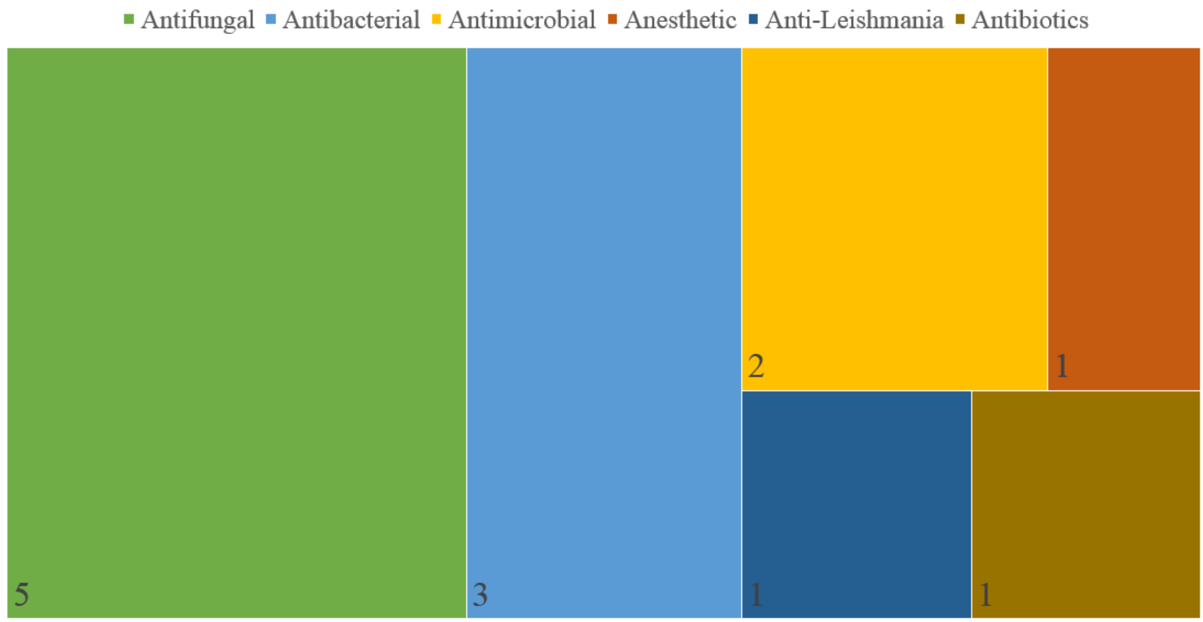

Figure 5 – Biological effects found in *Aloysia. gratissima* essential oils according to the number of studies.

Da Silva et al. (2014) investigated the antifungal effect of *A. gratissima* leaf EO on *O. eucalypti*. They identified that the oil contained active compounds capable of controlling the infection in the plant, and that *A. gratissima* had the additional advantage of inducing systemic resistance in eucalyptus plants. Thus, it can be concluded that the oil was effective in the local control of powdery mildew in eucalyptus even at the lowest concentrations tested (0.25%) and can be used to control the fungus. Galvez et al. (2018) described that the EO of *A. gratissima* leaves showed moderate antifungal activity against strains of *Fusarium* sp (minimum inhibitory concentration [MIC]=0.6–1.2 mg/mL) and had a weak effect on strains of *Aspergillus sp*, that is, it was inactive.

Pansera et al. (2021) assessed the antifungal activity of the oil from the branches and leaves of *A. gratissima* against the pathogenic fungi *Alternaria alternata* (Fr.) Keissl., *Fusarium oxysporum* Schlecht. emend. Snyder and Hansen, *Botrytis cinerea* (de Bary) Whetzel, *Sclerotinia sclerotiorum* (Lib.) de Bary, and *Colletotrichum gloeosporioides* Penz. After 14 days of exposure to a concentration of 0.10% v/v EO, the percentage of inhibition ranged from 3.27 to 33.87%. This result showed that the EO of *A. gratissima* can be used to control the fungi *A. alternata, F. oxysporum, B. cinerea, S. clerotiorum, and C. gloeosporioides.*

Regarding the antiprotozoal activity of *A. gratissima*, Garcia et al. (2018) conducted a study in which they evaluated the effect of the oil from the leaves and branches of *A. gratissima* on the parasite *Leishmania amazonensis* Lainson & Shaw, which causes the disease leishmaniasis, and observed structural changes in the kinetoplast, plasma membrane, and mitochondrial matrix. The authors correlated these changes with the chemical composition of the oil, the main compound being guaiol. It was also shown that there were inhibitions of 31, 44, and 85% of parasite growth 72 hours after treatment with 1, 10, and 100 $\mu g\ mL^{-1}$ of *A. gratissima* EO, respectively, resulting in IC50s at 48 h and 72 h for exposure to the compound of 25 and 14 $\mu g\ mL^{-1}$, respectively. The same study also examined the effect of the oil on the load of intramacrophage amastigotes, a protozoan vector of the disease leishmaniasis, and found an 85% reduction in the intracellular load of amastigotes with 2 $\mu g\ mL^{-1}$ of *A. gratissima* oil, similar to the reduction promoted by 1 $\mu g\ mL^{-1}$ of amphotericin B. The data from this research established, for the first time, that guaiol inhibits amastigotes survival with an IC50 of 0.01 $\mu g\ mL^{-1}$ against amastigotes, suggesting that the effects of *A. gratissima* oil may be due to the greater presence of guaiol in the composition of the oil, although the effect of other similar sesquiterpenes (for example, bulnesol and germacrene) cannot be definitively excluded. The authors also speculated that guaiol may be metabolized directly by the parasites, producing toxic products, or by host cells, producing leishmanicidal metabolites.

Referring to antibiotic activity, Santos et al. (2021b) investigated the potential of EO from leaves, twigs, and flowers of *A. gratissima* to enhance the activity of conventional antibiotics against resistant strains of *S. aureus, Escherichia coli* Migula, and *P. aeruginosa*, which cause serious infections such as endocarditis, urinary and intestinal infections, respectively. It was found that EO from *A. gratissima* potentiated ($p<0.0001$) the activity of norfloxacin against all the bacterial strains analyzed in the study. However, during the work it was found that EO caused significant changes in the activity of gentamicin and erythromycin against the bacterial strains studied. This suggests that *A. gratissima* EO selectively modulates resistance to different classes of antibiotics.

Conclusions

As a conclusion of this review, it was evidenced that *A. gratissima* EO could be used in a wide range of treatments, once they have promising potential as a source of compounds that can act as anti-inflammatory, antifungal, antibacterial, and antibiotic agents. It is possible to realize that there was a great variation between the compounds found in the oils evaluated and that there was no characteristic that standardized the compounds identified in the highest concentrations. The chemical compounds most frequently identified when extracting croup were terpenes, with 1,8-cineole, β-pinene, guaiol, sabinene, ß-caryophyllene, α-pinene, bicyclogermacrene, spathulenol, trans-pinocamphone, and trans-pinocarveol acetate being the main ones, but none of them was found in more than 5 papers.

Another point is that the extraction method used in the studies did not have a significant effect on the chemical composition of the extracts, but the period and time of harvesting could have an influence. Some of the main compounds identified were found in only one study and were identified in high concentrations, which may be due to the time of harvesting the plants. All studies were carried out in South American countries, as *A. gratissima* is a native species and has a wide distribution in this region.

Finally, the results of the study indicate that the possibility of utilizing the EO of *A. gratissima* is still insufficient in relation to its full potential in the field of phytomedicines and, in order to gain a better understanding of the subject, it would be fundamental to conduct several additional studies.

Contribution of authors

AVILA, L. M. Conceptualization, Data curation, Formal Analysis, Methodology, Project administration, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. TREVISAN, A. C. D. Conceptualization, Data curation, Formal Analysis, Methodology, Project administration, Software, Supervision, Validation, Visualization, Writing – review & editing. PEREIRA, A. B. Supervision and Writing – review

References

Andrade, B.; Dröse, W.; Alves de Aguiar, C.; Teixeira Aires, E.; Janisch Alvares, D.; Barbieri, R.L.; De Carvalho, C.J.B.; Bartz, M.L.C.; Becker, F.G.; Bencke, G.A.; Beneduzi, A.; Bernardo Silva, J.; Blochtein, B.; Boldrini, I.I.; Boll, P.K.; Bordin, J.; Silveira, R.M.B.; Borges-Martins, M.; Bosenbecker, C.; Braccini, J.; Braun, B.; Brito, R.; Brown, G. G.; Büneker, H. M.; Buzatto, C. R.; Cavalleri, A.; Zanini Cechin, S.; Colombo, P.; Constantino, R.; Fernanda da Costa, C.; Dalzochio, M. S.; Gehlen de Oliveira, M.; Antunes Dias, R.; Amaral dos Santos, L.; Da Fonseca Duarte, A.; Lessa Pinto Duarte, J.; Durigon, J.; Escobar da Silva, M.; Porto Alegre Ferreira, P.; Ferreira, T.; Ferrer, J.; G Ferro, V.; Fontana, C.; Freire, M. D.; Freitas, T.; Galiano, D.; Garcia, M.; Santos, T.; Gomes, L.R.P.; Gonzatti, F.; Silva Gottschalk, M.; Graciolli, G.; Granada, C.; Grings, M.; Santos Guimarães, P.; Heydrich, I.; Iop, S.; Jarenkow, J.A.; Jungbluth, P.; Käffer, M.; Kaminski, L.A.; Costa Kenne, D.; Dutra Kirst, F.; Kütter Krolow, T.; Ferreira Kruger, R.; Busnello Kubiak, B.; Leal-Zanchet, A.; Loebmann, D.; Borges Lucas, D.; Lucas, E.; Luza, A.L.; Machado, I.F.; Madalozzo, B.; Maestri, R.; Malabarba, L.R.; Maneyro, R.; Marinho, M.A.T.; Marques, R.; Da Silva Marta, K.; Da Silveira Martins, D.; Da Silva Martins, G.; Rambo Martins, T.; Mello, A.; Luciano Mello, R.; De Souza Mendonça, M.; Barros de Morais, A.B.; Moreira, F.F.F.; Bairos Moreira, L.F.; De A. Moura, L.; Helena Nervo, M.; Ott, R.; Paludo, P.; Passaglia, L.M.P.; Périco, E.; Sant'Anna Petzhold, E.; Marques Pires, M. ; Lucas Poppe, J.; Quintela, F.; Raguse-Quadros, M.; Ramos Pereira, M.J.; Renner, S.; Ribeiro, F.B.; Inacio Ribeiro, J.R.; N. L. Rodrigues, E.; Rodrigues, P.E.S.; Piccoli Romanowski, H.; Petersen Ruschel, T.; da Silva Alves Saccol, S.; Savaris, M.; Schmidt Silveira, F.; José Schmitz, H.; Emilia Siegloch, A.; Siewert, R.; Silva da Silva Filho, P.J.; Soares, A.G.; Somavilla, A.; Sperotto, P. ; Spies, M. ; Tirelli, F. ; Marques Tozetti, A.; Verrastro, L.; Vogel Ely, C.; Zafalon da Silva, Â.; Zank, C. ; Zefa, E., 2023. 12,500+ and counting: biodiversity of the Brazilian Pampa. Frontiers of Biogeography, v. 15, (3), e59288. https://dx.doi.org/10.21425/F5FBG59288

Arze, J.B.L.; Collin, G.; Garneau, F.X.; Jean, F.I.; Gagnon, H., 2013. Essential Oils from Bolivia. XI. Verbenaceae: *Aloysia gratissima* (Gillies & Hook.) Tronc. and Boraginaceae: *Cordia chacoensis* Chodat. Journal of Essential Oil-Bearing Plants, v. 16, (4), 545-550. https://doi.org/10.1080/0972060X.2013.831553

Baptista-Silva, S.; Borges, S.; Ramos, O.L.; Pintado, M.; Sarmento, B., 2020. The progress of essential oils as potential therapeutic agents: a review. Journal of Essential Oil Research, v. 32, (4), 279-95. https://doi.org/10.1080/10412905.2020.1746698

Benovit, S.C.; Silva, L.L.; Salbego, J.; Loro, V.L., Mallmann, C.A.; Baldisserotto, B.; Flores, E.M.M.; Heinzmann, B.M., 2015. Anesthetic activity and bioguided fractionation of the essential oil of *Aloysia gratissima* (Gillies & Hook.) Tronc. in silver catfish *Rhamdia quelen.* Anais da Academia Brasileira de Ciências, v. 87, (3), 1675-1689. https://doi.org/10.1590/0001-3765201520140223

Bersan, S.M.; Galvão, L.C.; Goes, V.F.; Sartoratto, A.; Figueira, G.M.; Rehder, V.L.; Alencar, S.M.; Duarte, R.M.; Rosalen, P.L.; Duarte, M.C., 2014. Action of essential oils from Brazilian native and exotic medicinal species on oral biofilms. BMC Complement Altern Medicine, v. 14, (451). https://doi.org/10.1186/1472-6882-14-451

Calsamiglia, S.; Busquet, M.; Cardozo, P. W.; Castillejos, L.; Ferret, A., 2007. Invited review:essential oils as modifiers of rumen microbial fermentation. Journal of Dairy Science, v. 90, (6), 2580-2595. https://doi.org/10.3168/jds.2006-644

Da Silva, A.C.; de Souza, P.E.; de Resende, M.L.V.; da Silva, M.B.; Ribeiro, P.M.; Zeviani, W.M., 2014. Local and systemic control of powdery mildew in eucalyptus using essential oils and decoctions from traditional Brazilian medicinal plants. Forest Pathology, v. 44, (2), 145-153. https://doi.org/10.1111/efp.12079

Da Silva, C.B.; Silva, K.B.; Oliveira, E.L.S.; Soares, V.F.; Costa, J.G.; Santos, A.F., 2017. A importância da ação antioxidante de óleos essenciais em benefício da saúde. Diversitas Journal, v. 2, (1), 52-55. https://doi.org/10.17648/diversitas-journal-v2i4.483

Elguy, L.G.P.; Trevisan, A.C.D.; Coelho, M.P.; Oliveira, Y.E.C.; Madureira, L.A.S., 2021. Manejo de biomassa e compostos majoritários de óleos essenciais de espécies do Bioma Pampa. Revista Fitos, v. 15, (3), 333-345. https://doi.org/10.32712/2446-4775.2021.1065

Fernandes, G.W.; Vale, M.M.; Overbeck, G.E.; Bustamante, M.M.C.; Grelle, C.E.V.; Bergallo, H.G.; Magnusson, W.E.; Akama, A.; Alves, S.S.; Amorim, A.; Araújo, J.; Barros, C.F.; Bravo, F.; Carim, M.J.V.; Cerqueira, R.; Collevatti, R.G.; Colli, G.R.; da Cunha, C.N.; D'Andrea, P.S.; Dianese, J.C.; Diniz, S.; Estrela, P.C.; Fernandes, M.R.M.; Fontana, C.S.; Giacomin, L.L; Gusmão, L.F.P.; Juncá, F.A.; Lins-e-Silva, A.C.B.; Lopes, C.R.A.S.; Lorini, M.L.; de Queiroz, L.P.; Malabarba, L.R.; Marimon, B.S.; Junior, B.H.M.; Marques, M.C.M.; Martinelli, B.M.; Martins, M.B.; Medeiros, H.F.; Menin, M.; de Morais, P.B.; Muniz, F.H.; Neckel-Oliveira, S.; Oliveira, J.A.; Oliveira, R.P.; Penha, F.P.J.; Podgaiski, L.R.; Rodrigues, D.J.; Scariot, A.; Silveira, L.F.; Silveira, M.; Torres, W.M.; Vital, M.J.S.; Pillar, V.D., 2017. Dismantling Brazil's science threatens global biodiversity heritage. Perspectives in Ecology and Conservation, v. 15, (3), 239-243. https://doi.org/10.1016/j.pecon.2017.07.004

Franco, A.L.P.; Oliveira, T.B.; Ferri, P.H.; Bara, M.T.F.; De Paula, J.R., 2007. Avaliação da composição química e atividade antibacteriana dos óleos essenciais de *Aloysia gratissima* (Gillies & Hook) Tronc. (alfazema), *Ocimum gratissimum L.* (alfavaca-cravo) e *Curcuma longa L.* (açafrão). Revista Eletrônica de Farmácia, v. 4, (2). https://doi.org/10.5216/ref.v4i2.3063

Gachkar, L.; Yadegari, D.; Rezaei, M.B.; Taghizadeh, M.; Astaneh, S.A.; Rasooli, I., 2007. Chemical and biological characteristics of *Cuminum cyminum* and *Rosmarinus officinalis* essential oils. Food Chemistry, v. 102, (3), 102:898-904. https://doi.org/10.1016/j.foodchem.2006.06.035

Galvez, C.E.; Jimenez, C.M.; Gomez, A.L.A.; Lizarraga, E.F.; Sampietro, D.A., 2018. Chemical composition and antifungal activity of essential oils from *Senecio nutans*, *Senecio viridis*, *Tagetes terniflora* and *Aloysia gratissima* against toxigenic *Aspergillus* and *Fusarium* species. Natural Product Research, v. 34, (10), 1442-1445. https:// doi.org/10.1080//14786419.2018.1511555

Garcia, M.C.F.; Soares, D.C.; Santana, R.C.; Saraiva, E.M.; Siani, A C.; Ramos, M.F.S.; Danelli, M.D.G.M.; Souto-Padron, T.C.; Pinto-da-Silva, L.H., 2018. The in vitro antileishmanial activity of essential oil from *Aloysia gratissima* and guaiol, its major sesquiterpene against *Leishmania amazonensis*. Parasitology, v. 145, (9), 1219-1227. https://doi.org/10.1017/S0031182017002335

Garlet, T.M.B., 2019. Plantas medicinais nativas de uso popular no Rio Grande do Sul [recurso eletrônico]. Santa Maria, RS: UFSM, PRE, 2019. 1 e-book: il. – (Série Extensão) ISBN 978-85-67104-45-4 (Accessed September 21, 2023) at:. https://www.ufsm.br/app/uploads/sites/346/2019/12/Cartilha-Plantas-Medicinais.pdf

Hasenack, H.; Weber, E.J.; Boldrini, I.I.; Trevisan, R.; Flores, C.A.; Dewes, H., 2023. Biophysical delineation of grassland ecological systems in the State of Rio Grande do Sul, Southern Brazil. Iheringia, v. 78, e2023001. https://doi.org/10.21826/2446-82312023v78e2023001

Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES), 2019. Global assessment report on biodiversity and ecosystem services of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (Version 1). Zenodo. https://doi.org/10.5281/zenodo.6417333

Khan, A.; Ahmad, A.; Akhtar, F.; Yousuf, S.; Xess, I.; Khan, L.A.; Manzoor, N., 2011. Induction of oxidative stress as a possible mechanism of the antifungal action of three phenylpropanoids, FEMS Yeast Research, v. 11, (1), 114-122. https://doi.org/10.1111/j.1567-1364.2010.00697.x

König, F.; Gonçalves, C.E.P.; Aguiar, A.R.; Silva, A.C.F., 2014. Bioma Pampa: Interações entre micro-organismos e espécies vegetais nativas. Revista de Ciências Agrárias, v. 37, (1), 3-9. https://doi.org/10.19084/rca.16792

MapBiomas, 2022. Destaques do mapeamento anual de cobertura e uso da terra no Brasil entre 1985 a 2021. Coleção 7 (Accessed January 16, 2023) at:. https://mapbiomas-br-site.s3.amazonaws.com/Fact-Sheet-Colecao7.pdf

Menezes, L.S.; Ely, C.V.; Lucas, D.B.; Silva, G.H.M.; Vélez-Martin, E.; Hasenack, H.; Trevisan, R.; Boldrini, I.I.; Pillar, V.D.; Overbeck, G.E., 2022. Reference values and drivers of diversity for South Brazilian grassland plant communities. Annals of the Brazilian Academy of Sciences, v. 94, (1), e20201079. https://doi.org/10.1590/0001-3765202220201079

O'Leary, N.; Moroni, P., 2020. *Aloysia in* Flora e Funga do Brasil. Jardim Botânico do Rio de Janeiro (Accessed November 14, 2022) at:. https://floradobrasil.jbrj.gov.br/FB135457

Pansera, M.R.; Silvestre, W.P.; Gonzatti, F.; Pauletti G.F.; Sartori, V C., 2021. Chemical composition and antifungal activity of the essential oils from native species of the 'Campos de Cima da Serra' region, South Brazil. Journal of Essential Oil Research, v. 33, (5), 488-501. https://doi.org/10.1080/10412905.2021.1928558

Paul, P.K.; Sharma, P.D., 2002. *Azadirachta indica* leaf extract induces resistance in barley against leaf stripe disease. Physiological and Molecular Plant Pathology, v. 61, (1), 3-13. https://doi.org/10.1006/pmpp.2002.0412

Paulo, C.L.R.; Santos, E.L.; De Araújo, A.C.J.; Freitas, P.R.; De Almeida, R.S.; De Araújo Neto, J.B.; Tintino, S.R.; Coutinho, H.D.M., 2021. Atividade antibacteriana e potencializadora do óleo essencial de *Aloysia gratissima* (Gillies & Hook) sobre cepas multirresistentes. VI Semana Universitária da Urca - XXIV Semana de Iniciação Científica da Urca (Accessed September 19, 2023) at:. https://siseventos.urca.br/assets/pdf/sub_trabalhos/351-937-4875-102.pdf

Risso, O.A.; Posadaz, A.; Leal, M.; Ardanáz, C.; Suárez, S.A.; Ojeda, M.S., 2022. Caracterización in situ de poblaciones de *Aloysia gratissima* var. *gratissima* recolectadas del noreste de la provincia de San Luis, Argentina. Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas, v. 21, (4), 431-445. https://doi.org/10.37360/blacpma.22.21.4.26

Risso, O.A.; Suárez, S.A.; Posadaz, A.C., 2021. *Aloysia gratissima* (Gillies & Hook. ex Hook.) Tronc. var. *gratissima*. In: Máthé, Á., Bandoni, A. Medicinal and Aromatic Plants of South America. v. 2. Medicinal and Aromatic Plants of the World. v. 7. Springer, Cham. https://doi.org/10.1007/978-3-030-62818-5_3

Santos, F.M.; Pinto, J.E.B.P.; Bertolucci, S.K.V.; Alvarenga, A.A.; Alves, M.N.; Duarte, M.C.T.; Sartoratto, A., 2013. Chemical composition and antimicrobial activity of the essential oil from the leaves and flowers of *Aloysia gratissima*. Revista Brasileira de Plantas Medicinais, v. 15, (4), 583-588. https://doi.org/10.1590/S1516-05722013000400015

Santos, T.G.; Laemmle J.; Rebelo, R.A.; Dalmarco, E.M.; Cruz, A.B.; Schmit, A.P.; Cruz, R.C.B.; Zeni, A.L.B., 2015. Chemical composition and antimicrobial activity of *Aloysia gratissima* (Verbenaceae) leaf essential oil. Journal of Essential Oil Research, v. 27, (2), 125-130. https://doi.org/10.1590/S1516-05722013000400015

Santos, E.L.; Freitas, P.R.; Araújo, A.C.J; Almeida, R.S.; Tintino, S.R.; Paulo, C.L.R.; Silva, A.C.A.; Silva, L.E.; Amaral, W.; Deschamps, C.; Junior, J.P.S.; Filho, J.M.B.; de Sousa, G.R.; Ribeiro-Filho, J.; Coutinho, H.D.M., 2021a. Enhanced antibacterial effect of antibiotics by the essential oil of *Aloysia gratissima* (Gillies & Hook.) Tronc. and its major constituent beta-caryophyllene. Phytomedicine Plus, v. 1, (4). https://doi.org/10.1016/j.phyplu.2021.100100

Santos, E.L.; Freitas, P.R.; Araújo, A.C J; Almeida, R.S.; Tintino, S.R.; Paulo, C.L.R.; Ribeiro-Filho, J.; Silva, A.C.A.; Silva, L.E.; Amaral, W.; Deschamps, C.; Junior, J.P.S.; Filho, J.M.B.; de Sousa, G.R.; Coutinho, H.D.M., 2021b. Phytochemical characterization and antibiotic potentiating effects of the essential oil of *Aloysia gratissima* (Gillies & Hook.) and beta-caryophyllene. South African Journal of Botany, v. 143, 1-6. https://doi.org/10.1016/j.sajb.2021.07.046

Schreiner, G.E., 2019. Extração e caracterização de metabólitos secundários de *Aloysia gratissima* (Gillies & Hook.) Tronc. Trabalho de Conclusão de Curso, Licenciatura em Ciências Biológicas, Universidade Federal da Fronteira Sul, Cerro Largo. (Accessed October 12, 2022) at:. https://rd.uffs.edu.br/handle/prefix/3571

Silva, M.J.; Batista, E.N.S.; Souza, L.E.V.; Filho, L.F.C.O.; Morgado, M.M.; Silva, T.B.; Santana, A.C.; Lorenzo, V.P.; Vilar, F.C.R., 2023. Uso de plantas medicinais no controle de pragas e doenças. In: Vilar, F.C.R.; Silva T.B. (Orgs.), Plantas medicinais e suas potencialidades. Editora Científica Digital, pp. 159-168. https://doi.org/10.37885/230111698

Tomazzoni, M.I.; Negrelle, R.R.B.; Centa, M.L., 2006. Fitoterapia Popular, a Busca Instrumental Enquanto Prática Terapêutica. Texto e contexto de Enfermagem, v. 15, (1), 115-121. https://doi.org/10.1590/S0104-07072006000100014.

Torchelsen, F.P.; Cordero, R.L.; Overbeck, G.E., 2020. Conservation of species-rich subtropical grasslands: traditional management vs. legal conservation requirements in primary and secondary grasslands. Acta Botanica Brasilica, v. 34, (2), 342-351. https://doi.org/10.1590/0102-33062019abb0306

Urrútia, G.; Bonfill, X., 2010. PRISMA declaration: a proposal to improve the publication of systematic reviews and meta-analyses. Medicina clínica, v. 135, (11), 507-511. https://doi.org/10.1016/j.medcli.2010.01.015

Zuanazzi, J.A.S; Mayorga, P., 2010. Fitoprodutos e desenvolvimento econômico. Quim Nova, v 33, (6), 1421-1428. https://doi.org/10.1590/S0100-40422010000600037