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Comparative Gas Chromatography-Mass Spectrometric Analysis of Biologically Active Volatile Phytochemical Constituents of Aerial Parts and Roots of *Coronopus didymus*

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Coronopus didymus is a medicinal plant belonging to family Brassicaceae. Volatile phytochemical constituents from various extracts of *Coronopus didymus* were investigated by gas chromatography–mass spectrometry to analyze the biologically active constituents of the herb. A total of 69 compounds were identified including 34 from the aerial parts and 35 from roots extract. Despite the parts used, this herb contained mainly volatile esters and sulfur containing compounds. In the *n*-hexane extract of the aerial parts, ethyl (9*Z*,12*Z*,15*Z*)-octadecatrienoate (22.33 %), ethyl 9*Z*,12*Z*-octadecadienoate (9.04 %) and ethyl hexadecanoate (8.80 %) were the major constituents, while in the roots extract benzyl isothiocyanate (20.51 %) and ethyl hexadecanoate (8.44 %) were found to be the major constituents. It was found that aerial parts are quite rich in various biologically active acetates while the roots are quite rich in benzyl isothiocyanate.

Keywords: ***Coronopus didymus*, GC-MS, Phytochemical constituents, Benzyl isothiocyanate, Pharmaceutical applications.**

INTRODUCTION

Almost all plants comprise many different compounds and can indeed be highly complex in composition involving several hundred types of volatile molecules. Volatile components are generally volatile at typical ambient temperature and are to be detected in the fragrance profiles of plants. The types of volatile components produced by plants are chemically highly diverse comprising major groups such as esters, alcohols, terpenoids, sulfur containing compounds, aldehydes, ketones and the benzoates, as well as many other minor groups. The natural volatile compounds produced by plants play key roles in the long-term fitness and survival of the plants as well as being of direct/indirect benefit to man. They have an even more important role in plant survival. Many such components are known to have a certain bio-activity in that they may act as anti-pest or anti-grazing agents by having an unpleasant effect on attacking organisms. Volatile compounds from medicinal and aromatic plants have also been known since ancient times to possess many biological activities, especially antibacterial, antifungal and antioxidant properties [1].

Coronopus didymus Linn (Brassicaceae) is an annual herb, commonly known as lesser swinecress. It is found throughout Pakistan, known by various names *i.e.*, Janglihaloon in Rawalpindi [2] and Gandabotay in Khyber Pukhtoon Khwa [3]. It is a low spreading plant with many long stems and deeply lobed leaves and small white flowers with a strong and very distinctive aroma that is found along roadside, in wastelands as well as a weed of cultivated areas [2]. Traditionally *C. didymus* in fresh form is usually eaten in salads or crushed and used as medicine to purify the blood and to cure various diseases like cough, bronchitis, rheumatism, gastric and urinary illnesses, muscular pain, wounds, external ulcers, *etc*. [4].

Perusal of literature reveals that biologically active volatile organic compounds (VOCs) of *C. didymus* were not reported. Over the years gas chromatography-mass spectrometry (GC-MS) has been widely applied to address the volatile organic compounds of herbs. Hence, the objective of this study is to explore the volatile constituents of *C. didymus* by subjecting the *n*-hexane extracts of the aerial parts and roots of the plant to GC-MS analyses, to develop a comprehensive profile of constituents and to ascertain the rationale for its use in traditional medicine. The resulting volatile profile was compared with constituents already reported in other plant studies.

EXPERIMENTAL

Coronopus didymus plant was collected from Sector I-8/1 Islamabad, Pakistan in April 2014. The identity of plant was authenticated by Prof. Dr. Mir Ajab Khan of Department of Plant Sciences, QAU, Islamabad, Pakistan. A voucher specimen (No. 74) has been deposited in the herbarium of the same Department.

Extraction of the plant material: Air-dried 20 g each of aerial parts and roots of *C. didymus* were extracted with *n*-hexane (150 mL) separately and allowed to stand at room temperature for 24 h accompanying occasional shaking and stirring. The next day extracts were ultrasonicated for 0.5 h using ULTRASONIC LC 30 H. Later, these extracts were filtered and the resulting filtrates were concentrated under reduced pressure to as low as 1 mL using rotary evaporator (BÜCHI Rotavapor R-200) and refrigerated at 4 °C.

GC-MS analysis: GC-MS analysis was performed using an Agilent 6890N gas chromatograph and an Agilent 5973 mass spectrophotometer with electron impact (EI) ionization mode, having ionization voltage: 70 eV; start stop masses (*m/z*): 0-1000 *a.m.u.*; MS time: 35 min. Separation was achieved with a fused silica capillary column DB-5MS with a 5 % phenyl methylpolysiloxane as a stationary phase (30 m × 0.25 mm i.d. × 0.25 µm film thickness); injection mode: splitless performed manually; injection volume: 5 µL; injection pressure: 60-85 *p.s.i.*; ramping temperature range: 120-280 °C with regular increase of 12 °C per min; carrier gas flow rate: helium at 1 mL/min; gas temperature: 120 °C; analyzer: quadruple; detector: photomultiplier tube; software used: Enhanced Chem Station; Library used: National Institute of Standard and Technology (NIST) Ver. 02.L-year 2002.

Identification of components: The task of identification of phytochemical constituents was made by comparing their recorded mass spectra with those stored in the Database of NIST Library of the GC-MS data system or with published mass spectra.

RESULTS AND DISCUSSION

Volatile compounds of various extracts of *C. didymus* were characterized by their strong aroma. Gas chromatography-mass spectrometry (GC-MS) analyses of various extracts of *C. didymus* led to the identification of 69 different phytochemical compounds. Total ion current (TIC) chromatogram of aerial parts (Fig. 1) provided evidence for 34 phytochemical constituents while in roots (Fig. 2) 35 compounds could be identified. Total ion current time appearing on TIC profile gave an idea about the volatility of the compounds. The compounds eluting at low retention time were considered to be more volatile.

The key categories of volatile compounds found in *C. didymus* were; esters, alcohols, aldehydes, ketones and sulfur-containing compounds. Identified phytochemicals in the aerial parts and roots with their retention time (Rt), molecular weight (m.w.), molecular formula, compound name, library match percentage and peak area percentage are listed (Tables 1 and 2, respectively). It was found that constituents of roots are more diversified than aerial parts. Benzyl isothiocyanate was

TABLE-1
IDENTIFIED PHYTOCHEMICAL COMPOUNDS FROM *C. didymus* AERIAL PARTS

S. No.	Retention time (min)	Compound name	m.f.	m.w. (a.m.u.)	Library match (%)	Peak area (%)
1	2.127	1-Methyl-2-(1-methylethyl)benzene	$C_{10}H_{14}$	134	96	1.53
2	2.190	Benzyl alcohol	C_7H_8O	108	76	0.83
3	2.201	3-Methyl-phenol	C_7H_8O	108	72	0.52
4	2.264	2-(1,1-Dimethylethyl)-1,4-benzenediol	$C_{10}H_{14}O_2$	166	55	1.00
5	2.436	3-Amino-2-methylbenzyl alcohol	$C_8H_{11}NO$	137	46	1.17
6	2.567	Benzyl thiol	C_7H_8S	124	64	1.96
7	2.716	Phenylethyl alcohol	$C_8H_{10}O$	122	72	1.70
8	2.739	1-Methyl-3-(1-methylethyl)benzene	$C_{10}H_{14}$	134	50	1.19
9	2.899	2-Methylbenzonitrile	C_8H_7N	117	86	1.56
10	3.112	Benzyl nitrile	C_8H_7N	117	96	1.13
11	3.791	Ethyl benzoate	$C_9H_{10}O_2$	150	86	1.47
12	5.053	Benzyl isothiocyanate	C_8H_7NS	149	87	8.16
13	5.423	Vanillin	$C_8H_8O_3$	152	49	0.91
14	5.760	2-Methyl-5-butylpyridine	$C_{10}H_{15}N$	149	81	1.76
15	8.558	1,2-Benzisothiazol-3(2*H*)-one	C_7H_5NOS	151	47	1.64
16	8.656	4-Amino-9-fluorenone	$C_{13}H_9NO$	195	38	1.37
17	9.245	*N*-(Phenylmethylene)benzenemethanamine	$C_{14}H_{13}N$	195	87	0.25
18	9.531	3-Acridinol	$C_{13}H_{19}NO$	195	49	1.87
19	10.773	Methyl 13-methylpentadecanoate	$C_{17}H_{34}O_2$	270	38	1.32
20	10.790	Methyl hexadecanoate	$C_{17}H_{34}O_2$	270	83	1.61
21	11.208	3-Methylene-1,6-heptadiene	C_8H_{12}	108	42	3.47
22	11.442	Ethyl hexadecanoate	$C_{18}H_{36}O_2$	284	91	8.80
23	11.448	Ethyl cyclohexanepropionate	$C_{11}H_{20}O_2$	184	53	2.57
24	12.438	1,2-Pentadiene	C_5H_8	68	43	1.92
25	12.501	1,3-Cyclooctadiene	C_8H_{12}	108	76	2.28
26	12.507	3*E*-Tetradecen-5-yne	$C_{14}H_{24}$	192	47	1.21
27	12.564	*Bis*(phenylmethyl)disulfide	$C_{14}H_{14}S_2$	246	64	3.43
28	12.575	Phytol	$C_{20}H_{40}O$	296	86	4.20
29	13.033	Ethyl 9Z,12*Z*-octadecadienoate	$C_{20}H_{36}O_2$	308	95	9.04
30	13.107	Ethyl 9Z,12*Z*,15*Z*-octadecatrienoate	$C_{20}H_{34}O_2$	306	99	22.33
31	13.302	Ethyl 15-methylheptadecanoate	$C_{20}H_{40}O_2$	312	81	3.47
32	15.139	5-(4-Ethoxy-phenyl)-5-methyl-imidazolidine-2,4-dione	$C_{12}H_{14}N_2O_3$	234	35	1.44
33	16.204	3-Methyl-1,8-naphthyridine	$C_9H_8N_2$	144	91	2.78
34	20.065	10-Methylnonadecane	$C_{20}H_{42}$	282	43	0.63

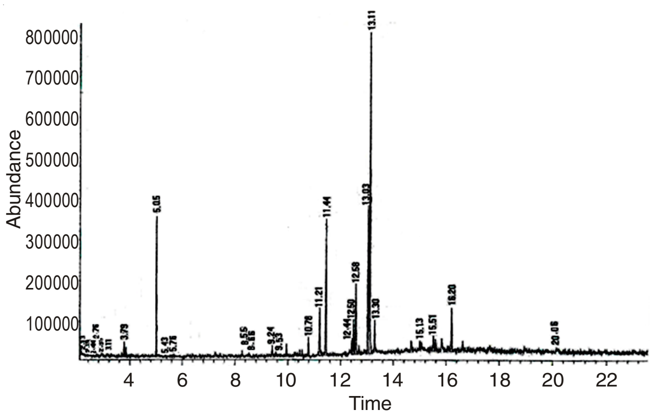

Fig. 1. TIC Chromatogram of *n*-hexane aerial parts extract of *C. didymus*

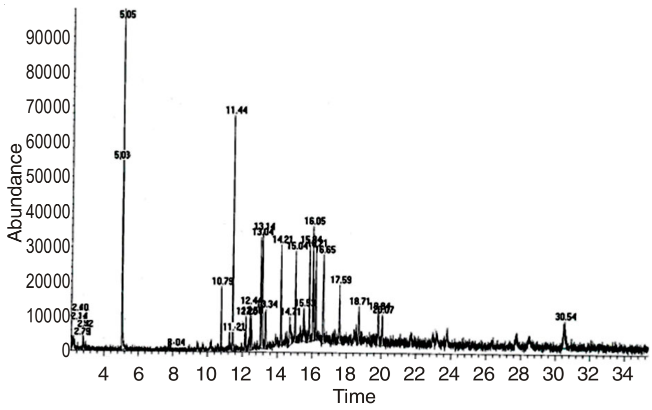

Fig. 2. TIC Chromatogram of *n*-hexane roots extract of *C. didymus*

present in higher amounts in roots. However, acetates were present in large amounts in aerial parts.

The identified compounds have interesting biological properties relevant to this study some of them are listed here. Volatile compounds explored in *C. didymus* were mostly found belonging to esters, in addition to some sulfur containing compounds. Volatile sulfur compounds are natural compounds contributing to the overall flavour and aroma of plants. The compound **12** (Table-1) and compound **8** (Table-2) was identified as benzyl isothiocyanate. It is obvious from the TIC profiles (Figs. 1 and 2) that this compound is very rich in the roots. The accumulation of benzyl isothiocyanate (20.51 %) in roots can be regarded as part of stress undoing mechanism evolved by this plant. The presence of benzyl isothiocyanate in *C. didymus* is the characteristic of the Brassicaceae plants. Benzyl isothiocyanate, also reported from different plants is

TABLE-2
IDENTIFIED PHYTOCHEMICAL COMPOUNDS FROM *C. didymus* ROOTS

S. No.	Retention time (min)	Compound name	m.f.	m.w. (a.m.u.)	Library match (%)	Peak area (%)
1	2.127	Benzylamine	C_7H_9N	107	93	1.53
2	2.401	1,2-Dihydro-1,2,5-trimethyl-3*H*-pyrazol-3-one	$C_6H_{10}N_2O$	126	43	1.27
3	2.728	α-Methyl-benzeneethanamine	$C_9H_{13}N$	135	50	1.17
4	2.779	Benzyl isocyanate	C_8H_7NS	133	64	1.21
5	2.905	2-Methyl-benzonitrile	C_8H_7N	117	97	1.26
6	2.922	Benzyl nitrile	C_8H_7N	117	96	1.04
7	5.039	Phenyl methyl thiocyanate	C_8H_7NS	149	72	7.82
8	5.052	Benzyl isothiocyanate	C_8H_7NS	149	86	20.51
9	8.038	3,5-Octadiyne	C_8H_{10}	106	83	1.64
10	8.041	5-(1-Methylethylidene)-1,3-cyclopentadiene	C_8H_{10}	106	16	0.37
11	10.784	Methyl 13-methylpentadecanoate	$C_{17}H_{34}O_2$	270	90	3.22
12	11.213	*n*-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	74	1.57
13	11.431	Ethyl hexadecanoate	$C_{18}H_{36}O_2$	284	96	8.44
14	12.192	3-(3,4-Methylenedioxyphenyl)-1-(2-quinolyl)-2-propen-1-one	$C_{19}H_{13}NO_3$	303	59	1.33
15	12.352	1-Hexyl-3-methyl-cyclopentane	$C_{12}H_{24}$	168	43	0.56
16	12.438	Methyl 7,10-octadecadienoate	$C_{19}H_{34}O_2$	294	56	2.52
17	12.443	Methyl 9*E*,12*E*-octadecadienoate	$C_{19}H_{34}O_2$	294	90	2.43
18	12.501	Methyl 9*Z*,12*Z*,15*Z*-octadecatrienoate	$C_{19}H_{32}O_2$	292	93	2.20
19	12.987	9*Z*,12*E*-Tetradecadien-1-ol, acetate	$C_{16}H_{28}O_2$	252	65	0.32
20	13.039	13-Tetradece-11-yn-1-ol	$C_{14}H_{24}O$	208	81	4.92
21	13.102	Ethyl 9*Z*,12*Z*,15*Z*-octadecatrienoate	$C_{20}H_{34}O_2$	306	83	1.08
22	13.136	Ethyl *E*-11-hexadecenoate	$C_{18}H_{34}O_2$	282	50	5.30
23	13.336	5-Propyl-decane	$C_{13}H_{28}$	184	59	1.38
24	14.189	1-Eicosene	$C_{20}H_{40}$	280	95	1.67
25	14.206	Heneicosane	$C_{21}H_{44}$	296	50	4.73
26	14.521	*cis*-9-Hexadecenal	$C_{16}H_{30}O$	238	58	1.09
27	15.036	Tetratriacontane	$C_{34}H_{70}$	478	74	3.44
28	15.213	5-(1-Methylethylidene)-1,3-cyclopentadiene	C_8H_{10}	106	46	0.69
29	15.838	Methoxymethoxycyclooctane	$C_{10}H_{20}O_2$	172	35	1.45
30	15.848	2-Tridecyl methoxyacetate	$C_{16}H_{32}O_3$	272	72	3.74
31	15.854	Heptadecylhexanoate	$C_{23}H_{46}O_2$	354	46	1.62
32	16.649	Heptadecane	$C_{17}H_{36}$	240	86	3.52
33	17.593	9-Octyleicosane	$C_{28}H_{58}$	394	58	2.70
34	17.748	2,3-Dihydroxypropyl 9*Z*,12*Z*-octadecadienoate	$C_{21}H_{38}O_4$	354	72	0.63
35	19.081	1-Chloro-3-methyl-benzene	C_7H_7Cl	126	35	1.63

highly active against Gram-negative bacteria [5]. A high dietary intake of benzyl isothiocyanate is also associated with a reduced risk of cancer in humans [6]. This could well explain the importance of sulfur containing compounds in the traditional medicine.

Acetates were present in greater amounts in this plant and are responsible for the sweet odor basically. Esters brought to the surface in different plant extracts have commercial applications [7] like use in fragrances, cosmetics, detergents, flavours and pharmaceuticals. Esters may also be used as biological additives [8]. Among the other identified compounds benzyl alcohol (compound **2**, Table-1), phenyl ethyl alcohol (compound **7**, Table-1) and ethyl benzoate (compound **11**, Table-1) showed antimicrobial and antioxidant activity [9]. Benzyl thiol (compound **6**, Table-1) used as flavouring agent. Both *n*-hexadecanoic acid (compound **12**, Table-2) which is also reported by researchers [10] in some other plant and ethyl hexadecanoate (compound **22**, Table-1 and compound **13**, Table-2) act as antioxidant, hypocholesterolemic, nematicide, pesticide, lubricant, antiandrogenic, flavour, hemolytic, 5-α-reductase inhibitor. Ethyl 9*Z*,12*Z*-octadecadienoate (compound **29**, Table-1) has anticancer property. Phytol (compound **28**, Table-1) is an important component of this plant that show antimicrobial, anti-inflammatory and anticancer activity. Phytol has significant antibacterial activity [11] and shows adjuvant and immune stimulatory activity [12].

The results obtained in this study thus suggest that the identified phytochemical constituents may be the bioactive constituents responsible for the efficacy of the aerial parts and roots of the plant. Due to the presence of above mentioned compounds in aerial parts and roots of *C. didymus,* this plant extract may be used in various pharmaceutical applications.

Conclusion

Detailed profiles of the various extracts of *C. didymus* assisted in the identification of 69 volatile compounds with esters, alcohols, aldehydes and sulfur containing compounds as the most dominant chemical classes contributing to aroma of the herb. It was found that most of the biologically active volatile phytochemicals were present in the *n*-hexane extract of roots. Roots contained mainly benzyl isothiocyanate in relatively higher concentration, while aerial parts were swarmed with acetates. The identified compounds have antimicrobial, anticancer, antioxidant, antidiabetic and anti-inflammatory effects. From this study it can be concluded that the *C. didymus* may serves as a new potential source of medicines due to the presence of these phytochemicals and bioactive compounds. This study is the pioneer contribution providing a better understanding of the use of this plant for various applications. Hence this study lures the researchers for preparative isolation and characterization of these useful phytochemical constituents from this useful plant.

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