



ISSN 2320-3862

JMPS 2015; 3(2): 61-64

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Received: 15-01-2015

Accepted: 01-02-2015

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Changes in content and chemical composition of *Dracocephalum moldavica* L. essential oil at different harvest dates

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Abstract

Dracocephalum moldavica L. (Lamiaceae = Labiatae) is one of the most important medicinal plants has powerful biological activity and pharmacological properties. The chemical constituents of essential oil by hydro distillation from the aerial parts of *Dracocephalum moldavica* L. from Egypt were identified by gas chromatograph-mass spectrometer (GC-MS) demonstrated the presence of 33 compounds. The volatile oil content has been analyzed by GC/MS. The GC/MS revealed the presence of geranyl acetate [(28.81, 27.02 and 27.81%); geranial (citral a) (20.42, 19.37 and 19.74%); neral (citral b) (18.36, 17.85 and 17.86%) and geraniol (12.66, 16.05 and 16.68%) which represents the main compounds of volatile oil extracted from *Dracocephalum moldavica* fresh herb. However, geranyl acetate compound contents were the highest in the volatile oil, followed by geranial, then neral and finally geraniol in all harvest dates.

Keywords: *Dracocephalum moldavica*, harvest date, essential oil content, essential oil composition? geranyl acetate, geranial, geraniol, neral.

1. Introduction

Dracocephalum moldavica L. known as Moldavian balm or Moldavian dragonhead is an annual herb belonging to the *Lamiaceae* (*Labiatae*) family, investigated primarily for essential oil composition [1]. It is native to central Asia and is naturalized in eastern and central Europe [2, 3]. Due to its pleasant lemon scent and medical effects, has acquired increasing impact as functional food [4]. In fact, dragonhead is traditionally used for medical purposes for coronary diseases as well as for pain relief [5]; Recently, extracts of *D. moldavica* were found to act as efficient and biologically safe insect repellent for food storage [6, 7]. Due to the aromatic nature of this plant, the chemical analysis of essential oils has been in the focus and neral, citral, geraniol, geranial, and other monoterpenes had been identified as leading components [8, 9, 10, 11]. As an herbal drug, it is used in stomach and liver disorders, headache and congestion [12]. An extract of this herb has been used for its antitumor [13], antioxidant [2], and Antimutagens properties [1]. It is traditionally used as a heart tonic, reconstituent, sedative, flatulence, vermifuge, diaphoretic, for snakes bites and stings, nausea, gastroenteritis and as gargle for stomatitis and fungal infections [14]. In clinical tests, it has been found that a decoction of this herb is effective in curing children's pyelonephritis [15].

The biosynthesis of secondary metabolites, although controlled genetically, is strongly affected by the environmental influences of a particular growing region, and also by the agronomic conditions, harvesting time and the type of processing [16, 17]. Harvesting time of medicinal and aromatic plants is essential to obtain higher essential oil content and better quality [18], the oil composition is altered during harvesting process. In addition, for maximum oil production, long days and high light intensities are required during the maturation period [19]. However, Murray *et al.* [20] and Court *et al.* [21] noticed that harvest date of the peppermint is a major factor in the composition of the essential oil and optimizing the date of harvest is essential for maximizing the production of oil of suitable quality.

However, there are no publications concerning harvest (cut) dates in dragonhead plants. In view of this fact it was of our interest to evaluate the influence of harvesting time on content and chemical composition of the essential oil of dragonhead (*Dracocephalum moldavica* L.) growing in Egypt.

2. Materials and Methods

2.1 Plant Material

Seeds of dragonhead were introduced from Conservator El-Jerdins Botanicus D-Nancy in France and have been adapted under Egyptian conditions for many years. The seeds of basil were sown in the nursery on 1st November, 2012. On 1st

January 2013, uniform seedlings were transplanted into the experimental farm of the Faculty of Pharmacy, Cairo University, Giza, Egypt, which represents clay-loamy soil. The flowering fresh herb was collected at the three harvest dates, 1st March, 1st April and 1st May. Meteorological data at Giza, during the two growing seasons are shown in (Table 1).

Table 1: Meteorological data at Giza (CLAC, Egypt)

Month	2012/2013 season			
	T(°C) Min.	T (°C) Max.	RH %	WS km/h
November, 2012	9.9	32	66	2
December, 2012	1.1	30.6	61	1.7
January, 2013	5.4	28.4	61	2.3
February, 2013	6.6	28.8	56	2.2
March, 2013	8.8	37.6	48	2.3
April, 2013	11.5	38.3	47	2.1
May, 2013	15.7	43.8	44	2

Source: Meteorological data of Giza (CLAC, Egypt), average values; T (°C) Max. And Min. are monthly average, maximum and minimum temperatures; RH is monthly average relative humidity; WS is monthly average relative wind speed

2.2 Gas chromatography/mass spectrometry (GC/MS)

Essential oil percentage of the fresh herb was determined according to the method described in the British Pharmacopoeia [22] by using Clevenger apparatus and expressed as (ml 100 g⁻¹ fresh herb).

The GC-MS analysis of the essential oil samples was carried out in the second season using gas chromatography-mass spectrometry instrument stands at the Department of Medicinal and Aromatic Plants Research, National Research Center with the following specifications. Instrument: a TRACE GC Ultra Gas Chromatographs (THERMO Scientific Corp., USA), coupled with a THERMO mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer). The GC-MS system was equipped with a TG-WAX MS column (30 m x 0.25 mm i.d., 0.25 µm film thickness). Analyses were carried out using helium as carrier gas at a flow rate of 1.0 mL/min and a split ratio of 1:10 using the following temperature program: 40 C for 1 min; rising at 4.0 C/min to 160 C and held for 6 min; rising at 6 C/min to 210 C and held for 1min. The injector and detector were held at 210 °C. Diluted samples (1:10 hexane, v/v) of 0.2 µL of the mixtures were always injected. Mass spectra were obtained by electron ionization (EI) at 70 eV, using a spectral range of m/z 40-450. Most of the compounds were identified using mass spectra (authentic chemicals, Wiley spectral library collection and NSIT library).

3. Results and Discussion

3.1 Essential oil content

Essential oil product from the *Dracocephalum moldavica* plant varies depending on the date of harvest Where the highest proportion of volatile oil (0.11%) resulting from the harvest in April, followed by the harvest when May (0.09%) and the lowest percentage volatile oil (0.06%) was obtained from the harvest in March.

3.2 GC/MS of essential oil

The essential oil of *Dracocephalum moldavica* growing in Egypt was subjected to detailed GC/MS analysis. Exactly 42 compounds, mostly aromatic, were identified, representing 99.69, 99.71 and 99.99% of the total oil at first, second and

third time of harvest. The fourth major compounds were geranyl acetate [(28.81, 27.02 and 27.81%); geranial (citral a) (20.42, 19.37 and 19.74%); neral (citral b) (18.36, 17.85 and 17.86%) and geraniol (16.68, 12.66 and 16.05%)] in the first, second and third time of harvest, respectively. Thus previously was found that geranyl acetate compound contents was the highest in the volatile oil, followed by geranial, then neral and finally geraniol in all harvest times. We also note that the highest percentage of the four main compounds were obtained from the harvest in March followed by harvest in May and the lowest percentages of these compounds as a result of the harvest in April, as shown in Table (2). Said-Al Ahl and Abdou [3] and Said-Al Ahl *et al.* [11] reported that *essential* oil content was 0.04-0.07% in flowering herb and geranial, geraniol and geranyl acetate are three main components in dragonhead grown in Egypt. Also, Janmohammadi *et al.* [23] found that geranial, geraniol and geranyl acetate was the major components in dragonhead grown in two locations in Iran. Omidbaigi *et al.* [24] noticed that geranyl acetate, geranial, neral and geraniol were major components in Iran.

Some compounds have the same behavior as the major compounds, the highest content of sulcatone compound from the harvest in March (3.15%) followed by in May (1.22%) and least (1.08%) at the harvest in April. However, higher content of linalool compound was obtained from the harvest in May (2.74%), followed by the harvest in March (2.67%) and the lowest percentage (1.93%) from the harvest in April also.

Other important compounds were bicyclogermacrene [(0.30, 1.14, 0.49%); trans-caryophyllene (1.15, 2.82, 1.31%); nerol (1.49, 3.31, 1.88%) and neryl acetate (2.79, 5.39, 4.35%)] in the first, second and third time of harvest, respectively. Thus we found that neryl acetate compound contents was the highest in the volatile oil, followed by nerol, then trans-caryophyllene and finally bicyclogermacrene in all harvest times. These compounds behavior reverse major compounds behavior where the highest percentage of the these compounds were obtained from the harvest in April followed by harvest in May and the lowest percentages of these compounds as a result of the harvest in March, as in Table 2.

Table 2: Principal constituents of *Dracocephalum moldavica* essential oil at three harvest dates.

Compound	Rt	First Harvest 1 st March	Second Harvest 1 st April	Third Harvest 1 st May
β -pinene	5.32	-	-	0.37
α -phyllandrene	6.67	-	-	0.31
β -myrcene	6.71	0.09	-	0.21
limonene	7.42	0.18	0.19	0.29
sabinene	7.66	-	-	0.19
2-hexenal	8.41	0.12	-	-
γ -terpinene	8.68	-	0.18	0.17
β -ocimene	8.97	0.55	-	-
3-octanone	9.29	0.29	0.49	0.44
p-cymene	9.49	-	-	0.21
tri-decane	10.80	-	0.70	-
sulcatone	11.88	3.15	1.08	1.22
1-octen-3-yl-acetate	13.08	0.11	-	0.17
3-octanol	13.53	0.11	0.29	0.27
1-octen-3-ol	15.29	0.44	0.65	0.64
copane	15.83	0.36	0.77	0.26
dihydroedulan II	16.04	-	0.19	-
1-undecyne	16.16	0.19	0.19	-
(-)- β -bourbonene	16.72	0.20	0.55	0.28
2,2-dimethylocta-3,4-dienal	16.99	0.30	0.57	0.32
trans-3-pinanone	17.19	-	-	0.62
isopinocampone	18.10	-	-	0.99
linalool	18.37	2.67	1.93	2.74
trans-caryophyllene	19.09	1.15	2.82	1.31
trans-2-decenal	21.14	0.13	0.26	0.20
α -caryophyllene	21.27	0.10	0.34	-
(+)-nerolidol	21.93	-	0.18	-
citral b (Neral)	22.53	18.36	17.85	17.86
(+)- α -terpineol	22.80	0.15	-	-
bicyclogermacrene	23.15	0.30	1.14	0.49
cis-3-hexenyl acetate	23.43	0.27	0.21	-
neryl acetate	23.60	2.79	5.39	4.35
cadinene	23.81	0.12	-	-
Citral a (geranial)	24.02	20.42	19.37	19.74
Geranyl acetate	24.54	28.81	27.02	27.81
nerol	25.74	1.49	3.31	1.88
anethole	26.48	-	-	0.22
geraniol	27.12	16.68	12.66	16.05
caryophyllene oxide	30.61	-	0.64	0.19
cubenol	33.22	0.16	0.31	0.19
(+) spathulenol	36.30	-	0.22	-
carvacrol	39.07	-	0.21	-
Total Identified compounds		99.69	99.71	99.99
Essential oil content		0.06%	0.11%	0.09%

As can be seen from (Table 1), β -pinene, α -phyllandrene, sabinene, p-cymene, trans-3-pinanone, isopinocampone and anethole compounds are present only at harvest in May, while, tri-decane, dihydroedulan II, (+) nerolidol, (+) spathulenol and carvacrol compounds found only at harvest in the month of April, but there are only β -ocimene, (+) α -Terpineol and cadinene compounds at harvest in March as compounds in minor proportions.

On the other hand, we see that γ -terpinene and caryophyllene oxide compounds only absent in the harvest in March, and β -myrcene and 1-octen-3-yl-acetate absent only at harvest in April. While, 1-undecyne, α -phyllandrene and cis-3-hexenyl acetate compounds absent only at harvest in May. While some compounds, such as limonene, 3-octanol, 1-octen-3-ol, copane, β -bourbonene, 2, 2-dimethylocta-3, 4-dienal, trans-2-decenal and cubenol existed in the three harvest dates in different proportions.

4. Conclusion

Harvest dates of *Dracocephalum moldavica* are essential to obtain higher essential oil content and better quality. It is

affected on the essential oil and its chemical compositions. In addition, for maximum oil production, second harvest at 1 April during flowering plants. Also, harvest date of the *Dracocephalum moldavica* is a major factor in the composition of the essential oil and optimizing the date of harvest is essential for maximizing the production of oil of suitable quality.

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