https://doi.org/10.31925/farmacia.2020.2.20 **ORIGINAL ARTICLE**

ALLICIN DIVERSITY IN SOME WILD POPULATION OF SIX *ALLIUM* SPECIES FROM IRAN

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Abstract

Allium is a large genus of flowering plants with potential medicinal values. In this study, the potential use of wild *Allium* species as medicinal plants was evaluated by determining the allicin diversity in nine *Allium* populations from six species of subgenus *Melanocrommyum* sect., *Acanthoprason* and *Asteroprason*. The assessments involved HPLC analysis. In addition, the fresh weight of bulbs and the allicin yield of each population were examined. The results showed that allicin ranged from 4.82% to 17.55% in the bulbs of different populations. Such amounts of allicin are higher than the British pharmacopoeia level. The average of bulb fresh weight and allicin yield varied from 2.08 g to 4.85 g and from 0.20 g to 0.81 g, respectively. Cluster analysis by the UPGMA method led to the classification of the nine wild populations of *Allium* into three major clusters. The study of diversity here provides a basis for future utilization of genetic resources in these populations and for their breeding.

Rezumat

Allium este un gen mare de plante cu flori, cu potențiale utilizări medicinale. În acest studiu, utilizarea potențială a speciilor sălbatice de *Allium* ca plante medicinale a fost evaluată prin determinarea diversității de alicină la nouă populații de *Allium* din șase specii din subgenurile *Melanocrommyum* sect. *Acanthoprason* și *Asteroprason*. Evaluările au implicat analiza HPLC. În plus, s-a determinat greutatea proaspătă a bulbilor și randamentul de alicină pentru fiecare populație. Rezultatele au arătat că alicina a variat de la 4,82% la 17,55% în bulbul diferitelor populații. Aceste cantități de alicină sunt mai mari decât nivelul stipulat în Farmacopeea Britanică. Media de greutate a bulbului proaspăt și randamentul de alicină a variat de la 2,08 g la 4,85 g, respectiv de la 0,20 g la 0,81 g. Analiza clusterului prin metoda UPGMA a condus la clasificarea celor nouă populații sălbatice de *Allium* în trei grupe majore. Studiul oferă o bază pentru utilizarea viitoare a resurselor genetice din aceste populații și pentru reproducerea lor.

Keywords: allicin, *Allium*, diversity, *Melanocrommyum*

Introduction

Medicinal plants have received considerable attention since ancient times due to their therapeutic properties. They are widely used for treating diseases. Further to the long history of medicinal plants and their usage, most medicinal plants are still being harvested from the wild. Nearly 15,000 species of wild harvested medicinal plants are now at the impending risk of extinction as a result of overharvesting, population decline and the destruction of their natural habitats [11]. Fluctuations in quality and quantity of secondary metabolites, along with a loss of genetic diversity, are characteristic of medicinal plants that are harvested from their natural habitats. The conservation, domestication and cultivation of medicinal plants can serve as effective solutions and appropriate responses to the increasing demand for phytomedicines.

Cancer, cardiovascular disease, stroke and diabetes are among the top 10 causes of death across the globe [34]. They have been the cause of death for more than 25 million people in 2015 [34]. Many cases of research have indicated that *Allium* species are effective in the prevention of those diseases [1, 6, 9, 12, 13, 15, 23, 28-31, 37]. In addition, *Allium* species are known to have antioxidant, anti-inflammation, anti-microbial and anti-obesity properties [3, 8, 16, 19, 21, 22, 26, 27, 32, 35]. The therapeutic effects of *Allium* are mostly attributed to sulphur compounds, especially allicin [7, 24]. Allicin is responsible for the specific aroma of *Allium* species. It is produced by the reaction of alliinase with alliin [10]. The amounts of allicin in *Allium* species are variable due to genetic and environmental factors. Allicin has beneficial effects that can contribute to the cure of human diseases. Accordingly, it can generate useful applications in medicine. In fact, allicin contents have been evaluated in the genetic resources of *Allium* species in previous research [5, 25, 33].

Iran is a central location of *Allium* diversity. It is home to several *Allium* species that are distributed across its natural reserves [14]. These species have received more attention as understood in the increasing number of studies on the numerous benefits of *Allium* species on human health, as well as a higher level of attention given to germplasm conservation over the past several years. Fristch and Abbasi [14] published a taxonomical review of the *Allium* subgenus *Melanocrommyum* in Iran. Furthermore, various studies have been conducted regarding the morphological, cytogenetic, molecular and phytochemical diversity of native *Allium* species [2, 4, 17, 18, 20, 36].

The current study aimed to determine allicin diversity in nine *Allium* populations from six species of the subgenus *Melanocrommyum* sect. *Acanthoprason* and *Asteroprason*. The ultimate objective is to provide useful information for a better exploitation, conservation and breeding of these valuable medicinal plants.

Materials and Methods

Plant material

Nine *Allium* populations were collected from the wild. In total, they were identified as six species of subgenus *Melanocrommyum* sect. *Acanthoprason* and *Asteroprason*. The populations were from different habitats in Iran according to Fritsch and Abbasi [14]. Randomly, ten bulbs with two replications were used in each population. All samples were collected at the flowering stage, weighed and frozen in liquid nitrogen immediately after harvesting. They were stored at -80ºC. The species of samples and the sites from which they were collected are listed in Table I.

Table I

Allium species and collection sites of nine natural *Allium* populations belonging to six species of subgenus *Melanocrommyum* sect. *Acanthoprason* and *Asteroprason* included in the analysis

Pop. no.	Section	Species	Location (Province)	Latitude (N)	Longitude (E)	Altitude (m)
1	*Asteroprason*	*A. elburzanse*	Ghabre Oros (Tehran)	′51 618º35	′25 25º51	2821
2	*Asteroprason*	*A. elburzanse*	Kandovan Tunnel (Mazandaran)	′9 56º36	′19 16º51	2672
3	*Asteroprason*	*A. pseudobodeanum*	Shen Jari (Tehran)	′45 0º35	′50 372º52	2290
4	*Acanthoprason*	*A. derderianum*	Vali Abad (Mazandaran)	′18 856º36	′11 1º51	2421
5	*Acanthoprason*	*A. derderianum*	Kochka (Mazandaran)	′18 232º36	′04 53º51	2248
6	*Acanthoprason*	*A. derderianum*	Vandarin (Mazandaran)	′22 55º36	′1 41º51	2926
7	*Acanthoprason*	*A. kurdistanicum*	Taze Abad Oryeh (Kurdistan)	′7 42º35	′40 309º47	2332
8	*Acanthoprason*	*A. minutiflorum*	Dehdasht (Kohgiluyeh and Boyer-Ahmad)	′50 315º30	′33 067º50	1920
9	*Acanthoprason*	*A. subakaka*	Jame Shoran (Kurdistan)	′5 733º35	′39 175º47	2318

Determining the allicin content

Allicin content was measured according to a method previously used by Baghalian *et al*. [5]. Accordingly, 20 mL of distilled water was added to 800 mg of bulb powder which was obtained from each sample. This solution was placed in an ultrasonic bath at 4ºC for 5 min. The homogenates were incubated for 30 min at room temperature and subsequently centrifuged at 6000 g for 30 min. Then, 10 mL of the supernatant was added to the vial containing 15 mL of A mix (1% (v/v) solution of anhydrous formic acid and methanol (4:6)). This was centrifuged again at 6000 g for 5 min. The extracts were analysed promptly following the extraction. 20 mg of butyl parahydroxybenzoate was dissolved in 100 mL of methanol:water (50:50) for use as an internal standard. Then, 0.5 mL of the internal standard was added to 9.5 mL of the supernatant. A sample volume of 20 µL was injected into the HPLC. The analyses were performed with a Knauer HPLC system (Berlin, Germany) equipped with a Knauer C18 column (25 cm × 4.6 mm) and a PDA detector. The mobile phase comprised methanol:water (50:50) and was characterized by a flow rate of 0.7 mL/min. The detection was carried out at 254 nm. Finally, the following formula was set to calculate the percentage of allicin:

$$\text{Allicin (\%)} = \frac{s_1 m_2 * 22.75}{s_2 m_1},$$

where s_1 and s_2 are the area of the peak corresponding to allicin and the internal standard, m_1 and m_2 represent the mass of the *Allium* powder and the butyl para-hydroxybenzoate in the internal standard solution, respectively.

The mean value of allicin yield in each population (*per* plant) was calculated by using the average of allicin (%) and the average of bulb fresh weight. The "Gorgan" garlic ecotype was used as the control because of its high allicin content (according to Baghalian *et al*. [5]).

Statistics

A Pearson correlation was used for determining the correlations between allicin content and bulb fresh weight. The cluster analysis of populations was carried out based on the allicin content and the bulb fresh weight using the un-weighted pair group method with arithmetic averages (UPGMA). The statistical analyse of data were performed using the SPSS statistical programme (SPSS Inc., Chicago, USA).

Results and Discussion

The evaluation of allicin content by HPLC (Figure 1) revealed that there were differences between populations in terms of their bulb allicin content, ranging from

4.82% in *A. derderianum* (Vandarin population) to 17.55% in *A. kurdistanicum* (Taze Abad Oryeh population). The allicin contents of all populations are provided in Figure 2.

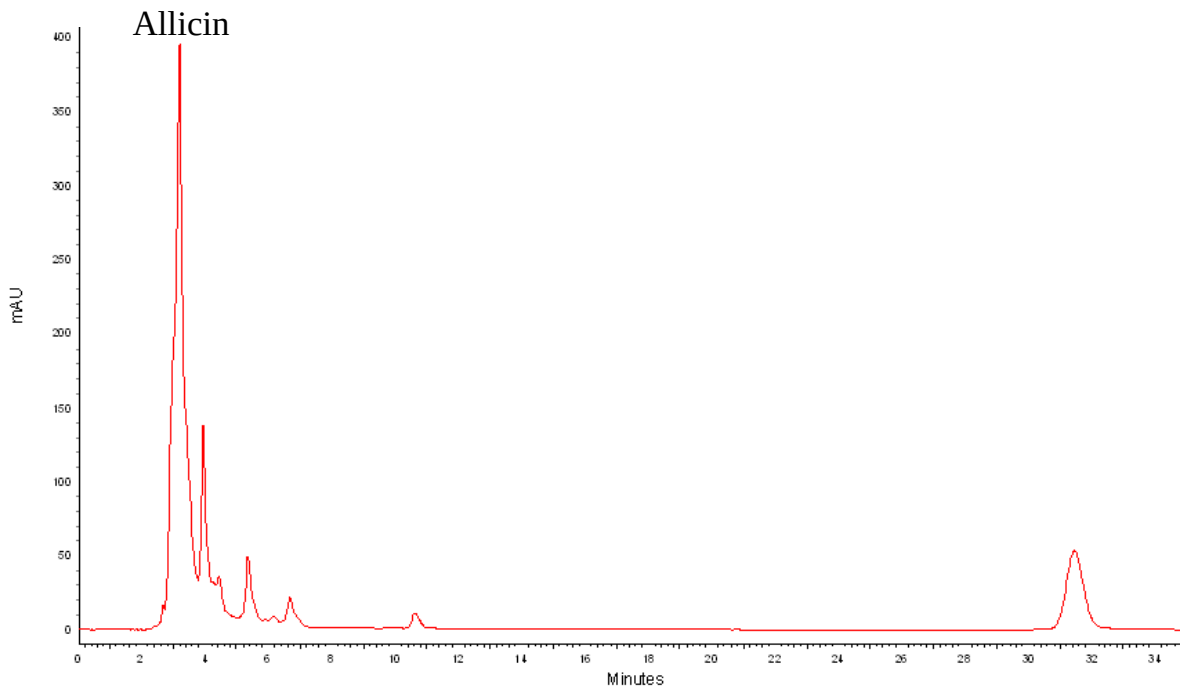

Figure 1.
HPLC chromatogram of allicin in test sample

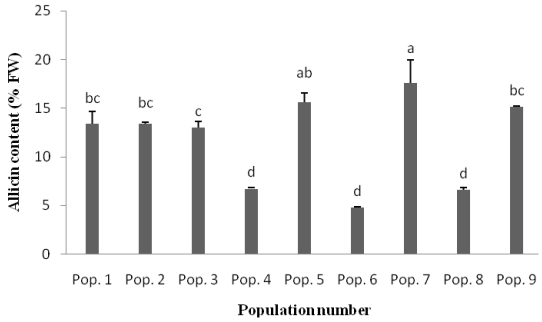

Figure 2.
Representation of the measured allicin content of bulbs of nine natural *Allium* populations from Iran. Bars are means ± SD.

Apparently, each and every population has an allicin content that is higher than the British pharmacopoeia level (0.45%) and, in some cases, they surpassed the percentage of allicin recorded in genotypes and ecotypes of garlic in different countries [5, 25, 33] and in the Gorgan garlic ecotype (9.11%) which was used as the control. Accordingly, all nine populations can be deemed suitable for the pharmacy industry. Variations in the allicin content of samples can be explained by genetic and environmental factors. Such reasons for variation are consistent with the results of previous studies [5, 25, 33]. The bulb fresh weight is also influenced by genetic and environmental factors.

The average of bulb fresh weight in the nine populations varied from 2.08 g in Ghabre Oros (*A. elburzanse*) to 4.85 g in Jame Shoran population (*A. subakaka*) (Figure 3). In this regard, the available literature cites variations in bulb fresh weight in previous works on *Allium* [18, 33].

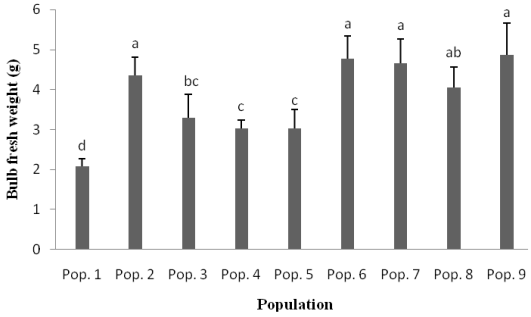

Figure 3.
Average of bulb fresh weight of nine natural *Allium* populations from Iran. Bars are means ± SD.

No correlation was found between allicin content and bulb fresh weight. Due to the variations in bulb weight, the allicin yield was calculated in order to determine the potential of each population for allicin production.

The allicin yield of bulbs in each population (Figure 4) revealed that the Taze Abad Oryeh (*A. kurdistanicum*) and Vali Abad (*A. derderianum*) populations had the highest (0.81 g) and the lowest (0.20 g) average of allicin yield of bulbs, respectively.

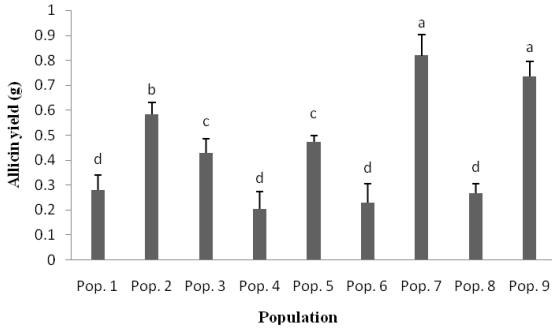

Figure 4.

The average of bulb allicin yield of nine natural *Allium* populations from Iran. Bars are means ± SD.

Cluster analysis grouped the nine wild populations of *Allium* into three major clusters. Each cluster hosted three populations (Figure 5). The first cluster consisted of two sub clusters including Kandovan Tunnel (*A. elburzanse*), Taze Abad Oryeh (*A. kurdistanicum*) and Jame Shoran (*A. subakaka*) populations. The second major cluster of the dendrogram comprised two sub-clusters, including Shen Jari (*A. pseudobodeanum*), Kochka (*A. derderianum*) and Ghabre Oros (*A. elburzanse*) populations. Vandarin (*A. derderianum*), Dehdasht (*A. minutiflorum*) and Vali Abad (*A. derderianum*) populations were grouped in cluster III. However, clustering could not completely separate the populations based on species and geographical origins.

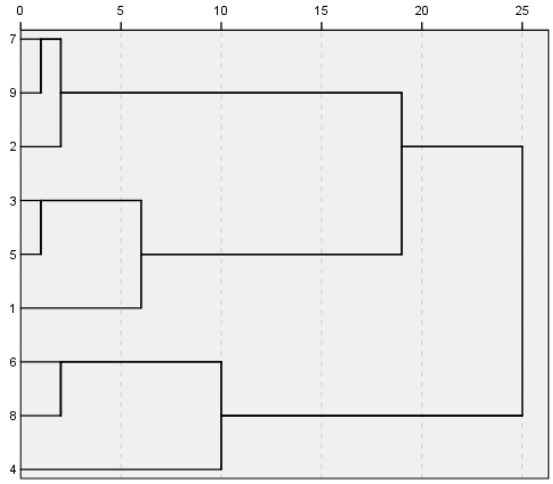

Figure 5.

Dendrogram obtained by cluster analysis of nine natural *Allium* populations from Iran using UPGMA method.

Conclusions

Allicin is a major compound in the genus *Allium*, especially in *A. sativum*, and most of the benefits that are attributed to garlic usually emanate from this metabolite. In this study, allicin yield was evaluated in some wild relatives of garlic. The results indicated that these populations have notable allicin content. Two populations of different species from the Kurdistan province of Iran (Taze Abad Oryeh and Jame Shoran) had the highest allicin amounts and can be used as an alternative to garlic in future applications of medicinal research. The conservation, evaluation and breeding of wild crop relatives not only provide a great opportunity to exploit their therapeutic benefits but also play an important role in the genetic improvement of their domesticated counterparts in cultivation. The study of diversity reported herein provides a basis for the future exploitation of genetic resources in these populations.

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Conflict of interest

The authors declare no conflict of interest.

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