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**RESEARCH ARTICLE**

# Volatile Constituents and Antimicrobial Activity of *Hirtellina lobelii* (DC.) Dittrich

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**Abstract**

The volatiles obtained by hydrodistillation of *Hirtellina lobelii* (DC.) Dittrich (Asteraceae) collected from Turkey was analyzed by gas chromatography and gas chromatography/mass spectrometry (GC/MS), simultaneously. Main constituents were identified as fokienol (% 7.4), cadinol (% 6.8), and β-caryophyllene (% 6.7), respectively. The *H. lobelii* volatiles were screened for antimicrobial properties against various pathogenic bacteria. The volatile showed a relatively good inhibitory concentration with 0.06 mg/mL against *Staphylococcus aureus*.

**Keywords**: *Hirtellina lobelii, Antimicrobial, Volatile*

## Introduction

Many Asteraceae species, widespread in the world and Anatolia exert biological and pharmacological activity. The phytochemistry of the family mainly consists of diterpenes, flavonoids as well as sesquiterpene lactone type secondary metabolites which show biological activities such as antibacterial, antifungal, anthelminthic, anti-inflammatory, insecticide, antitumor among many others (Shing et al, 2002).

*Hirtellina* (Cass.) Cass. (Asteraceae), is represented by 4 species in the world (Davis, 1975). *Hirtellina lobelii* (DC.) Dittrich is the only species found in Turkey. This species is recorded within the genus *Staehelina* L. in Flora of Turkey. Later, its status was changed to *Hirtellina* (Dittrich, 1996).

There is only one very recent report on the phytochemical composition and bioactivity on this species (Khoury et al., 2019). To the best of our knowledge, this is the first report on the chemistry of the volatiles and its antibacterial evaluation.

## Materials and Methods

### Plant material and isolation of essential oil

The plant sample was collected from Antalya, İbradi, Altınbeşik cave in May, 2018 in Turkey. Voucher specimen is kept in the Faculty of Pharmacy Herbarium, Anadolu University, Eskişehir, Turkey.

The air-dried aerial parts were hydrodistilled for 3 h using a Clevenger-type apparatus to produce a small amount of essential oil, which was trapped in *n*-hexane. It wasthen analysed by GC-FID and GC/MS.

### GC/MS analysis

The analysis was carried out with an Agilent 5975 GC-MSD system. Innowax FSC column (60 m x 0.25 mm, 0.25 µm film thickness) was used with helium as carrier gas (0.8 mL/min). GC oven temperature was kept at

60 °C for 10 min and programmed to 220 °C at a rate of 4 °C/min, and kept constant at 220 °C for 10 min and then programmed to 240°C at a rate of 1°C/min. Split ratio was adjusted at 40:1. The injector temperature was set at 250 °C. Mass spectra were recorded at 70 eV. Mass range was from *m/z* 35 to 450.

### GC analysis

The GC analysis was carried out using an Agilent 6890N GC system. FID detector temperature was 300 °C. To obtain the same elution order with GC/MS, simultaneous auto-injection was done on a duplicate of the same column applying the same operational conditions. Relative percentage amounts (%) of the separated compounds were calculated from FID chromatograms. The analysis results are given in Table 1.

### Identification of the components

Identification of the essential oil components were carried out by comparison of their relative retention times with those of authentic samples or by comparison of their relative retention index (RRI) to series of *n*-alkanes. Computer matching against commercial (Wiley GC/MS Library, MassFinder Software 4.0) (1,2) and *in-house* "Başer Library of Essential Oil Constituents" built up by genuine compounds and components of known oils were conducted.

### Antibacterial activity

Antibacterial effect of the volatiles of *H. lobelii* was screened by using partly modified CLSI (formerly NCCLS) microdilution broth methods M7-A7 standard protocol (Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically) (CLSI (NCCLS) M7-A7, 2006). Standard strains such as *Escherichia coli* NRRL B-3008, *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhimurium* ATCC 13311, *Serratia marcescens* NRRL B-2544 and *Klebsiella pneumoniae* NCTC 9633 were used as test bacteria. Ampicillin and chloramphenicol were used as standard antibacterial agents. *n*-Hexane and water were used also as controls. The experiments were repeated in dublicates, and MIC values were reported as mean.

## Results and Discussion

### Volatile Composition

48 compounds representing 89.5% of the essential oil trapped in hexane were characterized. Fokienol (% 7.4), cadinol (% 6.8), and β-caryophyllene (% 6.7) were found as main components. α-Bisabolol (34.5%), fokienol (12%) and T-muurolol (6.8%) were found as main constituents in the only previously published report (Khoury et al., 2019). Fokienol was a major constituent in both oils.

Table 1. The Volatile Composition of *H. lobelii*

| RRI | Compound | % |
|---|---|---|
| 1225 | (*Z*)-3-Hexenal | 5.9 |
| 1400 | Nonanal | 0.5 |
| 1466 | α-Cubebene | 0.8 |
| 1495 | Bicycloelemene | 0.2 |
| 1497 | α-Copaene | 1.0 |
| 1535 | β-Bourbonene | 0.8 |
| 1553 | Linalool | 1.8 |

| | | |
|---|---|---|
| 1589 | β-Ylangene | 0.3 |
| 1600 | β-Elemene | 0.3 |
| 1597 | β-Copaene | 0.4 |
| 1612 | β-Caryophyllene | 6.7 |
| 1638 | β-Cyclocitral | 0.5 |
| 1639 | Cadina-3,5-diene | 0.6 |
| 1677 | *epi*-Zonarene | 0.6 |
| 1681 | (*Z*)-3-Hexenyl tiglate | 5.5 |
| 1687 | α-Humulene | 1.1 |
| 1704 | γ-Muurolene | 1.1 |
| 1706 | α-Terpineol | 1.0 |
| 1722 | Bicyclosesquiphellandrene | 0.8 |
| 1726 | Germacrene D | 1.8 |
| 1740 | α-Muurolene | 2.0 |
| 1742 | β-Selinene | 2.8 |
| 1755 | Bicyclogermacrene | 0.6 |
| 1773 | δ -Cadinene | 5.9 |
| 1776 | γ -Cadinene | 4.4 |
| 1798 | Methyl salicylate | 0.4 |
| 1799 | Cadina-1,4-diene (=*Cubenene*) | 0.5 |
| 1807 | α-Cadinene | 0.8 |
| 1849 | Calamenene | 1.0 |
| 1900 | *epi*-Cubebol | 1.0 |
| 1941 | α-Calacorene | 1.0 |
| 1957 | Cubebol | 1.0 |
| 1958 | (*E*)-β-Ionone | 1.4 |
| 1984 | γ-Calacorene | 0.5 |
| 2008 | Caryophyllene oxide | 1.0 |
| 2050 | (*E*)-Nerolidol | 0.8 |
| 2057 | Ledol | 0.8 |
| 2080 | Cubenol | 1.6 |
| 2104 | Viridiflorol | 0.9 |
| 2148 | (*Z*)-3-Hexen-1-yl benzoate | 1.4 |
| 2161 | Muurola-4,10(14)-dien-1-ol | 0.9 |
| 2174 | Fokienol | 7.4 |
| 2187 | T-Cadinol | 6.1 |
| 2209 | T-Muurolol | 3.4 |
| 2219 | δ-Cadinol (=alpha-muurolol) | 1.9 |
| 2255 | α-Cadinol | 6.8 |
| 2300 | Tricosane | 1.0 |
| 2568 | 14-Hydroxy-α-muurolene | 0.5 |
| | **Total** | 89.5 |

RRI: Relative retention indices calculated against *n*-alkanes. % calculated from FID data.

Table 2. Antibacterial effects of *H. lobelii* essential oil (MIC, mg/mL)

| ***Bacteria*** | **Source** | ***H. lobelii*** | **Ampicillin** | **Chloramphenicol** |
|---|---|---|---|---|
| *Escherichia coli* | NRRL B-3008 | 0.5 | 0.002 | 0.001 |
| *Staphylococcus aureus* | ATCC 6538 | 0.06 | 0,001 | 0,008 |
| *Pseudomonas aeruginosa* | ATCC 27853 | >1 | 0.128 | 0.064 |
| *Salmonella typhimurium* | ATCC 13311 | 0.25 | 0.001 | 0.001 |
| *Serratia marcescens* | NRRL B-2544 | 0.12 | 0.016 | 0.004 |
| *Klebsiella pneumoniae* | NCTC 9633 | 0.25 | 0.001 | 0.004 |

MIC: Minimum inhibitoy concentration

The essential oil showed weak to moderate inhibitory effects against the tested pathogens between the concentration of 0.5 to 0.06 mg/mL (MIC). The oil was rather inactive towards the growth of *P. aeruginosa* at the maximum test concentration of 1.0 mg/mL. *S. aureus* was susceptible with a MIC value of 60 µg/mL as shown in Table 2. Supporting our study, Khuory et al. (2019) showed the antibacterial effects of the α-bisabolol-rich *H. lobelia* essential oil, where *S. aureus* was inhibited at the concentration of 32 µg/mL (MBC; 64 µg/mL), and the *E. coli* was inhibited at > 500 µg/mL, in agreement with our findings.

The initial findings on the chemistry and biological activity of *H. lobelia* suggest further evaluations and experimentations.

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