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Antioxidant Activity of Essential Oil of *Lallemantia iberica* in Flowering Stage and Post-Flowering Stage

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Abstract: In this study, the essential oils from the arial parts of *Lallemantia iberica* (Lamiaceae), collected in 2 stages (flowering and post-flowering) from plants that caltivated in Institute of Medicinal Plants (ACECR) in Hashtgerd of Iran were obtained by hydrodistillation in Clevenger type apparatus. The chemical components of the essential oils were examined by GC and GC-MS then 36 components were characterized in flowering stage with β-cubeben (19.55%), Linalool (18.71%), spathulenol (18.04%), β-caryophyllene (11.11%), geraniol (3.50%) and bicyclogermacrene (3.46%) as the major constituents. All constituents are representing 97.39% of the essential oil, contained monoterpenes (33.85%) and sesquiterpens (63.54%). About 39 components of essential oil of post-flowering stage were introduced which caryophylene oxide (38.77%), linalool (15.15%), Germacrene-D (7.03%), Trans-caryophylene (5.61%), β-bourbonene (4.96%) and Trans-geraniol (4.34%) as the major constituents of it. All components are representing 95.74% of the essential oil contained monoterpens (26.51%) and sesquiterpens (69.23%). The studied essential oils showed antioxidant activities as calculated by 2 *in vitro* assays; DPPH radical scavenging and Ferric Reducing Power Assay (FRAP).

Key words: *Lallemantia iberica*, Lamiaceae, essential oil composition, GC, GC-MS, antioxidant activity, DPPH, FRAP

INTRODUCTION

Lallemantia iberica belongs to the tribe Stachyoideae-Nepeteae, family Lamiaceae and this family has 46 genera and 410 species and subspecies in Iran (Naghibi *et al*., 2005). *Lallemantia iberica* originated from Caucasian region that has been found in Asia (Syria, Iran and Iraq) but it now appears in central and Southern Europe. The Lallemantia genus has 5 different species which are distributed in different places of Iran (North, East North, East South, Alborz and other areas). *Lallemantia iberica* is introduced with popular name Balangu and traditional name Balangue shahri and with other synonyms *Lallemantia sulphure*a, *Dracocephalum ibericum* (Bieb.) (Amin, 1991; Mozaffarian, 1996). People use leaves, oil, seed (Hedrick, 1972) and it has traditional uses as reconstiuent, stimulant, diuretic and expectorant (Aynechi, 1986; Naghibi *et al*., 2005). It is seeds contain mucilage that it used in the treatment of various disorders such as some nervous, hepatic and renal diseases and also used as general tonic, aphrodisiac and expectorant remedies in Iranian Folk medicine (Amin, 1991; Emad, 2000). *Lallemantia iberica* cultivated for its seeds from which and oil is extracted, the seed contains up to 30% of a drying oil (Usher, 1974).

MATERIALS AND METHODS

Plants: The aerial parts of cultivated *L. iberica* were collected in May, 2009 (flowering stage) and in July, 2009 (post-flowering stage) from the Karaj, Iran. Identification of the plant as *L. iberica* was confirmed by the Herbarium Department of the Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran. A voucher specimen of the plant was deposited in the Herbarium of the Department of Pharmacognosy, School of Pharmacy and Pharmaceutical Sciences, Tehran University of Medical Sciences, Tehran, Iran.

Chemicals: All reagents and solvents were of analytical grade or of pure quality which were purchased from Merck, Sigma, Aldrich and Fluka.

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Extraction of essential oil: The aerial parts of the plant (100 g) dried in the shade and then powdered after that the volatile oils were isolated by hydrodistillation for 3 h according to the method recommended by the European Pharmacopoeia. The oil was dried over anhydrous sodium sulphate and stored in a refrigerator (4°C).

Gas chromatography-mass spectrometry: Analytical gas chromatography was carried out using a Termoquest 2000 GC with capillary column DB-5 (30 m×0.25 mm i.d., 0.25 μm film thikness); carrier gas, He; split ratio, 1:25 and using a flame ionization detector. The column temperature was programmed at 50°C for 1 min and then heated to 265°C at a rate of 2.5°C min^{-1} and then kept constant at 265°C for 20 min GC-MS was performed on a Thermoquest 2000 with a qudrupole detector, on capillary column DB-5 (GC); carrier gas, He; flow rate, 1.5 mL min^{-1} the column was held at 50°C for 1 min and programmed up to 265°C at rate of 2.5°C min^{-1} then kept constant at 256°C for 20 min. The MS operated at 70 eV ionization. Retention indices were calculated by using retention times of n-alkanes that were injected after the oil at the same chromatographic conditions. Quantitative data was obtained from the electronic integration of the FID peak areas. The components of the oils were identified by comparison of their mass spectra and retention indices with Wiley library and those published in the literature.

Antioxidant activity

DPPH assay: This assay is based on the Spectrophotometric method. A test sample was added to a concentration of methanolic 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH). Then, the mixture was incubated in the dark at room temperature for 30 min and the absorbance was measured at 517 nm. The difference between the initial DPPH radical adsorption and the adsorption of the sample after reaction was determined as antioxidant activity. The IC_{50} values (concentration of the test samples providing 50% scavenging) were calculated from the graph-plotted scavenging percentage against the oil concentration. A lower IC_{50} value means a higher antioxidant power of the examined compound. Scavenging percentage of the DPPH stable radical was calculated in following way (Ramamoorthy and Bono, 2007; Rohman *et al*., 2010):

$$\text{DPPH scavenging activity (\%)} = 100 - [(A_0 - A_s)/A_0]\times 100$$

where, A_0 is the absorbance of the control (The DPPH solution without sample solution) and as is the absorbance in the presence of sample. The DPPH antioxidant activity was assessed by the method of Sanchez-Moreno *et al*. (1999). Butyl Hydroxyanisole (BHA) and α-tocopherol were used as positive controls. All values are shown as the mean of 3 measurements (Huang *et al*., 2005; Tofighi *et al*., 2009; Monsef-Esfahani *et al*., 2010).

Ferric Reducing Antioxidant Power (FRAP) assay: The FRAP assay was done according to the method of Benzie and Strain (1996). This assay is based on the ability of sample to reduce Fe^{3+} in the presence of a Tripyridyltriazine (TPTZ) solution. After forming the Fe^{2+} ion, the blue colored complex Fe^{2+}-tripyridyltriazine was produced. An increase above a complex concentration signals the reducing power of the sample. Solution absorbance was determined at 593 nm (Benzie and Strain, 1996; Huang *et al*., 2005; Monsef-Esfahani *et al*., 2010).

Statistical analysis: All tests repeated 3 times and data was expressed as mean±SD. Statistical analysis, plots and fittings were carried out by using Excel 2007.

RESULTS AND DISCUSSION

The hydrodistillation of the flowering aerial parts of *L. iberica* gave yellow oil with a sharp odour in the yield of 0.2% (w/w) based on dry weights. About 36 components were identified in it representing 97.39% of total oil. The identified components and their percentages are shown in Table 1. The oil of *L. iberica* was characterized by a high content of β-cubeben (19.55%), linalool (18.71%), spathulenol (18.04%), β-caryophyllene (11.11%), geraniol (3.50%), bicyclogermacrene (3.46%). It was characterized by high amount of sesquiterpene hydrocarbons (43.91%) and monoterpene oxygenated (28.01%) (Table 1).

The aerial parts of *L. iberica* in post-flowering stage yielded 0.1% (w/w) of a yellowish oil with a strong aroma. About 39 components were characterized, representing 95.74% of the total oil components detected. They are shown in Table 2 with their percentage.

The major constituents of the oil were caryophylene oxide (38.77%), linalool (15.15%), Germacrene-D (7.03%), Trans-caryophylene (5.61%), β-bourbonene (4.96%) and Trans-geraniol (4.34%). It was characterized by high amount of sesquiterpene oxygenated (49.3%) and monoterpene oxygenated (24.93%) (Table 2). Previous investigations on the oil of some species of Lallemantia showed various results.

In a study on *L. iberica* that collected from Larijan, Iran, p-Cymene (22.1%), isophytol (19.8%), T-cadinol (11.1%), 3-octanol (8.1%), caryophyllene oxide (7.4%) and

Table 1: Chemical composition of essential oil of *Lallemantia iberica* (%) in flowering stage

Components	RI	Percent
α-pinene	855.00	0.24
β-pinene	897.00	2.38
β-myrcene	912.00	0.17
Limonene	944.00	0.41
Trans-α-ocimene	954.00	0.27
Trans-β-ocimene	965.00	2.22
α-terpinolene	998.00	0.15
Linalool	1017.00	18.71
Trans-pinocarveol	1038.00	0.29
Geijeren	1048.00	0.09
Pinocarvone	1050.00	0.15
Myrtenal	1078.00	0.33
β-fenchyl alcohol	1087.00	2.97
Myrtenol	1091.00	0.27
Geranyl alcohol*	1123.00	1.04
Geraniol	1149.00	3.50
Eugenol	1234.00	0.17
Trans-β-damascenone	1275.00	0.49
α-copaene	1286.00	0.89
β-bourbonene	1296.00	2.59
β-elemene	1302.00	0.74
β-caryophyllene	1323.00	11.11
Germacrene-D	1327.00	0.42
α-caryophyllene	1341.00	0.43
β-farnesene	1351.00	1.68
β-cubeben	1364.00	19.55
Eremophilene	1367.00	2.09
Bicyclogermacrene	1372.00	3.46
Germacrene A	1376.00	0.19
γ-cadinene	1380.00	0.14
δ-cadinene	1387.00	0.62
Spathulenol	1439.00	18.04
Isospathulenol	1494.00	0.24
α-cadinol	1508.00	0.61
Valerenol	1520.00	0.48
Aromadendren epoxide	1575.00	0.26
Monoterpenes hydrocarbons	5.84	-
Monoterpenes oxygenated	28.01	-
Sesquiterpenes hydrocarbons	43.91	-
Sesquiterpenes oxygenated	19.63	-
Unknown	2.61	-
Total identified	97.39	-

Table 2: Chemical composition of essential oil of *Lallemantia iberica* (%) in post-flowering stage

Components	RI	Percent
α-pinene	855.00	0.05
Pentyl vinyl ketone	885.00	0.10
β-pinene	895.00	0.52
Furan, 2-pentyl	908.00	0.15
Myrcene	911.00	0.12
Limonene	944.00	0.32
Trans-β-ocimene	953.00	0.14
Terpinolene	998.00	0.18
Nonanal	1005.00	0.13
Linalool	1017.00	15.15
Trans-pinocarveol	1038.00	0.26
Pinocarvone	1050.00	0.09
4-terpineol	1073.00	0.07
β-fenchyl alcohol	1087.00	2.44
Myrtenol	1091.00	0.23
β-cyclocitral	1104.00	0.04
Cis-geraniol	1124.00	1.22
Trans-geraniol	1129.00	4.34
Eugenol	1235.00	0.19
Iso geraniol	1252.00	0.21
β-damascenone	1276.00	0.56
β-bourbonene	1299.00	4.96
β-elemene	1303.00	0.46
Trans-caryophylene	1322.00	5.61
Germacrene-D	1327.00	0.21
(E)-Farnesene	1335.00	0.24
α-caryophyllene	1341.00	0.32
β-farnesene	1349.00	1.10
Germacrene-D	1361.00	7.03
Eremoligenol	1365.00	1.70
Bicyclogermacrene	1370.00	2.45
δ-cadinene	1387.00	0.43
2-dodecen-4-yne (z)	1401.00	2.20
Caryophylene oxide	1447.00	38.77
Aromadendren oxide	1508.00	1.62
α-cadinol	1511.00	0.34
Patchoulane	1523.00	1.63
β-oplopenone	1530.00	0.16
Monoterpenes hydrocarbons	1.58	-
Sesquiterpenes hydrocarbons	19.93	-
Monoterpenes oxygenated	24.93	-
Sesquiterpenes oxygenated	49.30	-
Unknown	4.26	-
Total identified	95.74	-

Table 3: Antioxidant activities of essential oils of aerial parts of *Lallemantia iberica*

Stage	DPPH (μg mL^{-1})	FRAP (μmol^{-1} Fe^{2+} g^{-1} DW)
Flowering	100	70±3.3
Post-flowering	70	100±3.6

Data presented is mean±SD from 2 different experiments; radical scavenging assay (DPPH); Ferric Reducing Power Assay (FRAP); in flowering stage and post-flowering stage

terpinen-4-ol (5.7%) were mentioned as the main constituents (Morteza-Semnani, 2006). According to another study, the essential oil of leaves and stems of *Lallemantia peltata* (L.) (Baser *et al*., 2000) (Labiatae, collected from Turkey) was analysed by GC/MS. About 13 compounds were identified representing all of the components detected.

Germacrene D (27.4%), (E)-β-ocimene (20.1%) and geijerene (12.0%) were the major constituents of the oil (Baser *et al*., 2000). In the other study, water-distilled essential oil from aerial parts of *Lallemantia royleana* (Benth. in Wall.) grown in Isfahan province, Iran was analysed by GC and GC-MS. About 46 compounds were identified that constituting 94.5% of the total detected components, among them verbenone (16.4%) and trans-carveol (9.8%) were the major components of the oil (Ghannadi and Zolfaghari, 2003). In this research, the chemical constituents of two stages are different with each other and with other results that we have about essential oil of genus Lallemantia. About Lallemantia iberica , we compared the results with the result of study from Larijan, they are different because cultivar variations, geographical differences, times of plant growing and preparation procedures may have influenced oil compounds either at the qualitative or quantitative level (Javidnia *et al*., 2007; Masoudi *et al*., 2009).

DPPH radical scavenging activity: In the DPPH assay, the ability of the examined essential oils to perform as

a giver of the hydrogen atom or electron in transforming the purple-colored radical DPPH into the yellow-colored DPPH-H with a reduced shape was studied. All samples possessed inhibitory activity. Essential oil in post-flowering exhibited the highest radical scavenging potential (IC_{50} = 70 μg mL^{-1}) followed by essential oil in flowering stage (IC_{50} = 100 μg mL^{-1}). The greatest effect was obtained by essential oil in post flowering stag (IC_{50} = 70 μg mL^{-1}) though it was more effective than BHA (IC_{50} = 100 μg mL^{-1}) and less effective than α-tocopherol (IC_{50} = 40 μg mL^{-1}). There are not any results for camparing with the results.

FRAP assay: The reducing capacities of essential oils of *L. iberica* were calculated according to the FRAP assay. An aqueous solution of ferrous sulphate (50-500 μmol mL^{-1}), y = 0.005x - 0.0234, R^2 = 0.997) was prepared as acalibration curve. The results were expressed as μmol Fe^{2+} equivalents 9 g^{-1} DW and are shown in Table 3. FRAP values point to a considerably higher reducing power of post-flowering (100±3.6 μmol Fe^{2+} g^{-1} DW) compared with flowering (70±3.3 μmol Fe^{2+} g^{-1} DW).

CONCLUSION

The present study showed that it the chemical composition of the essential oil of aerial parts of *L. iberica* for possible use in foods and cosmetics products and their antioxidant effects of them because there are few texts about Lallemantia genus. This study will open new way to research on special effect of this plant.

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