Industrial Crops and Products 37 (2012) 247–252

Contents lists available at SciVerse ScienceDirect

Industrial Crops and Products

journal homepage: www.elsevier.com/locate/indcrop

# Geographical origin and drying methodology may affect the essential oil of *Lippia alba* (Mill) N.E. Brown

Simone Teles[a], José Alberto Pereira[b,*], Carlos H.B. Santos[a], Renata V. Menezes[a], Ricardo Malheiro[b], Angélica M. Lucchese[c], Franceli Silva[a,**]

[a] *Centre for Agricultural, Environmental and Biological Sciences, Federal University Recôncavo da Bahia/UFRB, University Campus, Cruz das Almas/BA, Zip code: 44380-000 Bahia, Brazil*

[b] *Mountain Research Centre (CIMO)/School of Agriculture, Polytechnic Institute of Bragança, Campus de Sta Apolónia, Apt. 1172, 5301-854 Bragança, Portugal*

[c] *Laboratório de Química de Produtos Naturais e Bioativos, Departamento de Ciências Exatas, Universidade Estadual de Feira de Santana, Campus Universitário, CEP: 44031-460 Feira de Santana, Bahia, Brazil*

ARTICLE INFO

*Article history:*
Received 1 September 2011
Received in revised form 8 December 2011
Accepted 18 December 2011
Available online 11 January 2012

*Keywords:*
*Lippia alba* (Mill) N.E. Brown
Geographic origin
Drying methodology
Essential oil
Volatiles

ABSTRACT

*Lippia alba* (Mill.) N.E. Brown, the ginger grass, is a medicinal and aromatic plant widely used in Latin America. In this work the effect of the geographical origin and different drying methodologies on *L. alba* leaves production, essential oil content and composition were evaluated. Field experiments were conducted in three different municipalities (Amargosa, Cruz das Almas and Santo Antônio de Jesus) from Bahia region (Brazil) and two drying methodologies (traditional and artificial with controlled conditions) were assessed. Fresh and dried leaves essential oils were extracted by hydro-distillation and their volatiles were determined by gas chromatography.The geographical origin affected the mass production, essential oil content and both qualitative and quantitative fractions of their composition. Leaves from Cruz das Almas had higher yield in essential oil content. Overall, 26 oil constituents were identified. Carvone, germacrene D and limonene were the main oil constituents. The drying methodologies combined with the geographic origin influenced essential oil composition showing that *L. alba* constituents were influenced by post-harvest treatments. Some constituents were sensitive to both methods applied, while some enhanced their content like germacrene D.



## 1. Introduction

*Lippia alba* (Mill.) N.E. Brown, the ginger grass, is an aromatic shrub which belongs to the Verbenaceae family and it is widespread through the Central and South America, as well in Africa, being also abundant in the south of the United States of America (Hennebelle et al., 2008). *L. alba* is one of the most important medicinal plant used by Latin American people (U.N., 2005). In Brazil it is known as "erva-cidreira" and is widespread through the territory being extensively used in the folk medicine. *L. alba* is more often used as infusions that are good tranquilizers, painkillers and are also used to relieve gastrointestinal problems (Vale et al., 2002). It is also applied as a compress against hemorrhoids, and as a macerate is used for topical application against toothache (Oliveira et al., 2006). It can also be used as a febrifuge during baths and to aid healing wounds (Oliveira et al., 2006). The properties of *L. alba* could be related with the fact that plant exhibit antifungal, antimicrobial and antioxidant properties (Aguiar, 2006; Shukla et al., 2009; Stashenko et al., 2004).Many of the health benefits reported and bioactive properties are directly associated to the composition of the essential oils of *L. alba*. However large variations are observed in their composition, suggesting the existence of a high number of chemotypes. Several factors are associated to the observed variance, such as, the part of the plant employed in the distillation, the state of plant's development and the geographical origin. Concerning the geographical origin, soil characteristics and composition, climate and other local conditions contribute as well to the high variability of the essential oils from *L. alba*. So far, respecting the essential oil composition and according to Hennebelle et al. (2008) seven chemotypes are considered for *L. alba* plant. Belong to chemotype I those oils which possess citral, linalool, β-caryophyllene as their main constituents (four subtypes within this chemotype). The oils included in the chemotype II have tagetenone as their main constituent. Those who possess limonene in high quantities with a variable amount of carvone or monoterpenic ketones instead of carvone are included in chemotype III (two subtypes). The remaining chemotypes are characterized by specific main constituents in

* Corresponding author. Tel.: +351 273303277; fax: +351 273325405.
** Corresponding author. Tel.: +55 7536213857.
*E-mail addresses:* jpereira@ipb.pt (J.A. Pereira), franceli@ufrb.edu.br (F. Silva).

0926-6690/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.indcrop.2011.12.029

their composition: chemotype IV (myrcene), V (γ-terpinene), VI (camphor-1, 8-cineole) and VII (estragole) (Hennebelle et al., 2006).

The drying step is of critical importance in the quality of medicinal plants and in their active principles. After harvest, the drying process facilitates the transportation, storage and handling of the plants. This process aims to minimize the loss of active principles and to delay their deterioration by reducing enzymatic activities. Consequently, the drying methods allow improving the conservation power of the plants allowing their use and commercialization for longer periods.

In this context to study the effect of the geographical origin and drying methods in the yield and composition of *L. alba* essential oils is of major importance. In the present study our goal is to study the effect of geographical origin in the oil content and essential oil composition as well to study the effect of drying methodology applied in the oil content and volatile composition. For this purpose, samples of *L. alba* were cultivated in three different municipalities (Amargosa, Cruz das Almas and Santo Antônio de Jesus) from Bahia (Brazil). Leaves from the plants were collected and submitted to traditional and artificial drying. After that, oil content and essential oil composition of the samples from different municipalities were determined by GC/MS.

## 2. Experimental

### 2.1. Sample cultivation and collection

The experiments were held in three locations: in the experimental field of the Federal University of Recôncavo da Bahia, in Cruz das Almas, and in the experimental fields of the Agriculture Secretary of the Santo Antônio de Jesus and the Amargosa municipalities. The climatic conditions of each region are presented in Table 1.*L. alba* plants from the same plant origin were propagated by cuttings, to guarantee the genetic homogeneity of the material, and grew in a greenhouse during 45 days before transplantation to the experimental field. At the transplantation all plants presented new leaves and sized between 15 and 20 cm. On August 2008, *L. alba* plants were transplanted for the experimental field, previously tilled and fertilized, in a compass of 0.30 m × 0.30 m. Fourteen independent plots of 12 plants each were constituted with a total surface of 2.7 $m^2$/plot. The soil analysis of each experimental site is reported in Table 1.After 150 days of cultivation in the field, plants were harvested when were in pre-flowering stage. At this time all plants were collected and leaves were separated from the stem. From each plot three sub-samples were constituted. In one, fresh leaves were used directly for essential oil extraction, as described in Section 2.3. And, the other two sub-samples were used for the studies of drying methodologies as described in Section 2.2.All plants are from the same vegetative origin. Voucher material was incorporated in the Botanic Herbarium of the Centro de Ciências Agrárias, Ambientais e Biológicas of the Federal University of Recôncavo da Bahia, with number 1242.

### 2.2. Drying methodologies

*L. alba* leaves from the different municipalities were submitted to two different drying methodologies: the traditional and to an controlled methodology.

The traditional methodology consisted in a natural drying process, by putting the leaves well scattered in trays at shadow. The artificial methodology was applied by using a dehumidifier (circulation capacity of 500 $m^3$) in a room with controlled moisture and temperature. At the drying rooms the mean temperature was kept at 28.2 ± 2.1 °C and relative humidity at 70 ± 11%. In both methods the samples were dried until a constant weight. After drying the samples were put in plastic bags and stored at darkness at room temperature before essential oil extraction.

### 2.3. Essential oil extraction

Fresh and dry leaves, subjected to the different drying methodologies, were subjected to essential oil extraction. 70 g of material were milled and extracted by hydro-distillation during 3 h in a Clevenger-type apparatus. The oils extracted were dried with anhydrous sodium sulphate and concentrated under reduced pressure by rotator evaporator, until water evaporation. The extraction yield was calculated in mL of oil per 100 g of dried leaves. The collected oil was then stored in sealed vials at −20 °C and protected by the light with aluminum foil after any analysis.

### 2.4. Determination of essential oil composition

The determination of the essential oils of *L. alba* leaves was analyzed by GC/FID and by GC/MS. Before injection, 10 mg of essential oil were diluted in 500 μL of trimethylpentane, and 0.2 μL of the volume was injected.

#### 2.4.1. Gas chromatography with flame ionization detection (GC/FID) analysis

The GC used was a Varian CP-2280 model, with a FID detector. The analysis were performed with a Chrompack CP-SIL 5, 30 m × 0.5 mm i.d., DF = 0.25 μm (Chrompack). The temperatures of the FID and of the injector were 240 °C and 220 °C respectively. The oven temperature was programmed at 60 °C with an increase of 3 °C/min until 240 °C, and maintained for 20 min. The carrier gas was helium, at a flow rate of 1 mL/min. The analysis was performed in split mode with a ratio of 1:100.

#### 2.4.2. Gas chromatography with mass spectrometry detection (GC/MS) analysis

The GC used was a Shimadzu GC-2010 model, coupled to a GC/MS-QP 2010 Shimadzu mass detector model. A DB-5 ms (30 m × 0.25 mm × 0.25 μm) capillary column was used. The injector port was heated to 220 °C, being the injections performed in split mode with a ratio of 1:30. The oven temperature was programmed at 60 °C with an increase of 3 °C/min until 240 °C, and maintained for 20 min. The carrier gas was helium, at a constant flow of 1 mL/min. The temperature of the ionization source was maintained at 240 °C, the ionization energy at 70 eV, and the ionization current at 0.7 kV.

Constituents were identified by comparing their retention times and their mass spectrum with those of authentic compounds analyzed under the same conditions, and by comparison of the retention indices (as Kovats indices) with literature data (Adams, 2007; Joulain and König, 1998).

The results are expressed in relative percentage of each constituent, calculated by normalization of the chromatographic peak areas.

### 2.5. Analysis of variance

An analysis of variance (ANOVA) with Type III sums of squares was performed using the GLM (General Linear Model procedure) of the SPSS software, version 19.0 (IBM Corporation, New York, U.S.A.). The fulfilment of the ANOVA requirements, namely the normal distribution of the residuals and the homogeneity of variance, were evaluated by means of the Kolmogorov–Smirnov with the Shapiro–Wilk's test, and the Levene tests, respectively. All dependent variables were analyzed using a one-way ANOVA with or without Welch correction, depending if the requirement of the homogeneity of variances was fulfilled or not. The main factors