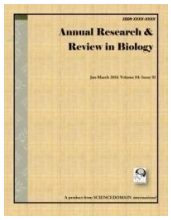

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Chemical Compositions of Essential Oils of Flower, Leaf, Stem and Root of *Phlomis cancellata* Bunge. from Mazandaran, Iran

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: *Phlomis cancellata* is an aromatic and native plant that is a member of Labiatae. Not only a traditional medicine but also the plant is considered in modern medicine and different industries because of its essential oil characteristics. The features of essential oils in aromatic plants are based on the part of plant from which they are extracted. In the present study the chemical composition of the essential oils of different parts of the *Phlomis cancellata* were evaluated.
Place and Duration of Study: Plant was collected from its natural habitat in Mazandaran province, Iran, in July 2012, its parts were separated and then dried in laboratory (Nour Branch, Islamic Azad University, Iran) and essential oils were extracted by hydrodistillation.
Methodology: The essential oil of *P. cancellata* obtained by hydrodistillation and analyzed by GC-FID and GC/MS.
Results: The chemical analysis has resulted in identification of 25, 20, 11 and 2 constituents,

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comprising about 99.8, 97.6, 99.8 and 99.0% of the total constituents in oils of flower, leaf, stem and root, respectively; additionally sesquiterpenes hydrocarbones components (82.3, 82.6 and 89.8%, respectively) were the major components in oils of flower, leaf and stem, whereas nonterpene component (99.0%) was dominant in the oil of the plant root. The major constituents in the oil of flower of *P. cancellata* were tetradecane, β-(E)-caryophyllene, (E)-farnesene, bicyclogermacrene, germacrene D, and germacrene B; while leaf's oil predominantly contained germacrene D, β-(E)-caryophyllene, bicyclogermacrene and 6,10,14-trimethyl-2-Pentadecanone. Also germacrene D, β-Elemene, β-(E)-caryophyllene, bicyclogermacrene, germacrene B and 6,10,14-trimethyl-2-Pentadecanone were identified as the major compounds in stem's oil; while only two nonterpene constituents' were identified in the root oil: linoleic acid and bicyclo [10.1.0] tridec-1-ene.

Conclusion: According to high outputting, presence of valued compounds and sesquiterpenes the most portion extracted essential oil from flowers and leaves have the best quality and are used in different industries.

Keywords: Essential oil; Phlomis cancellata Bunge.; Germacrene D.

1. INTRODUCTION

Phlomis genus belonging to the family Lamiaceae and 70 species of perennial herbaceous aroma that only limited parts of Asia, including Iran, Turkmenistan, Afghanistan and Iraq are distributed [1]. 17 medicinal species, native to Iran [2]. Among the various species of this genus, *Phlomis cancellata* Bunge. with Persian names Gushbarre sefid and Gushbarre irani that have been introduced. It is a perennial and annual plant distribution ranges dramatically in Khorasan, Mazandaran and Golestan. Despite its high medicinal value and antibacterial properties [3,4], less attention has been paid. The literature shows that some researches on the identification of chemical compounds of essential oil of this plant are done. A study was carried out in Rangeland Vaz, Semnan province, Iran, suggests that the 53 combinations exist in this plant's oil including germacrene D and α-pinene [3]. In another study essential oil from aerial parts of *P. cancellata* Bge., from Khorasan, Iran, was analyzed and eighteen compounds were distinguished, germacrene D, ß-caryophyllene, caryophyllene oxide, α-thujene and bicyclogermacrene were the major components [5]. Essential oil of aerial parts of *P. cancellata* from Gaduk area in Mazandaran province, Iran was analyzed 44 components were identified: hexadecanoic acid, germacrene D, eudesmol, octacosane and (E)-caryophyllene were as the major components [6]. Also essential oils obtained from leaves and stems and by diethyl ether extract of aerial parts of *P. cancellata* Bunge. were analyzed and oils obtained from the leaves of *P. cancellata* were rich in especially germacrene D and β-caryophyllene, whereas the stem oil contained mainly β-selinene, germacrone, germacrene B and γ-elemene. The major components of the diethyl ether extract were germacrene D, hexadecanoic acid and 6,10,14-trimethylpentadecan-2-one [7]. But in other studies, essential oils *of* different species of *Phlomis* have been evaluated. Kirimer et al. [8] showed among 18 combinations identified in the oils of *P. nissolii*, germacrene D and bicyclogermacrene were the indicator components. In another study chemical composition of the essential oil of flowering aerial parts of the *P. ferruginea* in northern Italy contained mostly β-(E)-Caryophyllene, hexadecanoic acid, germacrene D and caryophyllene oxide [9]. Khalilzadeh et al. [10] also evaluated other species, bicyclogermacrene and germacrene D were the major constituents in the oil obtained from medicinal plants *P. persica*, *P. oliveri*. Identification of chemical compounds in the essential oils obtained from *P. herba-venti* implies that in the Mazandaran sample, germacrene D and hexadecanoic acid have the highest rates in comparison with other compounds [11]. In another study, germacrene D has been observed as dominant component in oil of *P. chorassanica* [12]. Another study stated that germacrene D and α-pinene were the two major compounds found in the essential oil of *P. bruguieri* [13]. The major components of the essential oil of *P. pungens* were germacrene D and α-pinene [14]. This study aimed to compare the chemical composition of the essential oil obtained from flowers, leaves, stems and roots of medicinal plants *P. cancellata*.

As far as we know, there are few studies on the antioxidant, as well as its antimicrobial activity. A study was carried out in antimicrobial activities of oils and extracts of *P. cancellata* in Mazandaran, Iran. *In vitro* antimicrobial activity of essential oil

of *P. cancellata* against a panel of four strains of bacteria has been investigated. It has shown larger growth inhibition zone diameters against the gram-positive tested bacterial strains compared with gram-negative bacteria. Antimicrobial activities of essential oil was also tested by MIC and MBC method [6]. Also the medicinal properties of genus *Phlomis* investigated by the antimicrobial activity of the methanolic extracts of *P. bruguieri*, *P. herba-venti*, *P. olivieri*. The methanolic extracts of the aerial parts of applied *Phlomis* genus exhibited concentration-dependent antibacterial activity against all tested bacteria. The methanolic extracts were more active against gram-positive microorganisms. In another study total extracts of three species of *Phlomis*, *P. olivieri*, *P. anisodonta* and *P. persica* were tested for their antinociceptive effects using the visceral writhing test model in mice [13]. Antibacterial activities of *P. caucasica* from Iran were evaluated against a range of gram positive and negative bacterial strains [15].

2. MATERIALS AND METHODS

2.1 Plant Material

Phlomis cancellata at the full flowering stage of natural habitats located in the West of Mazandaran province (latitude between +35؛19' and +36؛23' N, longitude between +52؛5' and +52؛10' E), accumulation were collected. Voucher specimens of the plant have been deposited in the herbarium of the Department of Botany, Mazandaran University of Medical Sciences (Herbarium No. 335). Samples of various organs removed and separate from each other, were tested *in vitro*. The dried samples in order to create maximum surface contact with the water in the balloon device by Asian suck and 100 grams of powder from each of the organs studied by adding certain volume of distilled water to steam distillation method and device type Clevenger The Pharmacopoeia of Great Britain up to 5 hours of essential oils and essential oil (volume% dry weight) was calculated based on three times [18]. After decanting, the obtained essential oils were dried over anhydrous sodium sulfate and after filtration, stored in refrigerator at -4°C until tested and analyzed.

2.2 Gas Chromatography

The oils were diluted in pentane and 1 µl was used for analysis. Gas chromatography-mass spectrometry (GC-MS) analyses of the essential oils were analyzed on an Agilent Technologies 7890A GC system coupled to a 5975C VLMSD MS with an injector 7683B series device. An Agilent (9091) 413:325ºC HP-5 column (30m × 320 µm × 0.25 µm) was used with helium as carrier gas at a flow rate of 3.35 ml/min. The GC oven temperature was initially programmed at 50ºC (hold for 1 min) and finally at 300ºC (hold for 5 min) at a rate of 8ºC/min while the trial temperature was 37.25ºC.

The column heater was set at 250ºC in a split less mode while the pressure was 10.2 psi with an average velocity of 66.5 cm/s and a hold-up time of 0.75 min. MS was run in the electron impact mode (EI) at 70 eV. The percentage compositions were obtained from electronic integration measurements using flame ionization detectore (FID), set at 250ºC.

2.3 Gas Chromatography-Mass Spectrometry

The essential oils were analyzed by GC-MS on an Agilent Technologies 7890A GC system coupled to a 5975C VLMSD mass spectrometer with an injector 7683B series device. An Agilent (9091) 413:325 oC HP-5 column (30m × 320 µm × 0.25 µm) was used with helium as carrier gas at a flow rate of 3.35 ml/min. GC oven temperature and conditions were the same as previously described. The injector temperature was at 250ºC. Mass spectra were recorded at 70 eV. Mass range was from 30 to 500 m/z.

2.4 Identification of Components

Identification of components was based on their retention indices determined by reference to a homologous series of n-alkanes, and by comparison of their mass spectral fragmentation patterns with those reported in the literature [17] and stored on the MS library (NIST 08.L database/ chemstation data system) with data previously reported in literature [18,19]. The percentages of each component are reported as raw percentages based on total ion current without standardization. The chemical compositions of essential oils of different parts of *P. cancellata* Bunge. are shown in Table 1.

Table 1. Chemical composition (%[a]) of the essential oils of different parts of *Phlomis cancellata* Bunge identified by KI[b] and GC-MS[c]

No.	Compound	KI	Flower	Leaf	Stem	Root
1	α-Pinene	910	2.0	-	-	-
2	Limonene	1010	2.1	-	-	-
3	α-Terpinolen	1075	0.5	-	-	-
4	1,5,5-Trimethyl-6-methylene-cyclohexene	1338	0.5	-	-	-
5	α-Cubebene	1342	1.2	-	-	-
6	α-Copaene	1373	-	1.8	-	-
7	β-Cubebene	1382	-	1.8	-	-
8	β-Bourbonene	1387	1.5	3.2	2.4	-
9	β-Elemene	1388	2.0	3.0	4.3	-
10	γ-Elemene	1400	2.8	-	-	-
11	β-(E)-Caryophyllene	1419	13.3	7.4	9.6	-
12	(E)-Farnesene	1445	6.7	2.3	3.6	-
13	α-Humulene	1447	3.0	1.2	-	-
14	Germacrene D	1485	35.2	55.0	59.2	-
15	Bicyclogermacrene	1494	6.3	3.0	4.6	-
16	Germacrene A	1502	-	0.8	-	-
17	δ-Cadinene	1513	1.9	2.0	2.0	-
18	β-Ionone	1520	-	1.3	-	-
19	Germacrene B	1567	8.4	-	4.1	-
20	Spathulenol	1579	0.6	-	-	-
21	Caryophyllene oxide	1581	0.8	-	-	-
22	t-Muurolol	1668	1.5	2.3	-	-
23	Heptadecane	1693	1.7	-	-	-
24	Dehydroaromadendrene	1732	-	1.1	-	-
25	2-Pentadecanone, 6,10,14-trimethyl	1799	3.1	4.1	4.7	-
26	Nonadecane	1893	0.5	2.3	2.0	-
27	Isobutyl phthalate	1929	-	0.6	-	-
28	Hexadecanoic acid	1960	2.0	-	-	-
29	Dibutyl phthalate	2035	0.6	2.0	3.3	-
30	Phytol	2054	-	1.4	-	-
31	Linoleic acid	2144	-	-	-	66.0
32	Bicyclo[10.1.0]tridec-1-ene	2384	-	-	-	33.0
33	Pentacosane	2491	1.0	-	-	-
34	Heptacosane	2690	0.6	1.0	-	-
Number of identified compounds			25	19	11	2
Yield of the oil (w/w%)			0.26	0.22	0.18	0.14
Monoterpene Hydrocarbons			5.1	-	-	-
Sesquiterpenes Hydrocarbons			82.3	82.6	89.8	-
Oxygenated sesquiterpenes			2.9	3.7	-	-
Nonterpene compounds			9.5	11.3	10.0	99.0
Total Identified			99.8	97.6	99.8	99.0

[a] %, Peak area of essential oil components;
[b] KI, Kovats indices on HP-5 capillary column in reference to C_9-C_{31} n-alkanes [17];
[c]components were identified on KI and GC-MS (gas chromatograph coupled with mass spectrometry) and listed according to their elution on HP-5 MS capillary column (30m).

3. RESULTS AND DISCUSSION

The results of the study in three parts are comparable. The amount of essential oil samples can be confirmed by values obtained from flowers, leaves, stem and roots, respectively, 0.26, 0.22, 0.18 and 0.14% based on the dry weight of the plant. On the other hand, the comparison of the number of chemical components identified in four studied samples indicated that the number of these components varied from 25 in the oil of the flower to 2 in the root's oil. The number of chemical compounds in the leaves and stems were 19 and 11, respectively. In the qualitative part, four combinations: germacrene D, β-(E)-caryophyllene, bicyclogermacrene and (E)-farnesene as the dominant compounds in aerial parts have been observed in the following proportions: germacrene D (59.2%) in stem, over leaves (55%) and flowers (35.2%), while the rates of three combinations: β-(E)-caryophyllene, bicyclogermacrene and (E)-farnesene were predominant in essential oil the leaf. It is worth noting that the chemical components of essential oil extracted from roots included linoleic acid and bicyclo [10.1.0] tridec-1-ene which are not seen in other samples. Finally, the chemical properties of the compounds have been investigated. Based on this study, the essential oil obtained from the roots of *P. cancellata*, only showed nonterpene compounds but in oils of flowers, leaves and stems, sesquiterpenes were the major ones. In addition the oxygenated sesquiterpenes had a slight share in the essential oil obtained from the leaves and flowers. Also monoterpenes are present in very small quantities and only in the essential oils of flowers.

On the other hand, the analysis of the findings of the present study clearly states that the volatile oil distribution in different organs of the plant affect the quality of these compounds [13], in addition to influencing the quantitative component. If the two chemical compounds germacrene D and α-pinene in *P. cancellata* essential oils are concerned, it is recommended to use the aerial parts instead of flowers, leaves and stems separately, since they have a major contribution in the oils (37.2% to 59.2%). Qualitative study of the essential components of *P. cancellata* roots, showed remarkable differences with other samples from the study. In this treatment, only two compounds, linoleic acid and bicyclo [10.1.0] tridec-1-ene were introduced which were not observed in the air samples, and the first report on the chemical components of essential oils extracted from plants of the essential of *p. cancellata*. Looking at the chemical composition of three treatments, flowers, leaves and stems, which shows β-bourbonene, β-elemene, β-(E)-caryophyllene, (E)-farnesene, germacrene D, bicyclogermacrene , nonadecane, di-butyl phthalate and 6,10,14-trimethyl,2-pentadecanone that each member of the germacrene D, β-(E)-caryophyllene and bicyclogermacrene, we conclude that they had a larger share than others. In all other researches aiming to identify the chemical components of essential oils obtained from different species of the genus *Phlomis*, germacrene D was the basic compound [5-7]. In the other study, germacrene D and bicyclogermacrene identified as the dominant components in oils of *P. nissolii* [8]. On the other hand, some chemicals such as hexadecanoic acid observed in the chemical components in the essential oils extracted from plants such as various species of this genus have *P. ferruginea* [9]. In the present study, the contribution of this compound was only 2%. Finally, the last section analyzes the results to determine the structure and classification of the chemical constituents investigated. Based on this study, in the essential oil obtained from the roots of *P. cancellata*, non-terpene combination was dominant, but in oils of flowers, leaves and stems, sesquiterpenes were dominant. In the other compounds in addition to non-terpenein oil of underground organs of the plant, a small proportion of oxygenated sesquiterpenes were obtained from the oils of leaves and flowers.

Furthermore the amounts of hexadecanoic acid and germacrene D among the other chemical components in this study has harmony with Morteza-Semnani's et al. [3] investigating. They introduced germacrene D among 53 identified components as indicate components. Also, the consideration of chemical components in extracted essential oil of *P. herba-venti* plant from Mazandaran province, Iran, points that germacrene D and hexadecanoic acid has the most amounts in the compare of other components [11]. Also *P. chorassanica* species essential oil is full of germacrene D [12]. In another research germacrene D and bicyclogermacrene has reported as the indicate components in essential oils of *P. oliveri, P. nissolii* and *P. persica* species too [8,10]. Analysis of essential oil of *P. pungens* plant shows that germacrene D and α-pinene are as the major constituents [14]. According to Basta et

al. [20] germacrene D is the main component in obtained essential oil from *P. cretica*'s root and leaf. Also germacrene D and hexadecanoic acid were dominant in essential oil of flowered browses of *P. ferruginea* [9].

4. CONCLUSION

According to high outputting, presence of valued compounds and sesquiterpenes the most portion extracted essential oil from flowers and leaves have the best quality and are used in different industries.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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