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Composition and antioxidant potential of essential oil of *Geranium macrorrhizum* L. from different regions of Bulgaria

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The common geranium (*Geranium macrorrhizum* L.) is the economically most important species of the genus *Geranium*, and is highly valued for its fragrance and medicinal properties. Object of the present study is the essential oil (EO) prepared by hydro-distillation of aerial parts and rhizomes of common geranium from four floristic regions of the country. Its quality was determined by GC-MS analysis. The antioxidant potential of *G. macrorrhizum* was evaluated by the DPPH method. Forty-two compounds were identified. In the EOs from the aerial parts, the compound in the most quantity was the sesquiterpene germacrone (from 51.41 to 62.58 %). In the EOs from the rhizomes, this compound was only 5.80-8.94%. The most common ingredient of the rhizome EO was another sesquiterpene: cis-β-elemenone in a quantity from 45.20 to 50.64%. Other nine compounds were present only in the rhizome samples, among which the sesquiterpene globulol was found in good quantities: 15.71 – 15.90%. Monoterpenes like α-terpinene and phellandrene, and sesquiterpenes like eudesm-11-en-4α,6α-diol and eudesm-7(11)-en-4-ol acetate were detected only in some EOs from the aerial parts. The radical scavenging potentials of the EOs were from 33.15 to 41.79 μmol TE/100 μg EO, the rhizome samples showing weaker antioxidant potential compared to the aerial parts. Pearson correlation coefficients between the main ingredients of the EOs from the aerial parts and the radical scavenging potential pointed to the strongest impact of germacrene A on the antioxidant value. The large differences in the results are due to the different agro-ecological conditions under which the plants were grown.

Key words: Essential oil; *Geranium macrorrhizum* L.; Germacrone; Radical scavenging potential; Sesquiterpenes

INTRODUCTION

Common geranium *(Geranium macrorrhizum)* is a perennial plant of the *Geraniaceae* family. Natural habitats of the species are the mountains and semi-mountain regions in the country from 300 to 2500 m above sea level [1]. According to Yankulov [2] and Stoeva [3], common geranium is particularly widespread in the Rhodopes, the Rila and the Balkan Mountains. It is cold hardy and drought tolerant, likes semi-shaded areas and is not demanding of the soil. *G. macrorrhizum* is widely used as an ornamental plant, and finds application in many Bulgarian rituals and customs. The common geranium is also a nectariferous plant. Its extracts possess a wide range of antimicrobial, antiviral, hypotensive, antispasmodic, sedative, astringent, cardiotonic, antioxidant and antiatheromatous properties [4-6]. The phyto-therapeutic effect of geranium drug is also associated with its tannin content. The highest tannin content in the aboveground mass was found in the budding phase, and in roots - in autumn [7]. In Bulgarian traditional medicine, this species is used to treat skin diseases, as a disinfectant bath and a poultice of the affected area; it is also used to relieve pruritus, itching and skin lesions [8], and as a remedy for malignant diseases of the hematopoietic organs [3]. In the folk veterinary medicine of Italy, this species has found application in the treatment of diarrhea [9].

Essential oils of *Geraniaceae* species act as inhibitors of α-amylase, α-glucosidase and pancreatic lipase [10], and can be used to treat diabetes [11]. Common geranium is the economically most important species of the genus *Geranium*, highly valued for its fragrance and medicinal properties, and the plant is mainly used for the production of essential oil [3]. Bulgarian geranium oil is well known on the international market and is highly valued. Until recently, our country was one of the few in the world that produced it. The main constituent of the essential oil is germacrone (50-65 %), as well as terpinene, pinene, cymol, caryophyllene, borneol, murulene, elemenone, geraniol and others [12, 13].

The present study aimed to evaluate the quality and the antioxidant potential of the essential oil from aerial parts and rhizomes of common geranium from different regions of the country.

MATERIALS AND METHODS

Plant material and sample preparation

The plant material, object of the present study, were the aerial parts and rhizome of *G. macrorrhizum* collected from four floristic regions

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of Bulgaria, grown as an ornamental plant (Table 1). Aerial parts were collected during the full flowering period (May – June 2022), and the rhizome – in the autumn of the same year. From each population, 4 batches were collected, and 200 g average sample of population was prepared. Each sample was extracted by hydro-distillation for 4 hours with a Clevenger-type apparatus according to the European Pharmacopoeia 7th edition [14]. The organic layer obtained on top of the aqueous distillate was separated. After drying with anhydrous sodium sulfate (Na_2SO_4) the essential oil (EO) was kept in sealed airtight glass vials at 4 °C until used.

GC analysis

The EO samples were analyzed on an Agilent 7890A gas chromatograph (Agilent Technologies, CA, USA) with FID and Agilent 5975 Inert MS quadrupole detection with electron capture ionization (70 eV). The chromatographic conditions were: DB-5 MS column CA, USA (5 % phenyl methyl siloxane 30 m × 0.32 mm i.d., film thickness 0.25 µm), carrier gas helium (0.8 ml/min), 1 µl injection volume, split mode 100:1, injector and FID temperature of 250 ºC, 2 s scan time, and m/z=40-450 scanning range of MS detector. The start column temperature was 65 ºC and at the end of the 34 min single run it was set at 230 ºC with a rate of 1 ºC/min. Before injecting, the EO sample was diluted with methanol in a ratio of 1:20 (v/v).

The EO components were identified by comparing the registered mass spectra with those of the NIST 08 database (National Institute of Standardization and Technology, Gaithersburg, MD, USA) or by comparison of their mass spectra and retention indices with those reported in literature [16, 17].

DPPH test

The method described by Mileva *et al.* [18] was applied to measure the radical scavenging potential of the EO samples. In brief, to 2 mL of 100 M solution of DPPH in ethanol, 20 µL of 100 mg/mL ethanolic solution of EO sample was added. Two parallel samples of each extract were analyzed. Absorption at 517 nm was measured on a Thermo Scientific Evolution 300 spectrophotometer after 30 min. Since the composition of the extracts is complex, the results for their radical scavenging capacity were compared with Trolox and calculated by regression analysis from the linear dependence between concentration of Trolox and absorption at 517 nm. The results were expressed as µmol Trolox equivalent (TE) in 100 µg EO and as inhibition of DPPH in percentage (I, %) calculated by the formula:

$$I\ (\%) = [(Abs_0 - Abs_{sample})/(Abs_0)]*100$$

Table 1. *G. macrorrhizum* populations from different regions of Bulgaria and EO yield

Sample ID	Sample	Location, latitude	Elevation, m a.s.l.	Soil type*	EO quantitative yield, % w/w
A1	Aerial parts	Thracian Plain, green areas around Trakia University, town of Stara Zagora 42.4006 N, 25.5711 E	303	Anthrosols	0.035 ± 0.020
A2	Aerial parts	The Rhodopes (Eastern), town of Ivaylovgrad 41.5272 N, 26.1328 E	180	Anthrosols	0.061 ±0.008
A3	Aerial parts	The Balkan Range (Central), town of Kazanlak 42.6349 N, 25.3884 E	425	Fluvisols	0.042±0.002
A4	Aerial parts	Sredna Gora (Eastern), Pryaporets village, 42.4632 N, 25.5379 E	618	Anthrosols	0.062±0.004
A5	Aerial parts	The Balkan Range (Western), town of Varshets 43.2044 N, 23.2937 E	395	Chernozem	0.012±0.006
R1	Rhizomes Sept. 2022	Thracian Plain, green areas around, Trakia University, Stara Zagora 42.4006 N, 25.5711 E	303	Anthrosols	0.007±0.002
R2	Rhizomes Oct. 2022	Thracian Plain, green areas around Trakia University, Stara Zagora 42.4006 N, 25.5711 E	303	Anthrosols	0.008±0.002

*according to FAO 2001 [15]

IC_{50} was defined as the quantity of substance necessary to decrease the initial DPPH by 50%.

Data were obtained from the plotted graph of scavenging activity of each sample. Lower IC_{50} value means higher antiradical activity. Each experiment was carried out in triplicate and data were presented as a mean of the three values. Pearson's correlation was evaluated.

RESULTS AND DISCUSSION

The quantitative yields of the essential oil obtained from the aerial parts of *G. macrorrhizum* varied from 0.012% (A5) to 0.06% (A4 and A2) (Table 1). There was a six-fold difference between the lowest and the highest value. Similar biases in EO yields among different populations were estimated by other researchers [19, 20]. The quantities of the essential oil obtained from the rhizomes were much lower (0.007% and 0.008%). The differences between the EOs extracted from aerial parts and rhizomes were not only as regards the yield values. There were deviations also in the quality of the EOs.

The chemical compositions of the EOs obtained as described in Materials and Methods section are presented in Table 2.

From the results obtained in the present study, the main components in the EO samples from the aerial parts of the collected plants were the sesquiterpene germacrone (from 51.41% to 62.58%); in the EOs from the rhizomes, this compound was only 5.80-8.94%. Ivanov [21] carried out the first studies on the composition of geranium oil from Bulgarian populations. The author found that the oil mainly contained sesquiterpene hydrocarbons. The liquid part of geranium oil consists of about 10% terpene compounds and about 20% sesquiterpene hydrocarbons. The most significant achievement in geranium oil research was the solution of the question of the structure of germacrone, the crystalline component of the oil. Its structure was confirmed by the synthesis of the hydrocarbon germacrone, which is identical to the hydrocarbon produced by complete hydrogenation of germacrone.

The hydrogen monoterpene limonene was quantified between 0.17% and 0.78% in four of five EOs extracted from the aerial parts. In the group of oxygenated monoterpenes linalool dominates in contents from 0.11 and 0.35%. This compound was not found in the rhizome samples. The most abundant oxygenated sesquiterpene was the germacrene, which is identified in three isomers: germacrene A, germacrene B and germacrene D. Germacrene D was in the lowest amounts and ranged between 0.6 and 2.13%. Values of germacrene D more than 30% are toxic. In the present study, the highest concentrations were found for germacrene B between 2.7 and 6.77% (Table 2). The β-eudesmol is another toxic compound, which is more than 40% in the EO sample [22]. In the tested samples this compound was found in EOs extracted from aerial parts and rhizomes in the range from 0.78 to 3.00%. Monoterpenes like α-terpinene and phellandrene, and sesquiterpenes like eudesm-11-en-4α,6α-diol and eudesm-7(11)-en-4-ol acetate were found only in some EOs from the aerial parts (Table 2).

Navarro-Rocha *et al.* [20] studied the biological activities of *G. macrorrhizum* and identified β-elemenone (30.53%), thymol (18.52%) and germacrone (15.54%) as main ingredients of the EOs obtained from wild populations, and linalool (26.45%) and linalyl acetate (25.11%) as major components of the EOs from cultivated populations. A similar composition has been reported for collected wild individuals of this species in Hungary [23]. The essential oil of common geranium grown as an ornamental plant was dominated by linalool (26.45%) and linalyl acetate (25.11%). Ameline *et al.* [24] reported that the major components of the EO from common geranium were germacrone and β-elemene. So, the Bulgarian populations of common geranium are distinguished by a high content of germacrone - a substance with anticancer activities [25, 26].

Table 2. Chemical composition of EO from the tested *G. macrorrhizum* populations, %

Peak No	RT*	RI**	Name	A1	A2	A3	A4	A5	R1	R2
1	12.16	1002	α-Phellandrene	nd***	nd	nd	0.68	nd	nd	nd
2	12.51	1011	α-Terpinene	nd	nd	nd	0.12	nd	nd	nd
3	12.75	1018	p-Cymene	0.20	0.13	0.51	0.66	0.82	nd	nd
4	12.90	1022	Limonene	0.78	0.34	0.17	0.72	nd	nd	nd
5	13.47	1041	trans-β-Ocimene	0.11	0.15	0.10	0.3	nd	nd	nd
6	13.84	1058	γ-Terpinene	0.10	0.12	0.08	0.74	nd	nd	nd
7	14.70	1083	Terpinolene	0.18	0.10	0.09	0.88	nd	nd	nd

8	15.16	1090	Linalool	0.65	0.24	0.46	0.11	0.35	nd	nd
9	16.73	1141	trans-Verbenol	nd	nd	nd	nd	nd	0.11	0.45
10	17.32	1206	Verbenone	nd	nd	nd	nd	nd	0.57	1.29
11	17.57	1217	trans-Carveol	0.48	0.18	0.14	0.13	0.12	0.51	0.67
12	17.60	1220	2,3-dimethyl-Benzofuran	nd	nd	nd	4.72	nd	nd	nd
13	18.06	1228	cis-p-Mentha-1(7),8-dien-2-ol	0.50	0.22	0.13	0.50	0.22	nd	0.23
14	18.76	1256	Canrenone	nd	nd	nd	nd	nd	0.27	0.39
15	22.04	1340	trans-Carveyl acetate	0.81	0.94	1.12	0.35	0.16	0.48	0.87
16	23.12	1371	Isoledene	nd	nd	nd	nd	nd	0.30	0.48
17	23.46	1390	β-Elemene	0.25	0.36	0.49	0.32	0.34	nd	nd
18	23.89	1403	Italicene	0.20	0.47	0.25	0.46	0.10	nd	nd
19	24.23	1414	α-Gurjunene	nd	nd	nd	nd	nd	0.39	0.57
20	24.48	1421	γ-Elemene	0.40	0.58	0.95	0.83	0.51	nd	nd
21	24.94	1440	cis-β-Famesene	nd	nd	nd	nd	nd	0.72	1.24
22	25.69	1452	ar-Curcumene	1.08	6.35	0.3	5.89	0.80	nd	nd
23	25.71	1473	γ-Muurolene	nd	nd	nd	nd	nd	1.23	2.56
24	25.77	1477	γ-Curcumene	0.80	2.75	0.15	1.24	0.50	nd	nd
25	26.00	1482	trans-β-Guaiene	nd	nd	nd	nd	nd	7.88	9.91
26	27.34	1495	Germacrene D	2.13	1.20	0.60	0.92	0.21	1.19	0.33
27	27.72	1514	Germacrene A	3.03	4.96	4.55	3.85	2.54	0.65	0.53
28	28.61	1555	Germacrene B	3.36	2.70	5.59	2.76	6.77	0.71	1.44
29	28.66	1570	Globulol	nd	nd	nd	nd	nd	15.90	15.71
30	29.21	1580	Germacrene D-4-ol	1.34	1.83	1.52	1.68	0.91	0.31	1.38
31	29.37	1588	Viridiflorol	1.02	1.95	2.24	1.45	0.50	1.13	1.74
32	29.83	1601	cis-β-Elemenone	1.43	1.73	4.10	0.91	0.75	50.64	45.20
33	29.94	1610	5-epi-7-epi-α-Eudesmol	3.63	4.42	8.92	1.72	6.20	1.37	1.42
34	30.01	1622	10-epi-y-Eudesmol	1.43	2.96	5.81	6.07	0.84	1.26	1.14
35	30.15	1633	γ-Eudesmol	5.24	2.35	1.22	2.55	3.80	1.09	0.98
36	30.36	1647	β-Eudesmol	3.00	1.38	1.97	2.18	1.60	0.78	2.11
37	30.49	1654	α-Eudesmol	1.36	0.33	1.89	2.11	1.44	0.84	0.73
38	30.65	1666	7-epi-α-Eudesmol	2.72	3.34	2.20	2.80	4.03	0.63	1.15
39	31.01	1695	Germacrone	62.58	56.78	53.32	51.41	61.70	8.94	5.80
40	32.14	1751	(2E,6E)-Farnesol	0.31	0.26	0.55	0.17	0.92	1.16	0.82
41	33.40	1810	Eudesm-11-en-4α,6α-diol	nd	nd	nd	nd	1.40	nd	nd
42	33.85	1843	Eudesm- 7(11)-en-4-ol, acetate	nd	nd	nd	nd	1.65	nd	nd

*retention time; **relative index; ***not detected.

Table 3. Radical scavenging potential of EO from *G. macrorrhizum*

ID	R1	R2	A1	A2	A3	A4	A5
IC_{50}, mg/ml EO	0.237	0.236	0.205	0.192	0.196	0.208	0.217
µmolTE/100 µg EO	33.30	33.15	38.91	41.79	40.88	38.30	36.64

The EO extracted from the rhizomes of *G. macrorrhizum* were rich in the oxygenated sesquiterpenes cis-β-elemenone (from 45.2 to 50.64 %), and globulol (from 15.71 to 15.90%). The second one was not identified in the EOs from the aerial mass. Other eight compounds were present only in the rhizomes samples: trans-β-guaiene (7.88-9.91%); γ-muurolene (1.23-2.55%); cis-β-famesene (0.72-1.24%); verbenone (0.57-1.29%); α-gurjunene (0.39-0.57%)**;** canenone (0.27-0.39%); isoledene (0.30-0.48%); trans-verbenol (0.11-0.45%).

The antioxidant activity was evaluated as radical scavenging potential of the EOs extracted from the aerial parts and rhizomes of *G. macrorrhizum* plants collected from different populations, and the results for the EO from the aerial parts were from 36.64 (A5) to 41.79 (A2) μmol TE/100 μg EO, which is equal to IC_{50}, *ca*. 0.20 mg/ml EO. The results for the rhizomes were on average 33 μmol TE/100 μg EO, which is equal to IC_{50}, *ca*. 0.24 mg/mlEO (Table 3). So, the rhizome samples showed a weaker antioxidant potential compared to the aerial parts. The differences in the antioxidant potentials of the EOs from aerial parts and rhizomes are due to their different chemical composition.

Zeljković *et al.* [28] calculated the IC_{50} values for the essential oil extracted from aerial parts from *Geranium kikianum, which* ranged *ca*. 70 mg/mL using the DPPH method. The samples tested by the authors were also rich in germacrone. The mean content of this cyclic ketone was 45.6%. Compared to the results obtained in the present study, the EOs from *G. macrorrhizum* showed much stronger radical scavenging potential than EO from *G. kikianum.*

The calculation of the Pearson correlation coefficients between the main ingredients of the EOs from the aerial parts and the radical scavenging potential showed the strongest impact of germacrene A (0.923) on the radical scavenging activity. The coefficient of correlation between this parameter and germacrone was 0.391. The research on the impact of the ingredients on the antioxidant activities of the EO from Bulgarian geranium could lead to interesting results, Moreover, the plant shows promising anticancer activities.

CONCLUSION

In the EOs from the aerial parts of *G. macrorrhizum*, the compound in the most quantity was the sesquiterpene germacrone (from 51.41 to 62.58 %). In the EOs from the rhizomes, this compound was only 5.80-8.94%. The most common ingredient of the rhizome EO was another sesquiterpene: cis-β-elemenone in quantity from 45.20 to 50.64%. Other nine compounds were present only in the rhizome samples, among which the sesquiterpene globulol was found in good quantities: 15.71 – 15.90%. Monoterpenes like α-terpinene and phellandrene, and sesquiterpenes like eudesm-11-en-4α,6α-diol and eudesm-7(11)-en-4-ol acetate were determined only in some EOs from the aerial parts. The radical scavenging potentials of the EOs were from 33.15 to 41.79 μmol TE/100 μg EO, the rhizomes samples showing a weaker antioxidant potential compared to the aerial parts. The calculation of the Pearson correlation coefficients between the main ingredients of the EOs from the aerial parts and the radical scavenging potential showed the strongest impact of germacrene A (0.923) on the antioxidant value. The large differences in the results are due to the different agro-ecological conditions under which the plants were grown.

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