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DRIMYS BRASILIENSIS ESSENTIAL OIL AS A SOURCE OF DRIMENOL

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ABSTRACT

Drimys brasiliensis Miers is a native plant species to the Atlantic Forest, commonly known as cataia, and used as a stimulant, anti-diahrreal, antipyretic, among other properties. Dried and fresh leaves of cataia were collected in autumn/2012, submitted to hydrodistillation in a Clevenger graduated apparatus over a period of 4 hours after reaching the boiling point, then essential oil was collected. In oil from green leaves, 49 compounds were identified, being 65.0% sesquiterpenes, 12.0% monoterpenes and 23.0% other substances. In oil from dry leaves, 40 compounds were identified, being 76.1% sesquiterpenes, 2.0% monoterpenes and 21.9% other compounds. The main constituents in green leaves were germacrene D (8.9%), bicyclegermacrene (5.3%), epi-alpha-cadinol (5.1%), alpha-cadinol (6.0%), and drimenol (9.3%). In dry leaves the main constituents were germacrene D (6.3%), (E)-nerodidol (5.4%), spathulenol (9.5%), epi-alpha-cadinol (5.5%), alpha-cadinol (6.7%), and drimenol (11.6%). Due to its composition, the species may possibly present some biological activities like antifungical, antibacterial, insectifuge, molluscicide, and also the pharmacological properties that the species may present.

KEYWORDS: Cataia, Sesquiterpene, Monoterpene, Composition.

ÓLEO ESSENCIAL DE *DRIMYS BRASILIENSIS* COMO FONTE DE DRIMENOL

RESUMO

Drimys brasiliensis Miers é uma espécie nativa da Mata Atlântica, conhecida popularmente por cataia, utilizada como estimulante, antidiarreica, antifebril, dentre outras propriedades. Folhas frescas e secas de cataia foram coletadas no outono/2012, submetidas à hidrodestilação em aparelho graduado tipo Clevenger durante um período de 4 horas após ebulição, retirando-se em seguida o óleo. No óleo de folhas frescas foram identificados 49 compostos, sendo 65,0% sesquiterpenos, 12,0% monoterpenos e 23,0% de outras substâncias. Já no óleo de folhas secas identificou-se 40 compostos, sendo 76,1% sesquiterpenos, 2,0% monoterpenos e 21,9% de outros compostos. Os principais constituintes para as folhas frescas foram germacreno D (8,9%), biciclogermacreno (5,3%), epi-alfa-cadinol (5,1%), alfa-cadinol (6,0%) e drimenol (9,3%). Já para as folhas secas, os principais constituintes foram germacreno D (6,3%), (E)-nerodidol (5,4%), espatulenol (9,5%), epi-alfa-cadinol (5,5%), alfa-cadinol (6,7%) e drimenol (11,6%). Devido a sua composição, a espécie possivelmente pode possuir algumas atividades como antifúngicas, antibacterianas, além de insetífuga, moluscocida e com propriedades farmacológicas que a espécie pode possuir.

PALAVRAS-CHAVE: Cataia, Sesquiterpeno, Monoterpeno, Composição.

1 INTRODUCTION

Commonly known as “cataia”, *Drimys brasiliensis* is a species native that can be used for stimulant to the popular medicine because of the oil composition. Ribeiro *et al*. (2008) found that the essential oils found in the leaves was lethal to cattle ticks and canines.

Drimys stands out for display by several pharmacological effects secondary metabolites responsible scientifically proven, including anti-inflammatory activities, antibacterial, antifungal, cytotoxic, molluscicide piscicide and regulating plant growth. Among the major classes of substances found are the sesquiterpenes drimanos, flavonoids and aromadendranos (MORTON, 1981; SIMÕES *et al*., 1986; JANSEN; GROOT, 1991; ANDRÉ *et al*., 1999).

Thus, according to Gomes *et al*. (2013), the chemical study of the essential oil *Drimys brasiliensis* allows to know their biological activities and evaluate their toxicity, to find out more about its therapeutic potential and its possible adverse effects, thus increasing safety its use by the population.

There are several studies on the essential oil chemical composition of *Drimys brasiliensis* bark; however, according to Radomski *et al*. (2013), there is some difficulty in this study because it is not possible to collect on a large scale or at different times of the year in the same plant as the depletion of the bark on the stem of the same would result in impairment of the phloem sap flow, which the long-term, culminating with the death of the plant.

In order to study alternative for essential oil extraction recommendation *Drimys brasiliensis*, this study aimed to characterize the essential oil of fresh and dried leaves of this species and analyze your components.

2 LITERATURE REVIEW

Commonly known as “cataia” or “casca-de-anta”, *Drimys brasiliensis* is a species native to the Atlantic Forest Biome, occurring in the Brazilian states of Bahia, Minas Gerais, Espirito Santo, Rio de Janeiro, São Paulo, Paraná, Santa Catarina and Rio Grande do Sul (TRINTA & SANTOS, 1997).

In popular tradition is a medicine of internal use, known as a stimulant, antispasmodic, anti-diahrreal, antipyretic and antibacterial (ALMEIDA, 1993), due to the presence of flavonoids and terpenoids in its composition (WITAICENIS *et al*., 2007).

The term essential oil is used to describe volatile oily liquids with strong and in most cases pleasant aroma, extracted mainly from plants. It is believed that, during their development, plants synthesize essential terpenoids for their own growth and development (UGAZ, 1994; ARAUJO *et al*., 2001).

According to Cruz (2013), various authors reported quite common presence of drimanic sesquiterpenes in essential oils of this species, like drimanial, drimenol, valdivilolide, polygodial, 1-b-(*p*-methoxycinnamoyl) polygodial and 1-b-(*p*-coumaroyloxy) polygodial.

Drimenol is a very important representative of drimanic sesquiterpenoids. This terpenol served as a starting compound in the synthesis of various natural drimanes (JANSEN & GROOT, 1991;

VLAD, 2006). According to Jansen & Groot (1991), drimenol has the plant growth regulatory activity comparable with that of heteroauxin.

The importance of drimenol as a starting compound for the synthesis of drimanic compounds, on one hand, and the fact that its contents in natural sources is relatively low, on the other hand, have stimulated studies of its synthesis (JANSEN & GROOT, 1991).

Therefore, this work had the objective to characterize the essential oil extracted from fresh and dried leaves of *Drimys brasiliensis*.

3 MATERIAL AND METHOD

Leaves of 5 years old *Drimys brasiliensis* Miers, commonly known as "cataia", Winteraceae family, plants were used for the essential oil extraction. The plant material was obtained from plants grown at Embrapa Florestas, situated at south latitude 25º19'16" and west longitude 49º09'31". According to the Köppen classification, climate of the region is Cfb type, with average temperature of the hottest month above 10 °C, mild summers and winters with frequent icings, with tendency to rainfall concentrations during summer, however without a defined dry season. Dried samples of the species where stocked in the Embrapa Florestas herbarium with the HFC classification n° 7963, who was collected in Colombo (PR) by W. Mashio in January, 2005.

Extraction of the essential oil of *Drimys brasiliensis* was performed in the Plant Eco-physiology Laboratory of the Department of Phytotechnology and Phytosanitary Sciences of the Federal University of Parana (Universidade Federal do Paraná - UFPR), from leaves collected in autumn/2012.

The experiment was conducted using dried and fresh leaves, randomly collected from stock plants, lately submitted to hydrodistillation in a Clevenger type graduated apparatus (WASICKY, 1963), over a period of 4 hours after reaching the boiling point.

To extract the essential oil from dry leaves, the green material was left in an oven at 45°C over a period of 7 days, to obtain the needed dried material.

Essential oil was processed in a centrifuge for 2 minutes at 100 rpm. Essential oil samples were quantified and stocked at -20 °C, until the moment of the chromatographic analysis.

Quantification of the essential oil was performed using precision micropipettes (100-1000 µL). Samples of 30 µL taken from the total extracted oil were weighted and their weight was divided by the 30 µL volume, thus obtaining the density of the essential oil (L). Total weight of the sample was then divided by this density to obtain the total volume in g L^{-1}.

The essential oil samples were analyzed by gas chromatography coupled to mass spectrometry (GC / MS). The equipment was Varian gas chromatograph, Model CP 3800 with FID detector (CG_FID) capillary column Chrompack fused silica CP-SIL 8 CB, 0.25 mm internal diameter, 30m long and 0.25uM liquid film. Injector temperature: 250 °C, split 1:300, injected sample volume: 1.0uL . Carrier gas: helium 1mL/min constante. Gás makeup: synthetic air, nitrogen and hidrogênio. Temperatura FID detector: 300 °C. Oven Temperature Program: initial 50 °C temperature rise to 180 °C at a rate of 10 °C lasting for 20 minutes; temperature rise to 200 °C at a rate of 20 °C for 1 minute remains. Total run time: 35-40 minutes. Chemical characterization of the essential oil of *Drimys brasiliensis* was performed by Embrapa Agroindustria de Alimentos, located in Rio de Janeiro (RJ).

4 RESULT AND DISCUSSION

The essential oil yield from fresh leaves was 0.0236 µL/g of dry mass and yield from dried leaves was 0.0221 µL/g. The chemical composition of essential oil from dried and green leaves of *Drimys brasiliensis* is presented in Table 1.

In the oil of fresh and dried leaves, 49 and 40 compounds where respectively identified, with predominance of sesquiterpenes that were 65% in green leaves and 76.1% in dry leaves. Similar results were obtained by Limberger *et al*. (2007) in essential oil samples from fresh and dried leaves of *Drymis brasiliensis* in Rio Grande do Sul State, being 37.1% from fresh leaves and 65.4% from dried leaves. Lago *et al*. (2010) also reported predominance of sesquiterpenes equal to 57.31% in the oil from leaves of this species, collected in Campos do Jordão (SP).

In this study, polygodial sesquiterpene was not detected, although it is common in some studies with essential oils of *Drimys brasiliensis* (MUÑOZ-CONCHA *et al*., 2004). The sesquiterpene drimenol was the most abundant compound found in this study with 9.3% in the fresh and 11.6% in the dried material (Table 1).

According to Costa *et al*. (2005), the chemical composition and yield of essential oil is influenced by the plants drying process. The fact that percentage of drimenol increased in the composition of essential oil extracted from dry leaves may depend on the drying process. This process is intended to minimize loss of active principles and slow down their deterioration, and a very fast drying process could cause degradation. However, a very slow process could cause the establishment of undesirable microorganisms and, with water reduction, the quantity of active principles also increases (VON HERTWIG, 1991; SILVA & CASALI, 2000).

Besides the sesquiterpenes, monoterpenes were also found in fresh and dried leaves of *Drymis brasiliensis*, and the highest amount were found in the essential oil from fresh leaves (12%), when compared to dried leaves (2%) (Table 1).

One of the factors justifying the difference in the chemical composition of fresh and dried leaves oil is the fact that they have little stability, especially in the presence of air, light and heat (SIMÕES & SPITZER, 2004). Thus, it is argued that the temperature for drying the leaves influenced the chemical composition of essential oils when compared with the oil of fresh leaves .

Compounds with relative quantity > 5% in the essential oil of fresh material were germacrene D (8.9%), bicyclogermacrene (5.3%), epi-alpha-cadinol (5.5%), alpha-cadinol (6.0%) and drimenol (9.3%). For dry material the most represented compounds were germacrene D (6.3%), (E)-nerolidol (5.4%), spathulenol (9.5%), epi-alpha-cadinol (5.5%), alpha-cadinol (6.7%) and drimenol (11.6%) (Table 1).

TABLE 1: Compounds of the essential oil from green and dried leaves of *Drimys brasiliensis*.

Identification of the compound	Quantity in the Essential Oil from green material	Quantity in the Essential Oil from dried material
Alpha-pinene	1,5	0,3
Sabinene	1,0	-
Beta-pinene	2,2	0,5
Myrcene	0,3	-
Alpha-terpinene	0,5	-
Beta-phalandrene	3,3	0,5
Gamma-terpinene	1,0	-
Terpinolene	0,5	-
4-terpineol	1,5	0,7
Safrol	1,1	-
Alpha-cubebene	0,4	0,4
Beta-borbuonene	1,1	1,1
Beta-elemene	0,8	1,0
(E)-caryophyllene	1,0	1,1
Beta-copaene	0,5	0,5
Alpha-humulene	0,6	1,0
Gamma-muurolene	0,6	0,7
Germacrene D	8,9	6,3
Bicyclogermacrene	5,3	3,9
Alpha-Muurolene	0,5	-
Gamma-cadinene	0,7	0,8
Delta-cadinene	2,4	2,9
(E)-nerodidol	4,3	5,4
Spathulenol	3,8	9,5
Globulol	3,1	3,6
Viridiflorol	1,4	2,6
Rosifoliol	1,1	1,2
Junenol	1,0	1,1
n.i.	0,8	0,8
Epi-cubenol	2,4	2,4
Isospathulenol	1,3	2,0
Epi-alpha-cadinol	5,1	5,5
Alpha-Muurolol	1,6	2,0
Alpha-cadinol	6,0	6,7
Germacra-4(15),5,10(14)-trien-1[illegible]-ol	1,0	2,0
drimenol	9,3	11,6
ent-Rosa-5,15-diene	2,9	0,9
Drimenine	0,7	0,8
Kaurene	0,9	0,9
Total (%)	100,0	100,0
Monoterpenes	12,0	2,0
Sesquiterpenes	65,0	76,1
Others	23,0	21,9

Santos *et al*. (2013) collected leaves of *Drymis brasiliensis* in the São Paulo State and detected variations in the following compounds when compared to this work: 9.5% sabinene, 10.5% myrcene, 10.6% limonene and 28.3% cyclocolorenone. Gomes *et al*. (2013) identified 96.6% compounds in essential oil samples of *Drimys brasiliensis*. The main constituent was cyclocolorenone (18.3%), followed by terpinen-4-ol (8.4%) and myristicin (6.6%). Finally, Lago *et al*. (2010), extracted essential oil from leaves of *Drymis brasiliensis* collected in the Umauarama district, in the municipality of Campos do Jordão (SP) and identified sixteen volatile compounds, with predominance of sesquiterpenes (52.31%), being the main derivatives the cedrene (6.87%), bicyclogermacrene (5.31%), t-muurolol (7.75%) and drimenol (9.96%).

Those chemical differences found in the essential oil samples of this study, when compared to literature, may be due to the specific climatic conditions and to geographic conditions where plants had developed.

According to Kutchan (2001), secondary metabolites represent a chemical interface between plants and environment; thus, their synthesis is frequently influenced by environmental conditions. Roca-Pérez (2004), studying essential oil from leaves of *Digitalis obscura*, found lower cardenolides concentrations, like lanatoside A, in spring and with a rapid accumulation phase in summer followed by decrease in autumn. Wallaart *et al*. (2000) observed that metabolic stress caused by icing in *Artemisia annua* originated a 60% increase in artemisinin levels, coincident wih decrease of its biosynthetic precursor, the dihydroartemisinic acid.

According to Gobbo-Neto & Lopes (2007), production of essential oils of *Drymis brasiliensis*, such as production of others secondary metabolites, is strongly influenced by environmental factors.

The presence of the sesquiterpene drimenol is very strong in the essential oil composition of the species (9.3% and 11.6% respectively in green and dry leaves). This class of sesquiterpenes appears in a restricted group of angiosperms, like Winteraceae, Solanaceae, Canellaceae, Taxaceae and Polygonaceae, and has been raising particular interest due to the wide range of biological activities and sensory properties (ASAKAWA, 2004).

Sesquiterpenes with drimane structure may present biological activities like: antibacterial, antifungal, plant growth regulator, citotoxicity , mulloscicide, and others (JANSEN & GROOT, 1991).

Due to this potential, drimenol have been used as a base material to obtain other more active natural drimanes or semi-synthetic derivatives with an increased biological activity (KUCHKOVA *et al*., 2009).

Another important application of drimenol is as precursor for ambrox and isoambrox, which are the most important synthetic substitutes of ambergris, nowadays one of the most valuable and one of the few materials with animal origin used in the perfumery industry (BENITES *et al.,* 2006).

It is important to point out that some compounds identified in this work, like beta-elemene, (E)-caryophylene, alpha-humulene, gamma-muurolene, delta-cadinene, (E)-nerolidol, spathulenol, globulol, viridiflorol, rosifoliol, junenol, isospathulenol, epi-alpha-cadinol, alpha muurolol, alpha-cadinol, germacra-4(15),5,10(14)-trien-1-alpha-ol and drimenol, after drying of leaves, increased their percentage in the essential oil composition. This demonstrates that drying leaves at 45 °C has qualitative influence on the extracted essential oil of *Drymis brasiliensis.*

According to Silva *et al*. (1999), losses of active principles in essential oil of dry leaves are due to degradation caused by metabolic processes, hydrolysis, light, enzymatic, oxidation, fermentation, heat and microbiologic contaminations.

5 CONCLUSIONS

Under the conditions of the experiment that was conducted in this work it is possible to conclude that, for the composition of the essential oil of *Drymis brasiliensis* there was predominance of sesquiterpenes in the fresh material (65.0%) and in the dried material (76.1%). The main compounds of the green material were germacrene D (8.9%), bicyclogermacrene (5.3%), epi-alpha-cadinol (5.1%), alpha-cadinol (6.0%) and drimenol (9.3%), while for the dry material were germacrene D (6.3%), (E)-nerolidol (5.4%), spathulenol (9.5%), epi-alpha-cadinol (5.5%), alpha-cadinol (6.7%) and drimenol (11.6%). Because of its composition, the species may possibly present some biological activities like antifungical, antibacterial, insectifuge, pharmacological and molluscicide.

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