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Introducing of some species of genus *Allium* subgenus *Melanocrommyum* from Iran as new sources of allicin

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Introducing of some species of genus *Allium* subgenus *Melanocrommyum* from Iran as new sources of allicin

Abstract: Allicin is a sulfur compound found in genus *Allium* characterized by numerous biological and pharmacological properties. *Melanocrommyum,* the second largest subgenus of *Allium*, has about 10 sections and 82 species in Iran. In this study, allicin content of aerial part, aerial part fresh mass and allicin yield belonging to 17 wild populations of six species of *Allium* sect. *Acanthoprason* and *Asteroprason* growing in different region of Iran, were analyzed. Allicin content evaluation using HPLC method showed its variation between populations from 26.98 to 58.11 mg g^{-1} FW, also showing that all the tested populations of *Allium* are rich in allicin. The average of aerial part fresh mass and allicin yield varied between populations from 0.49 g to 1.66 g and from 14 mg to 78 mg, respectively. The populations were classified in four major groups using dendrogram generated by UPGMA method of cluster analysis. However, grouping of populations was not completely related to species and geographical regions. This study is the first evaluation of allicin content in wild populations of *Allium* sect. *Acanthoprason* and *Asteroprason* in Iran. High amount of allicin in these populations make them a new sources of allicin.

Key words: *Acanthoprason*; *Asteroprason*; medicinal plant; population; variation; allicin content

Uvajanje nekaterih vrst iz rodu *Allium*, podrodu *Melanocrommyum* iz Irana kot novih virov alicina

Izvleček: Alicin je žveplo vsebujoča snov v rodu *Allium* s številnimi biološkimi in farmakološkimi lastnostmi. Podrod *Melanocrommyum* je drugi največji podrod v rodu *Allium*, v Iranu z okoli 10 sekcijami in 82 vrstami. V raziskavi so bili analizirani nadzemni deli na vsebnost alicina in svežo maso pri 17 divjih populacijah šestih vrst iz rodu *Allium* ,sekcij. *Acanthoprason* in *Asteroprason*, ki rastejo na različnih območjih Irana. Vsebnost alicina, ovrednotena s HPLC metodo je pokazala razlike med populacijami v razponu od 26,98 do 58,11 mg g^{-1} na svežo maso, kar kaže, da so vse populacije preiskušenih vrst bogate na alicinu. Poprečna sveža masa in vsebnost alicina nadzemnih delov je med populacijami variirala od 0,49 g do 1,66 g in od 14 mg do 78 mg. Z generiranjem dendrograma po UPGMA metodi in klasterski analizi so bile populacije združene v 4 glavne skupne. Grupiranje populacij se ni popolnoma ujemalo z vrstami in geografskimi regijami izvora. Ta raziskava je prva v Iranu, ki je ovrednotila vsebnost alicina v divjih populacijah vrst iz rodu *Allium, Acanthoprason* in *Asteroprason*. Zaradi velike vsebnosti alicina so vrste iz teh popuacij lahko njegov nov naravni vir.

Ključne besede: *Acanthoprason*; *Asteroprason*; zdravilne rastline; populacija; variabilnost; vsebnost alicina

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1 INTRODUCTION

Plants of the genus *Allium* belonging to monocotyledonous flowering plants comprise more than 900 accepted species, with main center of diversity in the eastern Mediterranean area, Southwest and Central Asia (Fritsch & Abbasi, 2013). *Allium* species have been *used* for centuries as *vegetables,* as well as *medicinal plants. Several studies have been conducted showing Allium's therapeutic properties as well as numerous reports referring to their* antioxidant, antibacterial, antifungal, antiparasitic, antiseptic, anti-inflammatory, anticancer, antidiabetic, cardioprotective, antiatherosclerosis, hepatoprotective and immunomodulatory properties (Benkeblia, 2004; Galeone et al., 2006; Rizwani & Shareef, 2011; Feng et al., 2012; Lu et al., 2012; Nicastro et al., 2015; Sobolewska et al., 2015; Huang et al., 2016; Rad et al., 2017; Zeng et al., 2017). Most of medicinal properties of *Alliums* are mainly attributed to phenolic (like flavonoids: kaempferol, myricetin and quercetin derivatives) and sulfur-containing compounds and beneficial elements such as selenium (Omar & Al-Wabel, 2010; Nwachukwu & Slusarenko, 2014; Soto et al., 2016). Organic sulphur compounds like alliin, allicin, allyl sulfide, (E)-ajoene, (Z)-ajoene and 1,2-vinyldithiin are responsible for odor, flavor and most of biological activities of *Alliums* (Block, 1992; Benkeblia & Lanzotti, 2007). Among these, allicin (diallylthiosulfinate) has received more attention due to its significant human health benefits (Oommen et al., 2004; Rahman, 2007; Borlinghaus et al., 2014; Ye et al., 2016).

Allicin's structure and activities were described by Cavallito and Bailey in 1944 for the first time (Cavallito & Bailey, 1944). This unstable sulfur compound is composed from alliin by the action of alliinase released from vacuoles upon crushing or damaging *Allium* tissues (Jones et al., 2007). Allicin is now clearly accepted as a biologically active compound, and several documents have been published in this field (Ali et al., 2000; Li et al., 2010; Wallock-Richards et al., 2014; Gruhlke et al., 2017). Garlic (*Allium sativum* L.) is a main source of allicin among cultivated *Alliums*. Nevertheless, there are many wild *Allium* species that may have potentially some levels of allicin which needs investigation.

Melanocrommyum, the second largest subgenus of *Allium*, comprises about 10 sections and 82 species in Iran (Fritsch & Abbasi, 2013). Some species in *Acanthoprason* and *Asteroprason,* two sections in this subgenus, are used by folk peoples as wild leafy vegetable and medicinal herbs. These plants have specific smell like garlic. In spite of long local traditional usages, there is no research on their beneficial compounds like allicin. These species are threatened with *extinction* because of wild-harvesting as the only way to reach them. Awareness and knowledge about their potent in production of healthy metabolites is needed for domestication and breeding of these species.

In this study allicin content of 17 wild populations belonging to six species of *Allium* sect. *Acanthoprason* and *Asteroprason* which were collected from different regions of Iran, as a central part of diversity of this genus, were investigated.

2 MATERIALS AND METHODS

2.1 PLANT MATERIAL

Seventeen populations of *Allium* including six species of sect. *Acanthoprason* and *Asteroprason* from diverse geographical origin which were described by Fritsch and Abbasi (2013) were collected during the flowering stage. Table 1 gives the geographical location of populations. All plants were cut 1cm above ground, weighted and kept in a freezer at -80 °C.

2.2 SAMPLE PREPARATION

Allicin was extracted from randomly ten plants of each population in two replications according to Baghalian et al. (2005). In brief, each 800 mg powder sample was placed in an ultrasonic bath at 4 °C for 5 min with 20 ml of distilled water. Tubes were incubated for 30 min at room temperature. The supernatant were then separated by centrifuging at 6000 g for 30 min. The supernatant (10 ml) were added to 15 ml of solution which contains 1 % (v/v) solution of anhydrous formic acid and methanol (4:6) and centrifuged at 6000 g for 5 min. The extracts were analyzed as quickly as possible.

20 mg butyl parahydroxybenzoate in 100 ml of methanol–water (50:50) was used as internal standard. 0.5 ml of internal standard was added to supernatant and make up the volume to 10 ml and 20 µl of it was injected into the HPLC.

2.3 DETERMINATION OF ALLICIN

The allicin were determined according to the method of Baghalian et al. (2005). The HPLC analysis was carried out on a Knauer HPLC system (Berlin, Germany) equipped with a Knauer C18 column (25 cm × 4.6 mm) and a PDA detector. The mobile phase was methanol–water (50 : 50) at a flow rate of 0.7 ml min^{-1}. Elution was monitored at 254 nm. The percentage of allicin was calculated by using the following equation:

Allicin (%) = "s_1 m_2 * 22.75" /"s_2 m_1"

Where s_1 and s_2 are the area of the peak corresponding to allicin and internal standard and m_1 and m_2 are the mass of the *Allium* powder and butyl parahydroxybenzoate in internal standard solution, respectively. The allicin content was expressed as mg g^{-1} FM.

Mean allicin yield of each population (per plant) were calculated by using percentage of allicin and aerial part fresh mass.

2.4 DATA ANALYSIS

Pearson correlation and cluster analyses (UPGMA) were carried out on the data of allicin content and aerial part fresh weight using the statistical software SPSS (SPSS Inc., Chicago, USA).

3 RESULTS AND DISCUSSION

The aerial part allicin content of 17 populations of *Allium* belonging to *Acanthoprason* and *Asteroprason* sections collected from different regions of Iran are shown in Figure 1. The percentage of allicin content varied from 26.98 to 58.11 mg g^{-1} FM, where the highest content was found for Shen Jari population of *A. pseudobodeanum*, followed by Dehdasht of *A. minutiflorum* (57.95 mg g^{-1} FM), Pir Baba Ali of *A. subakaka* (56.87 mg g^{-1} FM) and Shirpala, another population of *A. pseudobodeanum* (55.94 mg g^{-1} FM), while the lowest content belonged to Taze Abad Oryeh population of *A. kurdistanicum*, followed by Vali Abad of *A. derderianum* (27.42 mg g^{-1} FM).

Variation in allicin content of different ecotypes of garlic as a main source of this valuable metabolite is reported in previous studies (Baghalian et al., 2005; Wang et al., 2014; Mostafa et al., 2015; Panahandeh et al., 2016). Allicin content of 212 accessions of garlic from different

Table 1: *Allium populations* including six species of sect. *Acanthoprason* and *Asteroprason* collected from various locations of Iran

Pop. no.	Section	Species	Location (Province)	Latitude (N)	Longitude (E)	Altitude (m)
1	*Acanthoprason*	*A. derderianum* Regel.	Dareh Oson (Tehran)	35°51′248"	51°25′786"	2645
2	*Acanthoprason*	*A. derderianum* Regel.	Vali Abad (Mazandaran)	36°18′856"	51°11′1"	2421
3	*Acanthoprason*	*A. derderianum* Regel.	Kochka (Mazandaran)	36°18′232"	51°04′53"	2248
4	*Acanthoprason*	*A. derderianum* Regel.	Vandarin (Mazandaran)	36°22′55"	51°1′41"	2926
5	*Acanthoprason*	*A. kurdistanicum* Maroofi & R.M. Fritsch	Taze Abad Oryeh (Kurdistan)	35°7′42"	47°40′309"	2332
6	*Acanthoprason*	*A. minutiflorum* Regel.	Dehdasht (Kohgiluyeh and Boyer-Ahmad)	30°50′315"	50°33′067"	1920
7	*Acanthoprason*	*A. subakaka* Razyfard & Zarre	Pir Baba Ali (Kurdistan)	35°6′17"	47°39′26"	2351
8	*Acanthoprason*	*A. subakaka* Razyfard & Zarre	Jame Shoran (Kurdistan)	35°5′733"	47°39′175"	2318
9	*Acanthoprason*	*A. subakaka* Razyfard & Zarre	Ghalelan (Kurdistan)	35°4′965"	47°39′245"	2618
10	*Asteroprason*	*A. elburzense* W.	Band e Yakhchal (Tehran)	35°50′648"	51°25′775"	2277
11	*Asteroprason*	*A. elburzense* W.	Emamzadeh Ebrahim (Tehran)	35°50′5"	51°25′10"	2120
12	*Asteroprason*	*A. elburzense* W.	Kamelat (Tehran)	35°44′514"	52°04′594"	2372
13	*Asteroprason*	*A. elburzense* W.	Abnik (Tehran)	35°51′353"	51°25′414"	2567
14	*Asteroprason*	*A. elburzense* W.	Ghabre Oros (Tehran)	35°51′618"	51°25′25"	2821
15	*Asteroprason*	*A. elburzense* W.	Kandovan Tunnel (Mazandaran)	36°9′56"	51°19′16"	2672
16	*Asteroprason*	*A. pseudobodeanum* R.M. Fritsch & Matin	Shen Jari (Tehran)	35°4′50"	52°50′372"	2290
17	*Asteroprason*	*A. pseudobodeanum* R.M. Fritsch & Matin	Shirpala (Tehran)	35°51′171"	51°25′458"	2515

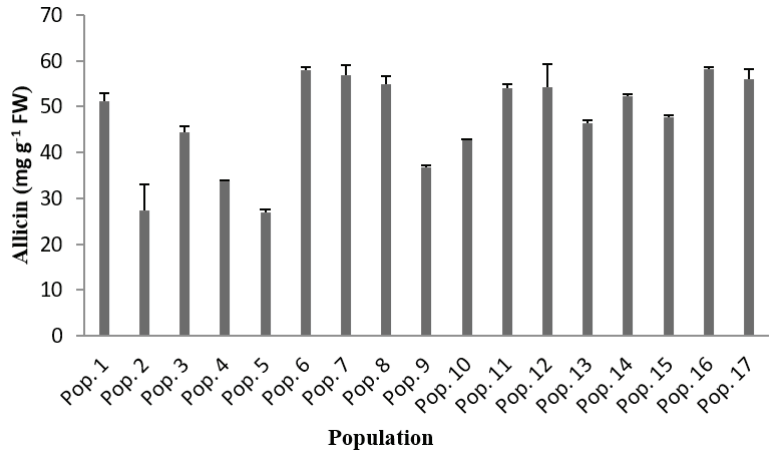

Figure 1: Schematic diagram representing the percentage of aerial part allicin in different populations of *Allium* belonging to *Acanthoprason* and *Asteroprason* sections from Iran

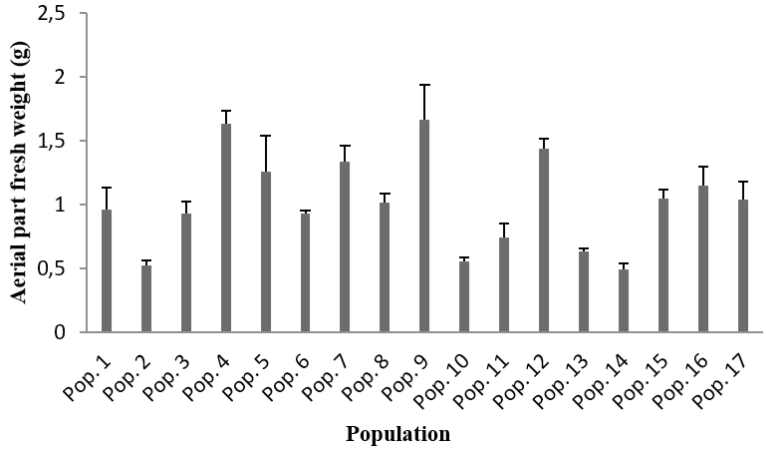

Figure 2: The average of aerial part fresh mass of different populations of *Allium* belonging to *Acanthoprason* and *Asteroprason* sections from Iran

provinces and areas of China ranged from 0.81 to 3.01 % (Wang et al., 2014). In an investigation of 24 Iranian garlic ecotypes from different areas, allicin was the highest in a local selected clone from northeast of Iran (13 % DW) (Baghalian et al., 2005). In the present work, high amount of allicin (2.69-5.81 % FM) was found in all the tested populations of *Allium*. So it was indicated that all studied populations are suitable for allicin production and pharmaceutical usage.

The average of aerial part fresh mass per plant in these populations ranged from 0.49 g in Ghabre Oros (*A. elburzense*) to 1.66 g in Ghalelan population (*A. subakaka*) (Figure 2). Variation in morphological parameters between species, populations and genotypes of *Alliums* is supported by previous literatures (Panthee et al., 2006; Karpaviciene, 2012; Khosa et al., 2014; Wang et al., 2014; Shiga et al., 2015; Silva et al., 2015; Hirata et al., 2016; Jafari et al., 2017).

Based on the obtained results, there was no correlation between percentage of allicin and aerial part fresh masst and these two characters were affected by species and environmental conditions.

Calculation of allicin yield of aerial part for each population shown in Figure 3 indicated that the average of allicin yield was the highest in Kamelat population (*A. elburzense*) (78 mg) and the lowest in Vali Abad (*A. derderianum*) (14 mg).

Due to variation of aerial part fresh mass among population, calculation of allicin yield appears to be a good parameter for evaluation of populations and finding the promising populations which can be selected for domesticating and breeding programs. Based on the results, Kamelat followed by Pir Baba Ali population have

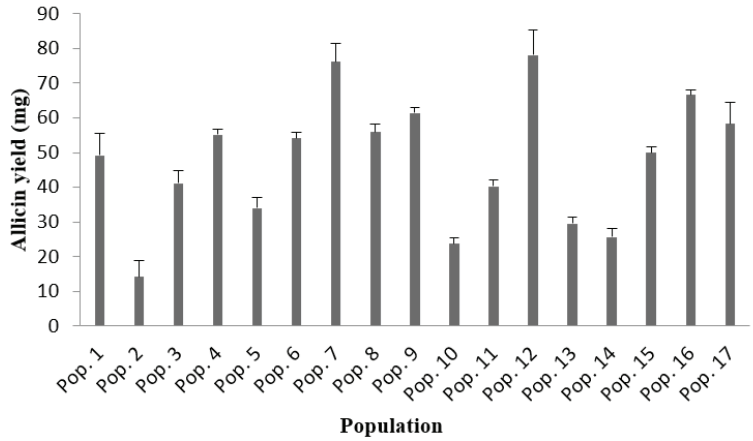

Figure 3: The average of aerial part allicin yield of different populations of *Allium* belonging to *Acanthoprason* and *Asteroprason* sections from Iran

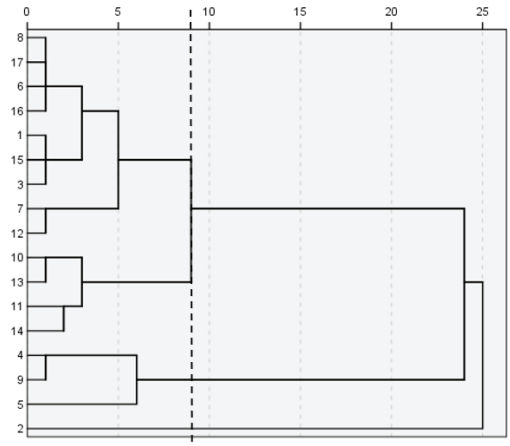

Figure 4: Cluster analysis of different populations of *Allium* belonging to *Acanthoprason* and *Asteroprason* sections from Iran using UPGMA method

the highest allicin yield and could be suitable candidates for breeding.

Dendrogram generated by UPGMA method of cluster analysis is presented in Figure 4. By applying cluster analysis, four main groups and some subgroups were evident. Jame Shoran (*A. subakaka*), Shirpala (*A. pseudobodeanum*), Dehdasht (*A. minutiflorum*), Shen Jari (*A. pseudobodeanum*), Dareh Oson (*A. derderianum*), Kandovan Tunnel (*A. elburzense*), Kochka (*A. derderianum*), Pir Baba Ali (*A. subakaka*), Kamelat (*A. elburzense*) populations were placed in cluster I. Four populations of *A. elburzense* from Tehran province (Band e Yakhchal, Abnik, Emamzadeh Ebrahim and Ghabre Oros) were assigned to cluster II. Cluster III was composed of Vandarin (*A. derderianum*), Ghalelan (*A. subakaka*) and Taze Abad Oryeh (*A. kurdistanicum*) populations. Finally, Vali Abad (*A. derderianum*) population formed cluster IV. Grouping of the populations were not completely related to species and geographical regions.

4 CONCLUSIONS

This study is the first evaluation of allicin content in wild populations of *Allium* sect. *Acanthoprason* and *Asteroprason* in Iran. Our results showed that these wild populations present considerable variation in percentage of aerial part allicin, aerial part fresh mass and allicin yield. High amount of allicin in these populations make them new sources of allicin. Conservation, domestication and breeding of studied populations are critical to exploitation and prevention of danger of their extinction. Allicin rich plants are desirable for medical industry and

Kamelat and Pir Baba Ali populations are good candidates for these purposes.

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