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COMPOSITION OF ESSENTIAL OIL AND ANTIOXIDANT PROPERTIES OF *GLAUCOSCIADIUM CORDIFOLIUM* (BOISS.) B.L.BURTT AND P.H.DAVIS PLANT ORGANS GROWING WILD IN TURKEY

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Abstract

Glaucosciadium cordifolium is distributed in Central Anatolia, the Mediterranean region and Northern Cyprus. It has been used in traditional medicine as an aphrodisiac. In the present study the essential oil content, oil components and antioxidant properties of *G. cordifolium* plant collected from the natural location were determined. It was observed that the essential oil content and oil components obtained from fresh and dry plant parts differed. The highest essential oil ratio (1.40%) was obtained from the dried leaf, while the lowest ratio (0.15%) was obtained from the fresh stem. Analysis results showed that the main components of the essential oil were 1-phellandrene (9.23-34.08%), α-pinene (10.23-31.95%), dl-limonene (10.39-22.21%) and cis-ocimine (6.84-12.45%). In addition, some of these main components were higher in dry plant parts, while others were higher in fresh plant parts. In terms of antioxidant properties, higher values were obtained in dry plant parts as well as in the essential oil ratio. While the maximum radical scavenging activity values (32.70%) were obtained in the dry stem, the lowest value (16.63%) was obtained in fresh leaves.

Introduction

The flowering plants belonging to *Apiaceae* consists of about 450 genera and 3700 species. It is widely distributed in temperate regions of both the northern and southern hemispheres and is highly diverse in Central Asia (Ozcan *et al.* 2021). This family is represented by 101 genera and 485 species (511 taxa) in Turkey and rich in commercial essential oils and has a wide traditional use. Yildiz *et al.* (2021) have investigated essential oils of many species belonging to *Apiaceae*.

Glaucosciadium cordifolium (Boiss.) B.L.Burtt and P.H. Davis has a characteristic smell and grow on stony river banks and chalky slopes and distributed in Central Anatolia, the Mediterranean region and Northern Cyprus. *G. cordifolium* has been used as an aphrodisiac in traditional medicine and is known as "sakarotu" or "çakşırotu" in Turkey. According to the Flora of Turkey, the genus *Glaucosciadium* Burtt & Davis is represented by one taxon in Turkey and two in the world (Davis 1982, Ozhatay and Koçak 2011, Karadag *et al.* 2019).

Karaman Ermenek District, both geographical and floristic in terms of climate, Central Anatolia is presented in the passage between the Mediterranean Regions, Davis (1982) enters the C4 frame according to his grid system for Turkey. Because Central Anatolia and Mediterranean climates meet on these habitats, the diversity in the vegetation of the region is understood automatically. Analysis of the flora of the region, showed that the ratio of Iran-Turan (21.20%) and Mediterranean (20.45%) elements is very close to each other and it may be considered as the result of this transition situation (Davis 1982, Maral *et al.* 2018).

Study on the essential oil composition and antioxidant activity of *G. cordifolium is not available.* Hence, the composition and antioxidant properties of the essential oil obtained from the fresh and dried fruit, stem and leaf of *G. cordifolium* were investigated.

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Materials and Methods

Fresh aerial parts of *G. cordifolium* were collected from Ermenek district of Karaman, Turkey in September 2020 and were dried at room temperature. For isolation, 25 g of samples were subjected to hydro-distillation with distilled water (1:10) by a neo-Clevenger type apparatus for three hrs in two replications. The oils were dried over anhydrous sodium sulfate and stored in dark vials at 4°C. The essential oil was obtained from fresh and dried stem, leaf and fruits.

A gas chromatography (GC) system (Agilent Technologies, 7890B) equipped with a flame ionization detector (FID) and connected to a mass spectrometer detector (MSD). Agilent Technologies, 5977A was used to identify the chemical components of EOs. The column for separation of the compounds was HP-Innowax (Agilent 19091N-116: 60 m × 0.320 mm inner diameter and 0.25 µm film thickness). The carrier gas was Helium (99.999%) with a flow rate of 1.3 ml min-1. The injection volume was set to 1 µl (20 µl EO dissolved in 1 ml n-hexane). The solvent latency was 8.20 min. The injection was carried out in split mode (40:1). Samples were analyzed with the column initially kept at 70°C after being injected with a 5 min retention time. Then the temperature was raised to 160°C with a 3°C min^{-1} heating ramp and 5 min holding time. Eventually, the temperature reached 250°C with a 6°C min^{-1} heating ramp and 5 min hold time. Detector, injector, and ion source temperatures were 270, 250, and 230°C, respectively. MS scan range was (Mz^{-1}): 50-550 atomic mass units (AMU) under electron impact (EI) ionization of 70 eV (Turkmen *et al.* 2022).

The retention indices (RI) were determined by injecting C7-C30 n-alkanes (Sigma-Aldrich) (GC/FID) into the system (Agilent Technologies, 7890B) under the same conditions of EO analyzes. The identification of EO components was determined by comparing retention indices, mass spectra to the US National Institute of Standards and Technology (NIST) computer library database, Wiley libraries, other published mass spectrum data (Adams 2007) and own database. The relative abundance (% area) is calculated based on the ratio between the peak area of each compound and the sum of the areas of all compounds. No response factors were calculated (Turkmen *et al.* 2022).

The DPPH method was used with some modifications for the antioxidant determination of the samples (Blois 1958, Brand-Williams *et al.* 1995). Accordingly, after the 20 µl sample was made up to 10 ml with methyl alcohol, 200µl of this solution was taken and 100 µl of 0.004/100ml DPPH's methanol solution was added. After the solution obtained was made up to 1 ml with methyl alcohol and kept in the dark for half an hour, the absorptions at 517 nm were read. The 0.9 ml of methyl alcohol and 0.1 ml of DPPH solution were mixed and read at 517 nm at 0 min and recorded as control. Methyl alcohol solution was evaluated as a blank sample. Radical scavenging activity (%) was calculated from the equation = $[(A_{Control}-A_{Sample}) / A_{Control}] \times 100$ (Maral *et al.* 2022).

The total phenolic content of fresh and dry plant parts was determined by using Folin-Ciocalteu assay (Singleton and Rossi 1965, Slinkard and Singleton 1977). In five different concentrations (0.2, 0.4, 0.6, 0.8 and 1 mg/ml) gallic acid solutions were prepared with 99.9% methanol. From the data, a plot of absorption versus concentration with R^2 value of 0.968 was obtained. Twenty µl of the extracted plant solution was diluted with methyl alcohol to 10 ml. Twenty µl of the solution was taken and 680 µl of distilled water, 400 µl of 0.5 N folin reagents (in water) and 400 µl of 10% Na_2CO_3 (in water) were added to the absorption values at 760 nm at the end of 30 min. The read absorption values were replaced by y in the obtained equation, and x values, in other words, phenolic equivalents in gallic acid were calculated.

Results and Discussion

The essential oil content of fresh and dried plant parts of *G. cordifolium* varied between 0.15-1.40%. The highest essential oil content was obtained in dry leaves with 1.40%, followed by dried fruit with 0.80% and dry stem with 0.75%. The lowest essential oil content (0.15%) was obtained from the fresh stem. The essential oil content of dry plant parts was higher than the essential oil of fresh plant parts (Table 1). Fresh plant parts lose 75-85% of their total weight during drying. Since the fresh leaf is distilled with the evaporated water during drying, it is an expected result that the essential oil ratio is relatively low (Aydın *et al.* 2019). Karadogan *et al.* (2015) reported that the essential oil content of *G. cordifolium*, collected from different flora, varied between 0.22-0.92%. Baser *et al.* (2000) reported 0.7% essential oil content of *G. cordifolium.*

GC-MS analysis of the essential oil of the fresh stem of *G. cordifolium* resulted in the identification of a total of 23 compounds amounting to 99.85 % of the total oil content. The major constituents of the essential oil were l-Phellandrene (28.64%), carvacrol (13.44%), α-Pinene (12.67%), dl-Limonene (12.26%) and cis-Ocimene (9.16%). Different classes of terpenoids were characterized. Monoterpene hydrocarbons had the highest (80.17%) contribution to this oil. Oxygenated monoterpenes were the second class of terpenoids detected in the oil obtained from the fresh stem and accounted for 14.04 % of the total oil content.

Analysis of the essential oil obtained from the dried stem resulted in the identification of a total of 17 compounds amounting to 98.47 % of the total oil composition. The oil was dominated by monoterpene hydrocarbons (92.13 %). dl-Limonene had the major contribution to this fraction (20.55%) and was followed by l-Phellandrene (20.53%), α-Pinene (18.70%), cis-Ocimene (12.45%) and β-Phellandrene (6.40%). The concentration of oxygenated monoterpenes had decreased to half its content as compared to the oil of the fresh plant (0.39, 14.04%, respectively).

A total of 18 compounds amounting to 99.40 % of the total oil content was obtained by GC-MS analysis of the essential oil of the fresh leaf of *G. cordifolium*. The major constituents of the essential oil were α-Pinene (31.95%), dl-Limonene (15.51%), β-Pinene (12.33%), carvacrol (11.31%) and l-Phellandrene (9.38%). Monoterpene hydrocarbons had the highest (84.39%) contribution to this oil. Oxygenated monoterpenes were the second class of terpenoids accounting for 11.40 % of the total oil content.

A total of 17 compounds amounting to 99.37 % of the total oil content of *G. cordifolium* was identified by GC-MS analysis of the essential oil of the dried leaf. The major constituents of the essential oil were α-Pinene (31.89%), dl-Limonene (22.21%), β-Pinene (15.15%), l-Phellandrene (9.23%) and cis-Ocimene (7.19%). Monoterpene hydrocarbons had the highest (93.59%) contribution to this oil.

In total, 25 compounds (98.34%) were identified for fresh fruit of *G. cordifolium* essential oil during chromatographic analysis. The major constituents of the essential oil were carvacrol (24.48%), l-Phellandrene (20.26%), dl-Limonene (10.39%), α-Pinene (10.23%) and cis-Ocimene (8.32%). Still, Monoterpene hydrocarbons had the highest (63.99%) contribution to this oil. Oxygenated monoterpenes were the second class of terpenoids accounting for 25.46% of the total oil content.

Twenty compounds (99.39%) were identified for dried fruit of *G. cordifolium* essential oil during chromatographic analysis. The major constituents of the essential oil were l-Phellandrene (34.08%), dl-Limonene (14.71%), α-Pinene (14.64%), cis-Ocimene (12.40%) and β-Phellandrene (8.89%). Monoterpene hydrocarbons had the highest (93.16%) contribution to this oil.

It was observed that there are visible qualitative and quantitative differences in the essential oil components of fresh and dried *G. cordifolium.* In addition to different climatic and soil characteristics, environmental factors such as temperature, light and day length might have a

Table 1. Essential oil components of fresh and dry plant parts of *Glaucosciadium cordifolium*.

RT	RI	Components	Fresh plant parts			Dry plant parts		
			stem	leaf	fruit	stem	leaf	fruit
8.670	1025	α-Pinene	12.67	31.95	10.23	18.70	31.89	14.64
9.677	1071	Camphene	0.15	0.30	0.12	0.21	0.31	0.17
10.753	1114	β-Pinene	2.29	12.33	1.67	4.41	15.15	2.35
11.068	1125	Sabinene	0.57	1.15	0.44	0.76	1.30	0.63
12.224	1162	β-Myrcene	1.30	1.73	1.07	1.75	1.80	1.56
12.470	1170	l-Phellandrene	28.64	9.38	20.26	20.53	9.23	34.08
13.585	1204	dl-Limonene	12.26	15.51	10.39	20.55	22.21	14.71
13.963	1214	β-Phellandrene	7.64	2.75	6.15	6.40	2.92	8.89
14.724	1234	cis-Ocimene	9.16	6.84	8.32	12.45	7.19	12.40
15.371	1251	cis-β-Ocimene	2.11	1.29	1.01	1.61	0.53	1.02
16.246	1275	ρ-Cimene	3.22	1.07	4.22	4.58	0.95	2.54
16.681	1286	α-Terpinolene	0.16	0.09	0.11	0.18	0.11	0.17
21.430	1403	Fenchone	0.13	nd	0.20	0.31	nd	0.16
29.430	1602	Caryophyllene	0.52	0.38	0.93	0.13	nd	0.22
29.538	1605	Isothymolmethylether	0.14	nd	0.34	nd	nd	nd
32.548	1684	β-Citral	nd	nd	0.11	nd	nd	0.10
34.15	1726	β-Bisabolene	0.20	0.22	0.55	nd	nd	nd
34.396	1733	Z-Citral	0.14	nd	0.16	nd	nd	0.20
35.392	1760	Citronellol	0.12	nd	0.24	nd	nd	nd
37.166	1806	α-Phellandrene	0.33	nd	0.39	nd	nd	nd
49.543	2176	Farnesol	nd	nd	nd	nd	1.56	nd
49.646	2180	Thymol	nd	0.09	0.12	nd	0.13	nd
49.817	2188	α-Cadinol	0.09	nd	0.33	nd	nd	0.36
50.309	2210	Carvacrol	13.44	11.31	24.48	0.08	nd	0.76
50.47	2218	α-Bisabolol	nd	nd	nd	nd	0.27	nd
56.254	2542	Butylidenephthalide	0.30	0.23	0.58	0.60	0.25	0.25
57.256	2602	3-Butylphthalide	4.27	2.78	5.92	5.22	3.57	4.18
Chemical grouped compounds (%)								
Oxygenated monoterpenes			14.04	11.40	25.46	0.39	0.13	1.22
Monoterpene hydrocarbons			80.17	84.39	63.99	92.13	93.59	93.16
Phthalides			4.57	3.01	6.50	5.82	3.82	4.43
Oxygenated sesquiterpenes			0.21	nd	0.57	nd	1.83	0.36
Sesquiterpene hydrocarbon			0.72	0.60	1.48	0.13	nd	0.22
Ether			0.14	nd	0.34	nd	nd	nd
Number of identified compounds			23	18	25	17	17	20
Total (%)			99.85	99.40	98.34	98.47	99.37	99.39
Essential oil content (%)			0.15	0.23	0.32	0.75	1.40	0.80

RT: Retention time; RI: Retention indices calculated against n-alkanes (C7-C30) on HP-Innowax column

major impact not only on essential oil composition but also on secondary metabolites. Other factors such as harvest time, drying method, extraction method, plant part investigated, all contribute to differences in oil composition. The main determinant of essential oil composition is certainly genetic factors and the determination of the chemotype of the species. In this study, it was revealed that in addition to the above factors, the fresh or dryness of the plant studied also affects the essential oil components.

Başer *et al.* (2000), reported that the main components of essential oil obtained from the aerial part of *G. cordifolium* are limonene (39.7%), α-pinene (12.3%) and β-pinene (10.3%).

In the present study, the highest inhibition value was observed in the dry stem with 32.70%, while the lowest value was observed in the fresh leaf with 16.63%. The radical scavenging activities of dried fruit, dried leaves, fresh stems and fresh fruit were determined as 28.37, 24.93, 21.19 and 18.88%, respectively. It was also determined that dry plant parts had higher radical scavenging activities than fresh plant parts (Table 2). When the samples are dried, their radical scavenging activities and accordingly the number of antioxidants transferred to the oil increase.

Table 2. Total phenolic content and radical scavenging activity of *Glaucosciadium cordifolium*.

Plant parts	Absorption values at 517 nm	Radical scavenging activity (%)	Phenolic equivalents in gallic acid (mg ml^{-1})
Fresh stem	0.30	21.19	7.80
Fresh leaf	0.38	16.63	7.37
Fresh fruit	0.31	18.88	7.96
Dry stem	0.25	32.70	7.31
Dry leaf	0.28	24.93	6.97
Dry fruit	0.27	28.37	7.36

From Table 2 it is apparent that the highest amount of phenolic acid was in fresh fruit (7.96 mg/ml), followed by the fresh stem (7.80 mg/ml) and fresh leaf (7.37 mg/ml), respectively. The amount of phenolic substance in terms of gallic acid in dry samples was lower than the fresh ones, and the order was the same (fruit, stem and leaf) as in the dry samples (Table 2). The antioxidant activity of monoterpene hydrocarbons was found to be higher than oxygenated monoterpenes, phthalides, sesquiterpene hydrocarbons or their oxygenated derivatives in *G. cordifolium*.

Quiroga *et al.* (2013), reported the γ-terpinene, β-phellandrene and terpinolene antioxidant activity. Foti and Ingold (2003), stated that terpenes and caryophyllene have antioxidant activity on lipids. Ozdemir *et al.* (2018) reported that γ-terpinene, α-terpinene, caryophyllene, l-phellandrene, p-cymene, α-pinene and β-pinene can contribute to antioxidant activity. In the present study, α-Pinene, β-Pinene, l-phellandrene, dl-limonene, β-phellandrene, cis-ocimene and carvacrol may have contributed to the antioxidant activity of *G. cordifolium* essential oils, and a synergistic effect may occur in the oil system.

The essential oil content, oil composition, total phenol content and antioxidant activity of *G. cordifolium* were strongly affected by the plant organs used and whether these organs were fresh or dry. It was observed that the dried plant parts contained a higher content of essential oil. The major components of the essential oil, α-pinene and carvacrol, were higher in fresh plant parts, while l-phellandrene and dl-limonene were higher in dry plant parts. The maximum radical scavenging activity values were higher in dried plant parts. Total phenol content was higher in

fresh plant parts in contrast to DPPH and essential oil content. It was determined that the studied plant parts contain significant amounts of phenolic compounds and have antioxidant properties. These data revealed the strong potential of these organs as a source of phenolic compounds with beneficial properties and a promising source of health products for the pharmaceutical industry. As a general result, it may be said that the use of dried plant parts was more suitable for optimum essential oil content, chemical properties and antioxidant activity, while fresh plant parts were determined to be more suitable for total phenol content. Thus, it may be concluded that it would be more appropriate to use fresh or dried plant parts according to the intended use.

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