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Research Article

Essential oil in *Phlomis tuberosa*

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Abstract

Phlomis tuberosa L. (Lamiaceae) is a flowering perennial plant, native to Eurasia, and sparsely distributed in Bulgaria. The species has been proved to accumulate flavonoids, iridoids, phenolic glycosides, as well as essential oil. To now, there is no information on the chemical content of Bulgarian essential oil from the plant. The aim was to study the chemical composition of essential oils, obtained from the species. Four different samples were studied – essential oils produced from leaves and herbs, collected in the fields, and from leaves and flowering parts, harvested from the mountainous location. It was found that *β*-copaene was the major in the essential oil from both the leaves and of the flowering parts collected from Popitza. In addition, the other major constituent in leaf essential oil from Kladnitza was *tau*-muurolol. The essential oil, produced from the species, harvested from the higher altitude had phytol as the main component. The sesquiterpenoid content in leaf essential oil suggests that this plant substance is appropriate for medicinal use, due to the pharmacological properties of these compounds.

Keywords

Phlomis tuberosa, GC-MS, essential oil, Clevenger, terpenes, phytochemical analysis

Introduction

Phlomis tuberosa L. (sect. *Phlomoides*, Lamiaceae family) is a perennial herbaceous plant with tuberous roots. The stem is erected, up to 150 cm; the lower leaves are triangular-spear shaped and the upper ones – ovate. Bracts are equal to the calyx, linear-subulate, pubescent with sparsely covered with distal hairs. The blossoming period is May-June, and the fructification occurs in July-August. It grows in steppes, in meadows and along stony mountain slopes. The species is found in the flora of Eastern Europe, the Mediterranean, Southwest and Central Asia (Stoyanov and Kitanov 1967). In Bulgaria, the plant is sparsely distributed, mainly in the Northern part of the country and in the Eastern Rhodopes (Asyov et al. 2012).

The plant is medicinal according to many traditional healing systems, primarily in Asia and Eastern Europe (Amor et al. 2009). An infusion from the aerial parts is used against diarrhoea, dysentery, pneumonia, bronchitis, fever, pulmonary tuberculosis. Its decoction is consumed in cases of hepatitis. The crushed leaves are applied locally on purulent wounds. A large number of applications of the roots are reported in Tibet and Mongolia – as a decoction against mastitis, rheumatism, arthritis, sore throat, gastritis, stomach ulcer, urethritis, etc. In addition, both the leaves and the tuberous roots are edible either fried or boiled (Amor et al. 2009). The species has been proved to contain: flavonoids (Vavilova and Gella 1973a, b), alkaloids (Khokhrina and Peshkova 1974), iridoids (Gella et al. 1972); (Calis et al. 2005); (Alipieva et al. 2000). The overground part accumulates phenylethanoid glycosides (Ersöz et al. 2001; Calis et al. 2005), neolignan glucosides (Calis et al. 2005), etc. The overground parts of genus *Phlomis* are reported to produce essential oil as well (Amor et al. 2009). The differences in the chemical content of essential oils are a direct result of the geographical location (longitude, altitude, and climate), soil composition (incl. contamination), and cross-pollination (intra population). Many variations have been assigned to differences of the extraction technique, of the phenological stage of development of the plants, and of the season, etc. It can be concluded that the composition of the oils depends largely on the geographical location (different species collected from the same area have a similar composition), mainly in terms of aliphatic constituents and terpenoids (Zhang and Wang 2008).

There are different essential oils from depending on the composition and the predominant main component, and *Phlomis* species can be divided into four main chemotypes (Amor et al. 2009): 1) rich in sesquiterpenes – the predominant components are germacrene D and caryophyllene (Sarkhail et al. 2005; Basta et al. 2006; Amor et al. 2009; Demirci et al. 2009); 2) rich in mono- and sesquiterpenes – the main constituents are *α*-pinene, limonene, linalool, germacrene D and *β*-caryophyllene (Ristić et al. 2000; Aligiannis et al. 2004; Celik et al. 2005; Liolios et al. 2007); 3) rich in aliphatic components – fatty acids, diterpenoid alcohols and higher fatty alcohols, with a higher percentage of hexadecanoic acid, trans-phytol and 9,12,15-octadecatrien-1-ol (Zhang and Wang 2008); 4) rich in terpenes, fatty acids and aliphatic compounds – hexadecanoic acid, *α*-pinene and germacrene D as the main representatives of fatty acids, monoterpenes and sesquiterpenes, respectively (Morteza-Semnani et al. 2004; Zhang and Wang 2008).

There are no studies of the essential oil from *Phlomis tuberosa*, grown in Bulgaria. Thus, the aim was to investigate the chemical content of essential oils from leaves and herbs of this plant.

Materials and methods

Plant material

It was collected twice in locations in Bulgaria as given in Table 1. One of us (I. K.) identified the plant and voucher specimens were deposited in the Herbarium of the Faculty of Pharmacy at the Medical University of Sofia (FF-180 and 181/2022). Immediately after collection, the samples were frozen at -40 °C.

Table 1. Plant material.

Sample	Location in Bulgaria	Altitude, m.a.s.l.	Collection	Specimen
Leaves	Popitza village	130	May 2022	FF180/2022
Leaves	Kladnitza village	1000	May 2022	FF181/2022
Overground parts	Popitza village	130	July 2022	FF180/2022
Overground parts	Kladnitza village	1000	July 2022	FF181/2022

Obtaining the essential oil

Each frozen sample (100 g) was chopped and then extracted in a Clevenger-type apparatus (500 mL of water) for 4 h. The essential oil obtained was separated, dried over anhydrous Na_2SO_4 and stored at -40 °C until analysis.

GC-MS analysis

The essential oil was diluted 1:10000 with n-hexane and subjected to GC-MS analysis. GC-MS was performed with an ExactiveOrbitrap GC-MS (ThermoFisher Scientific, Germany) system operating at 70 eV, ion source temperature 230 °C, transfer capillary temperature 260 °C, with split injection (1 µL, 20:1 ratio) at 230 °C injector temperature. A capillary column with 5% phenyl residues/95% methyl polysiloxane (TraceGOLD TG-5SilMS GC Column 30 m × 0.25 mm × 0.25 µm, Thermo) was used. The oven temperature program was: initial at 60 °C for 5 min, increased to 300 °C (rate: 6 °C/min), maintained at 300 °C for 5 min. Helium was used as the carrier gas (flow rate: 1 mL/min). The EI ionization mode and full MS-SIM scan were used (resolution 600, AGC target $1e^6$, maximum IT 200 ms, and scan range of *m/z* 50–450). Data collection, peak processing and compound identification were performed with Xcalibur 4.2.28.14 (Thermo Scientific, Germany). The GC column was calibrated by a standard procedure (Adams 2007; Rimayi et al. 2015) using a calibration mixture of n-alkanes (C8-C40 Alkanes Calibration Standard, Supelco, USA). Peaks were processed using the algorithm GENESIS. The same software was used to calculate the Kovats indexes and the relative percentage of constituents. Each compound was identified based on a comparison of its mass spectral fragmentation in the positive ionization mode with the records in two databases (Wiley Registry 10 and NIST 2014). Criteria for identification were the coincidence of the results from both databases, the coincidence of the confidence intervals (statistical %) for the compound in question from both databases and the coincidence of the Kovats index of the substance to that in the literature (Adams 2007; Babushok and Zenkevich 2009; Babushok et al. 2011).

Results and discussion

The hydro distillation of the four samples produced essential oils with an average yield (v/w) of 0.008% v/w). Leaves of *P. tuberosa*, collected in Popitza produced 0.016% essential oil, and those from Kladnitza – 0.013%. Overground parts from Popitza gave 0.0024% and those, collected from Kladnitza – 0.002% essential oil. A representative TIC chromatogram of the essential oil is shown on Fig. 1.

In the essential oil obtained from the leaves collected from the low altitude – Popitza, 13 compounds were identified, comprising 99.96% of its content. The main compound was the sesquiterpene *β*-copaene (19.97%). The

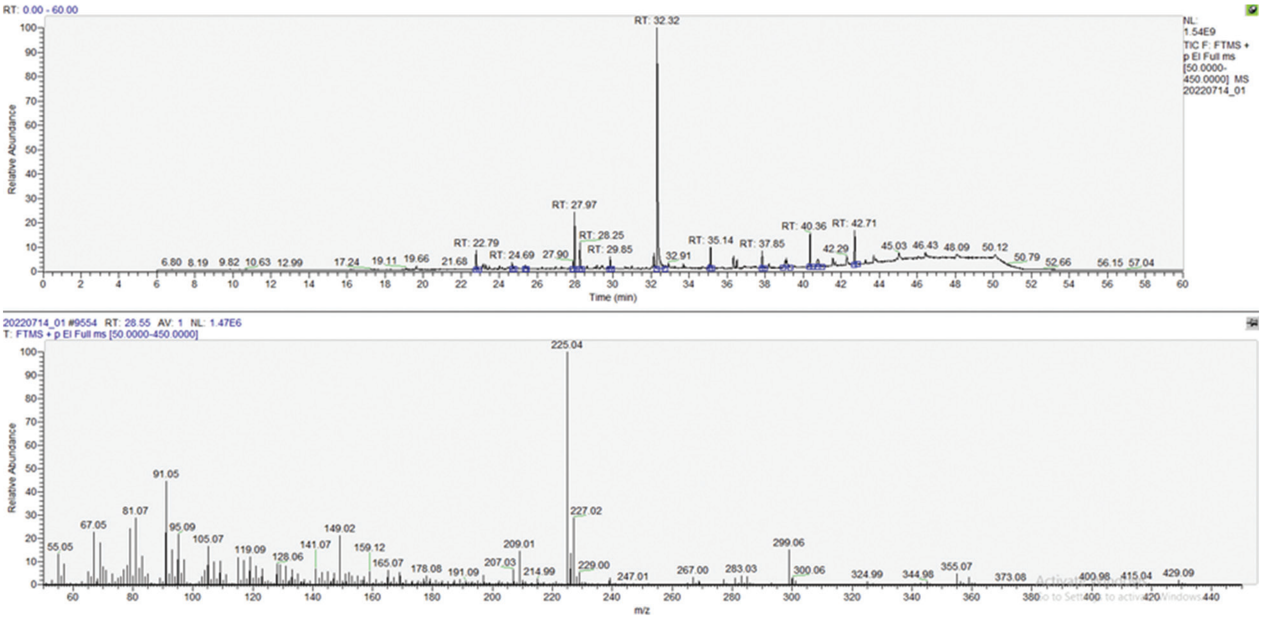

Figure 1. TIC chromatogram of the essential oil, obtained from herbs of *P. tuberosa*, collected from Kladnitza.

majority of volatiles found in the etheric oil were alkanes, comprising more than half of the content. This sample could be classified as alkane-type essential oil. The lowest content was of the sesquiterpene isochiapin B (4%), and the diterpene abietic acid (5%) was present as well (Table 2).

The essential oil from the leaves of the plant, collected from the mountainous region, had 14 compounds identified (99.95% of the total content), as presented in Table 3. The main substance was *tau*-muurolol, a sesquiterpene (15.81%), as the main ingredient. The other major component was β-copaene (15.64%), lower in quantity than in the oil from the leaves of Popitza. This sesquiterpene was the same in both samples of leaves. The essential oil could be classified as sesquiterpene chemotype. The lowest content was of 3-ethyl-5-(2-ethylbutyl)-octadecane, an alkane (3.14%) and of the limonoid deacetylgedunin (3.18%).

Table 2. Composition of the essential oil in leaves of *P. tuberosa*, collected from Popitza.

Peak №	Compound	Quantity, %	KI*
1.	Rhodopin	8.61	4025
2.	Butyl 4,7,10,13,16,19-docosahexaenoate	5.35	2821
3.	Doconexent	12.71	2511
4.	Gibberellin A9 methyl ester	6.07	2322
5.	Isochiapin B	4.15	1980
6.	Methyl eicos-8,11-dien-14-ynoate	7.39	2309
7.	β-Copaene	19.97	1378
8.	(-)-Abietic acid	5.06	2527
9.	2-(7-*t*-Butoxy-heptyl)-5-methoxy-cyclopent-2-enone	5.03	1965
10.	Dotriacontane	6.47	3203
11.	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	6.76	2413
12.	((3aS,6S,6aR,9aS,9bR)-6a-hydroxy-6-methyl-3-methylene-2,9-dioxodecahydroazuleno[4,5-b]furan-9a(4H)-yl)methyl 2-methylpropanoate	5.92	1873
13.	Geranyl isovalerate	6.47	1585
	Total	99.96	

* Kovats indexes were compared with literature data (Adams 2007; Babushok and Zenkevich 2009; Babushok, Linstrom and Zenkevich 2011).

Table 3. Composition of the essential oil in leaves of *P. tuberosa*, collected from Kladnitza.

Peak №	Compound	Quantity, %	KI*
1.	2-(7-*t*-Butoxy-heptyl)-5-methoxy-cyclopent-2-enone	5.29	1966
2.	4aα,4bβ-Gibbane-1α,10β-dicarboxylic acid, 4a-formyl-1-methyl-8-methylene-, dimethyl ester	7.71	2444
3.	β-Copaene	15.64	1216
4.	4,6-di-*tert*-Butyl-*m*-cresol	6.93	1668
5.	Mesityl(phenyl)methanol	5.53	1969
6.	β-Sitosterol	3.82	2731
7.	[10*R*,11*R*,12*S*]-Calanolide A	6.95	2917
8.	*cis*-2-Phenyl-1, 3-dioxolane-4-methyl octadec-9,12,15-trienoate	5.36	2793
9.	*tau*.-Muurolol	15.81	1580
10.	Methyl abieta-8,11,13-trien-18-oate	4.79	2324
11.	Deacetylgedunin	3.18	2553
12.	8-Isopentyl-3,3,5,5-tetramethyl-6,7-dihydro-2H-s-indacen-1-one	3.48	2045
13.	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	3.17	2413
14.	Dotriacontane	12.33	3202
	Total	99.95	

* Kovats indexes were compared with literature data (Adams 2007; Babushok and Zenkevich 2009; Babushok, Linstrom and Zenkevich 2011).

In the essential oil obtained from the flowering parts, collected from the low altitude (130 m.a.s.l.), 13 compounds were identified (99.96% of its content). The main compound was *n*-propyl 5,8,11,14,17-eicosapentaenoate (20.93%). The other major compound is *β*-copaene (20.33%). The oil could be classified as alkane-type, like that from the leaves from the same location. The low altitude had definitely an influence on the chemical composition of the essential oil. The majority of volatiles found in this etheric oil were alkanes, so this could be classified as alkane-type essential oil. The lowest content was of isochiapin B (3.64%), and of abietic acid (5%), as in the leaves from the same location (Table 4).

The overground parts of *P. tuberosa*, harvested from the mountainous region (Kladnitza) had essential oil in which 13 compounds were identified (99.98% of the sample). Unlike the herbs from Popitza, this sample had *trans*-phytol as the main compound (52.27%), more than half of the content. This is an alkane-type essential oil. The lowest quantity was of the alkenoid 2-(7-*t*-butoxy-heptyl)-5-methoxy-cyclopent-2-enone (0.52%), as shown in Table 5. Interestingly, this content was similar to that, reported previously (Olennikov et al. 2010).

The essential oil obtained from the leaves of the species,

Table 4. Composition of the essential oil in herbs of *P. tuberosa*, collected from Popitza.

Peak №	Compound	Quantity, %	KI*
1.	Phenylmethyl ester of (Z,Z,Z)-6,9,12-octadecatrienoic acid	4.72	2774
2.	Butyl 4,7,10,13,16,19-docosahexaenoate	4.57	2821
3.	*cis*-4,7,10,13,16,19-Docosahexanoic acid	13.42	2521
4.	Isochiapin B	3.64	1975
5.	*β*-Longipinene	3.81	1403
6.	*n*-Propyl 5,8,11,14,17-eicosapentaenoate	20.93	2515
7.	(-)-Abietic acid	3.36	2512
8.	*β*-Copaene	20.33	1216
9.	Dotriacontane	3.46	3202
10.	2-(7-t-Butoxy-heptyl)-5-methoxy-cyclopent-2-enone	6.47	1965
11.	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	4.60	2413
12.	Octadecane, 1-[2-(hexadecyloxy)ethoxy]-	4.50	3586
13.	Benzene, (2-decyldodecyl)-	6.15	2770
	Total	99.96	

* Kovats indexes were compared with literature data (Adams 2007; Babushok and Zenkevich 2009; Babushok, Linstrom and Zenkevich 2011).

collected from Buryatiya had phytol (35.5%) as the main constituent (Olennikov, Dudareva, and Tankhaeva 2010). In essential oil from aerial parts collected from Northern Kazakhstan phytol was in lower quantity (5.5–9.7%) (Kirillov et al. 2018). The essential oil obtained from flowering aerial parts obtained around Krasnoyarsk, contained again this compound in low quantity, 7.5% (Sokolova et al. 2012). In Bulgarian samples phytol was found not in leaves, but in the aerial parts collected from Kladnitza in a significantly larger quantity (52%), than previous reports.

Essential oil from the plant harvested around Irkutsk and Krasnoyarsk was rich in sesquiterpenes like chamazulene (6.5%), *δ*-cadinene (3.7%), *α*-bisabolol (2.9%), (*E*)-asarone (2.8%), *α*-cadinol (2.6%), *β*-elemene (2.4%), *τ*-muurolol (2.3%) (Polina and Efremov 2013). Kirillov et al. (2018) again proved sesquiterpenes – *β*-caryophyllene (3.3–4.9%)

Table 5. Composition of the essential oil in herbs of *P. tuberosa*, collected from Kladnitza.

Peak №	Compound	Quantity, %	KI*
1.	Formic acid, 3,7,11-trimethyl-1,6,10-dodecatrien-3-yl ester	3.15	1752
2.	*α*-Cadinol	1.06	1580
3.	2-(7-*t*-butoxy-heptyl)-5-methoxy-cyclopent-2-enone	0.52	1965
4.	6,10,14-trimethyl-2-pentadecanone	9.08	1842
5.	9-Undecenol, 2,10-dimethyl-	4.01	1477
6.	4a*α*,4b*β*-Gibbane-1*α*,10*β*-dicarboxylic acid, 4a-formyl-1-methyl-8-methylene-, dimethyl ester	2.02	2444
7.	Phytol	52.27	2102
8.	2-Hexadecanol	3.70	1702
9.	Dotriacontane	3.09	3202
10.	1,1'-[1,2-ethanediylbis(oxy)]bis-Hexadecane	5.27	3553
11.	Tetratetracontane	6.01	4395
12.	*β*-Resorcylic acid	3.68	1591
13.	7aH-Cyclopenta[a]cyclopropa[f]cycloundecene-2,4,7,7a,10,11-hexol, 1,1a,2,3,4,4a,5,6,7,10,11,11a-dodecahydro-1,1,3,6,9-pentamethyl-, 2,4,7,10,11-pentaacetate	6.12	3721
	Total	99.98	

* Kovats indexes were compared with literature data (Adams 2007; Babushok and Zenkevich 2009; Babushok et al. 2011).

and the distinctive bicyclic sesquiterpene valerianol in samples of Kazakhstan (Kirillov et al. 2018). Bulgarian samples were also rich in sesquiterpenes, but the main ingredient was *β*-copaene (15–20%), excl. the herbs of Kladnitza. Significant quantity of *tau*-muurolol (15%) was proved only in leaf essential oil from Kladnitza. Noteworthy, the content of sesquiterpenes in Bulgarian essential oils of this species was distinctively larger in leaves than previous reports.

Conclusion

Phlomis tuberosa grown in Bulgaria was studied for the presence of essential oils. It was found that *β*-copaene was the major in the essential oil from both the leaves and of the flowering parts collected from Popitza. In addition, the other major constituent in leaf essential oil from Kladnitza was *tau*-muurolol. The essential oil, produced from the species, harvested from the higher altitude had phytol as the main component. The sesquiterpenoid content in the essential oil from leaves and aerial parts, both collected from low altitude, suggests that these plant substances are appropriate for medicinal use. The highest sesquiterpenoid content (more than 40%) was in the herb essential oil from Popitza, so this is the most useful, due to the valuable pharmacological properties of this group of terpenes.

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