

SHORT COMMUNICATION

Chemical Composition and Effect of Hydrodistillation Times on the Yield of Essential Oil from *Eugenia pyriformis* Leaves

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Abstract:

This study aimed at determining the chemical composition and evaluating the influence of different times of hydrodistillation on the content of essential oil from *Eugenia pyriformis* (Myrtaceae) leaves. Four extraction times (60, 120, 180 and 240 minutes) were investigated by a completely randomized experimental design. The highest yield of oil was achieved in the 120-minute hydrodistillation time. The main compounds of the oil determined by GC-MS analyses were β -caryophyllene (18.0%), bicyclogermacrene (16.4%), spathulenol (10.0%) and α -cadinol (8.7%).

Key words: myrtaceae; β -caryophyllene; medicinal plant; sesquiterpenes

1. Introduction

Essential oils (EOs) have been defined as products obtained by steam distillation of plant raw material, by mechanical processes applied to the epicarp of citrus fruits and by dry distillation of wood [1]. EOs may then be separated from the aqueous phase by physical methods, such as filtration, decantation and centrifugation, which do not change their composition significantly. EOs are also considered sources of natural compounds that exhibit several biological activities, such as antioxidant, antibacterial, antifungal, antinociceptive, anti-inflammatory and insecticidal properties [1].

Eos *in natura* are complex mixtures, which are composed of low molecular-weight lipophilic compounds, mainly monoterpenes,

sesquiterpenes and phenylpropanoids, which have several functional groups. Since many are volatile and odoriferous, they bestow these properties on EOs [1], which are isolated from several parts of plants, such as leaves, bark, rhizome, fruits and flowers. The identity and relative quantity of these volatile compounds found in EOs are quite variable. The compounds perform different biological roles in the plant when they attract pollinizing insects, work as internal messengers and protect the plant against herbivores [1].

The genus *Eugenia* has large economic and pharmacological potential due to its species. It has been shown not only by the number of studies and publications, but also by the commercial exploration of its edible fruits and wood, besides its use as an ornamental plant and producer of EOs, which exhibit different

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biological properties. Many of the 400 species of the genus *Eugenia* distributed all over Brazil have high therapeutic potential [2].

Eugenia pyriformis Camb., known as *uvaia*, is a tree that belongs to the family Myrtaceae. It is a medium height tree naturally found in Brazil, from São Paulo to Rio Grande do Sul, and also likely to be found in Paraguay and Argentina [3]. The family Myrtaceae comprises 142 genera and 5,500 species of trees and shrubs, which are distributed mainly in tropical and subtropical regions of the world, particularly South America, Australia and Tropical Asia. It is one of the most prominent families in Brazil, represented by 23 genera and 1,000 species, which can be found in all over the country [4].

Essential oil extraction has been highlighted by scientific researches, which aim at discovering new compounds, and by the industry

that searches for acclaimed compounds of interest. However, few studies of the influence of time on essential oil extraction, and how it affects EO content, have been reported in the literature. Therefore, other studies on this topic are needed to carry out a previous evaluation of extraction times before starting a study which aims at extracting essential oil and optimizing the process in order to avoid waste and high costs [5].

Considering the information above and our current interest in studies of essential oils produced by species of the family Myrtaceae, as well as the influence of extraction times on the yield of essential oils [6], this study evaluated the chemical composition and the influence of different times of hydrodistillation on the content of essential oil from *E. pyriformis* leaves (Figure 1).



Figure 1. *Eugenia pyriformis* (Myrtaceae).

2. Results and Discussion

In order to evaluate the influence of time of hydrodistillation on the content of the essential oil from *E. pyriformis* fresh leaves, four extractions were carried out at four different times. It was shown that after 1 hour (60 minutes), the content of the essential oil extracted from the leaves was 0.13%. This was the lowest amount of EO obtained, which differed statistically from those obtained at the other times of hydrodistillation. After 2 hours (120 minutes), the highest yield (0.25%) was observed. Afterwards, amounts of the essential oil did not differ statistically: after 3

h, it was 0.22% and after 4 h, it was 0.21%. Similar results to those found in this study have been described in the literature, i. e., the method of extraction and times of hydrodistillation influenced the yield and the chemical composition of essential oils from *Rosmarinus officinalis* [7], *Zingiber officinale* [8] and *Pogostemon cablin* [9].

Despite the shortage of studies of the topic under investigation, the results obtained reveal that the essential oil yield, as well as the relative proportion of its chemical compounds vary significantly, depending on the species and both

extraction time and method. Concerning this fact, a gap is found in previous studies, since their EO extraction methods and times are not adequate to provide concise data on every medicinal species. As a result, reliable results of precision and maximization of EOs were not reached by studies that involve extraction processes [10].

Regarding the chemical composition, the essential oils obtained from *E. pyriformis* fresh leaves employing all four extraction times were mixed and analyzed by GC-MS. Twenty-eight constituents were identified, but the main ones were β -caryophyllene (18.0%), bicyclogermacrene (16.4%), spathulenol (10.0%) and α -cadinol (8.7%) (Table 1). Previous reports on the essential oil extracted from other *E. pyriformis* specimens showed that terpenes predominate and that their chemical composition

varies significantly, depending on the origin of the plant. The chemical composition found in this study is similar to that previously reported on a study that quantified and identified main chemical compounds of the volatile oil extracted from *E. pyriformis* grown in Rio Grande do Sul state [11]. However, the essential oil from this species extracted and evaluated by Stefanello et al. [12] exhibited significant differences, even concerning its major compounds

Several factors, such as the plant genetic diversity, the method of extraction, the habitat and the collection time, may influence the chemical composition of a volatile oil. Even though all plant organs may accumulate essential oils – as previously mentioned –, their chemical composition may also vary among organs [13].

Table 1. Chemical constituents of the essential oil from *E. pyriformis* fresh leaves

Compounds	RI _{lit}	RI _{exp}	% RA
α -Pinene	931	930	2.0
β -Pinene	939	938	2.0
β -Myrcene	991	992	5.3
α -Phellandrene	995	995	1.1
Limonene	1031	1033	1.3
<i>p</i> -Cymene	1023	1023	0.8
δ -Elemene	1331	1332	0.5
α -Cubebene	1355	1353	1.1
α -Copaene	1374	1375	2.3
β -Cubebene	1385	1385	1.2
β -Elemene	1392	1396	2.0
β-Caryophyllene	1420	1419	18.0
Aromadendrene	1439	1444	0.7
Germacrene-D	1484	1486	4.0
Bicyclogermacrene	1490	1491	16.4
α -Murolene	1500	1497	1.2
γ -Cadinene	1513	1511	2.0
<i>trans</i> -Calamenene	1516	1516	0.2
δ -Cadinene	1522	1520	1.0
Spathulenol	1574	1574	10.0
β -Caryophyllene-oxide	1583	1583	0.4
Globulol	1566	1566	0.2
Epi-globulol	1588	1588	3.0
1-Epi-cubenol	1601	1600	0.5
T-Cadinol	1664	1660	3.0
β -Eudesmol	1620	1620	2.0
α-Cadinol	1652	1651	8.7
α -Bisabolol	1685	1682	4.0
Total			95.9

RI_{lit}: Retention index found in the literature [14]. RI_{exp}: Retention index relative to *n*-alkanes (C₉-C₂₂) in the DB-5 column. % RA: Relative area (peak area relative to the total peak in the GC-MS chromatogram).

3. Material and Methods

Plant material

E. pyriformis leaves were collected in Pouso

Alegre, Minas Gerais, Brazil, in April 2018. The plant material was identified by the botanist Walnir G. F. Júnior, Ph.D., and a specimen voucher was taken to the Herbarium at the

Instituto Federal do Sul de Minas Gerais – Campus Machado (registration GERAES 08). Access activities were registered at SisGen (no. AEACDCA).

Extraction of volatile oil

The essential oil was extracted from *E. pyriformis* fresh leaves (100 g) by hydrodistillation (1, 2, 3 and 4 h) using a modified Clevenger-type apparatus. The oil was separated and dried over anhydrous sodium sulfate, stored in hermetically sealed glass containers and kept under refrigeration at 5 °C until GC/MS analysis. Total oil yield was expressed as percentage (g/100 g fresh plant material).

Identification of chemical components

A gas chromatography-mass spectrometry (GC-MS) analysis was carried out by a Shimadzu QP2010 with an AOC-20i auto-injector and a DB-5MS column (30 m x 0.25 mm, 0.25 mm in thickness). The carrier gas was He with pressure of 57.4 kPa and flow rate of 1.00 mL/min. The split ratio was 1/30, the injector temperature was 250 °C and the injected volume was 1 µL. Temperature programming was the following: 60 – 240 °C, increasing 3 °C/min. MS were recorded on the electron ionization (EI) mode, with ionization energy of 70 eV (scan time: 2 scans/s). Identification of the constituents was based both on the retention indices (RI) determined with reference to a homologous series of *n*-alkanes (C-9 to C-22) and on the fragmentation pattern observed in the mass spectra, which were compared to results found in the literature and the Wiley 7 and Nist 62 mass spectral library data [12].

Statistical analysis

The experimental design, which was completely randomized, evaluated four times of hydrodistillation (1, 2, 3 and 4 h) that were carried out in triplicate. Results were statistically analyzed by means of the analysis of variance ($F < 0.05$) and means were compared by the Tukey's test at 5% level of significance by the ASSISTAT software program, version 7.7 beta.

4. Conclusions

This study enabled to conclude that, in experimental conditions, times of hydrodistillation in a Clevenger-type hydrodistiller influenced the yield of the essential oil of *E. pyriformis* leaves. It was shown that the 120-minute extraction time is ideal to obtain the highest content of oil. Regarding its chemical composition, the oil under study exhibited the following terpenes as its major constituents: β -caryophyllene, bicyclogermacrene, spathulenol and α -cadinol. Despite the shortage of studies of the topic under investigation, the fact that essential oil yield and the relative proportion of its chemical compounds vary significantly, depending on the species and both extraction time and method, was confirmed in the present study. In sum, a gap was found in previous studies since their EO extraction methods and times were not adequate to provide concise data on every medicinal species. As a result, reliable results of precision and maximization of EOs are not reached by studies that involve extraction processes.

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