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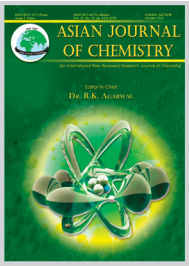

Chemical Composition and Antimicrobial Activity of the Volatile of *Gladiolus atroviolaceus* Boiss.

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In the present study, essential oil extracted by hydrodistillation from *Gladiolus atroviolaceus* Boiss. (Iridaceae) was analyzed by gas chromatography (GC) and GC-mass spectrometry (MS). A total of 52 compounds were identified, constituting over 93.8 % of the oil composition of *G. atroviolaceus*. Hexadecanoic acid (44.4 %) and heneicosane (6.6 %) were the major compounds in the volatile of *G. atroviolaceus*. Sesquiterpene hydrocarbons were shown to be the main groups of terpenoids in the oils (2.6 %). The major terpene constituents of the essential oil of *G. atroviolaceus* were limonene, γ-muurolene and linalool. The antimicrobial activity of the isolated essential oil of the plant was also investigated and it showed moderate antimicrobial and antifungal activities against *Staphylococcus aureus, Bacillus cereus, Mycobacterium smegmatis* and *Candida albicans*.

Key Words: *Gladiolus atroviolaceus*, Essential oil, Antimicrobial activity, GC-MS.

INTRODUCTION

The genus *Gladiolus* L. (Iridaceae) is represented with 9 species, 4 of them is endemics, in Turkey[1]. It is a perennial bulbous plant. It has very nice flowers and a typicall ovoid corm enclosed by several layers of fibrous tunics. Aerial parts of *G. atroviolaceus* Boiss., *G. italicus* Miller and *G. kotschyanus* Boiss. are collected and sold by local people in Turkey[2]. *G. atroviolaceus* Boiss. is mainly distributed in stony calcareous slopes, roadsides and cornfields between 650-2150 m in Inner Anatolia[1]. It is known as purple gladiolus in Turkey and corms are used as aphrodisiac and vomitory[3].

Previous phytochemical investigation of the genus *Gladiolus* L. (*G. atroviolaceu*, *G. gandavensis* and *G. segetum*) revealed the presence of anthocyanidins, flavonols, anthraquinones and saponin type natural compounds[4-12]. The biological activities of *Gladiolus* species have also been mentioned[13,14]. However, no previous reports dealing with any investigation on the composition and antimicrobial activity of the essential oil of *G. atroviolaceus* is available in the literature. The aim of this work is to perform a detailed compositional analysis of the volatiles isolated from the mentioned taxa originating from Turkey. Furthermore, we also tested the antimicrobial activity of the essential oil against a panel of microorganisms.

EXPERIMENTAL

G. atroviolaceus was collected in Yagmurdere valley, Gümüshane-Turkey (at heights of ~ 2020 m) in the northeastern part of Turkey in May, 2010. The plant was authenticated by Prof. S. Terzioglu. Voucher specimen was deposited in the Herbarium of the Faculty of Forestry, KATO (KATO: 9617), Karadeniz Technical University, Turkey.

Hydrodistillation apparatus and procedure: The fresh plant material (175 g) were grounded into small pieces and submitted to hydrodistillation (HD) using a Clevenger-type apparatus with cooling bath (-15 ºC) system (4 h) (yield (w/w): 0.033 %). The obtained oil was extracted with HPLC grade *n*-hexane (0.5 mL) and dried over anhydrous sodium sulphate and stored at -5 ºC in a sealed brown vial.

Gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) analysis: GC-FID and GC-MS analyses were done as described previously[15].

Identification of constituents: The components of the oil was characterized by comparison of their mass spectra with those of a computer library or with authentic compounds (α-pinene, limonene, linalool, α-terpineol, heptadecane, heneicosane, docosane, tricosane, tetracosane and pentacosane) and confirmed by comparison of their retention indices, either with those of authentic compounds or with data published in the literature[16-29].

Antimicrobial activity assessment: All test microorganisms were obtained from the Hifzissihha Institute of Refik Saydam (Ankara, Turkey) and were as follows: *Escherichia coli* ATCC 25922, *Yersinia pseudotuberculosis* ATCC 911, *Pseudomonas auroginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Bacillus cereus* 709 ROMA, *Mycobacterium smegmatis* ATCC607 and *Candida albicans* ATCC 60193. The oils were dissolved in hexane to prepare chemicals stock solution of 10.000 µg/400 µL.

Agar well diffusion method: Simple susceptibility screening test using agar-well diffusion method[30] as adapted earlier[31] was used. Each bacterium was suspended in Mueller Hinton (Difco, Detroit, MI) broth. The yeast like fungi was suspended in yeast extracts broth. Then the microorganisms were diluted approximately 10^6 colony forming unit (cfu) per mL. For yeast like fungi, sabouraud dextrose agar (SDA) (Difco, Detriot, MI) were used. Brain heart infusion agar (BHI) (Difco, Detriot, MI) was used for *M. smegmatis*. They were flood-inoculated onto the surface of Mueller Hinton and sabouraud dextrose agars and then dried. Five-millimeter diameter wells were cut from the agar using a sterile cork-borer and 40 µL of the extract substances were delivered into the wells. The plates were incubated for 18 h at 35 ºC. The *M. smegmatis* was grown for 3 days on brain heart infusion agar plates at 35 ºC[32]. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organism. Ampicillin (10 µg), streptomycin (10 µg) and fluconazole (5 µg) were standard drugs. Hexane was used as control solvent.

RESULTS AND DISCUSSION

The volatile oil obtained after hydrodistillation of the *G. atroviolaceus* gave average yield of 0.033 %. GC-MS analyze allowed the identification of 52 volatile compounds[16-29] accounting for 93.8 % of the detected GC peak areas. The list of the identified volatile constituents as well as their grouping into eight classes, namely monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpenes, terpene related compounds, aldehydes, hydrocarbons and others with the ratios are given in Table-1. The oil was characterized by high content of acid and ester (55.2 %) and hydrocarbons (19.4 %). Hexadecanoic acid (44.4 %) and heneicosane (6.6 %) were major constituents of the oil of *G. atroviolaceus*. Sesquiterpene hydrocarbones were found as the major group of terpene compounds in *G. atroviolaceus*, constituted 2.7 % of the oils. Limonene (2.2 %), γ-muurolene (1.0 %), viridiflorol (0.7 %) and linalool (0.6 %) were identified as the main components of terpenoids in the oil of *G. atroviolaceus*.

To our best of knowledge, no previous report is found dealing with any investigation on the chemical constituents of the essential oil of *Gladiolus* species. Therefore, we were not able to compare our result. Generally, the comparison of our data with those of literature with other species showed that

TABLE-1
IDENTIFIED COMPONENTS IN THE OIL OF *G. atroviolaceus*

Compounds	Area[a] (%)	Exp. RI[b]	Lit. RI
Monoterpene hydrocarbons			
α-Pinene[c]	0.1	939	939
p-Mentha-1(7),8-diene	0.1	1004	1004
Limonene[c]	2.2	1029	1031
Monoterpenoids			
Linalool[c]	0.6	1100	1097
α-Terpineol[c]	0.2	1188	1189
p-Menth-1-en-9-al	0.3	1216	1217
β-Cyclocitral	0.2	1217	1217
Sesquiterpene hydrocarbons			
α-Copaene	0.1	1377	1377
Aromadendrene	0.1	1442	1441
β-Copaene	0.3	1431	1432
Alloaromadendrene	0.2	1460	1460
γ-Muurolene	1.0	1479	1480
trans-Muurola-4(14),5-diene	0.3	1493	1494
γ-Amorphene	0.3	1496	1496
Epizonarene	0.4	1502	1502
Sesquiterpenoids			
Spathulenol	0.2	1579	1578
Viridiflorol	0.7	1593	1593
Epi-α-Muurolol	0.4	1644	1642
Cubenol	0.2	1647	1647
α-Cadinol	0.5	1654	1654
Mint sulfide	0.2	1742	1741
Terpene related compounds			
α-Ionene	0.7	1254	1255
1,6,8-Trimethyl-1,2,3,4-tetrahydro naphthalene	1.4	1284	MS1
1,1,5,6-Tetramethyl indane	0.4	1324	MS2
(6*E*)-6-Methyl-5-(1-methylethylidene)-6,8-nonadien-2-one	1.2	1332	MS3
1,1,6-Trimethyl-1,2-dihydronaphthalene	0.6	1352	1354
E-β-Damascenone	0.2	1385	1385
Geranyl acetone	0.2	1455	1455
E-β-Ionone	0.3	1486	1489
Hexahydro farnesylacetone	0.7	1847	1847

Compounds	Area[a] (%)	Exp. RI[b]	Lit. RI
Aldehydes			
Benzaldehyde	0.2	959	960
Benzene acetaldehyde	1.6	1041	1042
Nonanal	1.2	1101	1101
2*E*,6*Z*-Nonadienal	0.5	1155	1155
2*E*-Nonenal	1.0	1160	1162
2*E*,4*E*-Decadienal	0.4	1314	1317
Hydrocarbons			
Heptadecane[c]	0.5	1704	1700
Heneicosane[c]	6.6	2101	2100
Docosane[c]	6.0	2203	2200
Tricosane[c]	1.5	2300	2300
Tetracosane[c]	2.5	2399	2400
Pentacosane[c]	2.3	2500	2500
Others			
2-Methyl butyl acetate	2.0	882	881
2-Pentyl furan	0.4	992	993
Benzyl benzoate	0.4	1764	1760
Tetradecanoic acid	0.5	1769	1768
Pentadecanoic acid	0.7	1867	1866
Methyl hexadecanoate	1.3	1918	1922
Hexadecanol	0.1	1880	1876
Hexadecanoic acid	44.4	1981	1980
Heptadecanoic acid	4.3	2072	2070
Ethyl linoleate	1.1	2158	2161
			Number of compounds
Monoterpene hydrocarbons	2.4		3
Monoterpenoids	1.3		4
Sesquiterpene hydrocarbons	2.7		8
Sesquiterpenoids	2.2		6
Terpene related compounds	5.7		9
Aldehydes	4.9		6
Hydrocarbons	19.4		6
Others	55.2		10
Total	93.8		52

[a]Percentages obtained by FID peak-area normalization. [b]RI calculated from retention times relative to that of *n*-alkanes (C_6-C_{32}) on the non-polar HP-5 column. [c]Identified by authentic samples.; MS1: 174(24), 165(4), 160(12), 159(100), 144(16), 129(15); MS2: 174(32), 160(12), 159(100), 144(16), 105(5); MS3: 192(18), 159(20), 134(22), 121(38), 119(100), 105(42), 91(44), 65(10)

TABLE-2
SCREENING RESULT FOR ANTIMICROBIAL ACTIVITY OF THE ESSENTIAL OIL OF *G. atroviolaceus*

Samples	Stock (µg/ 400 µL)	Microorganisms and inhibition zone (mm)							
		Ec	*Yp*	*Pa*	*Sa*	*Ef*	*Bc*	*Ms*	*Ca*
G. atroviolaceus	10.000	-	-	-	7	-	7	25	10
Ampicillin	10	10	10	18	35	10	15	-	-
Streptomycin	10	-	-	-	-	-	-	35	
Fluconazole	5	-	-	-	-	-	-	-	25

Ec: E. coli, *Yp*: *Y. pseudotuberculosis*, *Pa*: *P. aeruginosa*, *Sa*: *S. aureus*, *Ef*: *E. faecalis*, *Bc*: *B. cereus* 702 Roma, *Ms*: *M. smegmatis*, *Ca*: *C. albicans*, (-): no activity

the main constituent of chemical composition of the investigated *G. atroviolaceus* oil was markedly different. The difference of the composition of the oil could be attributed to the family, climate and geographical origin.

The antimicrobial activity for the essential oils of *G. atroviolaceus* was tested *in vitro* using the agar-well diffusion method[30-32] with the microorganisms as given in Table-2. The essential oils showed moderate antibacterial activity against Gram-positive bacteria *S. aureus, B. cereus* 702 Roma, *M. smegmatis* and pathogenic fungi (*C. albicans*).

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