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# Essential oil content and composition of *Lippa citriodora* as affected by drying method in full flowering stages

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**ABSTRACT:** The aerial parts of *Lippa citriodora* (Verbenaceae) were collected at the full-flowering stage and dried by three different drying methods (sun-drying, shade-drying and oven-drying at 60 °C). The essential oils of every treatment were obtained by hydro-distillation method using clevenger apparatus. The oils were analyzed by GC and GC–MS. The main constituents of the oils in shade method were Limonene (4.0%), 1,8-Cineole (1.92%), trans-β-ocimene (3.2%), Neral (23.5%), Geranial (34.2%), (E)-caryophyllene (3.2%), GermacreneD (5.5%), bicyclogermacrene (4.1%), (E)-Nerolidol (1.8%) and Caryophyllene oxide (2.4%). The analysis of variance showed that the different drying methods had a significant effect on the quantity of essential oil. Finally it could be concluded that shade and sun drying method is suitable for highest essential oil quantity and Neral and Geranial concentration.

***Keywords:*** Essential oil; Drying methods; Shade drying; *Lippa citriodora***.**

## Introduction

The genus *Lippia* (Verbenaceae) includes approximately 200 species of herbs, shrubs and small trees. The genus Lippia shows a rich genetic diversity, enabling it to synthesize a variety of essential oil constituents in plants grown in different parts of the world (Catalan and De Lampasona 2002; Santos- Gomes et al., 2005). Lemon verbena *Lippia citriodora* is indigenous to South America and was introduced into Europe at the end of the 17th century. It is cultivated mainly due to the lemon- like aroma emitted from its leaves that are utilized for the preparation of herbal tea, which is reputed to have antispasmodic, antipyretic, sedative and digestive propertied. Lemon verbena has a long history of folk uses in treating asthma, spasms, cold, fever, flatulence, colic, diarrhea, indigestion, insomnia and anxiety (Carnat et al., 1999; Santos- Gomes, 2005). Distillation (water and /or steam) is a conventional method for the extraction of essential oils from plant materials, in which the plant materials are mixed (or not) with water followed by heating or by the introduction of water system. The resulting vapors are cooled by condenser and collected in a separator and essential oil separator and essential oil separates from water. However, distillation have several disadvantages including labor intensive/time consuming, loss of target compounds due to thermal degradation, etc (Vinatoru, 2003; Robert, 2004), have led to search for find new benefece and efficient alternatives for isolation methods. Among several recently introduced alternative techniques, the coupling of headspace solid-phase microextrction (HS-SPME) sampling with gas chromatography mass spectrometry (GC/MS) has been shown to be very fast, has been shown to be very fast, handy, reliable and inexpensive extraction tool for organic volatiles. Theoretical bases and various applications of SPME are presented by Pawlisyzn (Pawlisyzn, 2007)
as well as in numerous puplication. The chemical composition of the essential oil from the leaves of

*L.citriodora* has been previously reported (Catalan and De Lampasona 2002; Carnat et al., 1999; Pascual et al., 2001; Velasco-Negueruela et al., 1993 and Sartoratto et al., 2004). The post-harvesting process of medicinal plants has great importance in the production chain, because of its direct influence on the quality and quantity of the active principles in the product sold (Rocha et al., 2011), Drying of medicinal herbs that should take place as soon as possible; otherwise insects and mold make them unusable (Fargali et al., 2008). A literature search was undertaken on effects of different methods on essential oil content and chemical composition of the essential oil plants, The results showed that drying method had a significant effect on oil content and composition of aromatic plants (Ahmadi et al., 2008; Khangholi and Rezaeinodehi**,** 2008; Asekun et al., 2006; Okoh et al., 2008; Omidbaigi et al., 2004; Rao et al., 1998). Also duration of essential oil extraction affected on the quantity and quality of essential oil. it have been reported that essential oil percentage and essential oil component of fennel and Laurus nobilis L. were affected by duration of essential oil extraction (Jamshidi et al., 2004; Naderi et al., 2011). The objective of this study was evaluating the influence of drying method on essential oil yield and composition of Lippa citriodora in full flowering stages.

## Materials and methods

### Plant material

Samples of Lippa citriodora were collected in from greenhouse (controlled environment greenhouse at 25/13°C day/night temperature, and 65% relative humidity) in Sepidan city in Shiraz, Iran.

### Drying methods

To study the effect of drying method, three methods of drying, (sun-drying, shade-drying and oven-drying at 60°C for 24 hours) were investigated. The shade-drying occurred at room temperature.

### Essential oil isolation

The dried samples of *Lippa citriodora* were subjected to hydro-distillation using an all glass Clevenger-type apparatus, to extract essential oils, according to the method outlined by the European Pharmacopoeia (Anonymous, 1997). Extraction times were performed (3 hour) with three replications. The essential oils were separated from the aqueous layer, dried over anhydrous sodium sulfate and calculated average of essential oil yield. The extracted essential oils were dried over anhydrous sodium sulphate and stored in sealed vials at low temperature (4°C) before gas chromatography (GC) and gas chromatography-mass spectrometric (GC-MS) analysis. Essential oil content was defined as followed:

R (%) = (mass essential oil/mass of the dried leaves) × 100

### Identification of the oil components

Analysis was carried out using an Agilent-technology chromatograph with HP-5 column (30m ×0.32 mm i.d. × 0.25 µm). Oven temperature was performed as follows: 60° C to 210° C at 3°/min; 210° C to 240° C at 20 °/min and hold for 8.5 min, injector temperature 280° C; detector temperature, 290° C; carrier gas, $N_2$ (1 ml/min); split ratio of 1:50. GC-MS analysis was carried out using an Agilent 7890 operating at 70 eV ionization energy, equipped with a HP-5 MS capillary column (phenyl methyl siloxane, 30m × 0.25 mm i.d× 25µm) with He as the carrier gas and split ratio 1:50. Retention indices were determined using retention times of n-alkanes that were injected after the essential oil under the same chromatographic conditions. The retention indices for all components were determined according to the method using n-alkanes as standard. The compounds were identified by comparison of retention indices (RRI, HP-5) with those reported in the literature and by comparison of their mass spectra with the Wiley GC/MS Library, Adams Library, MassFinder 2.1 Library data published mass spectra data (Adams, 2007; McLafferty, 1989; Joulain et al., 2001)

### Statistical analysis

Treatments were arranged in a completely randomized factorial design with three replications. Analysis of variance was performed using the Minitab software and means were separated using Tukey's test ($p \leq 0.05$).

## Results and discussion

**The effect of drying methods on essential oil content**

The content of essential oil isolated from the aerial parts of *Lippa citriodora* which were dried under different conditions are shown in (Fig 1), result clearly shows that the oil content linked to the drying method. Indeed, in this survey the maximum mean of essential oil obtained from shade and sun-dried samples in 3 hours of extraction. The analysis of variance showed that the different drying methods (shade-drying, sun-drying and oven-drying) had a significant effect on the quantity of essential oil. In accordance with our results, there are similar reports from other researchers about other medicinal plants (Ahmadi et al., 2008; Khangholi and Rezaeinodehi**,** 2008; Asekun et al., 2006; Okoh et al., 2008; Omidbaigi et al., 2004; Rao et al., 1998).

**Chemical composition of the essential oil**

In the shade and sun-drying method, the number of compounds was the same but in the oven-drying method they were different. Thirty-eight compounds were identified in the essential oils in shade and sun drying method but Thirty-six compounds obtained in *Lippa citriodora* in oven-drying method. The identified constituents with their respective percentages and RIs are summarized in (Table 1, 2 and 3). The main constituents of the oils in shade method were Limonene (4.0%), 1,8-Cineole (1.92%), trans-β- ocimene (3.2%), Neral (23.5%), Geranial (34.1%), (E)-caryophyllene (3.2%), GermacreneD (5.5%), bicyclogermacrene (4.1%), (E)-Nerolidol (1.8%) and Caryophyllene oxide (2.4%). In our study, Neral and Geranial were also identified at high percentages .These results are in agreement with previous reports (Argyropoulou et al., 2007). Therefore, we investigated Neral and Geranial in three drying methods. Results showed that maximum Neral and Geranial percentage was obtained in shade and sun, and minimum Neral and Geranial percentage was shown in other method of drying (oven), changed considerably for Geranial and Neral decreasing from 34.2% to 28.3% and from 24.8% to 19.2%, respectively. All other components remained more or less unchanged. These results are in agreement with the previous reports about Roman chamomile (Omidbaigi et al. 2004), Yuan Zhang and Zhezhi Wang (2007) reported that in *Glechoma longituba* different drying methods caused some variation of the relative proportion of the components and the higher amount of Germacrene D (19.0%) was obtained by shade-drying. Finally it could be concluded that shade and sun drying method is suitable for highest essential oil quantity and Neral and Geranial concentration.

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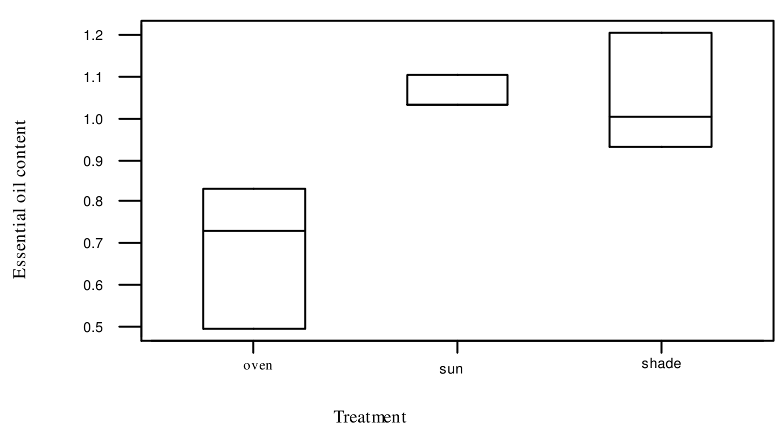


**Figure1. The effect of drying method on essential oil content of *Lippa citriodora*.**

**Table 1. The effect of shade drying method on essential oil content of *Lippa citriodora*.**

| Compounds | RI[a] | Area,% |
|---|---|---|
| α -pinene | *934* | 0.2 |
| Sabinene | *974* | 0.7 |
| 1- Octen-3-ol | *981* | 0.2 |
| 5-hepten-2-one-6-methyl | *991* | 1.4 |
| 3-octanol | *999* | 0.1 |
| limonene | *1030* | 4.1 |
| 1,8-cineol | *1036* | 1.9 |
| trans-β-ocimene | *1047* | 3.3 |
| cis-sabinene hydrate | *1072* | 0.2 |
| Linalool | *1103* | 0.3 |
| β-pinene oxide | *1153* | 0.2 |
| iso-Isopulegol | *1166* | 0.1 |
| Rosefuran epoxide | *1179* | 0.2 |
| trans-p-mentha 1(7),8-dien-2-ol | *1185* | 0.2 |
| □-terpineol | *1196* | 0.8 |
| Nerol | *1237* | 0.7 |
| Neral | *1254* | 23.5 |
| Geraniol | *1264* | 0.9 |
| Geranial | *1286* | 34.2 |
| □-Elemene | *1339* | 0.6 |
| Eugenol | *1361* | 0.1 |
| □-copaene | *1377* | 0.3 |
| Geranyl acetate | *1388* | 1.0 |
| □-cedrene | *1414* | 0.2 |
| (E)-caryophyllene | *1423* | 3.2 |
| □-humulene | *1456* | 0.2 |
| allo-Aromadendrene | *1463* | 0.3 |
| Geranyl propanoate | *1478* | 0.2 |
| Germacrene D | *1485* | 5.5 |
| □-zingiberene | *1496* | 0.9 |
| bicyclogermacrene | *1501* | 4.2 |
| □-curcumene | *1513* | 1.1 |
| □-cadinene | *1521* | 0.5 |
| (E)-Nerolidol | *1568* | 1.8 |
| Spathulenol | *1581* | 0.9 |
| caryophyllene oxide | *1585* | 2.4 |
| □-epi-cadinol | *1645* | 1.0 |
| □-cadinol | *1659* | 0.1 |

RI[a], retention indices

**Table 2. The effect of sun drying method on essential oil content of *Lippa citriodora*.**

| Compounds | RI[a] | Area,% |
|---|---|---|
| **α -pinene** | ***934*** | 0.3 |
| **Sabinene** | ***974*** | 0.9 |
| **1-Octen-3-ol** | ***981*** | 0.3 |
| **5-hepten-2-one-6-methyl** | ***991*** | 1.6 |
| **3-octanol** | ***999*** | 0.1 |
| **limonene** | ***1030*** | 5.2 |
| **1,8-cineol** | ***1036*** | 2.1 |
| **trans-β-ocimene** | ***1047*** | 3.5 |
| **cis-sabinene hydrate** | ***1072*** | 0.2 |
| **Linalool** | ***1103*** | 0.3 |
| **Cis - Limonen oxide** | ***1143*** | 0.1 |
| **β- pinene oxide** | ***1153*** | 0.3 |
| **iso-Isopulegol** | ***1166*** | 0.1 |
| **Rosefuran epoxide** | ***1179*** | 0.2 |
| **trans-p-mentha 1(7),8-dien-2-ol** | ***1185*** | 0.2 |
| **□-terpineol** | ***1196*** | 0.8 |
| **Nerol** | ***1237*** | 0.3 |
| **Neral** | ***1254*** | 24.8 |
| **Geraniol** | ***1264*** | 0.3 |
| **Geranial** | ***1286*** | 34.3 |
| **□-Elemene** | ***1339*** | 0.6 |
| **Eugenol** | ***1361*** | 0.1 |
| **□-copaene** | ***1377*** | 0.3 |
| **Geranyl acetate** | ***1388*** | 1.1 |
| **□-cedrene** | ***1414*** | 0.2 |
| **(E)-caryophyllene** | ***1423*** | 2.9 |
| **□-humulene** | ***1456*** | 0.2 |
| **allo-Aromadendrene** | ***1463*** | 0.2 |
| **Geranyl propanoate** | ***1478*** | 0.2 |
| **Germacrene D** | ***1484*** | 4.9 |
| **□-zingiberene** | ***1496*** | 0.8 |
| **bicyclogermacrene** | ***1501*** | 4.0 |
| **□-curcumene** | ***1513*** | 1.0 |
| **□-cadinene** | ***1521*** | 0.5 |
| **(E)-Nerolidol** | ***1568*** | 1.7 |
| **Spathulenol** | ***1581*** | 0.8 |
| **caryophyllene oxide** | ***1585*** | 2.3 |
| **□-epi-cadinol** | ***1645*** | 0.8 |

RI[a], retention indices

**Table 3. The effect of oven drying method on essential oil content of *Lippa citriodora*.**

| Compounds | RI[a] | Area,% |
|---|---|---|
| **α - thujene** | ***926*** | 0.03 |
| **α -pinene** | ***933*** | 0.3 |
| **Sabinene** | ***974*** | 1.1 |
| **5-hepten-2-one-6-methyl** | ***992*** | 2.8 |
| **limonene** | ***1031*** | 6.7 |
| **1,8-cineol** | ***1037*** | 2.8 |
| **trans-β-ocimene** | ***1047*** | 3.2 |
| **cis-sabinene hydrate** | ***1072*** | 0.3 |
| **Linalool** | ***1103*** | 0.5 |
| **β- pinene oxide** | ***1153*** | 0.2 |
| **iso-Isopulegol** | ***1166*** | 0.1 |
| **Rosefuran epoxide** | ***1180*** | 1.1 |
| **trans-p-mentha 1(7),8-dien-2-ol** | ***1185*** | 0.1 |
| **□-terpineol** | ***1196*** | 1.0 |
| **Nerol** | ***1237*** | 0.3 |
| **Neral** | ***1253*** | 19.2 |
| **Geraniol** | ***1264*** | 0.2 |
| **Geranial** | ***1285*** | 28.3 |
| **□-Elemene** | ***1338*** | 0.4 |
| **□-copaene** | ***1377*** | 0.4 |
| **Geranyl acetate** | ***1388*** | 1.4 |
| **□-cedrene** | ***1414*** | 0.2 |
| **(E)-caryophyllene** | ***1423*** | 3.9 |
| **□-humulene** | ***1455*** | 0.3 |
| **allo-Aromadendrene** | ***1462*** | 0.3 |
| **Geranyl propanoate** | ***1478*** | 0.3 |
| **Germacrene D** | ***1485*** | 6.5 |
| **□-zingiberene** | ***1496*** | 0.8 |
| **bicyclogermacrene** | ***1501*** | 4.5 |
| **□-curcumene** | ***1513*** | 1.0 |
| **□-cadinene** | ***1521*** | 0.6 |
| **(E)-Nerolidol** | ***1569*** | 2.2 |
| **Spathulenol** | ***1581*** | 1.4 |
| **caryophyllene oxide** | ***1586*** | 3.3 |
| **□-epi-cadinol** | ***1645*** | 1.4 |
| **□- cadinol** | ***1650*** | 0.1 |

RI[a], retention indices

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