Journal of Medicinal Plants Research Vol. 5(16), pp. 3667-3672, 18 August, 2011
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875 ©2011 Academic Journals

Full Length Research Paper

Chemical composition of essential oil and antibacterial activity of extracts from flower of *Allium atroviolaceum*

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Accepted 22 March, 2011

Essential oils of *Allium atroviolaceum* flowers collected from Mazandaran province in Kojur on late May 2010 were obtained by hydrodistillation and analyzed by gas chromatography coupled with mass spectrometry (GC/MS) for determining their chemical composition and identification of their chemotypes. Their antibacterial activity was studied *in vitro* on five bacterial strains: *Bacillus subtilis*, *Enterobacter cloacae*, *Klebsiella pneumonia, Proteus mirabilis* and *Staphylococcus aureus*. The major constituents of *A. atroviolaceum* flower oil were dibutylphthalate-1,2–benzenedicarboxylic acid (5/85%), crown (5.68%), disulfide methyl 2-propenyl (5.46%), eicosane (4.39%), pantadecanone (4/16%), dipropenyldisulfide (4.03), octadecanone (3/82%), methyl propenyl trisulfide (2.92), dimethyle trisulfide (2.80%), tetrathiephane (2/56%), cis propenyl propyl trisulfide(2/5%), tetracosane (2/48%), 3,6 – dibutyl-1,2 dihydro-1,2,4,5- tetrazine (2/37%), thrithiolane (2/19%), hydrazine (2/15%), dithio propionate (1/77%), hexadecanoic acid (1/68%), benzene (1/5%), isobutyl isothiocyanate (1/23%). The bacterial strains tested were found to be sensitive to methanol extracts studied which shows a very effective bactericidal activity with minimum inhibitory concentrations (MIC) range. The extracts from flower of *A. atroviolaceum* had slow activity against *B. subtilis* (16 mm diameter). The positive control, Valinomycine, Gentamicine and Chloramphenicol showed zone of inhibition inthicillin resistant all bacterial.

Key words: *Allium atroviolaceum*, essential oil, antibacterial activity.

INTRODUCTION

Medicinal plants have been traditionally used for pharmaceutical and dietary therapy in long history. A number of herbs and many relevant prescriptions have been screened and used for treating and preventing various tumors and inflammations as folk practices. Essential oils, which are used in traditional therapies, are generally aromatic oils which are obtained from the steam or hydro distillation of plants. Different parts of plants have been used to obtain essential oils. Essential oils and their components are widely used in medicine as constituents of different medical products in the food industry as flavoring additives and also in cosmetics as fragrances (Cowan, 1999) and pharmaceutical industries (Reische et al., 1998). These include flowers, leaves, seeds, roots, stems, bark and wood though secretionary parts. Essential oils are generally used in the cosmetic, medical and food industries. Bacterial resistance is a growing-problem worldwide (Cohen, 2002; WHO, 2001). One of the measures to combat the increasing rate of resistance in the long run is to have continuous investigation for new, safe and effective antimicrobials as alternative agents to substitute with no effective ones. Over the past 20 years, there has been a lot of interest in the investigation of natural materials as sources of new antibacterial agents. Different extracts from traditional medicinal plants were tested and some natural products were approved as new antibacterial drugs. However, there is still an urgent need to identify novel substances active against pathogens with higher resistance (Rieco, 1989; Cragg et al., 1997; Malika et al., 2004). Lot of works reports antibacterial and phytochemical constituents of medicinal plants and their use for the treatment of microbial infections (both topical and systemic applications) as possible alternatives to chemically synthetic drugs to which many infectious microorganisms

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have become resistant. During the last ten years the pace of development of new antibacterial drugs has slowed down, while the prevalence of resistance (especially multiple) has increased astronomically (Hugo and Russell, 1984). Literature reports and ethno-botanical records suggest that plants are the sleeping giants of pharmaceutical industry (Hostettmann and Hamburger, 1991) and provide natural source of antimicrobial drugs that provides novel compounds that may be employed in controlling some infections globally. *Allium* is the largest and important representative genus of the *Alliaceae* family and comprises 450 species, widely distributed in the northern hemisphere (Lonzotti, 2006). These species are characterized by a specific flavor and are used for cooking (Tada et al., 1988). In addition to their nutritional effects, the antibacterial and antifungal activities against the variety of Gram-negative and Gram-positive and continue to be extensively investigated (Whitemore and Naidu, 2000). However, *Allium* genus contains several species of which *Allium atroviolaceum* is only one. There is not any report about essential oil composition and antibacterial ability of this species of *Allium*. The aim of this investigation was to study the effect of aqueous extracts of *A. atroviolaceum* on bacterial test organisms and determination of its compounds.

MATERIALS AND METHODS

Plant materials

The flower of *A.atroviolaceum* were collected from Kojour-Nowshar (North of Iran), Iran, in the summer of 2010. The samples were identified by Dr. Bahman Eslami (Assistant Professor of Plant Systems, Islamic Azad University of Ghaemshahr, Iran). Voucher specimens are deposited with the Faculty of Biology Herbarium (No 720-722).

Isolation of the essential oil

The flowers of the plant collected were submitted for 3 h to water-distillation using a British-type Clevenger apparatus. The obtained essential oil was dried over anhydrous Sodium sulfate and after filtration, stored at 4°C until tested and analyzed.

Gas chromatography–mass spectrometry (GC–MS)

GC–MS was carried out using a Hewlett-Packard 5975B series instrument and an Agilent 19091J-433 HP-5 capillary column (30 m, 250 m i.d., film thickness 0.25 m) which was set at 50°C for 10 min, then increased 4°C/min to 300°C; using Helium as a carrier gas at a flow rate of 1 ml/min. The split ratio was 1:10; ionization energy was 70 eV; scan time was 1 s; acquisition mass range was m/z 40–400. The compounds were identified according to their retention indexes and by comparison of their mass spectra with those of a computer library or with authentic compounds. Apinene, Decane, Benzene and Limonene were identified by co-injection.

Preparation of methanolic extract

The flowers were dried at room temperature. Then 50 g of flowers was extracted at room temperature following the percolation method using methanol. The resulting extract was concentrated over a rotary vacuum until a crude solid extract was obtained (10.8%), which was then freeze-dried for complete solvent removal.

Assay for antibacterial activity of plant extract

Antibacterial activity of plant extract was determined by disc diffusion method as described by Bauer et al. (1966). Three Gram (-) bacteria (*Proteus mirabilis PTCC (1076)*; *Enterobacter cloacae PTCC (1003)*, and *Klebsiella pneumonia PTCC (1290))* and tow Gram (+) bacteria (*Staphylococcus aureus PTCC (1112)*) and *Bacillus subtilis PTCC (1023)*) were used for the present study. All the test bacteria were collected from Pastor Institute of Iran. Dried filter paper discs (4 mm in diameter) impregnated in known amount of test substances (200 µg/discs) were placed on Mueller-Hinton agar medium uniformly *seeded* with the test organisms. Valinomycine, Gentamicine and Cholrampnicol discs (30 µg/disc) soaked in respective solvent were used as positive control. These plates were then kept at low temperature (4ºC) for 2 - 4 h to allow maximum diffusion of compound. The diffusion occurred according to the physical law that controls the diffusion of molecules through agar gel the plates were then incubated at 37ºC for 24 h to allow maximum growth of the microorganisms. If the test materials have any antibacterial activity, it will inhibit the growth of the microorganisms giving the clear distinct zone around the disc called “Zone of Inhibition”. The antibacterial activity of the test material was determined by measuring the diameter of the zones of inhibition in millimeter with transparent scale.

RESULTS AND DISCUSSION

Chemical composition of the essential oil

The results obtained by the GC-MS analysis of the essential oil of the flower of *A.atroviolaceum* are presented in (Table 1). Eighty eight compounds were identified, representing 65% of the total oil. The oil yield of the plant was determined as 0.7% v/w. As determined from the GC-MS analysis, the major compounds were dibutyl phthalate- 1,2 – benzenedicarboxylic acid (5/85%), crown (5.68%), disulfide methyl 2- propenyl (5.46%), eicosane (4.39%), pantadecanone (4/16%), dipropenyl disulfide (4.03%), octadecanone (3/82%), methyl propenyl trisulfide (2.92%), dimethyletrisulfide (2.80%), tetrathiephane (2/56%) , cis propenyl propyl trisulfide(2/5%), tetracosane(2/48%) , 3,6–dibutyl- 1,2 dihydro-1,2,4,5-tetrazine (2/37%), thrithiolane (2/19%), hydrazine(2/15%), dithiopropionate (1/77%), hexadecanoic acid (1/68%), benzene (1/5%), isobutyl isothiocyanate (1/23%) (Table 1).

Assay for antibacterial activity

Crude extract of *A. atroviolaceum* showed moderate activity against *B. subtilis* with an inhibition zone of 16 mm. On the other hand, standard antibiotic valinomycine, gentamicine and cholrampnicol showed significant antibacterial activity against all tested Gram (+) and Gram

Table 1. Chemical composition of the essential oil from flower of *A.atroviolaceum*.

No.	K.I.	Components	Composition (%)
1	909	cis propenyl methyl disulfide	2.57
2	928	Methyl-trans-propenyl-disulfide 1,3-Dithiane	2.89
3	941	Dimethyl trisulfide	2.80
4	962	Furan, 2-pentyl	0.67
5	971	Octana1	0.92
6	1027	Disulfide, di-2-propenyl (CAS)	0.64
7	1038	dithio(1-propenyl)propionate	1.77
8	1042	Disulfide, di-2-propenyl (CAS)	1.62
9	1046	Nonanal (CAS)	1.74
10	1047	trans-propenyl propyl disulfide	0.64
11	1053	trans-propenyl propyl disulfide	0.70
12	1059	Disulfide, methyl (methylthio)methyl	0.62
13	1063	2-Ethyl[1,3]dithiane	0.47
14	1067	Trisulfide, methyl 2-propenyl (CAS)	2.92
15	1075	-Dibutyl-dihydro-tetrazine	2.37
16	1082	Hydrazine, (2-methylpropyl)	1.04
17	1085	1-Propene, 1-(methylthio)-, (Z)- Hydrazine,(2-methylpropyl)	1.11
18	1089	3-Methylcyclohexene oxide	0.37
19	1112	Decana1 (CAS)	0.81
20	1125	Spiro(4,5)decane-1,4-dione	0.59
21	1130	Benzene, [2-(methylsulfinyl)ethenyl]	0.57
22	1164	2,4-Dithiapentane	0.36
23	1168	2-Propenylthioacetonitrile	0.62
24	1175	Isobutyl isothiocyanate	1.23
25	1178	2-Methoxy-4-vinylphenol	0.93
26	1183	cis propenyl propyl trisulfide	2.50
27	1188	1,2,4-Trithiolane, 3,5-diethyl-cis propenyl propyltrisulfid	0.99
28	1189	2-Methylbenzene-1,3-dicarboxylicacid	0.77
29	1191	Trithiolane,	1.20
30	1204	1,2,4,6-Tetrathiepane (CAS)	2.56
31	1280	4,8-Dimethyl-1-oxaspiro[4.5]decan- 2-one	1.47
32	1286	Hexadecanal	0.52
33	1291	2-Hexene, 2,3-dimethyl- 3-cyclopropyl-2-(dichloro(cyclopropyl)methyl)cyclopropenone	0.81
34	1294	1,3-Benzenediol, 5-pentyl-	0.26
35	1309	2-Hydroxy-5-methylcyclohexa-2,5-di ene-1,4-dione1,3,5-Trithiane (CAS)	0.33
36	1349	Methanone, diphenyl- (CAS)	0.71
37	1361	Benzene, 1-methyl-3-(methylthio)- Cyclopentanepropanoic acid,	0.81
38	1386	1,3-(D2)MENTH-2-ENE	1.15

Table 1. Contd.

39	1395	Phenol, nonyl-	0.42
40	1428	1,3-diphenyl-1,3,5,5-tetramethylcyclotrisiloxane	0.63
41	1459	2-Pentadecanone, 6,10,14-trimethyl	4.16
42	1471	1,2-Benzenedicarboxylic acid, bis2-methylpropyl) ester (CAS)	1.04
43	1488	n-methylmethylenedioxyamphetamine	0.71
44	1494	1,2-Benzenedicarboxylic acid	0.35
45	1498	Hexadecanoic acid, methyl ester	1.68
46	1509	Isophytol	0.37
47	1517	Dibutyl phthalate	5.85
48	1523	3-Decen-5-one, 2-methyl-	0.75
49	1527	N-ethyl-1,3-dithioisoindoline	0.52
50	1545	3-Decen-5-one, 2-methyl-	1.23
51	1564	N-ethyl-1,3-dithioisoindoline	0.64
52	1580	8,11-Octadecadienoic acid, methyl ester	0.63
53	1583	9,12,15-Octadecatrienoic acid, methyl ester (CAS)	0.85
54	1589	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-	0.46
55	1594	1,4,7,10,13,16-Hexaoxanonadecane, 15-Crown-5	0.91
56	1606	Oxacycloheptadec-8-en-2-one (CAS)	0.29
57	1676	Eicosane (CAS)	0.88
58	1692	2-(Methoxyethoxy) -2-methylethyl 2-(hydroxyethoxy)ethyl ether	0.43
59	1721	1,4,7,10,13,16-Hexaoxanonadecane, 15-Crown-5	0.98
60	1768	Tetracosane	2.48
61	1788	Bis(2-ethylhexyl) phthalate	0.43
62	1796	5-Methylthio-7,8-dihydro-6H-benzocyclohepta[2,1e]pyrazolo[1,5-a]pyrimidine	0.38
63	1797	N-Methyl-1-adamantaneacetamide	0.42
64	1800	4,7-Dichloro-2-(1'-methyl-5'-nitro -2'-imidazolyl)quinoline	0.60
65	1803	2-(4'-nitro-2'-thienyl)pyrimidine	0.77
66	1805	Nonadecane	0.68
67	1807	3,5-Dimethyl-2,6-bis(trimethylsilo xy)pyridine	0.75
68	1810	1,4,7,10,13,16-Hexaoxanonadecane	0.71
69	1812	15-Crown-5	0.82
70	1827	21-KRONE-7	1.61
71	1829	21-KRONE-7	0.81
72	1834	Eicosane (CAS)	3.51
73	1859	N-Methyl-1-adamantaneacetamide	0.31
74	1862	1,1,1,3,5,5,5-Heptamethyltrisiloxane	0.49
75	1872	2-(Acetoxymethyl)-3-(methoxycarbon yl)biphenylene	0.34
76	1877	1-(2-trimethylsiloxy-1,1-dideuteri ovinyl)-4-trimethylsiloxy-benzene	1.25

Table 1. Contd.

77	1881	4,14-bis(hydroxymethyl)-[2.2]metacyclophane	0.87
78	1884	2-(4'-NITRO-2'-THIENYL)PYRIMIDINE	0.53
79	1893	Octadecane	3.82
80	1907	1,1,1,3,5,5,5-Heptamethyltrisiloxane	0.50
81	1910	4,14-bis(hydroxymethyl)-[2.2]metac yclophaNE	0.26
82	1912	21-KRONE-7	1.78
83	1967	3-[4-(2-Methoxy-ethoxymethoxy)-phe nyl]-acrylic acid, methyl ester	0.81
84	1990	15-Crown-5	0.66
85	2011	1,1,1,3,5,5,5-Heptamethyltrisiloxane	0.14
86	2049	1,4,7,10,13,16-Hexaoxanonadecane,18-(2-propenyl)-	1.19

Table 2. Antibacterial activity of *A.atroviolaceum* methanol extract expressed as minimum inhibitory concentrations (MICs) in _g/ml.

	***A. atroviolaceum* methanol extract (200 µg/disc) (mm diameter)**	**Gentamicine (30 µg/disc) (mm diameter)**	**Valinomycine (30 µg/disc) (mm diameter)**	**Chloramphenicol (30 µg/disc) (mm diameter)**
Proteus mirabilis PTCC(1076)	0	16	15	42
Enterobacter cloacae PTCC(1003)	0	21	13	12
Klebsiella pneumonia PTCC(1290)	0	18	0	13
Staphylococcus aureus PTCC(1112)	0	20	13	31
Bacillus subtilis PTCC(1023)	16	27	25	42

(-) bacteria (Table 2).

Results showed that the highest antibacterial activity of *A. atroviolaceun* was against *B. subtilis* with diameter of inhibition zone (DIZ) 16 mm and the lowest was against *P. mirabilis*, *E. cloacae*, *K. pneumonia* and *S. aureus* (n.a). Our results showed that tested plants had anti-bacterial activity against one tested bacteria. Many prior reports showed that garlic and onions have considerable antibacterial and antifungal effects (Benkeblia, 2004; Lee et al., 2004; Tsao and Yin, 2001). Since the studied plants have edible parts, our finding is important regarding using them as edible medicinal plants. In most of the plants, bulbous extracts were more effective regarding antibacterial effects. It seems that this ability is due to having Allicine (Cavallito and Bailly, 1944) and *Allium* species have been reported to accumulate the higher concentration of Allicine in their bulbs than other organs (Chehregani, 2007). The results indicated that standard antibiotic Gentamicine, Valinomycine, Chloramphenicol had no comparable activity to some plant extracts as shown in (Table 2). It seems that it is due to high resistance of the *P. mirabilis, E. cloacae, K. pneumonia, S. aureus* against plant extract. It is hoped that this study would lead to the establishment of some new and more potent antimicrobial drugs from natural origin and native plants.

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