

GC-mass analysis of *Lippia citriodora* L. stemAzadeh Boustani<sup>1,\*</sup>, Mansour Omid<sup>2</sup> and Sepide Torabi<sup>1</sup><sup>1</sup>Department of Plant Breeding, Science and Research Branch, Islamic Azad University, Tehran, Iran.<sup>2</sup>Department of Agronomy and Biotechnology and Plant Breeding, University of Tehran, Karaj, Iran.

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## ABSTRACT

Plants are important source of drugs for majority of the world population. The chemical components in *Lippia citriodora* help in treating various diseases especially about nervous system illnesses. The present investigation was carried out to determine the chemical compounds present in the stem cuttings of *L. citriodora* with using GC-MS analysis. The analysis revealed the presence of 26 compounds in the stem cutting. The major compounds were citral (10.63%), Geraniol (10.01%), neral (9.21%), Geranial (7.94%), 4-Phenyl undecan 4-ol (6.78%), 1-octen-3-ol (6.54%). The major and minor constituents were also observed.

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## Introduction

Herbal medicines have been the basis of treatment and cure for various diseases in traditional methods. Lemon verbena (*Lippia citriodora* L.) is an aromatic plant belonging to the *Verbenaceae* family, Common names for this plant include *Lippia triphylla*; *Aloysia triphylla*; *Aloysia citriodora* and *verbena triphylla* (Boulos, 2002). *Lippia* genus has more than 200 species which *L. citriodora* is the most important species (Omid beige, 1997). It is a shrub perennial medicinal plant, indigenous to South American countries and acts mainly on nervous system. The leaves and vegetative parts of this plant are refrigerant, anticonvulsant and gastrointestinal strengthening (Boulos, 2002; Pascual et al, 2001) also some antibacterial and antioxidant effects have found in this plant (Ponce-monter et al. 2010). So the mentioned properties of this plant were clarified the importance of it. Citral is the main chemical component found in *L. citriodora* leaves extract (Ponce-monter et al. 2010). Also according to a literature, limonene is the component found to occur in higher quantities in essential oils of the genus *Lippia* (Pascual et al., 2001; Terblanche and Kornelius, 1996). Gas chromatography-mass spectrometry (GC-MS) is the most popular method for the determination of essential oil composition (Milovanović et al, 2011) and lots of researches have been performed by this method. Even though there have been several papers published related to the chemical component in medicinal plants, there is no study about chemical composition of stem cuttings of *L. citriodora*. So the present study was aimed to apply GC-mass technique in order to evaluate the phytochemicals in Lemon verbena's stems.

## Material and methods

Stem cuttings were provided from Five year old plants which were collected from Sari (36.56° North latitude, 53.06° East longitude and 53 meters elevation above the sea) located in Mazandaran province, Iran in August 2012. These cuttings were cultured in greenhouse for 2 months, and then young stem cuttings in length of 10-15 cm were obtained from 2 month old plants. The plant material was dried naturally on laboratory benches at room temperature (23-27 °C), the leaves removed

and then the dried and crispy stems were become into powder with using of mixer. The dried materials were stored at -24 °C and then hydrodistilled by using Clevenger-type apparatus to extract its essential oil. Conditions of extraction were 50 g of samples, [1:10 plant material: water volume ratio], and 4 h distillation. The oil was dehydrated with anhydrous sodium sulphate (10 min) and immediately stored in airtight glassware in refrigerator at 4 °C. GC-MS analysis was carried out on a GC Clarus 500 Perkin Elmer system comprising of an Aoc-20i auto sampler and gas chromatograph interfaced to a mass spectrometer. GC-MS instrument employed the following conditions: column-Elite-1 fused silica capillary column (30 mm x 0.25 mm ID x1 μMdf), composed of 100% dimethyl poly siloxane, operating in electron impact mode at 70 eV; carrier gas: helium (99.999%) at a constant flow of 1 ml/min; injection volume: 0.5 μl (split ratio of 10:1); injector temperature: 250°C and an ion source temperature of 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min) with an increase of 10°C/min to 200°C, then 5°C/min to 280°C ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 45 to 450 Da. Total GC running time was 36 min. Unknown essential oil was identified by comparing its GC retention time to that of known compounds and by comparison of its mass spectra, either with known compounds or published spectra.

## Results

The extract of the plant was dark yellow with distinctive tang. Result of the GC-MS analysis of young stem cuttings is depicted in Tabale.1. The analyses of *L. citriodora* essential oil revealed that citral (10.63%), Geraniol (10.01%), neral (9.21%), Geranial (7.94%), 4-Phenyl undecan 4-ol (6.78%), 1-octen-3-ol (6.54%) were the main products.

## Discussion

Regarding the observations of the present study 26 compounds were found in Lemon verbena's extract. Monoterpenoids including citral (10.63%), Geraniol (10.01%), neral (9.21%), Geranial (7.94%) were the main components of the essential oil. The main components in the essential oils of *L.*

citriodora leaves collected from Armeni (Colombia), were geranial, neral and limonene (Olivero-Verbel et al., 2009; Terblanche' and Kornelius, 1996). The main constituents of the essential oil extracted from fresh leaves of *L. citriodora* from Greek origin, were geranial, neral and limonene (Argyropoulou et al., 2007). So it can be seen that Geranial and neral were existed as major constituents regarding the mentioned researches, and similarly in our study they were the major compounds.

**Table 1. Chemical composition of essential oils of *Lippia citriodora* stems.**

Compounds	Concentration (%)
Citral	10.63
Geraniol	10.01
Neral	9.21
Geranial	7.94
4-Phenyl undecan 4-ol	6.78
1-octen-3-ol	6.54
$\alpha$ - Curcumene	4.98
$\alpha$ - Cedrol	4.42
Caryophyllene oxide	4.02
$\alpha$ - Terpineol	3.89
Carveol	3.51
Linalool	3.22
$\alpha$ - pinene	3.05
Caryophyllene	2.73
Nealloocimene	2.66
Trans Caryophyllene	2.49
4- Terpineol	2.23
$\alpha$ - Terpineol Acetate	1.91
Nerolidol	1.82
Copaene	1.79
Trans Limonene oxide	1.66
$\beta$ - Bourbonene	0.87
Khusinol	0.74
Cis carveol	0.66
$\alpha$ - Cedrane	0.56
Verbenol	0.42
Other compounds	1.26

However, limonene was not seen in our study. Another analysis about Lemon verbenas collected from other localities of Iran, reported that 1,8-Cineole (23.66%),  $\alpha$ -curcumene (14.83%), geranial (13.74%), limonene (13.40%) and caryophyllene oxide (6.60%) were the component found to occur in higher quantities in essential oils of the *L. citriodora* leaves, respectively (Meshkatsadat et al., 2010). According to Meshkatsadat et al. literature the majority of extract of *L. citriodora* leaves was included 1,8-Cineole whereas it was not perceived in our experiment about this plant. Alavi et al., were reported in 2011 that AR-curcumene (14.1%) was the most important component of Lemon verbena's extract while it was not available in type of *L. citriodora* which was studied in our research. Furthermore, our results did not show the presence of spathulenol, which have been mentioned in other studies concerning *L. citriodora* (Meshkatsadat et al., 2010; Alavi et al., 2011; Olivero-Verbel et al., 2009). Mojab et al. in 1999 found that the extract of *L. citriodora* which are grown in Iran were mostly similar to French Lemon verbena (Montes et al., 1973) while the Iranian types had the maximum difference in comparison with Argentine species of *L. citriodora* (Zygadlo et al., 1994). Based on our knowledge, no research has been previously studied on chemical components of stem of *Lippia citriodora*, and regarding our investigation about this issue it could be concluded that citral, Geraniol and neral developed the

majority of *L. citriodora*'s extract. On the other hand it was not observed Limonene in stem oil whereas it was an important constituent in some other researches about essential oil of the plant's leaves (Khani et al., 2012; Meshkatsadat et al., 2010; Olivero-Verbel et al., 2009; Terblanche' and Kornelius, 1996; Argyropoulou et al., 2007). In conclusion our results show that this plant includes lots of vital medicinal compounds, so a breeding plan could be done as further study in order to promote the medicinal features of *L. citriodora*.

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