

Effect of natural and artificial drying of leaf biomass of *Psidium guajava* on the content and chemical composition of essential oil

Efeito da secagem natural e artificial da biomassa foliar de *psidium guajava* sobre o teor e a composição química do óleo essencial

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Abstract

Psidium guajava L. is native to Central and South America. It is widely distributed and well adapted to Brazil, a producer of essential oils rich in terpenes. The objective of this study was to evaluate the effects of natural and artificial drying on the content and chemical composition of the essential oil of guava leaves (*Psidium guajava* L.) grown in Rio Verde (GO). The two treatments consisted of drying fresh leaves either naturally in the shade or artificially at 40°C. Chemical composition was analyzed qualitatively and quantitatively by gas coupled with mass spectrometer chromatography (GC/MS) and gas chromatography using a flame ionizer (GC-FID), respectively. The method of drying changed the content and chemical composition of the essential oil of guava leaves. Drying in the shade reduced the content and altered the constituents of the essential oil, whereas drying in an oven at 40°C, despite having reduced the amounts of the constituents, exhibited the highest essential oil content and increased the concentration of certain major constituents as compared to that in the natural shade drying method. The major components found in the essential oil of leaves regardless of the drying processes were trans-caryophyllene, α -humulene, aromadendrene, α -selinene, and selin-11-en-4 α -ol. According to reports in the literature, these compounds possess fungicidal, insecticidal, antimicrobial, and anti-inflammatory activity, among others beneficial actions.

Key words: Hydrodistillation. Guava. Myrtaceae. Medicinal plants.

Resumo

Psidium guajava L. é uma planta nativa da América Central e do Sul, que está amplamente distribuída e bem adaptada no território brasileiro, produtora de óleo essencial rico em terpenos. O objetivo deste estudo foi avaliar o efeito da secagem natural e artificial sobre o teor e a composição química do óleo essencial das folhas de goiabeira (*Psidium guajava* L.), cultivada em Rio Verde (GO). Os tratamentos avaliados foram: folhas frescas; secagem natural à sombra e secagem em estufa a 40°C. A composição química qualitativa e quantitativa foi analisada por cromatografia gasosa acoplada ao espectrômetro de

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massa (CG/MS) e cromatógrafo gasoso com ionizador de chama (CG-FID), respectivamente. O tipo de secagem alterou o teor e a constituição química do óleo essencial das folhas de goiabeira. A secagem a sombra reduziu o teor e a quantidade de constituintes do óleo essencial, enquanto a secagem em estufa a 40°C apesar de ter reduzido a quantidade de constituintes, apresentou o maior teor de óleo essencial e aumentou a concentração de alguns constituintes majoritários em relação à secagem natural a sombra. Os componentes majoritários encontrados tanto no óleo essencial das folhas in natura quanto no óleo essencial obtido das folhas submetidas aos processos de secagem foram *trans*-cariofileno, α -humuleno, aromadendreno, α -selineno e o selin-11-en-4 α -ol, e segundo relatos na literatura estes compostos possuem atividades fungicidas, inseticidas, antimicrobianas, anti-inflamatórias, entre outras.

Palavras-chave: Hidrodestilação. Goiabeira. Myrtaceae. Plantas medicinais.

Introduction

Psidium guajava (L.), commonly known as “guava,” is a shrub or small fruit tree, belonging to the family Myrtaceae, genus *Psidium*, and is native to Central and South America. It is cultivated in all countries with tropical or subtropical climates, and is well adapted to and widely distributed in the Brazilian territory (GUTIÉRREZ et al., 2008; SHAH et al., 2011; TAVARES et al., 2002). In popular medicine, it is used to treat colic, colitis, diarrhea, dysentery, and stomach pain (KANERIA; CHANDA, 2011). Published data show that the extracts of leaves of this species present several pharmacological properties, such as antimicrobial, antispasmodic, hypoglycemic, anti-diabetic, antioxidant, antimutagenic, and anti-carcinogenic activity (BAKKALI et al., 2008; GUTIÉRREZ et al., 2008; SOMAN et al., 2013).

The leaves contain an essential oil that is rich in important insecticidal and pharmacologically active compounds, such as α -pinene, limonene, 1,8-cineole, *trans*-caryophyllene, and α -humulene (LIMA et al., 2009). Essential oils are natural products obtained from different parts of medicinal and aromatic plants through hydrodistillation (ISO, 1997), being designated as oil because of some of their physiochemical characteristics, such as being volatile, lipophilic, usually odorous, and liquid at room temperature (SIMÕES et al., 2007). These compounds are important in the preparation of products in the pharmaceutical, food, and cosmetic industries, as well as in agriculture for the biological control of pests and diseases (SOUZA et al., 2010; TELES, 2010).

Several factors can cause alterations in the content of essential oils and their therapeutic and flavoring properties, including as soil characteristics, climate, and even the post-harvest processing of the plant material. In terms of post-harvest processing, the factor that most greatly affects the content and chemical composition of the essential oil is drying. Drying consists of the withdrawal of free water present in plant tissues, preventing enzymatic degradation from occurring, and thus maintaining the quality of the plant materials (ISENBERG; NOZAKI, 2011; ROSADO et al., 2011). The leaves of medicinal and aromatic plants are often dried before the extraction of essential oils to reduce their moisture content; however, during this process many of the more volatile compounds are lost because they are removed during water evaporation. Thus, the drying method has a significant effect on the quality and quantity of essential oils of such plants (ASEKUN et al., 2007).

Studies have shown that the method of drying can affect the content and chemical composition of the essential oil (ANTAL et al., 2011; SHAHHOSEINI et al., 2013). For example, some medicinal and aromatic plants exhibited a reduction in the content of essential oil when subjected to natural drying at ambient temperature: 45–36% for basil (*Ocimum basilicum*), 33–23% for marjoram (*Origanum majorana*), and 17–6% for oregano (*Origanum vulgare*) (SHAHHOSEINI et al., 2013). Khangholi and Rezaeinodehi (2008) verified that the content of essential oil of *Artemisia annua* decreased after drying in an oven at higher temperatures. Sellami et al. (2011), when studying the influence of different

drying methods on *Laurus nobilis*, found that natural drying at ambient temperature and infrared drying at 45°C significantly increased the content of essential oil of the studied species.

Asekun et al. (2007) studied the effect of sun drying, shade drying at ambient temperature, and drying in an oven at 40°C on the quantity and chemical quality of the essential oil of wild-mint leaves (*Mentha longifolia*). They concluded that the monoterpenoids in the essential oil underwent a significant chemical transformation because of the drying process. When assessing the influence of drying temperature on content and chemical composition of the essential oil of Guaco (*Mikania glomerata* Spreng.), Radünz et al. (2010) also concluded that there was a change in the chromatographic identification of the essential oil caused by the drying process in comparison with the plant *in natura*. However, research related on the drying process is still insufficient and there is an increasing need for specific studies on medicinal species, because their behavior under various drying processes is unique. For this reason, the definition of more appropriate drying methods for each species is essential to ensure the amount of essential oil as well as the active substances (BORSATO, 2006; CORRÊA et al., 2004; KHATER et al., 2011; MACHADO et al., 2013).

Therefore, the choice of the best drying method and essential oil extraction technique is necessary to increase the efficiency and reduce loss of active substances. Thus, the objective of the present study was to identify the effect of different drying methods on the quantity and quality of the essential oil of *Psidium guajava* leaves.

Materials and Methods

Plant material

The experiment was conducted at the Natural Products Chemistry Laboratory at the Goiás Institute of Education, Science and Technology, IF

Goiano Campus Rio Verde, using fresh leaves of *Psidium guajava*. The leaves were collected in the city of Rio Verde, GO, at 17°48'28" S, 50°53'57" W at an altitude of 720 m, between 0600 h and 0800 h in July 2014. The plant material was identified, and samples were deposited as voucher specimens in the Herbarium of the State University of Montes Claros MG, under the identification number 4481.

The leaves were collected from the top and bottom of the plant, packed in plastic bags, and brought to the Natural Products Chemistry Laboratory of the IF Goiano, Rio Verde Campus. After collection, the leaves underwent a screening process, and leaves in poor condition were discarded.

Determination of moisture content

The moisture content of the leaves was determined before and after drying, in accordance with the methodology for forage and similar materials plants and leaves (ASAE, 2000). To determine the moisture content, the leaves were placed in an oven with forced air at a temperature of $103 \pm 2^\circ\text{C}$ for 24 h. Three identical samples were evaluated. The initial moisture content of leaves was 63.69% wet-basis (% wb). During the drying process, the samples were weighed periodically until a moisture content of approximately 10.02% was achieved.

Drying

Drying was performed using two treatments: drying in an oven at 40°C with forced air circulation and shade drying at ambient temperature ($27.0 \pm 4^\circ\text{C}$) and 61% average relative air humidity. In both treatments, leaves were dried to a constant weight using six replicates of 100 g. The leaves were placed in Kraft paper bags for oven drying and on Kraft paper bags for shade drying at ambient temperature. The reduction in the water weight during the drying process was monitored by the gravimetric method (weight loss), based on the initial water content of

the product, until constant weight and desired water content was attained. Monitoring of mass reduction during drying was performed using an analytical balance with resolution of 0.0001 g. The air temperature was monitored using a mercury-column thermometer with a range of -10°C to 300°C for oven drying and a digital thermo hygrometer with a precision of 3% for drying at room temperature.

Essential oil extraction

Extraction of essential oil was performed by hydrodistillation in a Clevenger apparatus with a 1-L volumetric flask; hydrodistillation continued for 2 h from the start of boiling. The extractions were performed from 100 g samples with six replicates of the following treatments: fresh leaves (extraction performed immediately after collection of plant material); leaves dried in the shade at ambient temperature; and dried leaves at 40°C in an oven. The essential oil was extracted from the aqueous phase using dichloromethane (3x 10 mL; 20 min each extraction), and dehydrated with anhydrous sodium sulfate. After 30 min, the sulfate was removed by filtration. After complete evaporation of the solvent, the mass content of the essential oil was determined using an analytical balance with a resolution of 0.0001 g. The samples were stored at 4°C in a refrigerator for later analysis of chemical composition.

Quantification and identification of chemical composition

Quantification of the compounds in the essential oils was performed using a Shimadzu GC-17^a gas chromatograph equipped with a flame ionization detector (FID) and SPB-5 fused silica capillary column (30 m \times 0.25 mm, film thickness 0.25 μm). Chromatography was performed under the following conditions: carrier gas (N_2) flow rate 1.8 mL min^{-1} ; temperature of the injector 220°C ,

detector temperature 240°C ; and initial column temperature 40°C . Conditions were isothermal for 4 min, followed by heating at $3^{\circ}\text{C min}^{-1}$ to 240°C , and remaining isothermal for 15 min. Sample injection volume was 1.0 μL (10 mg mL^{-1} in CH_2Cl_2), split ratio was 1:10, and column pressure was 115 kPa.

The identification of the essential oil compounds was performed with a Shimadzu GC-17^a gas chromatograph equipped with a RTX-5 fused silica column (30 m \times 0.25 mm, film thickness 0.25 μm) coupled to a Shimadzu CGMS-QP5050A mass spectrometer. The chromatographic conditions were the same as used for the CG-FID, except the carrier gas was He and the column pressure was 100 kPa. With regard to the mass spectrometer, the ionization process was by electron impact (70 eV) and scan amplitude was 30 to 700 Da.

The quantification of the compounds was performed in triplicate and the concentration of each constituent was calculated as the percentage of the area of the corresponding peak relative to the total area of all peaks observed in the chromatogram. The identification of the compounds was made via comparison of their mass spectra and retention indices (RI) with those of the standard substances in system libraries (Wiley 7th edition) and in the literature (ADAMS, 2007). The RIs were obtained using a homologous series of n-alkanes. The identification of the constituents of the essential oil was performed at the Laboratory of Analysis and Synthesis of Agrochemicals, Federal University of Viçosa (UFV).

Statistical analysis

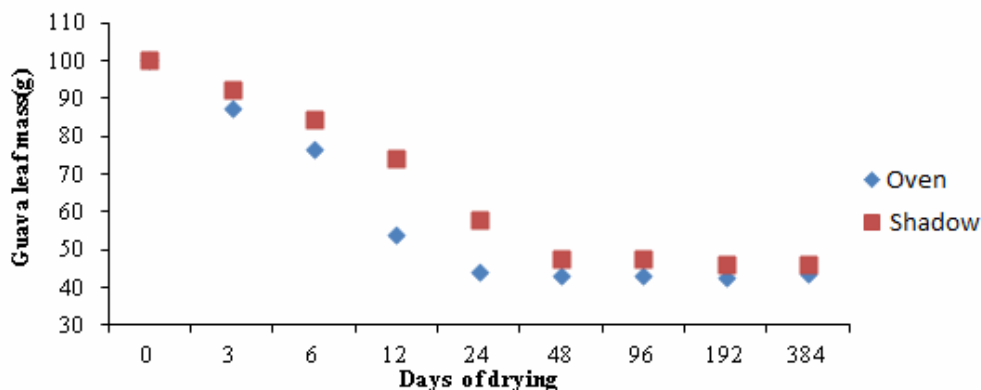
The experimental design was a completely randomized design with six replicates. An analysis of variance (ANOVA) was conducted with the data and the averages of the treatments were compared by Tukey's test at the 5% level of significance using ASSISTAT software.

Results and Discussion

The time to obtain constant weight of guava leaves is shown in Figure 1. The loss of water by plant tissues was more rapid during the first hours of drying and tended to stabilize by the fourth day. For oven drying, a constant weight was obtained after 8 days of drying, whereas for shade drying at ambient

temperature, constant weight was obtained at 16 days, at which time both drying processes were terminated. The moisture observed in the leaves at the end of the drying period was below 13% for both methods, which is in accordance with the recommendations of various pharmacopoeias (i.e., between 8 and 14% w.b.) (FARIAS et al., 2004).

Figure 1. Drying curve of *Psidium guajava* L. leaves subjected to natural shade drying and artificial drying in an oven at 40°C.



Oil content was modified by the drying method. The highest yield was obtained by artificial drying in an oven at 40°C, being 0.34% dry basis (d.b.). The yield of essential oil of the fresh leaves (0.27% d.b.) was lower than that of leaves dried artificially or by natural shade drying (0.30% d.b.). According to Martins et al. (2002), the lower water content in the leaves dried in an oven at 40°C allows the vapor stream generated during the hydrodistillation to more efficiently carry the volatile substances stored in the cells of the plant material when compared with the material *in natura*. According to Guenther (1972), because the plant material *in natura* has high water content, there is a strong agglutination of the essential oil, preventing the steam from penetrating plant tissues uniformly and efficiently. Similar results were found by Rosas et al. (2004) when studying the effect of different drying methods on the content of essential oil of the basil leaves, noting that drying in an oven at 35°C produced more essential oil in this species in comparison with drying with a dehumidifier.

Corrêa et al. (2004) studied the effect of various drying methods (oven at 35°C, solar drier at 32°C, mixed drying at 27°C, and shade drying at 25°C), and found less essential oil of *Vernonia polyanthes* Less. was produced following drying in the oven. Rosado et al. (2011) studied the influence of leaf processing and the type of drying on the essential oil content of basil and observed that oven drying resulted in more essential oil compared to plant material subjected to drying in a dehumidifier. In addition, Omidbaigi et al. (2004) studied the influence of the drying method on the content of essential oil of chamomile (*Chamaemelum nobile*). They compared natural shade drying, sun drying, and oven drying at 40°C and observed that the natural shade drying led to a higher yield of essential oil than the other methods evaluated, thus showing that for each species there is a specific drying method that will generate the highest content of essential oil from the plant material.

In the majority of the studies conducted on the drying of medicinal plants, the authors concluded that the drying temperature influenced the yield of essential oil, i.e., for each species there is a specific drying method that will yield the greatest amount of essential oil from the plant material.

Thirteen to 17 compounds were identified in the essential oil of *P. guajava*, depending on the drying method used, which corresponded to 91.44% of the total oil analyzed (Table 1). The method of biomass drying changed the chemical composition of the essential oil of guava leaves; 17 compounds were identified in the essential oil extracted from fresh leaves, whereas 13 and 15 compounds were identified in the oil from naturally and artificially dried leaves, respectively. The major constituents were trans-caryophyllene, α -humulene, aromadendrene, α -selinene, and selin-11-en-4 α -ol. They occurred at different proportions depending upon the drying method. In fresh leaves the proportions were trans-caryophyllene, 18.18%; α -humulene, 26.37%; aromadendrene, 7.63%; α -selinene, 7.35%; and selin-11-en-4 α -ol, 7.20%. In shade-dried leaves they were trans-caryophyllene, 9.04%; α -humulene, 10.21%; aromadendrene, 8.39%; α -selinene, 7.30%; and selin-11-en-4 α -ol, 12.52%. In oven-dried leaves they were trans-caryophyllene, 13.86%; α -humulene, 15.61%; aromadendrene, 11.16%; α -selinene, 10.32%; and selin-11-en-4 α -ol, 11.20%.

Thus, the process of natural and artificial drying of guava leaves increased the content of essential oil compared with the oil content of leaves *in natura*. However, there was a reduction in the concentrations of the major compounds trans-caryophyllene and α -humulene, and an increase in the concentrations of aromadendrene, α -selinene, and selin-11-en-4 α -ol with drying methods in comparison to that of the plant material *in natura*. Thus, artificial drying in an oven resulted the highest content of essential oil compared to the other treatments, but also decreased the concentration of trans-caryophyllene and α -humulene.

The special metabolites identified in the essential oil of the species studied are similar to previously published metabolites present in the essential oil extracted from the guava leaves. Craveiro et al. (1981), Cuellar et al. (1984), and Pino et al. (2001) characterized the essential oil of guava leaves and identified 21 compounds, including α -humulene, trans-caryophyllene, and selin-11-en-4 α -ol.

The major compounds identified as components of the essential oil of the guava leaves both *in natura* and those submitted to drying methods have biological activity supported by published data. Mevy et al. (2007) studied the essential oil of *Lippia chevalieri*, whose major constituent is trans-caryophyllene, and observed antibacterial and antifungal activity. *Lippia chevalieri* is traditionally used in the treatment of respiratory diseases of bacterial and fungal origins. In addition, Castro (2004) evaluated the insecticidal activity of the essential oil of yarrow (*Achillea millefolium* L.), and one of the major compounds was trans-caryophyllene, at a concentration of 3.52%.

According to Deus et al. (2011), the fungicidal effect of the essential oil of copaiba can be related to the concentrations of α -humulene and β -caryophyllene. Fernandes et al. (2007) concluded that α -humulene derived from the essential oil of *C. verbenacea* might represent an important tool for the management and/or treatment of inflammatory diseases.

Similarly, Freitas et al. (2016) assessed the fungicidal potential of essential oil of *Baccharis dracunculifolia* and observed that it inhibited the growth of *Fusarium* species by 100%. Its component compounds were nerolidol, spathulenol, δ -cadinene, and aromadendrene (ranging from 5 to 25% in this species), among others, and these compounds may be related with the antifungal activity of this oil (FERRONATTO et al., 2007).

Table 1. Average values of the levels (%) of chemical constituents of the essential oil of guava (*P. guajava*) leaves for each drying method.

Compounds of essential oil	RI	Treatments		
		1	2	3
Limonene	1024	2.22 ± 0.2	6.88 ± 0.2	4.75 ± 0.2
1,8-cineol	1026	1.50 ± 0.3	-	0.10 ± 0.3
α -copaene	1374	1.05 ± 0.3	0.18 ± 0.3	0.28 ± 0.3
<i>trans</i> -caryophyllene	1419	18.18 ± 0.4	9.04 ± 0.4	13.86 ± 0.4
α -humulene	1454	26.37 ± 0.2	10.21 ± 0.2	15.61 ± 0.2
4,11-selinadiene	1475	1.19 ± 0.2	-	-
γ -muurolene	1478	0.83 ± 0.2	0.77 ± 0.2	1.15 ± 0.2
Aromadendrene	1488	7.63 ± 0.2	8.39 ± 0.2	11.16 ± 0.2
α -selinene	1497	7.35 ± 0.3	7.30 ± 0.3	10.32 ± 0.3
α -panasinsene	1517	1.21 ± 0.2	0.22 ± 0.2	0.31 ± 0.2
<i>trans</i> -nerolidol	1566	3.38 ± 0.2	-	2.85 ± 0.2
caryophyllene oxide	1585	3.79 ± 0.2	5.95 ± 0.2	3.07 ± 0.2
α -humulene epoxide II	1612	4.18 ± 0.3	4.75 ± 0.3	2.80 ± 0.3
longipinene epoxide	1620	1.61 ± 0.2	1.63 ± 0.2	1.23 ± 0.2
epi- α -muurulol	1639	2.97 ± 0.2	-	3.52 ± 0.2
α -cadinol	1651	0.78 ± 0.3	0.51 ± 0.3	1.33 ± 0.3
selin-11-en-4 α -ol	1662	7.20 ± 0.2	12.52 ± 0.2	11.20 ± 0.2
Total Identified		91.44	68.35	83.54

1-Fresh leaves. 2-Natural drying. 3-Artificial drying in an oven at 40°C. – not identified. Data represent mean \pm standard deviation. RI = retention index. Means \pm standard deviation.

Santos et al. (2014) also studied the fungicidal action of essential oil of *Schinus terebinthifolius* Raddi, and achieved 100% inhibition of the development of the phytopathogens studied. According to the authors, this fungicidal action might have occurred because of its chemical composition. A major compound of the essential oil of the leaves of this plant was α -selinene (1.38%). There are no published reports concerning any biological activity of the compound selin-11-en-4 α -ol.

According to the results obtained, we conclude that drying methods altered the content and the chemical composition of the essential oil of guava leaves. Natural shade drying did not change the content of the essential oil when compared with the content of leaves *in natura*, whereas with oven drying the content of the essential oil was reduced. Oven drying reduced the relative amounts of major compounds in the essential oil and also their chemical variability; in contrast, it increased

the levels of essential oil and also increased the concentration of some bioactive constituents, such as *trans*-caryophyllene, α -humulene, aromadendrene, and α -selinene, when compared with shade drying. In the present study, the essential oil presented major compounds with fungicidal, insecticidal, antibacterial, and anti-inflammatory characteristics, similar to those reported in the literature. Thus, artificial drying of guava leaves is the preferred drying method to increase of the concentration of these compounds, but the use of leaves *in natura* is also recommended when the cost benefit relationship is assessed. Even with reduced oil content, it offers oil with higher concentrations of bioactive compounds, such as *trans*-caryophyllene and α -humulene. For this reason, the definition of appropriate drying methodologies for each species is necessary to ensure the appropriate levels of active substances, and the drying method should be defined according to the desired concentrations of active substances.

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