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Chemical composition of essential oil extracted from leaves of *Campomanesia adamantium* subjected to different hydrodistillation times

Juliana Dantas de Oliveira¹ Daniely Karen Matias Alves¹ Mayker Lazaro Dantas Miranda²
José Milton Alves¹ Marcelo Nogueira Xavier¹ Cristiane de Melo Casal³
Cassia Cristina Fernandes Alves^{1*}

¹Instituto Federal de Educação, Ciência e Tecnologia Goiano, Avenida Goiânia, Km 1, Zona Rural, 75901-970, Rio Verde, GO, Brasil. E-mail: cassiacfeftrv@gmail.com. *Corresponding author.

²Instituto Federal de Educação, Ciência e Tecnologia do Sul de Minas Gerais, Pouso Alegre, MG, Brasil.

³Instituto Federal de Educação, Ciência e Tecnologia do Sudeste de Minas Gerais, Barbacena, MG, Brasil.

ABSTRACT: *Campomanesia adamantium* is a native fruit species of the Cerrado and is used in food and medicines and as bee pasture. The chemical composition of essential oils obtained from plants of the same species have varying constituent proportions due to the influence of extractive factors, environmental, genetic and ontogenetic. This study aimed to identify the influence of hydrodistillation time on the content and chemical composition of essential oil extracted from the leaves of *C. adamantium*. Treatments consisted of five extraction times (1, 2, 3, 4, and 5h) using Clevenger with five replications in a completely randomized design. It was observed that after two hours of hydrodistillation, the essential oil content remains constant. Regarding the chemical constituents of essential oil, variation of the proportions of the compounds tested occurred at all hydrodistillation times. The compounds spathulenol oxygenated sesquiterpenes and caryophyllene oxide were the majority in the five hydrodistillation times.

Key words: essential oil, hydrodistillation time, sesquiterpenes.

Composição química do óleo essencial de *Campomanesia adamantium* extraído de folhas submetidas a diferentes tempos de hidrodestilação

RESUMO: A *Campomanesia adamantium* é uma espécie frutífera nativa do Cerrado que se destaca pela utilização na alimentação, na medicina e como pasto apícola. A composição química dos óleos essenciais obtidos de plantas de uma mesma espécie pode sofrer variação de seus constituintes por influência de vários fatores incluindo o método de extração do óleo essencial, fatores ambientais, genéticos e ontogenéticos. Neste sentido, o presente trabalho tem como objetivo identificar a influência do tempo de hidrodestilação sobre o teor e a composição química do óleo essencial das folhas de *C. adamantium*. Os tratamentos constituíram-se de cinco tempos de extração (1, 2, 3, 4 e 5 horas) utilizando aparelho Clevenger com cinco repetições, sendo o delineamento inteiramente casualizado. Foi observado que após duas horas de hidrodestilação o teor de óleo essencial permanece constante. Em relação aos constituintes químicos do óleo essencial ocorreu variação das proporções dos compostos em todos os tempos de hidrodestilação testados, os compostos sesquiterpenos oxigenados espatulenol e óxido cariofileno foram majoritários nos cinco tempos de hidrodestilação.

Palavras-chave: óleo essencial, tempo de hidrodestilação, sesquiterpenos.

INTRODUCTION

Campomanesia adamantium, commonly known as “gabirola” or “guabirola”, is a deciduous shrub between 0.5 and 1.5m high that flowers between September and October, and fructification between November and December (LORENZI et al., 2006).

C. adamantium belongs to the *Myrtaceae* family, which comprises approximately 4,000 species and 130 genera distributed across all continents, except Antarctica (D'ANGELIS et al., 2014). Its fruits are tasty, juicy, citric, and slightly sweet, constituting a very good aliment, and are consumed

in natura in the food industry and as flavoring agents in the beverage industry, liquors, juices, sweets, and ice cream (VIEIRA et al., 2011).

Leaves and fruits of *C. adamantium* have some medicinal properties, including anti-inflammatory, antidiarrheal, and antiseptic properties in the urinary tract. Leaves are utilized to treat flu, and fruits act on the intestine. The alcoholic extract of *C. adamantium* leaves, when tested, presented vasorelaxing activity on aortic rings isolated from mice (VIEIRA et al., 2011).

Studies on *C. adamantium* provided data for the identification of chemical compounds

found in its essential oil, leaf extracts, and fruit extracts, as well as for its pharmacology. However, no studies mention factors that may affect the content and chemical composition of essential oil extracted from gabioba.

COUTINHO et al., (2009) identified 95 compounds in essential oils extracted from *C. adamantium* leaves. During flowering, the major substances found were monoterpenes (limonene, α -pinene, and β -pinene), while during the vegetative state, the predominant substances were sesquiterpenes (bicyclogermacrene and globulol). When hexane and ethyl acetate extracts from *C. adamantium* leaves were evaluated, little variation was observed between their chemical compositions during plant development in different seasons, whereas the alcoholic extract had a significantly altered chemical composition (COUTINHO et al., 2010). When the biological activities of these organic extracts from *C. adamantium* were evaluated, they showed promising cytotoxic and antioxidant activities (RAMOS et al., 2007).

COUTINHO et al., (2008a) isolated five flavanones and three chalcones from *C. adamantium* leaves using HPLC. Extracts were obtained from leaves of this species harvested in four cities in Mato Grosso do Sul State, Brazil, revealing different amounts of total phenolic compounds and antioxidant activity. In another study performed with essential oil from its leaves and flowers, the chemical composition of volatile compounds from *C. adamantium* were determined: limonene was the major compound (21.9%) in essential oil from leaves, while ledol was the major compound (20.9%) in essential oil from flowers (COUTINHO et al., 2008b).

VALLILO et al., (2006) evaluated *C. adamantium* fruits harvested at different ripening stages, regarding to nutritional composition, essential oil profile, and the presence of organic substances. *C. adamantium* fruits presented an essential oil with a yellow color, and, through GC-MS analysis, 40 compounds were identified in the essential oil, corresponding to 93.3% of the compounds present. The major compounds reported in this essential oil were the monoterpene hydrocarbons α -pinene (10.6%), limonene (10.1%), and β -(z)-ocimene (9.2%).

According to the International Standard Organization (ISO), essential oils are defined as products obtained from plant tissue through steam distillation, as well as products obtained by pressing the pericarp of citrus fruits (NOGUEIRA et al., 2007).

Several international groups commercialize essential oils, many of which are used as raw

materials in the production of flavors and fragrances. Brazil has an important role in the production of essential oils, and, together with India, China, and Indonesia, is among the four biggest producers in the world. Brazil's position in this ranking is a result of the essential oils obtained from *Citrus* as by products of the juice industry (BIZZO et al., 2009).

The importance of studies with essential oils results from the fact that they present several biological activities reported in the literature, used, for instance, in the control of phytopathogens responsible for plant diseases that lead to significant production losses, grain destruction during storage, loss of nutritional value, and, occasionally, the production of mycotoxins that are harmful to human beings and animals. Approximately 60% of essential oils present antifungal activity and 35% show antibacterial properties (SANTOS et al., 2010).

According to OLIVEIRA et al., (2012), some aspects influence the chemical composition of essential oils, such as genetic and environmental factors, and others affect the yield, such as method and extraction time. These aspects indicated the importance of a previous evaluation of essential oil extraction time for each plant species analyzed, taking into account the particularities of the chemical compositions of the essential oils, and the extraction methods employed to obtain them, in order to promote an optimization of the whole process.

Studies concerning the influence of extraction time on the yield and chemical compositions of essential oils are scarce, though fundamental for the maximization of essential oil yield. In this context, the present research aimed to evaluate the effect of different hydrodistillation times on the content and chemical composition of essential oil extracted from *C. adamantium*.

MATERIALS AND METHODS

Material harvest and selection

C. adamantium leaves were harvested in the Rio Verde region between 7 and 8 a.m., on October 10th, 2014, at properties belonging to the University of Rio Verde (coordinates, 17°47'14.8", W 50°57'59.1"; altitude, 769m). The plant material was identified by Professor Germano Guarin Neto Herbarium (Jataí, Brazil) according to Voucher HJ 6561. The leaves were then taken to the Laboratory of Chemistry and Natural Products for essential oil extraction.

Essential oil extraction

Essential oil extraction from gabioba leaves (400g) *in natura* was performed *via a*

hydrodistillation method using a Clevenger apparatus, in a process similar to that employed by SIQUEIRA et al., (2011). Extractions were carried out at five different time lengths (1, 2, 3, 4, and 5h) after ebullition, with five repetitions each. Following the five different hydrodistillation times, essential oil was extracted from the aqueous phase by partition with an organic solvent (dichloromethane) in three successive repetitions of 10mL each, and a resting time of 10 min between them in order to ensure phase separation. Anhydrous sodium sulfate was used to remove traces of water from the resulting mixture consisting of essential oil/dichloromethane, which was subsequently filtered. After the complete evaporation of the dichloromethane, the extracted essential oil was weighed using an analytical scale and stored at -4°C until analysis by GC-MS.

Essential oil chemical analysis

The essential oil was diluted in hexane and analyzed by gas phase chromatography (QP-5000 da Shimadzu[®]) for detection *via* mass spectrometry (GC-MS). The evaluation was performed in a fused silica capillary column model DB-5 (30m length x 0.25mm inner diameter x 0.25 μm film thickness) (Shimadzu[®]). Helium (He) was used as the carrier gas with a flow of 1.0mL/min. The 1:20 split injection was performed using an injector at 240°C . The temperature of the detector was 270°C , and the oven was programmed to heat from 60 to 180°C at a rate of $3^{\circ}\text{C}/\text{min}$. Detection was performed in electron ionization mode at 70 e V. Three repetitions were performed for each essential oil analyzed. Essential oil chemical composition was determined based on the mass spectra acquired by GC-MS. Volatile compounds were identified by comparing the retention times achieved in the experiment with the retention times of linear hydrocarbons (C_{10} - C_{29} homologous series), which were co-injected with the sample of interest. The retention rates and the mass spectra were compared with data from the spectral library of Wiley, Nist, and other literature (ADAMS, 2007).

Statistical analysis

A completely randomized design (CRD) was used in the experiment, with five hydrodistillation times (1, 2, 3, 4, and 5h) and five repetitions each. The results were statistically analyzed using an analysis of variance ($F < 0.05$) and the averages were compared using the Tukey's test at a 5% significance level with the software ASSISTAT version 7.7 beta.

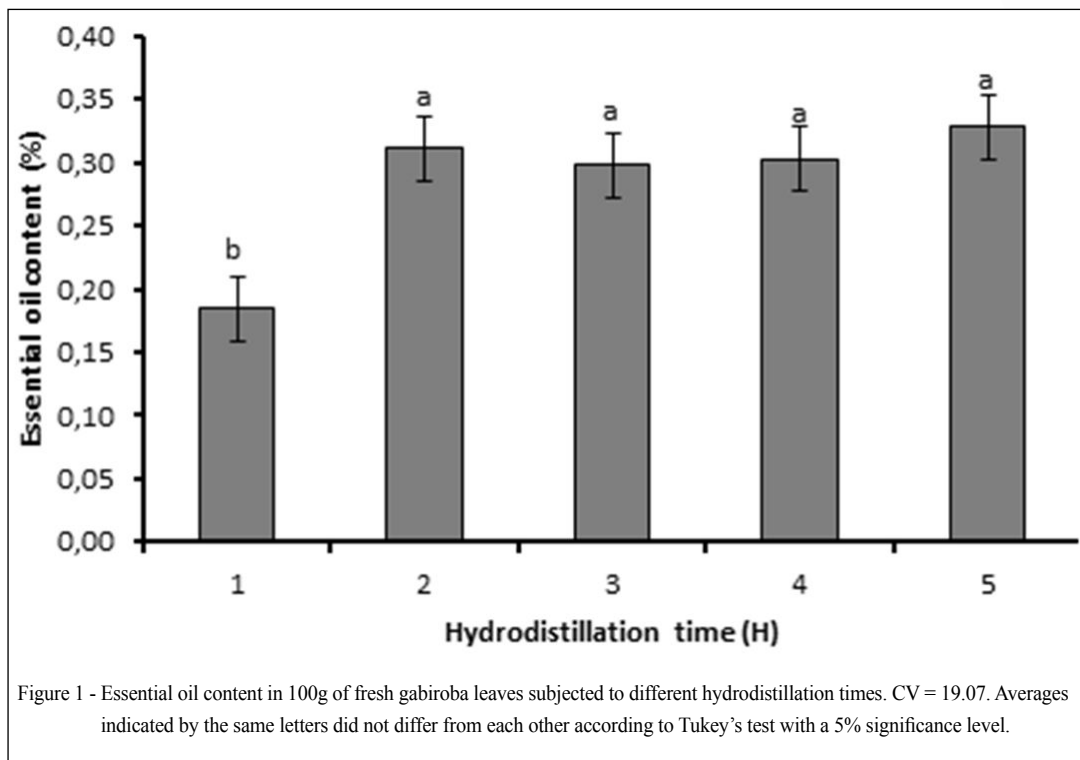
RESULTS AND DISCUSSION

To evaluate the effect of hydrodistillation time on the content and chemical composition of essential oils from *C. adamantium* leaves, several extractions were performed at different times, with the purpose of gathering information on the behavior of this species harvested at certain times and locations regarding the hydrodistillation time.

Figure 1 shows the content of essential oil extracted from *C. adamantium* leaves as a function of hydrodistillation time. After 1h of extraction, it was observed that the essential oil content was 0.18%, a value statistically lower than the amounts obtained at all other hydrodistillation times. After 2h, the essential oil content was 0.31%, after which longer hydrodistillation times did not result in statistically different amounts of essential oil; hydrodistillation for 3h, 4h, and 5h, resulted in contents of 0.29%, 0.30%, and 0.32%, respectively. The results obtained for *C. adamantium* were similar to those found by OLIVEIRA et al., (2012), who, after hydrodistillation for 2h in a Clevenger apparatus, was able to extract 0.5mL of essential oil from the leaves of mint plants, with stabilization of the extracted volume. According to SILVA et al., (2003), essential oil contents are affected by different relative humidity and temperature, even on the same day. Therefore, knowledge of the ideal hydrodistillation time in order to extract higher concentrations of active principles would be of fundamental importance when taking decisions on chemical and agronomical aspects of production.

When comparing the variation in essential oil yields observed in the present work, with the results from related studies, it was observed that there was indeed a large variation in essential oil yield according to the species, the method, and, time of extraction. Different methods and extraction times for essential oil from Peruvian pepper (*Schinus molle* L.) were also analyzed, and the authors observed that, in this species, the yield of essential oil extracted by hydrodistillation was directly proportional to the extraction time (4 h/4.61%). They also concluded that hydrodistillation was the most efficient extraction method when compared to the infusion and soxhlet methods, since it yielded a greater number of chemical compounds (MATTANA et al., 2015).

The hydrodistillation time resulting in the highest content of essential oil from *C. adamantium* leaves was similar to that found by EHLERT et al., (2006) for other medicinal plants: 130 min for *Cymbopogon citratus*; 150 min for *Cymbopogon*



winterianus, *Aristolochia* sp., *Hyptis pectinata*, and *Hyptis fruticosa*; 160 min for *Lippia sidoides*; and 230min for *Eucalyptus globulus*.

The variation in chemical composition of essential oil extracted from *C. adamantium* leaves when subjected to different hydrodistillation times is presented on table 1. Chemical analyses enabled the identification of 36 compounds after 1h of hydrodistillation. At other hydrodistillation times, the amount of compounds decreased, and, according to SIMÕES et al., (2007), the essential oils may have low stability in the presence of light, heat, air, and humidity. These factors may have affected the relative amounts of the compounds. Analysis of the chemical composition of the essential oil is studied based on relative ratio, not the molecular weights of the substance in the essential oil, increasing the proportion of other substances as time may result in the decrease of others. Another factor that may also be occurred is the breakdown of these substances, but more studies are needed (MATTANA et al., 2015).

According to STEFANELLO et al., (2010), compounds with relative amounts higher than 8% were considered major components. Therefore,

the major compounds found at all hydrodistillation times (1, 2, 3, 4, and 5h) were the oxygenated sesquiterpenes spathulenol (11.50–20.66%) and caryophyllene oxide (10.03–14.42%). These findings were similar to those achieved by LIMBERGER et al., (2001), who, when chemically analyzing essential oil from the leaves of *Campomanesia* species, observed the prevalence of sesquiterpenes spathulenol (27.7%) and β -caryophyllene oxide (29.0%) in *C. guazumifolia*; bicyclogermacrene (13.6%) and globulol (10.8%) in *C. rombea*, and *E*-nerolidol (28.8%) in *C. xanthocarpa*.

The highest occurrence of spathulenol (20.66%) and caryophyllene oxide (14.42%) was observed after hydrodistillation for 1h. Therefore, the concentration of oxygenated compounds from this species at the beginning of the extraction process reduced as the hydrodistillation time increased. This observation may result from the hydrofusion effect, i.e., a higher solubility of those molecules in water (MATTANA et al., 2015).

Variations in relative proportions were also observed for sesquiterpene hydrocarbon germacrene B, which produced higher

Table 1 - Substances identified in essential oil from leaves of gabiroba subjected to five different hydrodistillation times.

Substances	RI _{lit}	RI _{exp}	-----Hydrodistillation time (hours)-----				
			1	2	3	4	5
			-----%RA-----				
α -Pinene	917	926	-	0.35	-	-	-
β -Pinene	952	954	0.28	2.30	1.59	0.50	1.08
Ocimene	987	988	0.15	-	0.24	0.06	-
D-Limonene	995	991	0.48	-	0.51	0.19	0.51
Eucalyptol	997	994	0.25	-	0.25	0.08	-
Linalool	1060	1053	4.00	4.09	3.16	3.16	1.52
Fenchol	1063	1067	0.22	-	-	0.15	-
L-Pinocarveol	1083	1091	0.10	-	-	-	-
Camphol	1120	1118	0.51	-	0.40	0.30	-
Terpinen-4-ol	1200	1130	0.29	-	0.15	0.18	0.77
α -Terpineol	1135	1144	1.76	1.55	1.42	1.21	-
Myrtenol	1148	1150	0.15	-	-	-	-
α -Copaene	1376	1379	1.35	1.02	1.03	1.09	1.46
β -Elemene	1358	1356	0.37	-	0.98	0.92	0.72
α -Gurjunene	1395	1374	0.16	-	-	0.18	-
β -Caryophyllene	1382	1384	8.92	8.23	5.80	6.01	15.7
Isoledene	1373	1394	0.20	-	-	0.33	0.26
3,7(11)-Selinadiene	1400	1400	0.05	-	-	-	-
Aromadendrene	1410	1404	2.65	2.67	3.77	3.96	2.17
Humulene	1418	1419	2.18	1.70	1.47	1.49	2.91
Alloaromadendrene	1427	1427	2.15	2.07	2.37	2.26	1.64
γ -Murolene	1475	1443	0.75	-	0.83	0.99	1.14
β -Copaene	1448	1447	1.78	4.52	3.18	1.89	4.89
β -Eudesmene	1458	1453	0.21	-	0.33	0.18	0.17
Germacrene B	1460	1464	5.19	16.80	18.27	9.30	10.8
α -Murolene	1467	1467	0.71	0.68	0.72	0.82	1.23
γ -Cadinene	1472	1481	0.96	0.87	0.92	1.02	1.23
δ -Cadinene	1492	1490	0.94	1.43	1.74	1.42	2.45
Viridiflorene	1505	1504	0.25	-	-	-	-
Guaia-1(10),11-diene	1530	1523	0.07	-	-	0.16	-
Epiglobulol	1527	1525	0.28	-	-	0.37	0.22
Spathulenol	1548	1544	20.66	16.27	15.04	19.27	11.5
Caryophyllene oxide	1552	1550	14.42	10.19	10.03	12.37	12.2
Viridiflorol	1557	1557	3.28	3.75	4.17	4.98	12.2
Guaiol	1560	1559	1.23	1.49	1.81	1.99	1.52
Cubedol	1580	1592	0.46	-	-	-	0.37
δ -Cadinol	1600	1605	1.70	2.17	-	-	-
α -Cadinol	1618	1617	2.59	2.84	-	-	-
Monoterpenes			8.19	8.29	7.72	5.83	3.88
Sesquiterpenes			73.51	76.70	72.46	71.00	84.8
Total compounds			37	16	17	21	20

RI_{lit}: Retention index from the literature. RI_{exp}: Retention index determined relative to n-alkanes (C₁₀-C₂₉) on the DB-5 column. %RA: Relative area (peak area relative to the total peak in the GC-MS chromatogram), average of three replicates.

concentrations at hydrodistillation times of 2, 3, 4, and 5h. The oxygenated sesquiterpene viridiflorol showed a higher concentration at a hydrodistillation time of 5h. Another compound that had varying relative amounts was sesquiterpene hydrocarbon β -caryophyllene, with

higher concentrations at the following hydrodistillation times: 1h (8.92%), 2h (8.23%), and 5h (15.72%), due to the non-polar profile of its molecule.

There were indications that the extraction time affected the amount of each

substance extracted; however, specific studies are still needed, such as performance of the calibration curve for each compound using molecular patterns or molecule stability assays.

CHATZOPOULOU & KATSIOTIS (1995) and PRINS et al., (2006) evaluated the effect of extraction time on the chemical composition of the species *Juniperus communis* L. and *Rosmarinus officinalis* L., respectively, and observed a tendency for the concentration of oxygenated compounds at the beginning of the extraction process, in accordance with the results achieved here in for the essential oil extracted from *C. adamantium* leaves.

CONCLUSION

Despite the scarcity of studies related to this subject, it had already been shown that the yield of essential oils and relative proportion of their chemical substances varied according to the species, and the time and method of extraction employed. Nevertheless, there was still the need for information on the ideal method and time for essential oil extraction, so that there was concise data for each medicinal species, in order to provide trusted and precise results, allowing for the maximization of essential oil yield in all works involving extraction processes.

Under the experimental conditions, we conclude that the content and chemical composition of essential oil extracted from *C. adamantium* leaves were significantly different depending on how long the plant material was subjected to hydrodistillation. After 2h of hydrodistillation, the essential oil content stabilized, and the amount of major compounds present in the essential oil varied according to hydrodistillation time.

We identified oxygenated sesquiterpenes, such as spathulenol and caryophyllene oxide, as the major compounds present, regardless of hydrodistillation time. Considering the results of the present work, we can conclude that, for *C. adamantium*, the essential oil extraction time directly affects the yield and the proportion of the chemical compounds present in the essential oil. In order to achieve the maximum essential oil yield from this species, it is recommended to use an extraction time of 2h, since longer times did not lead to different results.

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