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Phytochemical Screening of the Aqueous Extracts of Iranian Onion (*Allium cepa* L.) Landraces

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ABSTRACT: Onion, as one of the most valuable plants in nature and human diets, includes various phytochemicals compounds. Due to the consumption of these biological compounds as edible bulbs or additives by humans, these compounds play critical roles in fighting several diseases. In the current study, three Iranian landraces onions were selected to assess their total phenolic content, flavonoid and antioxidant activity, comparing the onion bulb layer extractions. Total phenols were assessed using modified Folin-Ciocalteu method. Antioxidant activity was evaluated by scavenging stable free radical 2,2-diphenyl-1-prierylhydrazyl. The results showed that from the three factors, cultivars and layers of the onions included significant effects on phytochemical compounds. A wide range of responses (11.24–17.00 mg GAE/G DW) were achieved in onion layers with respect to total phenolic content. Considering that the onion bulb outer layers include the highest level of the highlighted characteristics, the highest and the lowest quantities of total phenolic content were respectively detected in Azar Shahr and Neyshabur cultivars and accordingly for other two traits. Although use of Cycocel foliar spray increased the value of total phenolic content and flavonoid, it did not include significant differences with the control. Thus, the aim of the current study was to provide helpful information with phytochemical compounds of the Iranian onion landraces.

Keywords: *Drugs, Free Radical, Medicinal Plant, Oxidation, Phytochemical Compound.*

Introduction

The common onion (*Allium cepa* L.), as the perennial and oldest cultivated plant, is usually used as vegetable and food flavoring worldwide. Furthermore, this plant is highly valued for its therapeutic characteristics. Onion has been used as a food remedy from millenniums ago. This plant consists of several varieties with a long history of medicinal uses. Widespread assessments have indicated that these species are rich sources of putative health-promoting phytochemicals such as flavonoids and organosulfur compounds. Up-to-date, the Allium family, as a source of leading vegetable crops in world with their biological and phytochemical characteristics, has comprehensively been studied (Sampath Kumar *et al*., 2010; Marrelli *et al*., 2019; Sadeghian *et al*., 2020). Due to the increasing demands for alternative compounds of current chemicals with

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pharmacological roles, researchers are fascinated to study the available traditional plants for their medicinal potentials (Karthishwaran *et al*., 2018). For the generation of energy by mitochondria, cells use oxygen and hence create free radicals as a catabolite of adenosine triphosphate (ATP) production (Pham-Huy *et al*., 2008). Free radicals with various chemical structures consist one or more unpaired electrons in their external orbitals and generally display a significant reactivity. For several decades, these structures have been suggested to include exclusively damaging effects. Nowadays, novel evidences have cleared that the living systems not only have been adapted to free radicals but also have developed mechanisms to turn these toxic substances to non-toxic ones using free radicals in critical physiological processes (Di Meo & Venditti., 2020).

Free radicals and oxidants play a dual role of toxic and beneficial compounds since they can be harmful or helpful to the body. The chemicals are produced from either normal cell metabolisms or external sources (e.g. pollution, cigarette smoke, radiation and medication) (Pham-Huy *et al*., 2008). Oxidative stress can be attributed to various diseases such as cancers, diabetes, aging, hypertension, atherosclerosis and neurodegenerative disorders (Feghhi-Najafabadi *et al*., 2019). Cell free radicals and reactive oxygen species (ROS) can be naturalized by a complex of natural compounds, called antioxidants. In various sciences, biological activity of these compounds has become as one of their most interesting characteristics (Zehiroglu & Sarikaya, 2019). Several compounds such as polyphenols and carotenoids, as natural antioxidants with a wide range of biological characteristics, are widely distributed in food and medicinal plants (Xu *et al*., 2017). Antioxidant activities in plants have contributed to natural cell reactions such as delaying, preventing and removing oxidative damages to target molecules (Ayodeji *et al*., 2018). In order to neutralize oxidative stress using antioxidants, the human body has several mechanisms which are natural or externally acquired through foods and other supplements (Pham-Huy *et al*., 2008). Therefore, numerous plants can be used in pharmaceutical industries as potential sources of natural antioxidants and phytochemicals (Karthishwaran *et al*., 2018).

Recently, popular interests on investigation of antioxidant plants and phytochemicals have increased to decrease risks of various diseases and improve the quality of life (Feghhi-Najafabadi *et al*., 2019). Edible plants such as Allium family have great potentials as functional compounds that are able to prevent or treat several human diseases (Marrelli *et al*., 2019). To investigate these potential sources and develop their uses in functional foods, pharmaceuticals and food additives, effective extraction and appropriate assessment of these compounds in medicinal plants are necessary (Xu *et al*., 2017). Moreover, plants are considered as sources of good incomes in pharmaceutical industries. Nowadays, increasing demands for medicinal plants due to their benefits such as easy availability and less side-effects seem logical. According to this idea, research on plant derivatives in various fields such as pharmaceuticals, foods and human health is not extensive (Karthishwaran *et al*., 2018). Considering the genetic origin of the onion plants in Iran and its neighbors, investigating chemical compounds of local varieties with more affections on human health can be challenging (Alemzadeh Ansari, 2007; Keighobadi *et al*., 2020). The current study has investigated cell chemical compounds linked to human health. The aim of this study was to develop methods for cell compound extraction and analysis.

Materials and Methods

- *Plant materials*

This study was carried out in Plant Improvement and Seed Production Research Center (PISPRC) laboratories, Isfahan (Khorasgan) Branch of Islamic Azad University, Isfahan, Iran. The mature seeds of three Iranian landraces of Azar Shahr, Neyshabur and Gholi Gheseh were obtained from local farms and Seed and Plant Improvement Institute, Isfahan, Iran. These were then screened and collected in one generation cultivation based on the agronomic descriptions. Selected seeds were sterilized in 70% ethanol for 90 s before they were immersed in sterile water. Treated seeds were planted in plastic pots and divided into two major groups. Grouped pots were sprayed with solutions, containing various concentrations of Cycocel (CCC) as plant growth regulator. Based on their physiological maturation and characteristics, onion plants were harvested at similar maturity stages after 50% of the leaves collapsed. Foliage was removed from the bulbs and cleaned. These bulbs were carefully washed under running tap water and dried with soft clothes. Table 1 presents brief descriptions on the onion cultivars.

- *Onion peels and preparation of extractions*

In order to extract polyphenols, three outer layers of the fresh bulbs were separated as external layers and the other layers were collected. Onion peels were separated, washed, cut into 4 × 4 mm pieces, frozen, lyophilized, ground in stainless steel blenders, mixed with distilled water at 1:2 (W/V), passed through 40-mesh sieves and then freeze-dried. Freeze-dried onions were ground using mortar to obtain fine powder and stored at 4 ^{o}C in dark at dry atmosphere. To avoid deterioration of onion peel extracts, appropriate methods such as avoidance of light exposure during the extraction processes were used (Santas *et al*., 2010; Park *et al*., 2007).

- *Chemicals*

Folin-Ciocalteu reagent (99.8%), chlorogenic acid (95%), quercetin (99%), rutin (99%), ascorbic acid (99%) and DPPH (1, 1-diphenyl-2-picrylhydrazyl) (92%) were purchased from Sigma-Aldrich, St Louis, MO, USA. Other chemicals were purchased from Merck, Darmstadt, (Germany).

- *Assessment of radical scavenging activity*

Based on the reduction of DDPH in methanol, assessment of the radical scavenging activity (RSA) was carried out, as described previously by Benkeblia (2005) with mild modifications. Briefly, 50 µL of each extract were added to the methanolic solution (2.5 µL) of DDPH and the mixture was vortexed. After 30 min in dark at room temperature, absorption was measured at 517 nm by UNICO S2100 Recording Spectrophotometer. The RSA toward DPPH was estimated from the following equation (Benkeblia, 2005):

$$\%\text{Inhibition} = (A_{\text{control}} - A_{\text{sample}}) / 100$$

Where, A was absorbance of the control and sample, respectively (Karthishwaran *et al*., 2018).

Table 1. A brief description on the onion cultivars collected from various geographical regions

Cultivar No.	Province	City	Latitude (o N)	Longitude (o E)	Bulb color
1	East Azerbaijan	Azar Shahr	37.7443	45.9872	Red
2	Razavi Khorasan	Neyshabur	36.2141	58.7961	White
3	Zanjan	Zanjan	36.6830	48.5087	Copper

Nos. 1, 2 and 3 represent Azar Shahr, Neyshabur and Gholi Gheseh cultivars, respectively

- ***Assessment of total phenolic and flavonoid contents***

Due to the origins and roles of phenolic compounds in antioxidant activities of plants, as well as the lack of permanent correlations between these activities with the presence of large quantities of phenols (Bozina *et al*., 2008), sets of data were necessarily studied. Onion extractions were investigated for their total phenol and flavonoid contents. Total phenol content (TPC) values of the extracts were assessed using Folin–Ciocalteu method, as described previously by Sadeghian *et al*. (2020). These values were assessed using calibration curve prepared with gallic acid (R^2 = 0.9802). Results were expressed as mean ±SD in mg of galic acid per g of dry weight. Flavonoid contents were assessed in the various fractions as described by Sadeghian *et al*. (2020), with modifications.

- ***Statistical analysis***

Parameters of the three onion cultivars belonging to each CCC treatment group were analyzed. The results are presented as means and standard deviation (SD). Analysis of variance was carried out and the least significant differences were calculated using the least significant difference (LSD) test ($p \leq 0.05$). The statistical analysis was carried out using SAS v.9.2 and Excel 2007 Software.

Results and Discussion

In the current study, effects of treatments on onion plant biological characteristics such as edible bulb antioxidants were assessed using plant growth regulators such as CCC. In order to carry out the aim, extracts of three famous onion cultivar bulbs were treated with CCC. The plant samples, abundantly cultivated in agricultural regions of Iran, were investigated for their total phenolic and flavonoid contents as well as antioxidant activities. Despite treatment of onion plants with growth hormones in the present study, results showed that the edible onion bulbs could be important sources to combat cell biological disorders. These plants (as integrate parts of nature) have served humans for hundreds of years. Of these plants, herbals are used for the treatment of several diseases with no significant harms. *Allium* species is one of the remedial herbs with bioactive parts, which acts as a medicinal plant and is used to cure diseases (Ahmad khan *et al*., 2017). The analysis of variance showed that the cultivars and onion layer treatments (as effective factors) included significant effects on all the biological characteristics. However, use of CCC treatment showed significant effects only on DPPH (Table 2). Based on the results from the current study, genetic variations in Iranian onion cultivars can play significant roles in their biological characteristics such as phenol and flavonoid contents and antioxidant activities. It seems that various compounds in these plant cells can help improve these activities. Onion landraces with numerous genetic, biological and morphological diversities have been cultivated in several regions of Iran (Alemzadeh Ansari, 2007). Results of further studies on the two-way interactions indicated that layer and cultivar interactions included significant effects on flavonoid and DPPH traits.

Results of various onion bulb layers with respect to the assessed Iranian cultivars demonstrated that the highest levels of total phenolic content (TPC) were in outer layer extractions. A wide range of responses (11.24–17.00 mg GAE/G DW) were seen in the three cultivars. Moreover, data showed that Azar Shahr, Gholi Gheseh and Neyshabur landraces respectively included the highest values of these compounds. The onion plant belongs to *Allium* as a genus of monocotyledonous flowering plants with hundreds of species. Onion as an edible and famous plant in this species is known to include sulphur amino acids as well as

several vitamins and minerals. Secondary metabolites such as flavonoids, phytosterols and saponins have also been found in onion (Marrelli *et al*., 2019). Large quantities of phytochemical compounds such as total polyphenols and quercetin have been reported in onion peels. As these compounds could suppress lipid oxidation *in vivo*, onion peels could be useful sources for the functional food industries (Park *et al.*, 2007). Further findings of various bulb parts demonstrated that the outer layers included more effective compound than the inner layers. Results of flavonoid content assessments revealed that the highest and the lowest levels (respectively 1.37 and 0.80 mg/g DW) were achieved in the outer layers of Azar Shahr and Gholi Gheseh, respectively. The highest (18.86%) and the lowest (2.25%) antioxidant activities were achieved in Azar Shahr, Gholi Gheseh and Neyshabur cultivars, respectively.

- Radical scavenging activity

Data showed that a wide range of responses to the antioxidant potentials were achieved in this experiment (Table 3). Statistical analyses showed distinct differences in antioxidant data from all trials when various levels of the investigated factors used. In comparisons of the cultivars, significant differences were presented from the lowest to the highest in Neyshabur, Gholi Gheseh and Azar Shahr landraces, respectively. Data illustrated that use of CCC as promoting plant growth regulator could improve plant antioxidant activity with significant differences between the treatments. In assessing two parts of the onion bulbs, significant differences were seen between the two layers and their changing of responses from the outer to the inner layers has decreased (Figure 1).

Table 2. Effect analyses of variance of various factors on phytochemical characteristics of the onion layer extractions

Source	df	Mean square on biological characteristics		
		Flavonoid	TPC	DPPH
Model	11	0.0402^{**}	0.5238^{**}	2.9791^{**}
CCC	1	0.0048^{ns}	0.0693^{ns}	0.6621^{**}
Cultivar	2	0.0749^{**}	0.8815^{**}	10.2681^{**}
Layer	1	0.1146^{**}	1.0212^{*}	9.1292^{**}
CCC × cultivar	2	0.0122^{ns}	0.6639^{*}	0.2703^{*}
CCC × layer	1	0.0440^{ns}	0.3424^{ns}	0.0648^{ns}
Layer × cultivar	2	0.0467^{*}	0.2591^{ns}	0.9114^{**}
CCC × layer × cultivar	2	0.0053^{ns}	0.3598^{ns}	0.0072^{ns}
Error	24	0.0106	0.1404	0.0726
Corrected total	35	-	-	-
CV	-	9.14	10.25	9.63

df, TPC, DPPH and CCC represent degrees of freedom, total phenolic content, 1,1-diphenyl-2-picrylhydrazyl and Cycocel, respectively. *, ** and ns represent significances at 0.05, 0.01 and no significance, respectively.

Table 3. Total phenolic content expressed as mg/g gallic acid equivalent (GAE), Antioxidant activity and flavonoid content in various cultivars and layers of onions (*Allium cepa* L.) on dry weight basis

Cultivars	Layer	AOA (%)	TPC (mg GAE/G DW)	Flavonoid content (mg/g DW)
Azar Shahr	Outer	18.86	17.00	1.37
	Inner	7.60	13.65	0.86
Gholi Gheseh	Outer	12.18	15.52	0.80
	Inner	6.31	11.24	0.85
Neyshabur	Outer	3.91	11.36	1.34
	Inner	2.25	11.29	0.97
LSD at $p < 0.05$	For layer	8.52	13.34	1.03
	For cultivar	9.25	13.38	1.11

AOA, antioxidant activity; TPC, total phenolic content; LSD, the least significant difference.

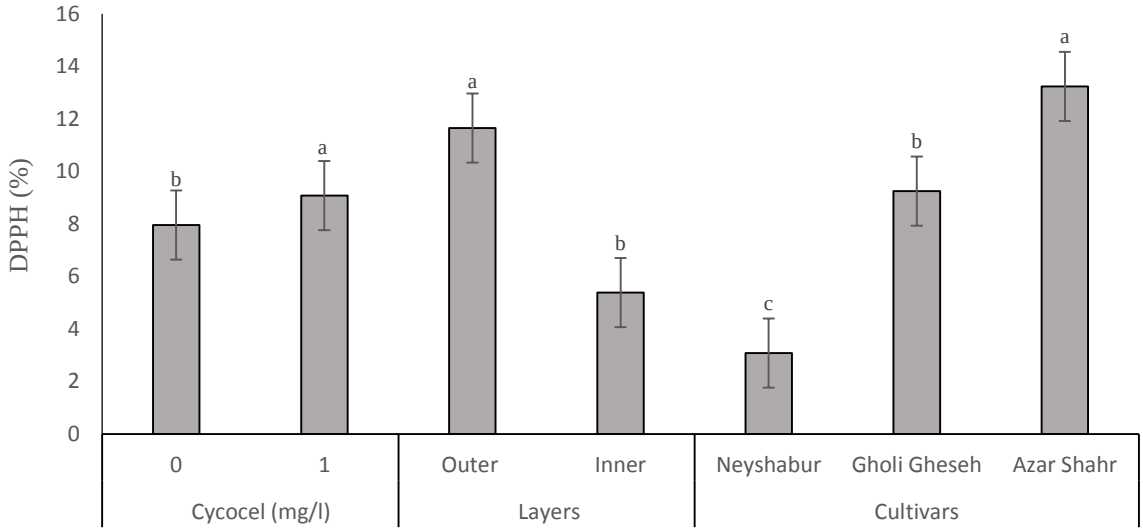

Fig. 1. Comparison of Cycocel, bulb layers and cultivars on antioxidant activities of the onion extracts.

For every assayed factor, letters were compared separately. Chart columns followed by a common letter are not significantly different at 5% levels based on the least significant difference test.

To inhibit free scavenging radicals, antioxidants are suggested as critical chemicals (Kaur *et al.*, 2016). Various plants are reported as important sources of phenolic compounds. They can be associated to antioxidant activity and free radical scavenging ability in human health (Sowndhararajan & Kang, 2013).

- Phenolic and flavonoid contents

The TPC in onion samples are presented in Table 3. The TPC ranged 11.24–17.00 mg GAE/G DW. The highest and the lowest levels of these compounds were reported in Azar Shahr and Gholi Gheseh cultivars, respectively. Based on the results, the Azar Shahr cultivar can be suggested as a better source of phenolic contents, compared to other cultivars. This variability of responses could be attributed to various genetic sources of the cultivars. Results of comparisons between the investigated factors showed no significant differences between responses of the phenolic contents in use of various quantities of CCC and onion layers. However, significant differences were seen between various cultivars used in this study. In assessment of this biological trait and despite significant differences, the Gholi Gheseh landrace did not included differences with other two cultivars (Figure 2). Due to destructive roles of free radicals in cells and occurrence of common disorders such as cancers, aging and autoimmune disorders (Pham-Huy *et al*., 2008), it seems that onion can play critical roles in controlling these disorders. Cells include several mechanisms to counteract oxidative stresses. An example of these mechanisms includes production of antioxidants, which are naturally produced *in situ* or externally supplied through foods and/or supplements (Pham-Huy *et al*., 2008).

In flavonoid content assessment, similar results were observed with those of the phenolic content assessment. The highest level of this biological compound was detected in the outer layers of bulbs, compared to those of the inner layers. The highest level of this compound was reported in Azar Shahr cultivar with no significant differences with that in Gholi Gheseh

cultivar (Figure 3). In addition to predominant use of onion in foods, a wide range of beneficial characteristics such as antioxidant, antimicrobial and antidiabetic characteristics have been investigated in this plant (Marrelli *et al*., 2019).

Conclusion

Onion is certainly one of the major sources of pharmaceutical and chemical compounds, including phenolic, flavonoid and antioxidant compounds based on multiple genetic structures from various onions, their compositions, concentrations and beneficial activities. Furthermore, plant growth regulators such as CCC which are effective in increasing or decreasing intracellular compounds can be used to improve the quality of onion bulbs. Due to the onion indispensability for these compounds and associated defense reaction mechanisms, regular consumption of onion seems necessary. Compounds within the edible layers of onions can affect various responses to TPC, flavonoids and antioxidants. Based on the promising findings from this study, further developments of the current assessment seem important.

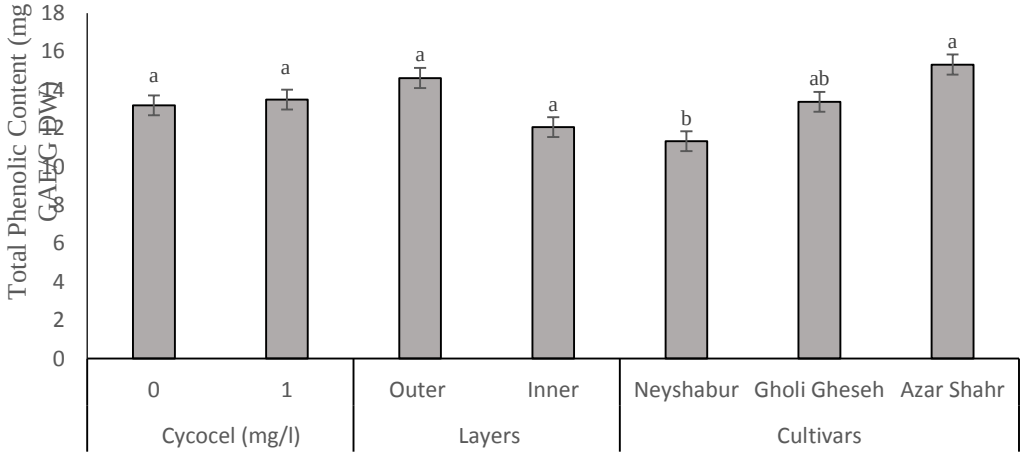

Fig. 2. Comparison of Cycocel, bulb layer and cultivars on total phenolic content of the onion extracts.

For every assayed factor, letters were compared separately. Chart columns followed by a common letter are not significantly different at 5% levels based on the least significant difference test.

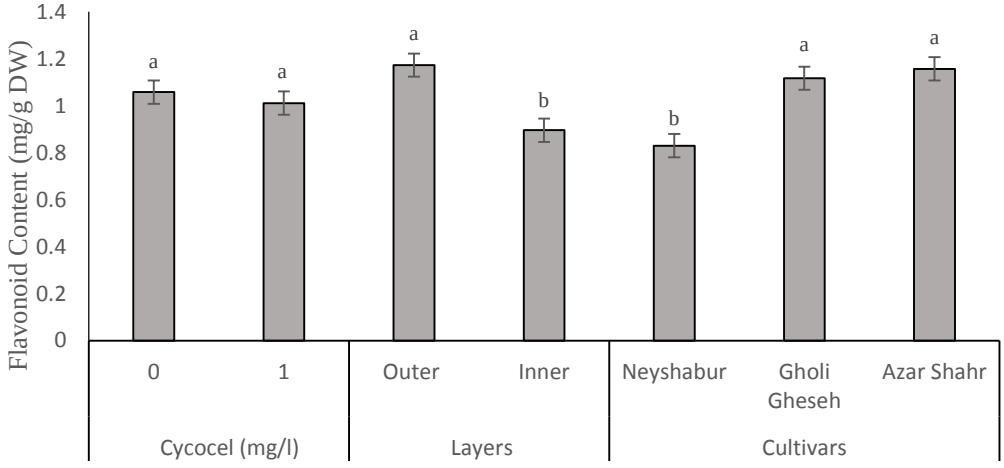

Fig. 3. Comparison of Cycocel, bulb layers and cultivars on flavonoid content of the onion extracts.

For every assayed factor, letters were compared separately. Chart columns followed by a common letter are not significantly different at 5% level based on the least significant difference test.

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