

## PHYTOCHEMICAL AND BIOACTIVITY DIVERSITY IN THE EXTRACTS FROM BULBS AND LEAVES OF DIFFERENT POPULATIONS OF *Allium jesdianum*, A VALUABLE UNDERUTILIZED VEGETABLE

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### ABSTRACT

*Allium jesdianum* Boiss. & Buhse (Yazdi onion) belonging the family *Alliaceae*, is an endemic species of Iran that grows wild in the Zagros Mountains range, southwestern Iran. The indigenous people of Iran use the leaves and bulbs of *A. jesdianum* for the treatment of colds and kidney problems. The bulbs and leaves of various populations of the plant were collected from the alpine regions in Chaharmahal va Bakhtiari province, southwestern Iran. The total phenolic content of ethanol extract was determined by Folin-Ciocalteu method, the antioxidant activity was evaluated measuring 1,1-diphenyl-2-picrylhydrazyl (DPPH), and the antibacterial activity of the extracts against four bacteria was determined by serial dilution assay. Results indicated that the total phenolic content in ethanol extracts from leaves and bulbs of *A. jesdianum* ranged between 27.83 to 98.23 mg GAE/g extract. A comparison of all plant extracts in the DPPH assay indicated that ethanol extracts from the populations of *A. jesdianum* leaves were the most effective free radical scavenging agents. The extracts indicated moderate-to-good inhibitory activities against four bacteria, especially against *B. cereus*. This finding suggests that the bulbs and leaves of *A. jesdianum* may be considered a natural source of antioxidants and antimicrobial agents.

**Key words:** antioxidant activity, antibacterial activity, endemic herb, Yazdi onion

**Abbreviations:** DPPH – 1,1-diphenyl-2-picrylhydrazyl, GAE – gallic acid equivalents, BHT – butylated hydroxytoluene, MHB – Mueller-Hinton broth, MIC – minimum inhibitory concentration, DMSO – dimethyl sulfoxide, MBC – minimum bactericidal concentration

### INTRODUCTION

Oxidation, deterioration, and microbial contamination that occur in food products can lead to sickness of consumers and economic loss to processors [Yanishlieva et al. 2006]. Herbs can contain a wide variety of free radical scavenging molecules, especially phenolic compounds such as phenolic acids, flavonoid, quinones, coumarins, lignans, stilbenes, and tannins that have antioxidant activity [Shahidi and Naczki 1995, Conforti et al. 2009, Ghasemi Pirbalouti et al. 2013, Bajalan et al. 2017, Vosoughi et al. 2018]. Primary

sources of naturally occurring antioxidants are whole grains, fruits, spices, and vegetables [Rahman et al. 2012]. For this reason, the herbs and their constituents have been used in the food industry for their flavoring and biological activities since ancient times [Katalinic et al. 2006]. Phenolic compounds present in spice plants, as dietary sources, possess bioactive properties protecting cellular systems against oxidative stress [Bajalan et al. 2017, Vosoughi et al. 2018]. Recently, interest in finding naturally occurring antioxidants to

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replace synthetic antioxidants in foods has increased considerably, primarily due to the possible carcinogenicity of synthetic antioxidants [Velioglu et al. 1998]. An antioxidant is a substance present at low concentrations compared to those of an oxide substrate, significantly delays or prevents oxidation of that substrate [Li et al. 2007].

The genus of *Allium* L. is the largest and important representative genus of the *Alliaceae* family comprising 700 species; each with different tastes, forms and colors; nonetheless, they are close in biochemical, phytochemical, and nutraceutical properties [Tepe et al. 2005]. *Allium* species are revered to possess antibacterial, antifungal, antiviral, antiprotozoal, and anthelmintic activities and they contain the powerful antioxidants, sulfur and other numerous phenolic compounds, that have aroused great interests for food industries [Griffiths et al. 2002, Benkeblia 2005, Ariga and Seki 2006, Taran et al. 2006]. The *Allium* species have been used for a long time as a medicinal for the prevention and treatment of certain diseases such as diabetes, arthritis, colds and flu, stress, fever, coughs, headache, hemorrhoids, asthma, arteriosclerosis, cancer, respiratory, gastrointestinal, rheumatic, and inflammatory disorders [Amin 1991, Hirsch et al. 2000, Kojuri et al. 2007, Bagheri et al. 2011]. Biological and medical functions of *Allium* species are due to their sulfur compounds, such as S-alk(en)yl-L-cysteine sulfoxides [Fritsch and Keusgen 2006], however, presence of phenolic compounds are also beneficial for human health [Corzo-Martinez et al. 2007].

*Allium jesdianum* is an endemic species of Iran that grows wild in the high altitude (1800–2600 m) in

the Zagros Mountains chain. Known as “Yazdi onion” or “Bonsorkh” in Persian, the leaves and bulbs of *A. jesdianum* are used for the treatment of abdominal pain, rheumatic pain, colds, and kidney problems by the indigenous people of Chaharmahal va Bakhtiari, southwestern Iran [Ghasemi Pirbalouti 2009]. Results of studies have shown analgesic effects of the extract of *A. jesdianum* [Yoshihiro and Minpei 1999]. Furthermore, there are some steroids in its bulb, which have shown cytotoxic and cytostatic effects against malignant tumor cells [Grases et al. 2009].

To our knowledge, there are no published reports on total phenolic content, antibacterial and antioxidant activities of various populations of *A. jesdianum*. The main objective of this study was to evaluate the content of phenolic compounds, antioxidants and antibacterial activities of ethanol extracts from the bulbs and leaves of *A. jesdianum*.

## MATERIAL AND METHODS

**Plant material.** Samples of bulb and leaves of *A. jesdianum*, collected from wild populations of plants growing in various alpine regions of southwestern Iran were used in this study (Fig 1. and Tab. 1). In total, three replicate samples of 30 plants were gathered from three natural habitats at the early flowering until April 30<sup>th</sup> to May 20<sup>th</sup> 2012. Plant identity was confirmed by Prof. V. Mozaffarian, and a representative voucher specimen (No. 100×) was been placed in the Herbarium of RCANR of Chaharmahal va Bakhtiari province, Iran.

**Table 1.** Geographical and climate of natural habitats of *Allium jesdianum*

Region	Altitude (m)	Latitude (UTM)	Longitude (UTM)	P (mm)	T (°C)	pH	E.C. (dS/m)	O.C. (%)	Sand (%)	Silt (%)	Clay (%)
Chalroghani	2609	0440446	3556437	779.9	12.6	6.68	0.35	1.014	16	38	46
Shirmard	2760	0526249	3476689	603.9	14.4	6.67	0.648	3.686	22	46	32
Dastena	2517	0476569	3542913	418.5	12.6	7.05	0.306	1.95	22	42	36
Saldaran	2504	0484154	3579016	406.0	11.9	7.64	0.633	2.301	23.5	38.5	38
Shaykh-Alikhon	2517	0410664	3597378	1025.1	9.7	7.21	0.389	1.092	26	32	42

P – annual precipitation (mm), T – average temperature (°C), E.C. – electrical conductivity (dS/m), O.C. – organic carbon (%)

Meteorological information was obtained from weather stations located within the study area and the surrounding zone; each value in the mean of 10 to 15 year data



Fig. 1. Aerial parts of *Allium jesdianum*

**Extract preparation.** Immediately following the collection, the separated leaves and bulbs of each *A. jesdianum* plant sample were bagged independently. The bulbs were rinsed with tap water and cut into small slices using a kitchen mixer. The tissue samples were subsequently air-dried in a shaded room at 30°C. A 100 g sample was extracted with 250 ethanol (99%, Merck, Darmstadt, Germany) at 45°C for 8 h followed by a Soxhlet apparatus. The ethanol was subsequently removed under reduced pressure on a rotary evaporator (Model Zirbus 302 W, Italy) at 40°C. The extracts were filtered using a Whatman No. 2.

**Determination of total phenolic content.** The total amount of phenolic compounds in each extract was determined using the Folin-Ciocalteu method following procedure of Singleton and Rossi [1965] with some modifications. Briefly, 0.5 ml of the sample was mixed with 2.5 ml of Folin-Ciocalteu's (Sigma-Aldrich Co., Steineheim, Germany) phenol

reagent for 5 min at 37°C, 2 ml of saturated Na<sub>2</sub>CO<sub>3</sub> (7.5%) (Merck Co., Darmstadt, Germany) was added and the mixture was brought to 10 ml with the addition of deionized, distilled water. The mixture was maintained at room temperature in the dark for 120 min and then the absorbance was measured at 765 nm against a reagent blank using a Perkin-Elmer Lambda UV/Vis spectrophotometer. Gallic acid (Merck Co., Darmstadt, Germany) was used as the reference standard and the total phenolic content was expressed as mg of gallic acid equivalents per gram of each extract on dry basis (mg GAE/g extract).

**Antioxidant test.** The DPPH radical scavenging activity of the ethanol extract was determined using the method proposed by Huang et al. [2005]. The extracts (100 µl) at concentrations of 8 to 500 µg/ml were mixed with 3.9 ml an equal volume of 0.2 mM ethanol solution of DPPH (Sigma-Aldrich Co., Steineheim, Germany). The disappearance of the DPPH after 30 min of incubation at room tempera-

ture was determined using a Perkin-Elmer Lambda UV/Vis spectrophotometer at 515 nm against a blank, i.e. without DPPH. Ethanol was used to zero the spectrophotometer and the absorbance of the DPPH radical without antioxidant and measure daily served as the control. The amount of sample necessary to decrease the absorbance of DPPH by 50% ( $IC_{50}$ ) was calculated graphically and the percentage inhibition was determined according to the equation:

$$\% \text{ inhibition} = \frac{AC_0 - AA_t}{AC_0} 100$$

where:  $AC_0$  is the absorbance of the control at  $t = 0$  min and  $AA_t$  is the absorbance of the antioxidant at  $t = 30$  min. The inhibitory concentration ( $IC_{50}$ ) of the extract needed to inhibit 50% of the DPPH radicals obtained from the standard curve was compared to that of standard/commercial antioxidants. The food preservative butylated hydroxytoluene (BHT) was used as positive control. All measurements were replicated three times.

**Antibacterial test.** Antibacterial activities of the extracts were tested using clinical isolates of four bacteria strains, the Gram-positive bacteria (*Bacillus cereus* and *Listeria monocytogenes*) and the Gram-negative bacteria (*Proteus vulgaris* and *Salmonella typhimurium*). The bacteria, originally obtained from chicken meat samples, were provided by the Food Microbiology Laboratory, Veterinary Medicine Faculty, I.A.U., Iran. The population of each bacterial strain was increased by culturing in an overnight Mueller-Hinton broth (MHB) at 37°C. To quantify the antibacterial activity of extracts, bacteria populations were prepared for testing by adjusting each population to 1.0 McFarland standards ( $1.0 \times 10^7$  CFU/ml), using a spectrophotometer. Minimum inhibitory concentrations (MIC) were determined using the broth-serial dilution method following standardized methods [CLSI 2012]. The extracts and the antimicrobial agents (ciprofloxacin, and flumequine) were each dissolved in 5% DMSO and then diluted to the highest test concentration. Subsequent test concentrations were made in a series of two-fold dilutions to develop concentration levels of 8 to 500 µg/ml in sterile, 10 ml test tubes containing MHB. A population of bacteria was subsequently

added to each tube containing extract and/or antimicrobial agent and then incubated at 37°C for 48 h. After the incubation period, the absorbance of each incubated solution was measured at 630 nm using a spectrophotometer as a measure of bacterial growth to indicate MIC values [Zampini et al. 2005]. The minimum bactericidal concentration (MBC) of each essential oil was determined according to the MIC values by transferring 5 µl from MIC tubes to agar plates and incubating at 37°C for 48 h. The MBC was recorded as the minimum concentration of extract, in which no viable bacterial growth was observed. All experimental tests were replicated three different times.

**Statistical analysis.** Data were analyzed by one-way analysis of variance with three replications using the SPSS 19.0 statistical software. Means of the total phenolic content and  $IC_{50}$  of various extracts for antioxidant assays were compared with Duncan test at  $p \leq 0.05$  level.

## RESULTS AND DISCUSSION

**Extraction yield.** The colors of the ethanol extracts from the leaves and bulbs of *A. jesdianum* were light red and dark red, respectively. Statistical analysis indicated that there was significant difference ( $p \leq 0.01$ ) among various populations for extract yield. The highest extract yield was obtained from the leaves of Dastena population of *A. jesdianum* with 61.7% w/w on dry weight basis (Tab. 2). No information on extracts yield of the leaves and bulbs of *A. jesdianum* is available.

**Total phenolic content.** Phenolic compounds are believed to account for a major portion of antioxidant capacity and antimicrobial activity in many plants [Yogesh et al. 2012]. A significant difference ( $p \leq 0.01$ ) for total phenolic content was measured among the extracts (Tab. 2). The maximum total phenolic content was obtained from the extract of *A. jesdianum* leaves collected from the Shirmard population with  $98.23 \pm 15.5$  mg GAE/g extract (Tab. 2). The lowest total phenolic content was achieved for the extract from leaves of *A. jesdianum* collected from Dastena with  $27.83 \pm 2.5$  mg GAE/g extract (Tab. 2). In a study, the amount of total phe-

nolic in the extracts from three *Allium* species ranged from 75 to 80000 mg/kg freeze-dried plant material [Nuutila et al. 2003]. Results of a study [Prakash et al. 2007] indicated that the total phenolic contents in extracts from four varieties of *A. cepa* varied from 4.6 to 74.1 mg GAE/g. Within the vegetable family, the composition and quantity of the phenolics vary significantly according to different intrinsic and extrinsic factors, such as plant genetics and cultivar, soil and growing conditions, maturity state and harvest conditions [Jeffery et al. 2003].

**Antioxidant test.** The potential antioxidant activity of extracts was determined by the scavenging activity of stable free radical DPPH. This is a quick, reliable and reproducible method to assess the *in vitro* antioxidant activity of pure compounds as well as plant extracts [Ghasemi Pirbalouti et al. 2014]. The effect of antioxidants on DPPH is based on their ability to donate a hydrogen atom to DPPH, thus converting the radical into a stable molecule [Diouf et al. 2009]. Lower IC<sub>50</sub> value indicates a stronger ability of the extract to act as a DPPH scavenger, while higher IC<sub>50</sub> value indicates a lower scavenging activity of the scavengers as more scavengers were required to achieve 50% scavenging reaction. In our study, the antioxidant activity of the extract from various populations of *A. jesdianum* was expressed as IC<sub>50</sub> with values from 0.33 to

2.15 mg/ml indicating that extracts act as moderate to good DPPH scavenger (Tab. 2). Significant differences ( $p < 0.01$ ) in IC<sub>50</sub> values were found for different parts and populations of *A. jesdianum*. A comparison of all plant extracts in the DPPH assay indicated that ethanol extracts from the leaves of three populations and the bulbs of a population of *A. jesdianum* (IC<sub>50</sub> values ranged from 0.32 to 0.48 mg/ml) were the most effective free radical scavenging agents (Tab. 2). The extracts from three populations of *A. jesdianum* leaves have the highest total phenolic content (80.31 to 89.23 mg GAE/g extract). The total phenolic in these plant extracts provided substantial antioxidant activity. The antioxidant activity of *Alliums* species have been reported by numerous investigators [Velioglu et al. 1998, Ghasemi Pirbalouti et al. 2015].

Chaithradhyuthi Gayathri et al. [2009] reported that the antioxidant properties of methanol extracts from some *Allium* species, with *A. ascalonicum*, had the maximum radical scavenging activity and total phenolic content. In present study, the extracts from leaves had higher antioxidant activity than bulbs. This study confirms the results obtained in previous researches [Nencini et al. 2007, Ghasemi Pirbalouti et al. 2015]; in fact, these studies showed that the wild-type species of *Allium* and in particular leaves are more active and efficient in respect to bulbs.

**Table 2.** Extract yield, antioxidant activity, and total phenolic content of the ethanol extracts from *Allium jesdianum*

Species	Part used	Populations	Extract yield (% w/w)	Total phenolic (mg GAE /g extract)	IC <sub>50</sub> (mg/g)
<i>A. jesdianum</i>	leave	Chalroghani	49.9 ±25.9 ab†	80.31 ±19.39 ab	0.32 ±0.03 a
<i>A. jesdianum</i>	leave	Shirmard	57.8 ±12.4 ab	98.23 ±15.58 a	0.47 ±0.30 a
<i>A. jesdianum</i>	leave	Dastena	61.7 ±29.7 a	92.65 ±4.47 a	0.46 ±0.15 a
<i>A. jesdianum</i>	bulb	Chalroghani	45.9 ±13.98 ab	36.03 ±2.45 c	0.48 ±0.06 a
<i>A. jesdianum</i>	bulb	Saldaran	42.83 ±12.55 ab	32.55 ±8.11 c	1.16 ±0.14 b
<i>A. jesdianum</i>	bulb	Dastena	45.91 ±16.22 ab	27.83 ±2.58 c	1.42 ±0.41 c
<i>A. jesdianum</i>	bulb	Shaykh-Alikhon	33.31 ±21.56 b	33.96 ±2.19 c	2.15 ±0.66 d
BHT	–	–	–	–	0.21 ±0.03 a
ANOVA			$p \leq 0.01$	$p \leq 0.01$	$p \leq 0.01$

†Values in column having similar letter are not statistically different at  $p \leq 0.05$

**Table 3.** Antibacterial activity (MICs and MBCs) of the ethanol extracts from *Allium jesdianum* against four bacteria

Species/ Antibiotics	Part used	Populations	<i>Bacillus cereus</i>		<i>Listeria monocytogenes</i>		<i>Pereteus vulgaris</i>		<i>Salmonella typhimurium</i>	
			MIC (µg/ml)	MBC (µg/ml)	MIC (µg/ml)	MBC (µg/ml)	MIC (µg/ml)	MBC (µg/ml)	MIC (µg/ml)	MBC (µg/ml)
<i>A. jesdianum</i>	leave	Chalroghani	250	500	62.5	250	250	500	125	250
<i>A. jesdianum</i>	leave	Shirmard	125	250	62.5	125	250	>500	250	500
<i>A. jesdianum</i>	leave	Dastena	250	500	125	500	250	500	125	250
<i>A. jesdianum</i>	bulb	Chalroghani	250	500	250	500	250	500	125	250
<i>A. jesdianum</i>	bulb	Saldaran	125	250	250	500	250	500	125	500
<i>A. jesdianum</i>	bulb	Dastena	125	500	250	500	250	500	250	500
<i>A. jesdianum</i>	bulb	Shaykh- Alikhon	250	500	250	500	250	>500	250	500
Ciprofloxacin	–	–	32.2	125	32.2	62.5	62.5	125	62.5	125
Ampicillin	–	–	62.5	125	62.5	125	125	250	125	250

**Antibacterial test.** Extracts from various populations of *A. jesdianum* demonstrated relative inhibitory activities against pathogenic bacteria tested, the MICs and MBCs of the tested samples are presented in Table 3. Results indicated that different bacteria species demonstrated different levels of sensitivity to the extracts. The MICs of extracts were within concentration ranges from 0.062 to 0.25 mg/ml, and respective MBCs were from 0.125 to >0.50 mg/ml. Generally, the ethanol extracts from leaves and bulbs of *A. jesdianum* indicated moderate to good inhibitory activities against four bacteria. The highest antibacterial activity was obtained from the extracts of leaves of the Chalroghani and Shirmard populations against *L. monocytogenes*. Probably in present study, the phenolic compounds are responsible of the antibacterial activity of the extracts from *A. jesdianum*, especially in the leaves of *A. jesdianum*. Amin and Kapadnis [2005] reported the antimicrobial activity of the water extract from *A. ascalonicum* (shallot). The mechanisms, due to which plant extracts can inhibit microorganisms vary. Phenolic compounds can act at two different levels: the cell membrane and cell wall of the microorganisms [Taguri et al. 2006]. They can interact with the membrane proteins of bacteria by means of hydrogen bonding through their hydroxyl groups, which can result in changes in membrane

permeability and cause cell destruction. Phenolic compounds can also penetrate the bacterial cells and coagulate the cell content [Tian et al. 2009]. Several investigators have observed antibacterial activity of *Allium* species extracts and have attributed this activity to thiosulfonates compounds, which arise from *Allium* species [Benkeblia et al. 2007, Ghasemi Pirbalouti et al. 2015]. These compounds are unstable and give rise to transformation products; however, polar compounds as polyphenols are more stable in cooking and storage [Ahmad and Beg 2001, Lanzotti 2006, Ghasemi Pirbalouti et al. 2014, Ghasemi Pirbalouti et al. 2015].

## CONCLUSIONS

The present study is apparently the first report of quantitative total phenol profile, antioxidant and antibacterial activities of the ethanol extracts from leaves and bulbs of *A. jesdianum*, as an Iranian endemic herb. This medicinal plant is ordinarily used for food and pharmaceutical purposes and as health foods in Iran. Results of this study indicated that the extracts from leaves of *A. jesdianum* had the highest antibacterial properties. The results of current study demonstrated that the ethanol extracts from *A. jesdianum* with the maximum total phenolic content espe-

cially in the leaves had the highest antioxidant activity. Phenolic compounds present in the plant are responsible for its effective free radical scavenging, antioxidant and antimicrobial activities. In conclusion, *A. jesdianum* could be an important dietary source of phenolic compounds with antioxidant capacity and antibacterial properties. Nonetheless, in order to gain better views on the antioxidant levels and activities in *Allium* species, further studies on purification, identification and quantification of each phenolic compound and other non-phenolic compounds are necessary in future.

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