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Antibacterial Activity And Composition Of Essential Oils Of Flower *Allium rotundum*

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ABSTRACT

The chemical composition and the antibacterial properties of the essential oils obtained from the flower of the *Allium rotundum* L. that is a wild important Allium species in Iran, were evaluated. The results obtained by the GC-MS analysis of the essential oil of the flower of *A. rotundum* L. are presented in (Table 1). Fifty four compounds were identified, representing 68% of the total oil. The oil yield of the plant was determined as 0.5% v/w. As determined from the GC-MS analysis, the major compounds were 14-Beta-H-Pregna (14.9%-as a total different component of pregnan), Demephion(8.30), Propenyl Propyl Disulfide (8.04), Trisulfide, dipropyl (4.27), Phenol, 2,4-bis(1,1-dimethylethyl) (3.99), phthalic thiothionoanhydride (3.81), Decane (3.75), Cyclododecane (3.00), 9-Eicosene (2.71), Nonanal (2.56). The essential oils were investigated for activity against bacteria (*Proteus mirabilis*, Enterobacter cloacae, Klebsiella pneumonia, Staphylococcus aureus, Bacillus subtilis were used. The essential oils inhibited all bacteria at concentrations of <1/200 (v/v). The results of this study confirmed the presence antibacterial activity of essential oils and the potency prevent the growth of this bacteria and extend the shelf life of processed foods.

Key words: Lamiaceae; Antibacterial activity; Essential oils

Introduction

A number of plant especially wild mountain herbs have been traditionally used as medicine and dietary therapy from thousand years in Iran. They have been screened and used for treating and preventing various causes such as heart problems, head and stomach pain, blood pressure etc. Traditional Iranian medicine has used a wide variety of plants to treat gastrointestinal disorders such as diarrhea and intestinal parasites which were particularly exist in rural areas of the country. The information about medicine plant obtained from oral and books of ancient scientists is a good option to have more investigation and protect these kind of plant that are useful for human health. To extract and isolate of essential oils that are used in medicine as constituents of different medical products various part of plan especially its flower and roots are used in Iran.

The use of essential oils was common as flavor additives and perfumes but recent investigations have also pointed out it is to use of the extraction of plant for antimicrobial and antioxidant activity [1] that are also used for preservation of food. Therefore the antimicrobial activity of essential oils in particular has formed the basis of many applications of different aromatic plants [2]. Plants and their essential oils, used since antiquity in folk medicine and for the preservation of food, are known sources of natural secondary metabolites having biological activity such as antimicrobial and antioxidant action among many others [1].

To study of medicinal plants enable us finding plants producing effective essential oils having a considerable range of applications. The differences in biological activities of the drug could be observed, related to the different compounds present in the plant material used. In general, the use of essential oils is a broad range of application as anti bacteria, anti fungi, anti viruses etc. The use of plant medicine not only have human application but also it is used in animal treatment. There is interaction between Environment (feed and disease) and genetic

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of fish [3]. Recently in orther to minimize the use of antibiotic, other complement such as pribiotic, probiotic or medicin plant such as garlic are used [4] Recently bacterial pathogens and their control are a serious problem in agriculture practice. The application of antibiotics and copper compounds, are used to control bacterial diseases, nowadays are not recommended. The use of antibiotics are forbidden in many countries and copper compounds, because of their general toxicity, exert a negative impact on both yield and the environment [5]. Therefore global interest in bio-preservation of food systems has recently been increased because of great economic costs of deterioration and poisoning of food products by food pathogens. Essential oils and extracts of various species of edible and medicinal plants, herbs, and spices constitute of very potent natural biologically active agents [6]. In this respect to prevent the disease through natural biological activity and to use the different application of plant, we had a plan of being tested the flower and roots of a wild famous medicine plant of *Allium rotundum* for their antimicrobial activity and essential oil components.

The round garlic (*Allium rotundum* L., family Liliaceae) has stems of up to 90 cm in height, bearing pink or purple flowers. Its name derives from the large rounded umbels, which are 2-3 cm across. The umbels lack bulbils, but the underground bulb is usually surrounded by purplish-black bulbils with long stalks [7]. Allium species occur in temperate climates of the northern hemisphere. They can vary in height between 5 cm and 150 cm. The flowers form an umbel at the top of a leafless stalk. 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 [8]. However, Allium genus contains *A. rotundum* collected in North of Iran. There is not any report about essential oil composition and antibacterial ability of this species. The results can be used to for the optimal harvesting part of this plant for relevant industries.

Material and Method

In the present article, the essential oils of *A. rotundum* were obtained from the flower and their antibacterial activity was investigated against one bacteria with agar disc diffusion and microbroth dilution methods. The compositions of the essential oils from the flower and were also analyzed by GC (RI) and GC/MS.

Plant materials:

The flower of *A. rotundum* were collected from Zagmarz area (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 786-247).

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 µm) 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. (9). 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 (4 °C) 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 37 ºC 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.

Cytotoxicity activity:

The *Allium rotundum* L. as medical plant also are tested to show potent cytotoxicity activity and having antitumor agents. We used a bioassay technique that appears capable of detecting a board spectrum of bioactivity present in crude extracts using the brain shrimp lethality bioassay (BSLT) [10].

Hatching shrimp:

Brine shrimp eggs *Artemia fransiscana* were hatched in artificial sea water prepared by dissolving 35g of sea salt in 1L of distilled water. The eggs were incubated at 28 °C for 48 h. The nauplii were gathered in one side of the jar with a light source and collected with pipette. Nauplii were separated from eggs by a liquating them three times in small beakers containing sea water.

Brine shrimp assay:

The bioactivity of the extracts were monitored by the method previously described by Meyer *et al.* [10]. The sample were dissolved in DMSO (up to 2% of final dosage) and diluted with sea water. 500 microliters of dissolved sample in sea water and DMSO was placed in all of 24-well microliter plat. Final concentration of plate extract was adjusted to 10, 100, 500 and 1000 microgram per ml. 500 microliters of suspension of nauplii containing about 15-20 larvae was added into each well. The nauplii then incubated for 24 h. The plates were then examined under a microscope (12.5X) and the number of dead nauplii in each treatment was counted. Lethality concentration fifties (LC_{50} values) for each assay were calculated by taking average of three experiments using probit analysis.

Statistical analysis:

Experimental results are expressed as means ± SD. All measurements were replicated three times. The data were analyzed by an analysis of variance ($p<0.05$) and the means separated by Duncan's multiple range tests. The LC_{50} values were calculated from linear regression analysis.

Results:

Chemical composition of the essential oil:

The results obtained by the GC-MS analysis of the essential oil of the flower of *A. rotundum* L. are presented in (Table 1). Fifty four compounds were identified, representing 68% of the total oil. The oil yield of the plant was determined as 0.5% v/w. As determined from the GC-MS analysis, the major compounds were 14-BETA-H-PREGNA (14.9%-as a total different component of pregnan), Demephion(8.30), CIS and TRANS-PROPENYL PROPYL DISULFIDE (8.04), Trisulfide, dipropyl (4.27), Phenol, 2,4-bis(1,1-dimethylethyl) (3.99), phthalic thiothionoanhydride (3.81), Decane (3.75), Cyclododecane (3.00), 9-Eicosene (2.71), Nonanal (2.56), Benzene,1,3-bis(1,1-dimethylethyl) (2.44), PROPENYL METHYL DISULFIDE (2.38), DODEMORPH (2.05) (Table 1).

Table 1: The analysis of essential oil of the flower Allium rotundum L

No	K.I	**Components**	**Composition (%)**
1	924>	Thiophene, 2,3-dimethyl	0.34
2	924>	Thiophene, 2,4-dimethyl	0.76
3	924>	CIS PROPENYL METHYL DISULFIDE	1.11
4	924>	TRANS PROPENYL METHYL DISULFIDE	1.27
5	924>	Furan, 2-pentyl	0.65
6	924>	Octanal	0.69
7	924	1-Hexanol, 2-ethyl	0.31
8	933	Decane, 3,7-dimethyl	1.18
9	936	Heptane, 2,3,4-trimethyl	0.44
10	939	Decane, 3,7-dimethyl	0.68
11	943	Nonanal	2.17
12	946	TRANS-PROPENYL PROPYL DISULFIDE	3.68
13	951	CIS-PROPENYL PROPYL DISULFIDE	4.36
14	954	1-Oxa-4,6-diazacyclooctane-5-thion	0.91
15	958	o-DICHLOROBENZENE	1.10
16	976	DODEMORPH	2.05
17	988	DEMETON	0.60
18	1021	Decanal	1.14
19	1028	Benzaldehyde, 4-ethyl	0.51
20	1054	Benzene, 1,3-bis(1,1-dimethylethyl)	2.44

21	1068	Tetradecane	0.75
22	1076	PROPAMOCARB	0.44
23	1082	Undecanal	0.96
24	1086	1,4-Dioxane-2,3-diol	0.50
25	1091	Trisulfide, dipropyl	4.27
26	1095	phthalic thiothionoanhydride	3.81
27	1098	DEMEPHION	8.30
28	1101	1-Propene, 3,3'-thiobis	1.43
29	1121	Tetradecane	0.53
30	1143	5,9-Undecadien-2-one, 6,10-dimethyl	0.40
31	1152	1-Decene	1.89
32	1160	beta.-Ionone	0.52
33	1163	Pentacosane	0.72
34	1172	Phenol, 2,4-bis(1,1-dimethylethyl	3.99
35	1205	TERBUTHYLAZINE	1.62
36	1228	Hexadecane	3.2
37	1296	Cyclododecane	3.00
38	1300	9-Eicosene	2.71
39	1324	Tetradecane	0.62
40	1349	2-Pentadecanone, 6,10,14-trimethyl	0.89
41	1396	Dibutyl phthalate	0.48
42	1430	(S)-4,4-Dimethyl-2-(4-methyl-3-cyclohexen-1-yl)-1,5-hexadiene	1.03
43	1482	Phytol	0.96
44	1798	Octadecane	0.53
45	1851	14-.BETA.-H-PREGNA	0.29
46	1886	14-.BETA.-H-PREGNA	0.90
47	1905	1-Hexacosene	1.09
48	1975	14-.BETA.-H-PREGNA	1.56
49	2035	Eicosane (CAS)	2.94
50	2065	14-.BETA.-H-PREGNA	2.23
51	2137	14-.BETA.-H-PREGNA	0.06
52	2482	14-.BETA.-H-PREGNA	2.95
53	2518	HAHNFETT	0.85
54	2521	14-.BETA.-H-PREGNA	1.98

Assay for antibacterial activity:

The in vitro antimicrobial activity of the essential oils of *Allium rotundum* L on Gram-positive and Gram-negative bacteria collected from Zagemarz localities of Mazandran province Iran, were studied. The results are shown in [Table 2]. The maximum activity was on B. subtilis PTCC(1023) (16 mm) and the minimum activity was on P. mirabilis, E. cloacae, K. pneumonia and S. Aureus (n.a). The inhibition zones, especially on Chloramphenicol-resistant S. aureus PTCC(1112) and Gentamicine resistant S. PTCC(1112) were 31 mm and 20 mm, respectively. Furthermore, the inhibition zones on Chloramphenicol resistant Proteus mirabilis PTCC(1076) were also very high and was 31 mm. On the other hand, the high activity was observed on Enterobacter cloacae (23mm), and Klebsiella pneumonia PTCC(1290) (25mm), against Chloramphenicol bacteria. The antibacterial activity of this plant on multiple antibiotic resistant strains did not showed very stong.

Table 2: Antibacterial activity of *Allium rotundum* L methanol extract expressed as minimum inhibitory concentrations (MICs) in g/ml

	A. rotundum 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	18	12	31
Enterobacter cloacae PTCC(1003)	0	19	0	23
Klebsiella pneumonia PTCC(1290)	0	15	0	25
Staphylococcus aureus PTCC(1112)	0	20	13	31
Bacillus subtilis PTCC(1023)	16	29	20	32

Assay for cytotoxicity activity:

Results of toxicity against brine shrimp of the extract are shown in table 3. The extracts of flower showed significant toxicity against brine shrimp with LC_{50} value 480 to 140 µg/ml, at two interval time of 5h to 24h respectively.

Table 3: Brine shrimp bioassay results of plant extract of *Allium rotundum* L.

Tested material	Mortality(%) in concentrated tested µg/ml						Level of mortality
Time	100	200	400	600	800	1000	LC_{50}
5h	1±1	3±1	10±4	25±8	46±12	80±15	890
24h	5±5	8±4	17±6	48±11	83±14	91±8	610

Discusion:

Chemical composition of essential oil and antibacterial activity of extracts from flower of *A. rotundum* L. were studied and fifty four compounds were identified in the present study, representing 98.8% of the total oil. The essential oil yield of *A. rotundum* L. was 1.2 percent from region of Zagmarz area in north of Iran. It is relatively higher than some other plants industrially exploited as a source of essential oils such as Lippia rotundifolia (0.01%) (11). Thymus (1%) [12], Tetraclinis (0.22%) [13], Menthe (0.5-1%), Néroli (0.5-1%) and Laurel (0.1-0.35%) (14), which are previously reported as medicine plant.

The chemical compositions revealed that this flower had compositions relatively similar to those of other Allium atroviolaceum essential oils analyzed by Dehpour et al. [15]. One of the highest component in flower of *A. rotundum* L. extracted was Phenol. Phenols and polyphenolic compounds, such as flavonoids, are widely found in food products derived from plant sources, and they have been shown to possess significant antioxidant activities [16]. Studies have shown that increasing levels of flavonoids in the diet could decrease certain human diseases [17]. Substances which are able to perform this reaction can be considered as antioxidants and therefore radical scavengers [18]. It was found that the radical-scavenging activity of the extracts increased with increasing concentration. High concentration of phenol (up to 4 %), illustrate a antioxidants activity of this plant.

Our results showed that tested flowers had antibacterial activity but not strongly. Some of Allium plant are consider as garlic and other as onion or the flowers of plant. Many prior reports showed that garlic and onions have considerable antibacterial and antifungal effects [19-21]. We studied a part of usually not edible parts of plant. In most causes the root, leaves, fruits and other edible parts of plant are investigated. Our finding is important regarding using a flower as not edible parts of medicinal plants but having the same composition which is important in medicine aspect. Allium species have been reported to accumulate the higher concentration of Allicine in their bulbs than other organs [22], however by relatively moderate antimicrobial activity of *A. rotundum* L. it seems the concentration of Allicine is comparable in flower of this plant. It was also considered that major components, such as Hexanol, Decane, Heptane and also Propenyl Propyl Disulfide have a possible interaction between this substances and phenol, could also affect the antimicrobial activities. 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.

Taken together, antimicrobial activities and antioxidants activities of these results suggest that the essential oils investigated here could find practical application in the prevention and protection of fungal infections of plants, animals and humans. Essential oils could safely be used as preservative materials on foods for protection to fungal infection, since they are natural, and mostly non-toxic to humans. The selected plant species are popular culinary herbs, and their essential oils have been used extensively for many years in food products, perfumery, dental and oral products.

There are few investigation reported about the medicin plant checked for their toxicity in Iran. Obtaining the LC_{50} value less than 1000 revealed that *A. rotundum* L. have an active potent of cytotoxic effect that is due to presence of unpolar component present in extract of this plant. The LC_{50} obtained in present work illustrate the cytotoxicity effect of *A. rotundum* L. can be compared with *Centratherum anthelminticum* (seed) *Acorus calamus* (rhizome), *commiphora mukul* (gum resin), *Albizia gummifera* (roots) and *Cyathula cylindrical* which have been reported previously [23,24] and many other medicine plant which are used in different country as plant disease trapy.

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