

Essential Oil Composition of *Clinopodium vulgare* L. subsp. *arundanum* (Boiss.) Nyman from Bingöl (Turkey)

Ömer KILIÇ^{*1}, Mehmet Ali KUTLU², Fethi Ahmet ÖZDEMİR³

¹Bingol University, Technical Science Vocational College, Department of Park and Garden Plant, Bingol-Turkey

²Beekeeping, Research, Development, Applications Centre Offices, Bingol University, Bingol-Turkey

³Bingol University, Faculty of Science and Art, Department of Molecular Biology and Genetics, Bingol-Turkey

Received: 29 April 2017 - Revised: 07 June 2017 - Accepted: 23 June 2017

Abstract: Many of the medicinal and aromatic plants from Lamiaceae taxa are uses presumed to be connected to the terpenic constituents of the essential oils. In this study aerial parts essential oil of *Clinopodium vulgare* L. subsp. *arundanum* was analyzed by HS-SPME. As a result thirty seven components were identified. β -caryophyllene (16.1%), γ -terpinene (15.4%), germacrene D (10.3%), *p*-cymene (8.6%) and thymol (6.4%) were detected the major constituents. With this study, chemotypes of studied sample were detected β -caryophyllene, γ -terpinene, germacrene D, *p*-cymene and thymol. In addition studied plant sample was found to be rich in respect to essential oils and the results discussed natural product, renewable resources and chemotaxonomy

Keywords: *Clinopodium*, essential oil, HS-SPME, Apiaceae

1. INTRODUCTION

Clinopodium vulgare L. is one of the two *Clinopodium* spp. (Lamiaceae) growing wild in Anatolia. In Flora of Turkey, two subspecies are defined: *vulgare* and *arundanum*. The latter is widespread in Anatolia [1]. Until recently, essential oils have been studied most from the point of view their flavour and fragrance chemistry for flavouring foods, drinks and other goods. Actually, however, essential oils and their components are gaining increasing interest because of their relatively safe status, wide acceptance by consumers, and exploitation for potential multi-purpose functional uses [2]. Many authors, in fact, have reported antimicrobial, antifungal, antioxidant and radical-scavenging properties by spices and essential oils and, in some cases, a direct food-related application has been tested [3]. *Clinopodium vulgare* is one of the curative plants used in Turkish folk medicine, mainly during wars for the purposes of healing wounds; this plant also showed a very strong action on bacteria [4]. Aqueous extract of *C. vulgare* showed strong antitumor activity [5]. A literature survey has shown that there is one report on the volatile constituents of *C. vulgare* subsp. *arundanum* Boiss. growing in Turkey [6].

*Corresponding Author E-mail: omerkilic77@gmail.com

In our study, essential oil composition of *C. vulgare* subsp. *arundanum* which was collected from Bingöl (Turkey) was undertaken for the first time.

2. MATERIAL and METHODS

2.1. Plant Material

C. vulgare subsp. *arundanum* was collected from Bingöl-Solhan, vicinity of Hazarşah village, dry slopes, 1700-1750 m, July 2015, O.Kilic. The taxonomic description of the plant sample was made according to volume 4 of Flora of Turkey [1]. Voucher specimen was deposited in the Bingöl University, Department of Park and Garden Plants.

2.2. HS-SPME Procedure

Dried aerial part powder of five grams plant samples were carried out by a head space solid phase microextraction method using a divinyl benzene/carboxen/polydimethylsiloxane fiber, with 50/30 μm film thickness; before the analysis the fiber was conditioned in the injection port of the gas chromatography (GC) as indicated by the manufacturer. For each sample, 5 g of plant samples, previously homogenized, were weighed into a 40 ml vial; the vial was equipped with a ‘‘mininert’’ valve. The vial was kept at 35°C with continuous internal stirring and the sample was left to equilibrate for 30 min; then, the SPME fiber was exposed for 40 min to the headspace while maintaining the sample at 35°C. After sampling, the SPME fiber was introduced into the GC injector, and was left for 3 min to allow the analytes thermal desorption. In order to optimize the technique, the effects of various parameters, such as sample volume, sample headspace volume, sample heating temperature and extraction time were studied on the extraction efficiency as previously reported by Verzera et al., [7].

2.3. GC-MS Analysis

A Varian 3800 gas chromatograph directly interfaced with a Varian 2000 ion trap mass spectrometer was used with injector temperature, 260°C; injection mode, splitless; column, 60 m, CP-Wax 52 CB 0.25 mm i.d., 0.25 mm film thickness. The oven temperature was programmed as follows: 45°C held for 5 min, then increased to 80°C at a rate of 10°C/min, and to 240°C. at 2°C/min. The carrier gas was helium, used at a constant pressure of 10 psi; the transfer line temperature, 250°C; the ionisation mode, electron impact (EI); acquisition range, 40 to 200 m/z; scan rate, 1 us^{-1} . The compounds were identified using the NIST library, mass spectral library and verified by the retention indices which were calculated as described by Van den Dool and Kratz [8]. The relative amounts were calculated on the basis of peak-area ratios. The identified constituents of *C. vulgare* subsp. *arundanum* is listed in Table 1.

Table 1. Essential oil composition of *Clinopodium vulgare* subsp. *arundanum* (%)

Compounds	RRI*	%
α -thujene	1016	1.5
α -pinene	1022	0.8
Camphene	1034	0.4
Sabinene	1052	0.1
β -pinene	1056	2.1
β -mrycene	1064	2.5
α -terpinene	1085	1.8

α -phelladrene	1090	2.2
Limonene	1096	0.1
<i>p</i>-cymene	1098	8.6
β -ocimene	1100	0.2
1,3,6- octatriene	1108	0.1
γ-terpinene	1117	15.4
Trans-sabinene hydrate	1126	0.1
Linalool	1148	0.2
Terpineol-4-ol	1166	0.4
Camphor	1182	0.1
Borneol	1200	1.3
Pulegone	1205	0.1
α -terpinolene	1220	2.5
Thymol	1295	6.4
Carvacrol	1300	3.3
α -copaene	1350	0.2
β -Caryophyllene	1382	16.1
β -cubebene	1400	0.3
Aromadendrene	1406	0.1
α -humulene	1418	3.4
Germacrene D	1435	10.3
Piperitenone	1441	1.2
β -bisabolene	1450	0.6
Bicyclogermacrene	1455	0.1
δ -cadinene	1462	0.1
Spathulenol	1495	0.2
Caryophyllene oxide	1497	4.1
α -muurolene	1520	1.1
Spathulenol	1620	0.1
Hexadecanoic acide	1702	0.4
RRI*: Relative Retention Index	Total	88.5

3. RESULTS and DISCUSSIONS

In this study, β -caryophyllene (16.1%), γ -terpinene (15.4%), germacrene D (10.3%), p-cymene (8.6%) and thymol (6.4%) were detected the major constituents of the essential oil plant. In a study was designed to examine the chemical composition essential oil of *Clinopodium vulgare* by GC-MS analysis of the oil resulted in the identification of 40 compounds, representing 99.4% of the oil; thymol (38.9%), γ -terpinene (29.6%) and p-cymene (9.1%) were the main components [9]; similarly in our study γ -terpinene (15.4%), thymol (16.4%), p-cymene (8.6%) and thymol (6.4%) were detected high percentages (Table 1).

In another research, a total of 34 components of the essential oil of *Clinopodium gracile* were identified and principal compounds of the essential oil were germacrene D (20.59%), nootkatone (8.22%), morillo (7.74%), β -elemene (7.38%), α -bergamotene (6.08%), cis- β -farnesene (5.47%) and caryophyllene (5.17%) [10]. It is noteworthy that, nootkatone and morillo were not detected in the essential oil of *C. vulgare* subsp. *arundanum* (Table 1). In another investigation, *C. vulgare* contains piperitone oxide (11.4%) [11]; this compound was not found in the oil of *C. vulgare* subsp. *arundanum* as main component (Table 1).

The differences in chemical composition of oil of present study and previous research may be because of the collection time, chemotypes, drying conditions, mode of distillation, geographic and climatic factors. The essential oil composition of *C. vulgare* subsp. *arundanum* collected from two different localities in Turkey, was analysed by means of GC and GC-MS; thirtyseven compounds were identified, representing 89.6-90.5% of the samples; and the main constituents of the oils were germacrene-D, β -caryophyllene and β -caryophyllene oxide [6]. Germacrene-D and β -caryophyllene also were found to be the main constituents of studied sample (Table 1).

4. CONCLUSION

In conclusion, the present work is the first report on the composition of essential oils obtained from the aerial parts of *C. vulgare* subsp. *arundanum* from Bingöl (Turkey). Sample extracts from *C. vulgare* subsp. *arundanum* was rich in essential oil compounds. The biologic activity of *Clinopodium* taxa may be related to its richness in secondary metabolites, especially essential oils. β -caryophyllene, γ -terpinene, germacrene D, p-cymene and thymol were found to be the reason of chemotypes of *C. vulgare* subsp. *arundanum*.

Acknowledgement

The authors acknowledge the Scientific and Research Council of Bingol University (BAP - TBMYO.2016.00.001) for support this study.

Conflict of Interests

Authors declare that there is no conflict of interests.

5. REFERENCES

- [1]. Davis, P.H. (1982). Flora of Turkey and East Aegean Islands. *Edinburgh, University Press*, 7.
- [2]. Ormancey, X., Sisalli, S., & Coutiere, P. (2001). Formulation of essential oils in functional perfumery. *Parfums Cosmetiques Actualites*, 157, 30–40
- [3]. Madsen, H.L., & Bertelsen, G. (1995). Spices as antioxidants. *Food Science and Technology*, 6, 271-277.
- [4]. Opalchenova, G., & Obreshkova, D. (1999). Antibacterial Action of Extracts of *Clinopodium vulgare* L. Curative Plant. *Drug Dev Indian Pharmacy*, 25, 323-328.

- [5]. Dzhambazov, B., Daskalova, S., Monteveva, A., & Popov, N. (2002). In vitro Screening for Antitumour Activity of *Clinopodium vulgare* L. (Lamiaceae). *Extracts Biological Pharmacy Bulletin*, 25, 499–504.
- [6]. Kökdil, G. (1998). Composition of the Essential Oil of *Clinopodium vulgare* L. spp. *arundanum* (Boiss.) Nyman Collected From Two Different Localities in Turkey. *Flavour and Fragrance Journal*, 13, 170–172.
- [7]. Verzera, A., Zino, M., Condurso, C., Romeo, V., & Zappala, M. (2004). Solid-phase microextraction and gas chromatography/mass spectrometry for the rapid characterisation of semi-hard cheeses. *Analytical Bioanalytical Chemistry*, 380, 930-936.
- [8]. Van Den Dool, H., & Kratz, P.D. (1963). A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. *Journal Chromatography*, 11, 463-471.
- [9]. Tepe, B., Sihoglu-Tepe, A., Daferer, D., Moschos, P., & Sokmen A. (2007). Chemical composition and antioxidant activity of the essential oil of *Clinopodium vulgare* L. *Food Chemistry*, 103(3), 766-770.
- [10]. Chen, X.B., Liu, X.C., Zhou, L., & Liu, Z.L. (2013). Essential Oil Composition and Larvicidal Activity of *Clinopodium gracile* (Benth) Matsum Aerial Parts against the *Aedes albopictus* Mosquito. *Tropical Journal of Pharmaceutical Research*, 12 (5), 799-804.
- [11]. De Pooter, H.L., & Schamp, N.M. 1986. *Progress in Essential Oil Research*, ed. E. J. Brunke, p. 139, Walter de Gruyter, New York.