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Essential oil composition of lemon verbena (*Lippia citriodora*) leaves cultivated in Mazandaran, Iran

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Abstract

In this study the chemical composition of the essential oil extracted from leaves of *Lippia citriodora* (Verbenaceae) grown under greenhouse conditions in mazandaran, Iran. The essential oil was obtained by hydro-distillation method using Clevenger apparatus. The oil was analyzed by GC and GC-MS. Thirty-four compounds were identified. The main constituents of the oils in this method were sabinene (1.34%), 6 methyl-5heptene-2one (3.46%), D-limonene (5.81%), 1,8-Cineole (2.51%), trans-beta ocimene (1.17%), Alpha terpineol (1.75%), neral (12.6%), geranial(15.07%), geranial acetate (1.15%), caryophyllene (4.02), D-germacrene (3.52), alpha-curcumene (4.17%), bicyclogermacrene (3.42%), nerolidol (1.59%), spathulenol (4.40%) caryophyllene oxide (2.04%), alpha-cadinol (1.06%).In our study citral was also identified at high percentages.

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Introduction

Plant oils and extracts have been used for a wide variety of purposes for many thousands of years (Jones, 1996). Since ancient times, herbs and their essential oils have been known for their varying degrees of antimicrobial activity. More recently, medicinal plant extracts were developed and proposed for use in food as natural antimicrobials. 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 *et al*., 2002; Santos-Gomes *et al.,* 2005) *Lippia citriodora* (ORT.) HBK (Verbenaceae) is an herbal species mainly used as a spice and medicinal plant. It grows spontaneously in South America and is cultivated in North Africa and Southern Europe. It has many synonymies: *Lippia citriodora*, *Lippia triphylla* and *Aloysia citriodora*. 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 properties. 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 *et al*., 2005).

Literature Review

There are few studies published about the chemical composition and pharmacological aspects and there is no defined standard quality profile of the *L. citriodora* essential oils. The chemical composition of the essential oil from the leaves of *L. citriodora* has been previously reported. The major components of the essential oils of plants cultivated in France neral, geranial, geraniol, limonene, citronellal, nerol, 1, 8 – cineol reported (Tutin,1981) and the major compounds in Morocco 1, 8 - cineole, granial, neral and 5-heptene-2-one-6-methyl (Blakhdar *et al*, 1993) have been reported. Using an appropriate method for the isolation of the essential oil can be useful for diagnostics and introduction components. 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. Using gas chromatography mass spectrometry (GC/MS) is also fast, reliable and inexpensive tool for diagnostics and introduction components.

This work aimed to identify the essential oil chemical composition of *L. citriodora* leaves grown under greenhouse conditions in mazandaran, Iran.

Material and methods

Study area

Plant Material and Isolation Procedure: The leaves of *L. citriodora* from shrubs grown in a greenhouse in Mazandaran province, Iran at full flowering stage in May 2012. The samplewere cleaned in shade condition to prevent hydrolyze of the existing materials and to keep the natural color of the sample fixed. Then they were dried in the temperature of the environment plant were obtained and were powdered and kept at appropriate conditions from the viewpoint of temperature and light until the essential oil taking stage. Afterwards, Hydro-distillation procedures were done according to the European Pharmacopoeia (2008).

Methods

Essential oil was taken from 30 grams of the powdered sample dissolved in 250 ml of distilled water in hydro- distillation method with the help of Clevenger set for 3 hours. Using volumes of 1.0 ml of n-hexane for retention of the hydro-distillate components. Following the sample oils were dried with anhydrous sodium sulfate and analyzed by gas chromatography-mass spectrometry (GC-MS). The oil yields were calculated on a dry weight basis as 1.2%.

The gas chromatography-mass spectroscopy method was done according to Moghtader (2009), by some modification.20 A Hewlett-Packard (HP) 7890 A gas chromatograph which was connected to an HP 5975C inert MSD was used for the identification of compounds from the oil. An HP-5MS fused silica capillary column (Hewlett-Packard, 30 m, 0.25 mm i.d., 0.25 μm film Thickness, cross-linked to 5% phenyl methyl siloxane stationary phase) was used. The entire system was controlled by MS Chem Station software (Hewlett- Packard, version A.01.01). Electron impact mass spectra were recorded at 70 eV. Helium was used as the carrier gas at flow rate of 1 mL/min. The injection volume was 1 μL and all the injections were performed in a split ratio of 1:50. Injector and detector temperatures were 270 and 280 °C, respectively. Column oven temperature was initially set at 50°C for 5 min, then increased to 270 °C (ramp, 5 °C/ min), and held for 10 min.

Result

Composition of the essential oil

The composition of the essential oil of *L. citriodora* obtained by hydro-distillation. Analysis of Essential Oil was done by using Gas Chromatography with Mass Spectrometer. The qualitative and quantitative analysis is done to know the constituents in the oil and the percentage of components present in the oil respectively, by doing so we can know the purity of that particular oil (Williams *et al.*, 1993). The chemical composition of the essential oil of *L. citriodora* is summarized in Table 1. Thirty-four compounds were identified.

Table 1. The chemical composition of essential oils obtained from *L.citriodora*.

N[a]	Compounds	RT[b]	RI[c]	Area[d] (%)
1	Alpha pinene	4.209	934	0.47
2	sabinene	5.046	974	1.34
3	1-Octen-3-ol	5.172	980	0.83
4	6 Methyl-5Heptene-2One	5.375	991	3.46
5	Beta-Myrcene	5.452	1011	0.26
6	D-Limonene	6.494	1029	5.81
7	1,8-Cineole	6.551	1036	2.51
8	Trans-beta-ocimene	7.032	1097	1.17
9	Trans-sabinene hydrate	7.596	1062	0.5
10	3-Methyl-2-(2-Methyl-2-butenyl)furan	8.595	1093	0.26
11	Linalool	8.695	1103	0.67
12	Cis,Cis-photocitral	10.026	1116	0.23
13	3,3-Dimethyl-hepta-4,5-dien-2-one	10.422	1132	0.28
14	Citronella	10.592	1147	0.36
15	Cyclohexane,ethenyl	11.734	1165	0.68
16	Alpha terpineol	12.010	1196	1.75
17	Nerol	13.508	1237	0.55
18	Neral	14.216	1240	12.60
19	Geranial	15.489	1242	15.07
20	Bicycloelemene	17.784	1330	0.22
21	Alpha-cubebene	19.305	1364	0.46
22	Geranyl acetate	19.859	1386	1.15
23	Caryophyllene	21.062	1421	4.02
24	Alpha-caryophyllene	22.334	1427	0.39
25	Aromadendrene	22.618	1439	0.45
26	D-Germacrene	23.492	1485	3.52
27	Alpha- Curcumene	23.723	1507	4.17
28	bicyclogermacrene	24.113	1509	3.42
29	Delta cadinene	25.170	1521	0.37
30	Nerolidol	26.874	1564	1.59
31	Spathulenol	27.235	1581	4.40
32	Caryophyllene oxide	27.352	1585	2.04
33	Iso-spathulenol	29.413	1629	0.68
34	Alpha-cadinol	29.525	1659	1.06

a. Numbers in the compounds correspond to peaks in Fig. 1.
b. Retention time
c. Retention index
d. Total percentage

The main constituents of the oils were sabinene (1.34%), 6 methyl-5heptene-2one (3.46%), D-limonene (5.81%), 1,8-cineole (2.51%), trans-beta ocimene(1.17%), alpha terpineol (1.75%), neral (12.6%), geranial (15.07%), geranial acetate (1.15%), caryophyllene (4.02%), D- germacrene (3.52), alpha-curcumene (4.17%), bicyclogermacrene (3.42%), nerolidol (1.59%), spathulenol (4.40%) caryophyllene oxide (2.04%), alpha-cadinol (1.06%). Geranial and neral are isomers citral. The E-isomer is known as geranial or citral A. The Z-isomer is known as neral or citral B. In our study citral (geranial and neral) was also identified at high percentage. In other research conducted in Iran by Khani *et al* (2012) The major compounds were citral (11.3%), limonene (10.6%), 4-phenyl undecan-4-ol (7.7%), α-curcumene (6.5%), α-cedrol (4.5%) and caryophyllene oxide (4.5%). Furthermore, lesser amounts of the other components include carveol (3.7%), linalool (3.5%), α-pinene (3.2%), caryophyllene (2.8%) and geranylacetat (1.8%) were existed in the essential oil.

Roseane O. de Figueiredo *et al* (2004) reported Geranial (29.54%) and neral (27.01%) have highest percentage essential oil composition of lemon verbena leaves cultivated in Brazil. In research conducted by Hanaa *et al* on leaves of lemon verbena, as well as the highest percentage citral was introduced (Hanaa *et al*, 2008). In a review on *A. triphylla* was characterized by the presence of citral A (37.15%), citral B (22.34%), cineole (15.43%), geraniol (10.6%) (Terblanché *et al.*, 1996).

Gas chromatogram of essential oil

Fig. 1. shows the essential oil chemical composition of L. citriodora obtained through gas chromatography connected to the mass spectrometer (CG-MS).

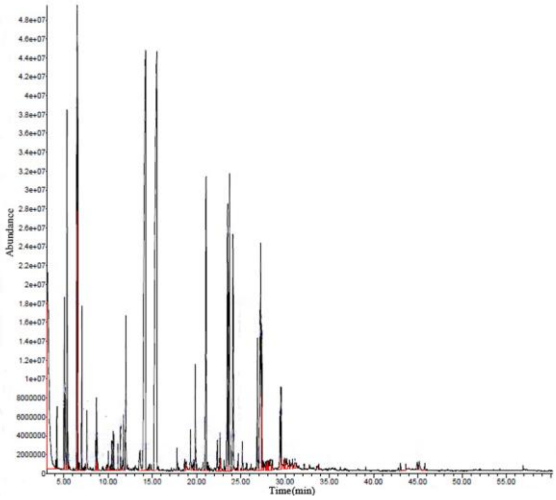

Fig. 1. Gas chromatogram of essential oil of *L. citriodora* from Iran.

Citral showed the highest percentage which is not in agreement with lower values found for geranial (9.9 %) and neral (6.9 %) from studies by Bellkhdar *et al* (1994). Zygadlo *et al* (1994) detected myrcenone, α-thujone and lippifoli-1(6)-en-5-one as the main components, limonene in low percentage and no citral. In *A. triphylla* grown in Morocco. Ozek *et al* (1996) identified 69 compounds in leaves and branches of *A. triphylla* in Turkey, where the most important ones are as follows: limonene (18.6% and 14.8 % respectively), geranial (11.9 % and 19.1 % respectively) and neral (6% and 8.1% respectively).
In another research, GC-MS analysis of essential oils revealed that 1, 8-cineole l (23.66%), α-curcumene (14.83%), geranial (13.74%), limonene (13.40%) and caryophyllene oxide (6.60%) were the main components of essential oils of *L. citriodora*, respectively (Meshkatalsadat *et al.*, 2010).

Conclusion

According to the sources reviewed lemon verbena essential oil, in different areas, show different combinations of types and amounts. The literature emphasizes that a variety of geographical and ecological factors can lead to qualitative and quantitative differences in the essential oil produced. At the same time, a number of other factors can influence its composition, such as the developmental stage of the plant, its physiology, the age of leaves and the growing conditions. In addition, chemical composition of the essential oils affected by the isolation procedure and analysis conditions.

Experimental parameters such as extraction time, irradiation power can be optimized for the particular aim of the distillation, either to obtain a high yield of essential oil, or to obtain essential oils of differing composition (Pawlisyzn *et al.*, 2007). However, distillation is capable to analyze the chemical composition with a small amount of sample, and sample preparation steps. Geranial has a strong lemon odor. Neral's lemon odor is less intense, but sweeter. Citral is therefore an aroma compound used in perfumery for its citrus effect. Citral is also used as a flavor and for fortifying lemon oil citral is used in the synthesis of vitamin A, ionone, and methylionone, and to mask the smell of smoke. Two studies have shown 1-1.7% of people to be allergic to citral, and allergies are frequently reported. Citral on its own is strongly sensitizing to allergies. Citral has been extensively tested and has no known genotoxicity, and no known carcinogenic effect, but animal tests show dose-dependent effects on the kidneys. Citral also has strong antimicrobial qualities (Onawunmi, 1989) and pheromonal effects in insects (Kuwahara *et al.*, 1983; Robacker *et al.*, 1977). The results of this study could be of interest for further phytochemical and biological investigation of lemon taking into account that citral oil showed marked antimicrobial activity.

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