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Analysis of essential oil from leaves and Bulbs of *Allium atroviolaceum*

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Abstract:

Introduction: Medicinal plants used in traditional medicine as prevention and treatment of disease and illness or use in foods, has a long history. Plants belonging to genera *Allium* have widely been acquired as food and medicine. In many countries, including Iran, a variety of species of the genus *Allium* such as garlic, onions, leeks, shallots, etc use for food and medicinal uses.

Methods and Results: The leaves and bulbs of *Allium atroviolaceum*, collected from Borujerd (Lorestan Province, Iran) in May 2015 and their essential oils of were obtained by hydro-distillation. The oils were analyzed by gas chromatography coupled with mass spectrometry (GC/MS) and their chemical composition was identified.

The major constituents of *A. atroviolaceum* leaves oil were dimethyl trisulfide (59.0%), ethyl linolenate (12.4%), phytol (11.4%) and in bulb oil were methyl methyl thiomethyl disulfide (61.3%), dimethyl trisulfide (15.1%) and methyl allyl disulfide (4.3%). The major constituents of both essential oils are sulfur compounds.

Conclusion: The results of the present study can help to increase of our information about composition of an edible herb in Iran. The major components of the oils can use as marker for standardization of the herbs.

Keywords: *Allium atroviolaceum*; Bulbs; Leaves; GC/MS; Essential oil

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1. Introduction

Medicinal plants used in some countries for their many actions and for relieve discomfort and help treat diseases; now the importance of medicinal plants has not, as well as the attention of the public are. 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 of some parts of plants by steam or hydro-distillation methods. They are widely used flavoring additives and/or fragrances in different products in the food industry cosmetics [1] and pharmaceutical industries [2].

The genus *Allium* is a very diverse and taxonomically complicated group belonging to the family Amaryllidaceae [3]. The genus *Allium* comprises of around 750 species [4] and is rich in flavonoids, saponins and volatile sulfur compounds. The later compounds are responsible for their characteristic pungent aroma and taste, and responsible for their medicinal activities, though they are unstable and easily transform to other compounds [5]. However, only a few of them have been used for their pungency and flavoring value and in some parts of the world, for religious connotations. Since ancient times, species of the *Allium* genus have been used in folklore of many cultures as food, preventive and therapeutic medicinal agents [6].

Allium species are reported to have several positive health effects including antibacterial, antifungal, antiviral, anticancer and practically cardiovascular activities along with immune functions. They directly affect vessel wall and produce hypotensive and cholesterol- and triglyceride-lowering properties [7].

The essential oil of some *Allium* species have studied by many researchers, previously, for instance essential oil of *A. vineale* is mainly formed allyl trisulfide (24 %) and

diallyl tetrasulfide (16 %), when *A. sativum* oil mostly composed of allyl trisulfide (45 %) and diallyl disulfide (22 %) in Algeria [8]. The main components of *A. sativum* essential oil from Cameroon were diallyl trisulfide (41.6 %), diallyl disulfide (19.7 %), allyl methyl trisulfide (12.9 %), diallyl sulfide (7.1 %) and diallyltetrasulfide (4.2 %). Those of *A. cepa* essential oil were diallyl trisulfide (22.2 %), dipropyl trisulfide (11.1 %), 2-methyl-3, 4-dithiaheptane (9.9 %), methyl propyl trisulfide (8.1 %), dipropyl tetrasulfide (8.1 %) and 2-propenyl propyl disulfide (5.2 %) [9].

The essential oil constituents of *A. stipitatum* (Syn. *A. hirtifolium*) or Persian shallot were investigated through gas chromatography/mass spectrometry (GC-MS) technique. 1-(methylthio)-1-(Z) Butene (18.2 %), methyl methyl thiomethyl disulfide (8.4 %), dimethyl tetrasulfide (6.5 %) and piperitenone oxide (4.5 %) were the most abundant components and comprised 37.64% of the oil. Mono-sulfur compounds are the major components in this oil [10].

Essential oil of the aerial parts of *Allium neapolitanum* Cirillo collected in Sicily was analyzed and the main components were found to be (E)-chrysanthenyl acetate (28.1%), (Z)-chrysanthenyl acetate (23.8%), (E)-β-farnesene (9.6%), dimethyl trisulfide (9.6%), camphor (7.4%), methyl allyl disulfide (6.8%) and 1-methyl-3-allyl trisulfide (5.8%) [11].

In oil of *Allium ursinum* leaves, some new compounds (allyl (methylthio)methyl, (methylthio)methyl (Z)/(E)-1-propenyl and allyl 1-(methylthio)propyl disulfides) were detected, in seeds heptyl methyl, methyl octyl, allyl hexyl, allyl octyl and propyl (propyl thio) methyl sulfides were reported, too [12]. Chemical characterization of *Allium roseum* var. *grandiflorum* essential oil has shown methyl methane thio sulfinate as major sulphurous compound [13].

Allium atroviolaceum Boiss., with local name of Varkouaz in Lorestan Province (Iran), is using as a kind of soup or dessert, locally. Some researches were studied on A. *atroviolaceum.* In 2018, Ghasemi et al., were reported tricin isolated from the herb, potentiated the effect of docetaxel on PC3 cell proliferation [14]. Khazaei et al., were reported flower extract of *A. atroviolaceum* triggered apoptosis, activated caspase-3 and down-regulated antiapoptotic Bcl-2 gene in HeLa cancer cell line [15].

In addition, Khazaei et al., promotion of HepG2 cell apoptosis by flower of *A. atroviolaceum* and the mechanism of action was reported [16].

In 2017, in vitro antiproliferative and apoptosis inducing effect of *A. atroviolaceum* bulb extract on breast, cervical, and liver cancer cells were reported by Khazaei et al. [17].

In other study, Lorigooini et al, were evaluated anti-platelet aggregation effect of *A. atroviolaceum* [18].

The sapogenin atroviolacegenin and its diglycoside atroviolaceoside were isolated from *A. atroviolaceum* broadleaf, too. These compounds are accompanied by three known spirostanol and furostanolsaponins. In addition, 4, 4′-dihydroxy-3-methoxychalcone, *p*-coumaroyl-*N*-tyrosine, and *p*-feruloyl-*N*-tyrosine have been found in the flowers and bulbs [19].

Khazaei et al., were reported cytotoxicity and proapoptotic effects of *A. atroviolaceum* flower extract by modulating cell cycle arrest and caspase-dependent and p53 - independent pathway in breast cancer cell lines, too [20].

Chemical composition of essential oil and antibacterial activity of extracts from flower of *A. atroviolaceum* were studied. The major constituents of the flower oil were dibutylphthalate-1, 2-benzenedicarboxylic acid (5.8%), crown (5.7 %), disulfide methyl 2-propenyl (5.5 %) [21].

Tepe, et al, were reported in vitro antioxidant activities of the methanol extracts of *A. atroviolaceum* from Turkey. IC_{50} of the herb in DPPH test was 79.0 ± 2.75 µg/ml. In the β-carotene/linoleic acid assay, the inhibition ratios of the oxidation of linoleic acid by A. atroviolaceumwas 71.2 ± 2.20% [22].

Stajner and Szöllosi Varga were evaluated antioxidant abilities of A. *atroviolaceum* in some different models, too [23].

In present study, the chemical composition of oil of *Allium atroviolaceum* leaves and bulb was undertaken and results are reported. As far as we know, the chemical composition of the oil has not been studied to date.

2. Materials & Methods

2.1. Plant material

Allium atroviolaceum Boiss. (The local name; Sirdang, Violet onion, Varkouaz, Fig. 1) were collected in May 2015 in Village Bondizeh, Borujerd (Lorestan Province, Iran). A voucher No (1566-AupF) has been deposited in the Herbarium of Pharmaceutical Sciences Branch, Islamic Azad University, Tehran, Iran (Fig. 1).

Figure 1. *Allium atroviolaceum*, voucher sample has been deposited in Herbarium

2.2. Essential oil extraction

Leaves and bulb of *A. atroviolaceum* were carefully gathered. The fresh organs were chopped separately and subjected to hydro-distillation for 4 h, using a Clevenger-type apparatus. The oils were dried over anhydrous sodium sulfate and stored at 4ºC.

2.3. Gas chromatography-Mass spectrometry analysis

The GC-MS analyses were carried out on a Hewlett Packard GC-MS system, model 5973, fitted with a 30m long, cross-linked 5% phenyl methyl siloxane (HP- 5MS 5% Phenyl Methyl Silox, Agilent 19091S-433) (30 m x 250 µm x 0.25 µm). The source temperature was 230°C, the quadrupole temperature 150°C, the initial oven temperature was 60°C; this was then raised to 260°C at 4°C/min and the final temperature maintained for 20 min. The injector and detector temperatures were 200°C and 250°C,respectively. The carrier gas was helium at 1.0 mL/min. The sample was injected using a split ratio of 1:100. The carrier gas helium, adjusted to a linear velocity of 34 m/s. The ionization energy was 70 ev, and the scan range 40-650 AMUat 3.9 scans/s. The injected volume was 1.0 µL of a 2% dilution of oil in *n*-heptane. The identification of the oil components was based on comparison of mass spectra with the literature and Wiley MS data library (6^{th} ed) (Fig. 2-3).

3. Results and Discussion

The results of GC-MS analysis from the bulbs and leaves oil of *A. atroviolaceum* are presented in Table 1 (according to elution from column). In the leaves oil, seven compounds representing 99.9%, were identified, while fifteen compounds were identified in the bulbs oil, representing 97.7%.

The yields of the both oil was determined 0.7% v/w. As determined from the GC-MS analysis, the major constituents of *A. atroviolaceum* leaves oil were dimethyl trisulfide (59.0%), ethyl linolenate (12.4%), phytol (11.4%); and the major constituents of *A. atroviolaceum* bulb oil were methyl methyl thiomethyl disulfide (61.3%), dimethyl trisulfide (15.1%), methyl allyl disulfide (4.3%). As can be seen, the major constituents of both essential oils are sulfur compounds. The results indicated that the highest amount of sulfur compounds is related to tri-sulfur compounds. The presence of sulfur compounds in the leaves and bulbs of *A. atroviolaceum* is significant. The role of dietary sulfur compounds as anticarcinogens, antimicrobials, and antioxidants has been and continues to be extensively studied [24]. There was any similarity between the components that characterized in leaves and bulbs; and the components reported in *A. atroviolaceum* flower, previously [21], except disulfide methyl 2-propenyl (5.5%) in flower oil and (1.3 %) in bulb oil. In our work, the major constituents of *A. atroviolaceum* leaves was dimethyl trisulfide (59.0 %) and methyl methyl thiomethyl disulfide (61.3 %) while in flower oil [21] were dibutyl phthalate-1, 2-benzenedicarboxylic acid (5.9 %).

Chemistry of the essential oil from *Allium* species is complex and it is rich of sulfur components. Some similarities and differences in the two oils can be seen. Dimethyl trisulfide that reported in our work (59% as a main component in leaves of *A. atroviolaceum* and 15.1% in its bulbs), was reported in *A. neapolitanum* [11] and *A. hookeri* [25] and *A. roseum* [26], previously. Methyl methylthiomethyl disulfide (as a main component in bulbs of *A. atroviolaceum* (61.3%)), was reported in Persian shallot (*A. stipitatum*), too [10].

*Allium*s and their sulfur constituents have been found to combat cardiovascular disease, cancer and infections [27]; maybe *A. atroviolaceum* and its constituents can combat these diseases. Moreover, antibacterial, antifungal, antiviral, anticancer and practically effects on cardiovascular diseases and immune functions are reported for *Allium* species [28-29].

Conflict of interest

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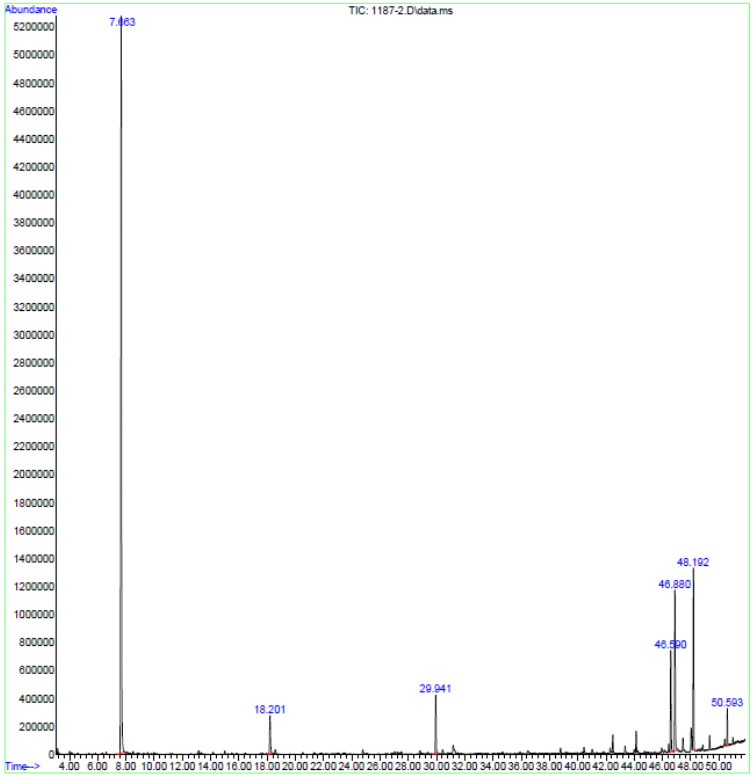

Figure 2. Gas chromatogram of leaf oil of *A. atroviolaceum*

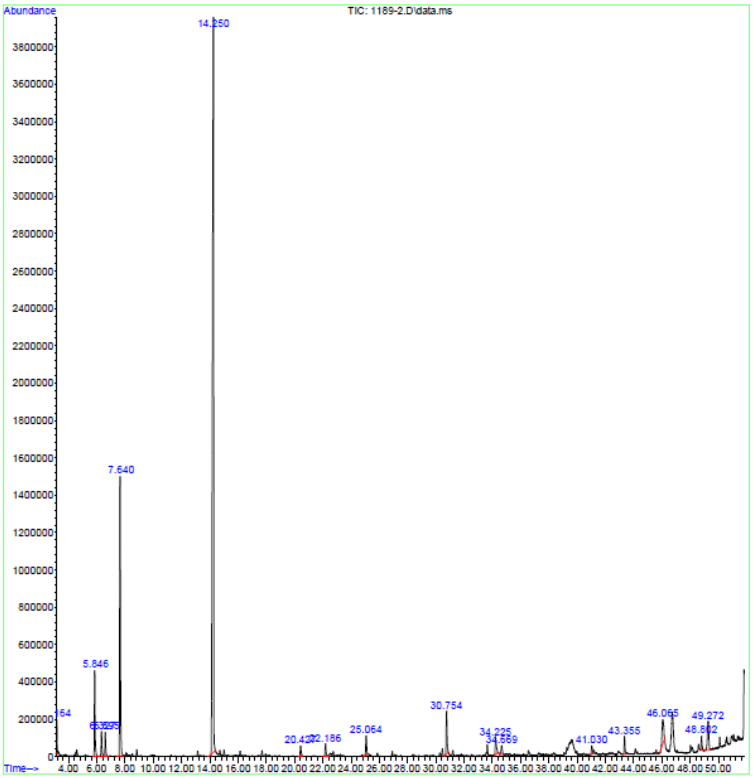

Figure 3. Gas chromatogram of Bulb oil of *A. atroviolaceum*

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

No.	Components	RT	KI	% (bulb)	% (leave)
1	Octane	3.2	800	1.0	-
2	Methyl allyl disulfide	5.9	918	4.3	-
3	methyl propyl disulfide	6.3	932	1.4	-
4	Methyl *E*- propenyl disulfide	6.7	940	1.3	-
5	dimethyl trisulfide	7.6	974	15.1	59.0
6	Methyl methyl thiomethyl disulfide	14.2	1147	61.3	-
7	Dimethyl tetrasulphide	18.2	1232	-	3.5
8	*n*-Methyl thioacetamide	20.4	-	0.7	-
9	1,4-Dioxane-2,3-diol	22.2	-	0.9	-
10	7,11-dimethyl-3-methylene 1,6,10-Dodecatriene	25.1	1458	1.1	-
11	β-Ionone	30.0	1490	-	4.5
12	2,4,6-trithiaheptane 2,2-dioxide	30.8	-	2.8	-
13	3,4-dihydroxy benzoic acid	34.2	1557	1.4	-
14	Asarone	34.7	1621	0.7	-
15	Isobutyl phthalate	41.0	1847	0.2	-
16	1-Eicosanol	46.1		2.7	-
17	Methyl linolenate	46.6	2098	-	7.3
18	Phytol	46.9	2128	-	11.4
19	Ethyl linolenate	48.2	2198	-	12.4
20	n-Tricosane	49.3	2300	-	1.9
21	1,2-Benzenedicarboxylic acid, diisooctyl ester	50.6	2700	2.9	-
Sum				97.7	99.9