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# Chemical compositions and antimicrobial activity of *Prunella vulgaris* L.

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**Abstract**

The popular medicinal plant *Prunella vulgaris* L., (Lamiaceae) is a perennial and an edible herbaceous plant which is widely distributed in the temperate zone and tropical mountains of Europe and Asia. Due to its medicinal and industrial importance, the demand for *P. vulgaris* has increased steadily in recent years. In the present study, the volatile compounds of the *P. vulgaris* were accumulated by Headspace-Solid Phase Microextraction (HS-SPME) technique, and, analysed by gas chromatography/mass spectrometry (GC/MS). The chemical composition of the methanolic extract (ME) and infusion (INF) of *P. vulgaris* were determined. Aerial parts of *P. vulgaris* INF of the major compounds were found hexanal (23.1%), ionol (10.7%), (Z)-3-hexenal (3.2%) and 3,5-octadien-2-one. The ME of *P. vulgaris* were characterized with α-fenchone (11.1%), hexanal (8.2%), 3,5-octadien-2-one (4.7%), methyl benzoate (4.5%) and selina-4,11-diene (3.1%). It was evaluated the antimicrobial activity of *P. vulgaris* extracts (ME, INF) in *in vitro* conditions against different kinds of microorganisms. The INF showed weak antimicrobial activity Minimum Inhibitor Concentration (MIC) against all tested microorganisms whereas ME showed weak antimicrobial effects *E. coli, S. aureus and Pseudomonas aeruginosa*; *S. pyogenes* (20 mg / mL) and *C. albicans* (15 mg / mL).

**Keywords:** *Prunella vulgaris*, HS-SPME, volatile compound, antimicrobial activity

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## Introduction

*Prunella* is a genus of perennial herbaceous plants in the Lamiaceae family. There are approximately 15 species worldwide, distributed widely in the temperate regions and tropical mountains of Europe and Asia (Bai et al., 2016). In the genus *Prunella*, *P. vulgaris* L. also known as "self-heal;" contains several active components, including oleanolic acid, betulinic acid, ursolic acid, flavonoids and rosmarinic acid (Lamaison et al., 1991). Some pharmacological activities such as the immunmodulatory effect (Han et al., 2009), anti-viral activity against HSV-1, HSV-2 (Zhang et al., 2007), HIV (Yao et al., 1992), antioxidant activity (Psotova et al., 2003; Osakabe et al., 2002) and anti hyperglycemic action (Zhang et al., 2007) were confirmed. It has been used as a traditional medicine in the clinical treatment of herpetic keratitis and for its antioxidative and antimicrobial activities. In spite of its traditional uses as an antiseptic agent for treatment of wounds and sore throat, there are a few literatures on its antimicrobial activity.

Various bioactive constituents, such as terpenoids (Qi et al., 2009), polyphenols (Feng et al., 2010), flavonoids (Lee et al., 2008), and polysaccharides (Chiu et al., 2004) have been identified in the extracts of *P. vulgaris*. Various methods, such as steam distillation, Soxhlet extraction, and solvent extraction (Guan et al., 2007; Wang et al., 2008) can be used for the determination of volatile components. With the advantages of better selectivity and higher efficiency, headspace solid-phase microextraction (HS-SPME) has been introduced as a modern alternative to the traditional sample preparation technology (Adam et al., 2005).

The aim of this research was to determined volatile composition of the methanolic extract (ME) and infusion (INF) of *P. vulgaris* by headspace solid-phase microextraction (HS-SPME) combined with gas chromatography-mass spectrometry (GC-MS) and examined antimicrobial activitis.

## Materials and Methods

The aerial parts of *P. vulgaris* was collected in July 2017 from Beşikderesi, Eskişehir (Turkey). The ME and 5% INF of *P. vulgaris* were prepared. Their volatile compounds were trapped with Headspace Solid Phase Micro Extraction (HS-SPME) and analyzed by Gas Chromatography-Mass Spectrometry (GC/MS). ME and INF were examined for antimicrobial activity by the microdilution broth susceptibility assay against *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* NRRL B-3008, *Streptococcus pyogenes* ATCC 13615 and *Candida albicans* ATCC 90028.

### Headspace-SPME

The manual SPME device (Supelco, Bellafonte, PA, USA) with a fiber-precoated 65 µm thick layer of polydimethylsiloxane/divinylbenzene (PDMS/DVB-blue) was used for extraction of the methanolic extract and

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infusion of *P. vulgaris* volatiles. The vial containing the plant extract was sealed with parafilm. The fiber was pushed through the film layer for exposure to the headspace of the extract for 15 min at 40 °C. The fiber was then inserted immediately into the injection port of the GC-MS for desorption of the adsorbed volatile compounds for analysis.

**Analysis of volatile compounds**

The ME and INF volatiles were analyzed by GC/MS using an Agilent 5975 GC-MSD system. Innowax FSC column (60 m x 0.25 mm, 0.25 m 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. The injector temperature was set to 250 C. Mass spectra were recorded at 70 eV. Mass range was *m/z* 35 to 450.

**Identification of Components**

Identification of the volatile 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) (McLafferty and Stauffer,1989; Hochmuth, 2008) and in-house "Başer Library of Essential Oil Constituents" built up by genuine compounds and components of known oils. Relative percentage amounts of the separated compounds were calculated from TIC chromatograms. The volatile compounds identified are listed in Table 1.

**Antimicrobial Activity**

ME and INF were examined for antimicrobial activity by the microdilution broth susceptibility assay against *Staphyloccoccus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* NRRL B-3008, *Streptococcus pyogenes* ATCC 13615 and *Candida albicans* ATCC 90028.

## Results and Discussion

The current study aimed the determination of the HS-SPME volatile profile of the methanolic extract (ME) and infusion (INF) of *P. vulgaris*. In addition, antimicrobial activities were also examined**.** HS-SPME was used to collect the volatile components of *P. vulgaris*. Aerial parts of *P. vulgaris* INF of the major compounds were found hexanal (23.1%), ionol (10.7%), (Z)-3-hexenal (3.2%) and 3,5-octadien-2-one (3.1%). The ME of *P. vulgaris* were characterized with α-fenchone (11.1%), hexanal (8.2%), 3,5-octadien-2-one (4.7%), methyl benzoate (4.5%) and selina-4,11-diene (3.1%) (Table 1). It was evaluated the antimicrobial activity of *P. vulgaris* extracts (ME, INF) in *in vitro* conditions against different kinds of microorganisms. The INF showed weak antimicrobial activity Minimum Inhibitor Concentration (MIC) against all tested microorganisms whereas ME showed weak antimicrobial effects *E. coli, S. aureus* and *Pseudomonas aeruginosa*; *S. pyogenes* (20 mg / mL) and *C. albicans* (15 mg / mL).

Phytochemical studies indicate that *P. vulgaris* contains oleanolic, betulinic, ursolic, 2α,3α- dihydroxyurs-12-en-28-oic and 2α,3α-ursolic acids, triterpenoids, flavonoids, tannins and anionic polysaccharide prunelline (Ruy et al., 2000). More recently, the organic fraction of *P. vulgaris* was found to exhibit antioxidative and antimicrobial activities (Psotova et al., 2003). *P. vulgaris* is rich in phenolic acids and its main component is rosmarinic acid (Lamaison et al., 1991; Psotova et al., 1998; Han et al., 2009).

Rosmarinic acid or α-O-caffeoyl-3-4-dihydroxyphenyllactic acid is the mulifunctional caffeic acid ester with antimicrobial activity against *Bacillus cereus*, *B. subtilis* and *B. polymyxa*. Gram-negative bacteria were previously reported to be highly susceptible to rosmarinic acid (Askun et al., 2009). The volatile compounds of *P. vulgaris* from different parts of the herb (cultivated in Jiangsu) and from different geographical regions were comparatively analyzed by HS-SPME combined with GC-MS. And the following 12 were found in all origins: 1-nonanol, dodecane, tridecane, *a*-bourbonene, tetradecane, geranyl acetone, pentadecane, caryophyllene oxide, hexadecane, tetradecanal, isobutyl phthalate, and n-butyl hexadecanoate (Yang et al., 2013).

In another study performed by Golembiovska et al. in 2014 (16) on *P. vulgaris* from 104 components were identified in flowers, leaves, stems and roots and the main constituents were observed squalene, myristic acid, spathulenol, viridiflorol, germacrone. In recent years, studies have found that *P. vulgaris* also exhibits certain anti-pathogen effects on plant pathogens. Antibacterial activity of the methanolic extract of *P. vulgaris* extracts was reported against *E. coli*, *S.aureus*, *S. typhimurium* and *K. pneumonae* (Rasool et al., 2010). Studies by Yoon et al. found that the methanol extract of *P. vulgaris* had a strong anti-fungal and antioomycete activity on *Phytophthora infestans*, rice blast fungus, red pepper anthracnose and wheat leaf rust fungus (Yoon et al., 2010). An Iranian study by Mahboubi et al. (2015) showed that the methanol extract of *P. vulgaris* exhibited the best activity against *St. mutans* (MIC 3.2 mg.7ml), *S. aureus*, *S. epidermidis*, *S. sobrinus*, S. *sanguis, S. salivarius, S. dysenteriae, S. flexeneri, P. aeruginosa* (MIC 3.2, 6.4 mg/ml). *S. saprophyticus, S. pneumoniae, S. pyogenes, E. faecalis, E. faecium, S. agalactiae, K. pneumoniae, E. aerogenes, A. flavus, A. niger* and *S. marcescenes* with MIC and MLC 6.4 and 12.8 mg/g had lower sensitivity to methanol extract. Methanol extract had cidal activity against *E. coli*, *B. subtilis*, *B. cereus*, *C. albicans*, and *C. glabrata*.

Several researchers have evaluated the composition of the essential oil of *P. vulgaris* growing in different geographic areas. These studies revealed some chemical differences in the oil compositions, probably related to the different subspecies and/or the geographical origin of the plants. In conclusion, using HP-SPME-GC-MS, it was possible to quantify different volatile compounds like as hexanal, α-phenchone in *P. vulgaris* which belong to different chemical classes. Results shows that the extracts of *P. vulgaris* tested exhibited significant antimicrobial activity. Our results indicated that the methanol extracts of *P. vulgaris* showed higher antimicrobial activity than INF of *P. vulgaris* against tested microorganisms. In addition, the extract of *P. vulgaris* were observed more efficiency against skin patogenes.

## Conclusion

By using headspace technology coupled with GC/MS, volatile profile of the methanolic extract (ME) and infusion (INF) of *P. vulgaris* were determined. The GC/MS analysis results of the samples led to identification of 74 compounds. Our results showed that the number of components were different in the other studies.

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The observed differences and variability of the essential oil of *P. vulgaris* of are likely due to different environmental and genetic factors. In addition, it was evaluated the antimicrobial activity of *P. vulgaris* extracts (ME, INF) in in vitro conditions against different kinds of microorganisms. Among the tested microorganisims, *S. pyogenes* and *C. albicans* were found to be more sensitive to the ME.

**Table 1.** The Volatile Composition of the methanolic extract (ME) and infusion (INF) of *P. vulgaris*

| RRI | Compound | ME % | INF % | IM |
|---|---|---|---|---|
| 1093 | Hexanal | **8.2** | **23.1** | RRI, MS |
| 1194 | Heptanal | - | 2.5 | RRI, MS |
| 1225 | *(Z)*-3-Hexenal | - | **3.2** | MS |
| 1260 | 1-Pentanol | - | 1.0 | MS |
| 1197 | Methyl hexanoate | 1.5 | - | RRI, MS |
| 1213 | 1,8-Cineole | 2.9 | - | RRI, MS |
| 1244 | 2-Pentyl furan | 0.9 | - | MS |
| 1296 | Octanal | - | 0.8 | RRI, MS |
| 1348 | 6-Methyl-5-hepten-2-one | 1.7 | 0.9 | MS |
| 1360 | 1-Hexanol | 0.5 | - | MS |
| 1391 | *(Z)*-3-Hexenol | - | 0.3 | MS |
| 1398 | 2-Nonanone | 0.4 | - | MS |
| 1400 | Nonanal | 2.4 | 2.3 | MS |
| 1406 | *α*-Fenchone | **11.1** | - | RRI, MS |
| 1416 | 3-Octen-2-one | 1.1 | - | MS |
| 1441 | *(E)*-2-Octenal | - | 1.5 | MS |
| 1452 | 1-Octen-3-ol | 1.1 | - | MS |
| 1463 | 1-Heptanol | - | 0.5 | MS |
| 1479 | *(E,Z)*-2,4-Heptadienal | - | 2.3 | MS |
| 1496 | 2-Ethyl hexanol | 2.1 | 2.2 | MS |
| 1497 | *α*-Copaene | 0.4 | - | MS |
| 1500 | Methyl nonanoate | 0.3 | - | RRI, MS |
| 1500 | Pentadecane | 0.6 | - | RRI, MS |
| 1506 | Decanal | 0.6 | - | MS |
| 1507 | *(E,E)*-2,4-Heptadienal | 0.6 | 2.5 | MS |
| 1520 | 3,5-Octadien-2-one | **4.7** | **3.1** | MS |
| 1532 | Camphor | 1.2 | 0.7 | RRI, MS |
| 1535 | *β*-Bourbonone | 1.8 | - | MS |
| 1541 | Benzaldehyde | 0.7 | 1.1 | RRI, MS |
| 1548 | *(E)*-2-Nonenal | - | 0.8 | MS |
| 1553 | Linalool | 1.4 | 1.4 | RRI, MS |
| 1562 | Octanol | 0.4 | 0.9 | RRI, MS |
| 1573 | *(E,E)*-3,5-Octadien-2-one | 1.8 | 1.1 | MS |
| 1582 | *cis*-Chrysanthenyl acetate | 0.5 | - | MS |
| 1597 | *β*-Copaene | 0.3 | - | MS |
| 1600 | Hexadecane | 1.0 | - | RRI, MS |
| 1604 | 2-Undecanone | 0.6 | - | MS |
| 1611 | Terpinen-4-ol | 2.6 | 1.2 | RRI, MS |
| 1638 | *β*-Cyclocitral | 0.7 | 0.9 | MS |
| 1641 | Methyl benzoate | **4.5** | - | RRI, MS |
| 1655 | *(E)*-2-Decenal | - | 0.6 | MS |
| 1664 | 1-Nonanol | - | 0.6 | MS |
| 1704 | *γ*-Muurolene | 2.3 | - | MS |
| 1706 | *α*-Terpineol | 0.5 | 0.4 | RRI, MS |
| 1715 | *(E,E)*-2,4-Nonadienal | - | 0.3 | MS |
| 1719 | Borneol | 0.3 | - | RRI, MS |
| 1740 | *α*-Muurolene | 0.8 | - | MS |
| 1744 | Selina-4,11-diene | **3.1** | - | MS |
| 1747 | 3,4-Dimethyl-2,5-furandione | 0.3 | - | MS |
| 1751 | Carvone | 0.8 | - | RRI, MS |
| 1773 | *δ*-Cadinene | 1.7 | - | MS |
| 1776 | *γ*- Cadinene | 1.1 | - | MS |
| 1798 | Methyl salicylate | 0.2 | - | RRI, MS |
| 1802 | Cumin aldehyde | - | 0.6 | RRI, MS |

| | | | | |
|---|---|---|---|---|
| 1849 | Calamenene | 1.3 | - | MS |
| 1868 | *(E)*-Geranyl acetone | 0.6 | 0.5 | MS |
| 1870 | Hexanoic acid | 1.3 | - | RRI, MS |
| 1878 | 2,5-Dimethoxy-*p*-cymene | 0.3 | 0.1 | MS |
| 1896 | Benzyl alcohol | 0.5 | - | RRI, MS |
| 1935 | Phenyl ethyl alcohol | 0.4 | - | RRI, MS |
| 1958 | *(E)*-*β*-Ionone | 0.6 | 0.8 | MS |
| 2009 | *trans*-*β*-Ionon-5,6-epoxide | 0.4 | - | MS |
| 2019 | 2,3,6-Trimethyl benzaldehyde | 0.3 | 0.4 | MS |
| 2045 | Carotol | 1.2 | - | MS |
| 2179 | 3,4-Dimethyl-5-pentyliden-2(5H)-furanone | tr | - | MS |
| 2228 | Acorenone B | 0.2 | - | MS |
| 2300 | Tricosane | 0.3 | 0.9 | RRI, MS |
| 2380 | Dihydroactinidiolide | 0.4 | - | MS |
| 2500 | Pentacosane | 0.5 | 1.5 | RRI, MS |
| 2600 | Hexacosane | 0.6 | 1.7 | RRI, MS |
| 2700 | Heptacosane | 0.8 | 1.6 | RRI, MS |
| 2800 | Octacosane | 0.9 | 1.7 | RRI, MS |
| 2900 | Nonacosane | 0.7 | 1.3 | RRI, MS |
| 2931 | Hexadecanoic acid | 0.6 | - | RRI, MS |
| | **Total** | **81.6** | **63.3** | |

RRI: Relative retention indices calculated against *n*-alkanes
*%: calculated from TIC data
tr: Trace (< 0.1 %)
IM: Identification method based on the relative retention indices (RRI) of authentic compounds on the HP Innowax column; MS, identified on the basis of computer matching of the mass spectra with those of the Wiley and MassFinder libraries and comparison with literature data

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