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Original Article

Chemical characterization of *Glaucosciadium cordifolium* (Boiss.) B. L. Burtt & P. H. Davis essential oils and their antimicrobial, and antioxidant activities

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ABSTRACT

Chemical composition of volatile oils obtained from the roots, fruits and aerial parts of *Glaucosciadium cordifolium* (Boiss.) B.L. Burtt&P.H. Davis (Apiaceae) were analyzed using gas chromatography-flame ionization detector/mass spectrometry, simultaneously. Furthermore, antimicrobial and antioxidant activities of *G. cordifolium* volatile oils were investigated for possible utilization. Total of 62 volatile compounds were identified in *G. cordifolium* essential oils, where the main component was characterized as α-pinene in all parts, commonly. The other main components were β-pinene (15.7%), (*Z*)-β-ocimene (14%) and sabinene (7%) in the volatile oil of the aerial part; sabinene (10.1%), β-pinene (10.1%) and α-phellandrene (5.3%) in the essential oil of the fruits; hexadecane (12.2%), tetradecane (11.9%), octadecane (7.4%) in the essential oil obtained from the root, respectively. The *in vitro* microdilution method was used for the antimicrobial activity testing against *Salmonella typhi* ATCC 6539, *Acinetobacter baumanii* ATCC 19606, *Bacillus cereus* ATCC 14579, *Staphylococcus aereus* ATCC 6538, *Listeria monocytogenes* ATCC 19115, *Helicobacter pylori* ATCC 43504 and *Mycobacterium avium* ATCC 25291. The best antimicrobial activity of the volatile oils was against *L. monocytogenes* among the tested microorganisms. In addition, DPPH•-ABTS• scavenging activity was tested, none of the essential oils showed any significant antioxidant activity.

Keywords: Apiaceae, *Glaucosciadium cordifolium,* antimicrobial, antioxidant, gas chromatography, mass specrometry

INTRODUCTION

Glaucosciadium cordifolium (Boiss.) B. L. Burtt & P. H. Davis was used as an aphrodisiac in traditional medicine and known as "sakar otu" or "çakşır otu" in Turkey (Özhatay and Koçak 2011). According to the Flora of Turkey, the genus *Glaucosciadium* Burtt & Davis is represented by one taxon in Turkey and two taxa in the world (Davis 1982).

G. cordifolium has a charasteristic smell and grows in stony river banks, chalk screes and slopes (Davis 1982). This species is distributed in Central Anatolia, Mediterranean region and Northern Cyprus. Although the volatile oil composition of *G. cordifolium* aerial parts has been investigated previously (Baser et al. 2000), so far the volatile oil compositions of *G. cordifolium* fruits and roots have not been analyzed. Here, we report the comparative essential oil compositions of the aerial parts, fruits and roots of *G. cordifolium* by using gas chromatography-mass spectrometry (GC-MS) and flame ionization detector (FID) systems. In addition, antimicrobial-antioxidant activities of aforementioned volatile oils were studied by DPPH and ABTS radical scavenging and broth microdilution methods, respectively.

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To the best of our knowledge, this is the first report on the roots and fruits volatiles and antioxidant-antimicrobial activities of the volatile oils from different parts of *G. cordifolium*. The volatile oils were obtained by hydrodistillation method followed by the *in vitro* biological evaluation using various human pathogens and DPPH and ABTS as targets.

MATERIALS AND METHODS

Plant Material

The aerial parts, fruits and roots of *G. cordifolium* were collected in the vicinity of Karaman-Ermenek in September 2018. The voucher specimen has been deposited at the Herbarium of the Selcuk University (KNYA), Konya, Turkey (Voucher specimen no: 28001).

Distillation

Air dried aerial parts, fruits and roots were coarsely crushed and hydrodistilled using a Clevenger apparatus, separately. The aerial parts, fruit and root oils obtained in 0.5%, 0.4% and 0.2% yield were dried using anhydrous sodium sulfate and kept at 4°C until GC and GC-MS analyses as well as biological assays, respectively.

Chromatospectral Analyses

The Agilent 5975 GC-MSD system was used for GC-MS studies. Innowax FSC column with 60 m x 0.25 mm, 0.25 µm film dimensions and helium with 0.8 mL/min rate were used. GC oven conditions were set as follows; 60°C for 10 minutes, 220°C with 4°C/min ascending rate, 220°C for 10 minutes and 240°C with 1°C/min ascending rate along with split ratio of 40:1 and 250°C injector temperature. Mass spectra measurements were performed at 70 eV with *m/z* 35 to 450 range.

An Agilent 6890N GC system was used for the GC-FID analyses. The temperature of the FID detector was set to 300°C. Concurrent auto-injection was performed in two identical columns using the same conditions in the GC/MS system. Relative percentages (%) were calculated using FID chromatograms (see Table 1). Relative retention indices were used to characterize the essential oil components. This process was held either by authentic samples or analyzing relative retention index (RRI) of n-alkanes, along with GC/MS Library, MassFinder 3 Library, in-house "Başer Library of Essential Oil Constituents"(ESO 1999).

Antimicrobial Activity

The antimicrobial activity of the essential oil was determined using the broth microdilution assay (CLSI 2006).

Salmonella typhi ATCC 6539, *Acinetobacter baumanii* ATCC 19606, *Bacillus cereus* ATCC 14579, *Staphylococcus aereus* ATCC 6538, and *Listeria monocytogenes* ATCC 19115 strains were grown in Mueller Hinton Broth (MHB, Merck, Germany). All microorganisms were standardized to 1×10^8 CFU/mL using McFarland No: 0.5 in sterile saline (0.85%). Serial dilutions were prepared from the sample. Each strain along with the diluted samples were added to the wells and then allowed to incubate at 37°C for 24 hours.

Helicobacter pylori ATCC 43504 were grown for 24 hours in Brucella broth containing %5 (v/v) horse blood Colombia agar and containing %10 (h/h) fetal bovine serum (FBS) at 37°C in an anaerobic incubator (%5 CO_2). After the plates had been incubated at 37°C, 100 µL of 1:10 diluted and density modulated *H. pylori*'s strain was added to each microtitration petris (EUCAST 2011; Whitmire and Merrell 2012).

Mycobacterium strains were inoculated in Middlebrook 7H11 agar (Sigma Aldrich), and incubated in aerobic conditions at 37°C for 4-5 days. The microorganism was transferred to the cation doped MHB and incubated for a further five days. Growing cultures were vortexed and allowed to collapse for 30 min. Diluted bacterial suspensions (10^6 CFU/mL) were added to each well and then allowed to incubate at 37°C for 5 days (CLSI 2003; Chung et al. 1995; Lee et al. 2007).

The minimum inhibitory concentrations (MIC) were calculated as mean of three repetitions.

Antioxidant Activity

DPPH radical scavenging assay

The antioxidant capacity was determined using 2,2-Diphenyl-1-picrylhydrazyl (DPPH·) scavenging activity (Blois 1958). The reaction mix contained 100 µM DPPH· and several concentrations of the crude extract. After 30 min, absorbance was read at 517 nm by using an UV–Vis spectrophotometer at 25±2°C and the radical scavenging activity (RSA) was determined as the percentage of radical reduction as follows:

$$DPPH^{\cdot}\ RSA\ \% = [(Absorbance_{control} - Absorbance_{test\ sample})/Absorbance_{control})] \times 100$$

Each experiment was performed in triplicate. Ascorbic acid was used as the reference (Okur et al. 2018).

ABTS radical scavenging assay

For the second method ABTS RSA is used to determine the antioxidant activity of the essential oils (Re et al. 1999). ABTS radicals were produced by reacting 7 mM aqueous ABTS radical and 2.45 mM potassium persulfate. The mixture was left at 25°C for 12 h in the dark. The colored ABTS· was diluted with ethanol. Absorbance was measured at 734 nm. The assay was performed in triplicate. Ethanol was used as the negative control. The assay was carried out on Trolox as a positive control, the water-soluble α-tocopherol analogue. The results were expressed as IC_{50} as follows:

$$ABTS^{\cdot}\ RSA\ \% = [(Absorbance_{control} - Absorbance_{test\ sample})/Absorbance_{control})] \times 100$$

RESULTS AND DISCUSSION

The air dried root material was hydrodistilled in a Clevenger-type apparatus for 6 hours to yield a dark yellow oil. Aerial part and fruit materials have light yellow oils and hydrodistilling procedures same as the roots. The *G. cordifolium aerial part, fruit and root* oils yield were 0.5% (v/w), 0.4% (v/w), 0.2% (v/w), respectively which were consquently analyzed both by GC-FID and GC-MS, simultaneously. Sixty-two compounds were identified in *G. cordifolium* essential oils obtained from different parts constituting

Table 1. Essential oil components of *G. cordifolium*

RRI	Compound	GcH %	GcF %	GcR %
1000	Decane	0.2	-	tr
1032	α-Pinene	27.7	60.8	18.4
1035	α-Thujene	1.1	0.3	tr
1076	Camphene	0.3	0.4	tr
1100	Undecane	-	-	0.3
1118	β-Pinene	15.7	6.8	0.4
1132	Sabinene	7.0	10.1	0.7
1174	Myrcene	3.0	2.8	1.2
1176	α-Phellandrene	6.0	5.3	0.3
1200	Dodecane	-	-	5.4
1203	Limonene	2.5	1.7	2.0
1218	β-Phellandrene	2.8	1.9	1.2
1246	(*Z*)-β-Ocimene	14.0	2.7	1.7
1255	γ-Terpinene	0.2	0.1	0.5
1266	(*E*)-β-Ocimene	2.9	0.3	0.5
1280	p-Cymene	1.8	1.5	2.3
1290	Terpinolene	0.2	0.1	1.6
1296	Octanal	-	-	0.3
1300	Tridecane	0.1	-	0.4
1400	Nonanal	-	-	0.3
1400	Tetradecane	-	-	11.9
1438	Tetradec-1-ene	-	-	0.5
1476	(*Z*)-β-Ocimene epoxide	0.2	-	-
1477	4,8-Epoxyterpinolene	-	-	0.3
1497	α-Copaene	-	0.3	-
1500	Pentadecane	-	-	1.0
1549	β-Cubebene	-	0.1	-
1600	β-Elemene	0.1	0.4	-
1600	Hexadecane	-	-	12.2
1611	Terpinen-4-ol	0.2	0.1	-
1612	β-Caryophyllene	2.1	0.3	-
1648	Myrtenal	0.2	-	-
1650	γ-Elemene	-	tr	-
1655	(*E*)-2-Decenal	-	-	0.9
1668	(*Z*)-β-Farnesene	-	0.2	-
1670	*trans*-Pinocarveol	0.2	0.1	-
1683	*trans*-Verbenol	-	0.3	1.0
1687	α-Humulene	-	0.2	-
1700	Heptadecane	-	-	0.1
1726	Germacrene D	-	0.6	-
1773	δ-Cadinene	0.2	0.7	-
1786	ar-Curcumene	-	0.2	-
1800	Octadecane	-	-	7.4
1804	Myrtenol	0.1	-	-
1823	*p*-Mentha-1(7), 5-dien-2-ol	0.1	0.2	-
1854	Germacrene-B	-	0.2	-
1864	*p*-Cymen-8-ol	-	-	0.8
1933	Tetradecanal	0.4	-	-
2000	Eicosane	-	-	3.4
2008	Caryophyllene oxide	0.5	-	-
2144	Spathulenol	0.1	0.2	-
2200	Docosane	-	-	1.3
2209	T-Muurolol	0.3	-	-
2273	Selin-11-en-4≈-ol	-	0.3	-
2296	Myristicine	-	-	0.3
2384	Dill apiole	-	-	0.5
2400	Tetracosane	-	-	0.4
2512	Benzophenone	-	-	2.2
2554	(*E*)-3-Butylidene phthalide	1.5	-	-
2609	(*Z*)-3-Butylidene-3, 4-dihydro phthalide (=(*Z*)-Ligustilide)	4.8	-	-
2655	Benzyl benzoate	-	-	0.7
2931	Hexadecanoic acid	-	-	1.8
	Monoterpene Hydrocarbones	85.2	94.8	30.8
	Oxygenated Monoterpenes	1.0	0.7	2.1
	Sesquiterpene Hydrocarbones	2.4	3.2	-
	Oxygenated Sesquiterpenes	0.9	0.5	-
	Fatty acid+esters	-	-	1.8
	Others	7.0	-	49.5
	Total	96.5	99.2	84.2

RRI: Relative retention indices calculated against *n*-alkanes
% calculated from FID data
GcH: *G. cordifolium* aerial part essential oil; GcF: G. *cordifolium* fruit essential oil; GcR: *G. cordifolium* root essential oil

Table 2. Antioxidant activity of *G. cordifolium* essential oils

	GcH	GcF	GcR	References
	IC50 ±SD (mg/mL)			
DPPH•	1.14±0.086	1.02±0.07	1.18±0.052	0.004±0.001 (Ascorbic acid)
ABTS•	0.94±0.075	1.01±0.069	1.09±0.075	0.015±0.008 (Trolox)

GcH: *G. cordifolium* aerial part essential oil; GcF: *G. cordifolium* fruit essential oil; GcR: *G. cordifolium* root essential oil

84.2-99.2% of the total oil. The essential oils were dominated by monoterpene hydrocarbons. These sixty-two volatile compounds are listed in Table 1 with their relative percentages. Main components were found as α-pinene (27.7%), β-pinene (15.7%), (*Z*)-β-ocimene (14%), sabinene (7%) for aerial part; α-pinene (60.8%), sabinene (10.1%), β-pinene (10.1%), α-phellandrene (5.3%) for

Table 3. Antimicrobial activity of *G. cordifolium* essential oils (MICs in mg/mL)

Bacteria / Sample	*St*	*Sa*	*Lm*	*Ab*	*Hp*	*Bc*	*Ma*
GcH	>10	>10	0.156	>10	>10	0.625	>10
GcF	>10	>10	0.078	>10	>10	0.312	>10
GcR	>10	>10	0.078	>10	>10	1.25	>10

(+ control) Antimicrobial: Chloramfenicol
(- control) DMSO.
GcH: *G. cordifolium* aerial part essential oil; GcF: *G. cordifolium* fruit essential oil; GcR: *G. cordifolium* root essential oil; *St: Salmonella typhii; Sa: Staphyllococcus aureus; Lm: Listeria monocytogenes; Ab: Acinetobacter baumanii; Hp: Helicobacter pylori; Bc: Bacillus cereus; Ma: Mycobacterium avium*

fruit; α-pinene (18.4%), hexadecane (12.2%), tetradecane (11.9%), octadecane (7.4%) for root essential oil, respectively. In a previous study, limonene (39.7%), α-pinene (12.3%) and β-pinene (10.3%) were found as main components of the oil (0.7%) obtained from the aerial part of *G. cordifolium* (Baser et al. 2000). It can be thought that this is due to the collection of plant materials from different locations. It can be seen from the results, location differences in plants can change the phytochemistry of plants and hence biological activities. However, the essential oil of the aerial part includes phthalides such as (*Z*)-ligustilide (1.5%) and (*E*)-3-butylidene phthalide (4.8%) which are the important volatiles of *Apium graviolens* and some other Apiaceae plants. These compounds provide the characteristic odor of the celery specific to the plant.

Results of DPPH-ABTS radical scavenging activities are shown in Table 2. In DPPH testing system, RSA IC_{50} value of *G. cordifolium* aerial part, fruit and root essential oils were determined as 1.14, 1.02, and 1.18 mg/mL, respectively. When checked, in the ascorbic acid results (0.004 mg/mL) the oils were less effective than those of the standard ascorbic acid. In addition, the ABTS radical scavenging activity was also found at moderate levels (0.94, 1.01, and 1.09 mg/mL) and the results were compared with the standard Trolox (0.015 mg/mL).

Some pathogenic Gram (+) and (–) bacteria are listed in Table 3, were challenged with *G. cordifolium* essential oils. Among the tested bacteria in this present study, *L. monocytogenes* was the more sensitive to the essential oils, while *H. pylori* and *M. smegmatis* appeared to be the most resistant. Growth of *L. monocytogenes* was remarkably inhibited by essential oil of *G. cordifolium* aerial part, root and fruit parts. This present study results indicated that these volatile oils can be natural, potential antimicrobial agents in the food industry and improve the microbial safety of foods. The results of this study were promising for the use of these oils as an antimicrobial ingredient for the safety foodborne microorganisms.

As a conclusion, to the best of our knowledge, this is the first comparative report on the volatiles and *in vitro* antioxidant-antmicrobial activities of *G. cordifolium* aerial part, root and fruit essential oils.

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