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Research Article

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Study on free radical scavenging activity of carrot (*Daucus carota* L.) grown in three different regions of Iran

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ABSTRACT

Carrot (Daucus carota L.) is one of the valuable vegetables belonging to Umbelliferae plant family and being greatly cultivated in Iran. Our previous study on carrot samples taken from different climatic regions of showed that; some weather changes could affect the carotenoids levels in carrot. Since carotenoids levels in carrot may have influence on the antioxidant activity of the plant; therefore the present study was aimed to determine the maximum free scavenging activity of carrot among Iranian carrot growing regions. Furthermore we studied the association between cartotenoids levels and scavenging activity in carrot. The samples were extracted with 60 % acetone and centrifuged and then the aqueous extracts were evaluated using DPPH (2, 2 - diphenyl - 1- picryl-hydrazyl) for free scavenging activities. IC_{50} of samples were finally compared to ascorbic acid. The highest and lowest inhibition of DPPH radical was found to be 47.20 ±10.42 and 263.86 ±39.53 which were related to carrots taken from Zarinshahr and Andimeshk regions in Isfahan and Khuzestan provinces, respectively. IC_{50} values obtained from these regions were ranged from 25.95 to 337.26. Strong scavenging activity of DPPH radicals was seen in carrots taken from Isfahan province with IC_{50} value of 1.20 times of Vit-C`s. There was not any significant correlation between carotenoid levels and scavenging activity. Based on this study we conclude that carrots from Zarinshahr in Isfahan have better antioxidant activity compared to other regions of Iran and its carotenoids levels could not play a prominent role in this property.

Keywords: Carrot, Free radical scavenging, Antioxidant, DPPH

INTRODUCTION

One of the most vegetables which grow in regions with temperate climate is carrot (*Daucus carota* L.) which belongs to Umbelliferae (Apiaceae) family [1, 2]. Carrot is a biennial plant which firstly found as wild form in southern west of Asia in places around Afghanistan and Iran countries [3, 4]. Presently, carrot is considered as one of vegetable that has the highest per capita consumption in comparison with the other vegetables [5, 6]. The nutritional value of this plant is very high; so that it concludes significant macromolecules such as carbohydrates, proteins, lipids those that the account as basis for lives [7]. Besides of that, carrots are rich in some minerals and pivotal vitamins like vitamin A, E and C [8, 9]. Carrot has also phenolic compounds and by these compounds may involve in improvement of some diseases such as cancer or aging decline and inflammatory disorders [10-12]. In carrots, there are various valuable resources of good natural antioxidant such as carotenoids, phenolic compounds and a series of the other compounds like flavonoids [13-15]. The natural antioxidants presents in fruits and vegetable

have been shown that have potential for attenuation of lipid peroxidation reactions which may occur in body of human beings if in otherwise not be antagonized its harmful effects by defense mechanism [16-19]. By focusing closely in pathogenesis of much disease it would be clear that free radicals through different mechanisms such as the oxidative stress have crucial role in formation and inductions of many diseases such as cancer and heart strokes [20, 21].

The carotenoids including α-carotene, β-carotene widely presents in kinds of carrots is important class of natural pigments and they have been demonstrated act as natural antioxidants [22, 23]. Since, based on scientific documents; there is an association between presence natural oxidant in fruits and vegetables with the reduced DNA damage; functional maintenance of immune system in humans those who consume largely of these plant resources, therefore nowadays the worldwide efforts have been focusing for finding plants or vegetable with better antioxidant properties [18, 22, 24,25]. Carrots in Iran are considered as a major and economical crop by which many of pharmaceutical, nutritional and beverage industries, in addition to agriculture area, gain benefit from that [2, 26, 27]. Because of wide variety of climatic in Iran, the carrot is a crop that can be planted and grown in some areas of the southern, Northern and central places of Iran [28]. Climatic difference in carrot cultivation places have caused to be produced in these regions types of carrots with various pigments like purple, yellow, and orange [29]. With regarding to these aforementioned differences; it seems that the chemical compounds present in the carrots based on different climatic conditions may be differed [30, 31]. Our before study on carotenoids level in carrots grown in some regions of Iran showed that , carrots taken from southern areas of Iran such Khuzestan province have higher carotenoids levels compared with other regions placed in central provinces such as Isfahan[32, 33]. Since some research have shown the carotenoids compounds can have an antioxidant role [34, 35] and the resultant scavenging activity may be due to this property, therefore the principal aim of this study was that the evaluation of scavenging activity of carrots grown in different parts of Iran and also its correlation with carotenoid levels which were obtained from our previous studies.

EXPERIMENTAL SECTION

Chemicals

The solvent and chemicals including Acetone, Methanol and Petroleum ether which were provided from Merck company (Darmstadt, Germany), 2,2 - Diphenyl-1- Picryl-hydrazil (DPPH) was purchased from Sigma company (St. Louis , MO, USA) and Ascorbic Acid (Vit-C) was provided by Daejung Chemical and Metals company (Shiheung, Korea). All chemicals were of the highest purity grade.

Sampling

Three regions of carrot cultivation which placed in central, southern and Northern provinces namely, Isfahan, Khuzestan and Gilan, respectively were selected and from each region thirty samples were taken (Fig1). The samples then were transferred to lab and cleaned from soil particles and kept at -75ºC till the date of analysis.

Plant extraction

Plant extraction and sample preparation were in accordance with a procedure which were adopted by Zhang and Hamauzu [16] along with some modifications which as follows. The carrot samples were chopped with a peeler and converted into a very small particles, then 10 gram of each minced sample was mixed with 15 ml of pure acetone and the mixture were left for 60 minutes.

Aqueous phase preparation of carrot samples

The prepared samples were then centrifuged at 7000g for 20 minutes. The supernatant of each sample were separated and the remained phase was again extracted with 20 ml of 60 % acetone within two successive steps. The whole supernatant was collected and the residue was discarded. The solvent were then evaporated under vacuum at 35 ºC. Then, the pigments and fatty acid were completely removed using petroleum ether with a ratio of 2/1 (V: V) with in two successive steps.

Free radical scavenging activity evaluation

Scavenging activity of carrot samples were done using DPPH free radical and based on Brand – Williams et al., [39] and with few modifications. Briefly 0.1mM solution of DPPH in methanol was prepared and 0.1 ml of the prepared aqueous phase of carrot extract was added to 2 ml of this solution. Then the mixture was incubated in darkness for

60 min. After this time the decrease in absorbance was read at 517 nm against blank which was methanol. Inhibition percentage (I %) was measured by following formula:

$$Inhibition\ Percentage\ (I\%) = \frac{Abs_{DPPH} - Abs_{Sample}}{Abs_{DPPH}} \times 100$$

Where: *Abs DPPH is the absorbance of DPPH when the extract was omitted; Abs $_{Sample}$ is the absorbance of carrot extracts. For each sample; the tests were carried out in three replications.*

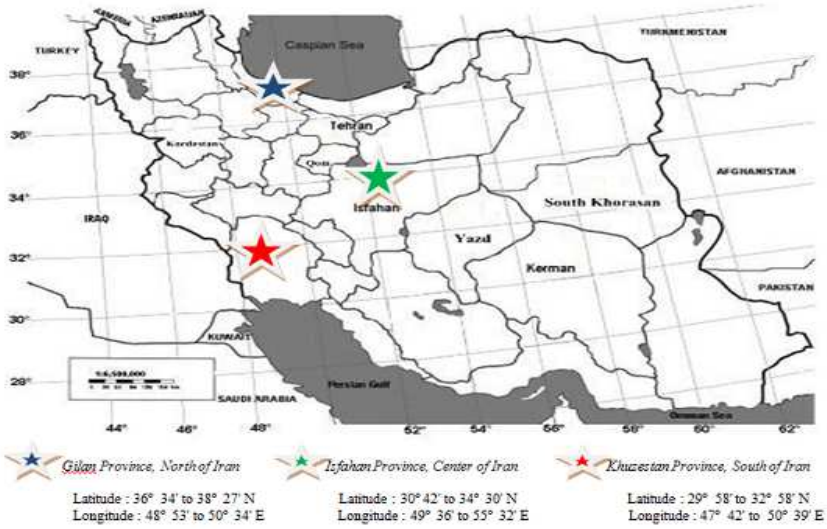

Fig 1. Map of Iran represents the places where carrots taken and depicted with asterisk
Longitudes and latitudes have been adopted from Archives of SID and the other geographical resources [36-38].

The IC_{50} value which showing the 50 % inhibition of DPPH radicals were calculated using SPSS software and the calculated values were compared with IC_{50} of Ascorbic acid which was considered as positive control

Association between carotenoid levels and scavenging activities
The correlation between carotenoids levels and DPPH free radical scavenging of carrots in regions of the study were determined by drawing a plot and making its equation.

RESULTS AND DISCUSSION

Free radical scavenging of carrots taken from different parts of Iran
The results of scavenging activity in various regions of carrot cultivations have been shown in fig 2. As shown in this figure, the highest mean of free radical scavenging activity was related to Zarinshahr region placed in Isfahan province which found to have the inhibition of 71.13 ± 3.81 % for DPPH radical. While the least free radical scavenging was among carrots taken from Andimeshk in Khuzestan province which had inhibition percent of DPPH free radical equal to 32.13 ± 2.30 %. There were seen significant differences ($P<0.001$) between carrots samples taken from Astaneh and Zarinshahr regions compared with the other regions namely Khomeinsahr, Dastgerd in Isfahan province and Dezful, Shoush and Andimeshk in Khuzestan province. All inhibitions obtained from various carrots cultivations were compared with that obtained from Vit –C value (Fig 2).

Free radicals are considered as unpaired components need to be taken electron from surrounding environment and also from macromolecules like DNA, RNA and proteins and lipids in human bodies [25]. It is obvious that the balance between the free radicals production left in body and antioxidants defences showing which pathway may be dominants [40]. And in this regard we need to strengthen to defence mechanisms against free radicals which produced daily in body of living system [41]. The plants cover the potential sources of natural antioxidants such as

flavonoids, phenolic compounds carotenoids,Vit - A,Vit - C and a series of the other compounds such as Xanthophylls which may have a protective role for cells in body against harmful and destructive effects resulted from free radicals [42, 43]. In this regards carrot is considered as one of the richest plant antioxidants and being cultured in different regions of Iran as main product [15, 26].

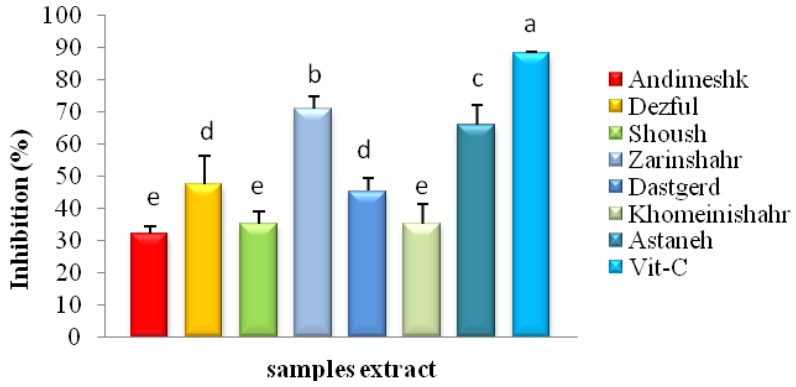

Fig 2. Comparison of DPPH free radical inhibition by aqueous extract of carrot (AEC) in some regions of Iran against Vit-C
[a-e] Mean values with different letters are statistically significant (P<0.001)

Our results concerning the antioxidant index for carrot in different areas was completely differed. Based on the results obtained from this study which were shown in Fig 2; the carrot samples taken from Zarinshahr in Isfahan province has allocated to itself the highest levels of antioxidants so as its free radical inhibition percent was found to be 71.13 ± 3.81 %. Therefore we expect that the carrots taken and consumed from this region have a better efficacy and can be candidate for protective tests against cancers in cell lines .Whilst the least amount of this property was related to Andimeshk in Khuzestan province (32.13 ± 2.13 %).

Determination of IC_{50} value for carrots taken from different regions of Iran

The powers of antioxidant activity for the fifty percent of inhibition of the free radicals by carrots were shown in Table 1. As shown in table 1 carrot samples taken from Zarinshahr had the lowest rate of IC_{50} which was equalled to 47.20 ± 10.42 µg/ml when compared to that of Vit - C which was 39.14 ± 0.35 µg/ml . While the carrots of Andimeshk had the highest mean value of IC_{50} equal to 263.86 ± 39.53 µg/ml. The acceptability level (lower and upper limits) of IC_{50} values for various carrots in Astaneh and Andimeshk regions were ranged from 25.95 to 337.26 µg/ml, respectively which were shown in Table 1.

Table 1. The capability of free radical scavenging activity (IC_{50}) in the aqueous extract of carrot resulted from some regions of Iran

Region	IC_{50} (µg/ml)	Lower limit	Upper limit	Sample / Vit-C IC_{50}
Andimeshk	263.86 ± 39.53^{e}	219.98	337.26	6.74
Dezful	154.71 ± 37.98^{d}	125.07	181.74	3.95
Shoush	233.29 ± 53.55^{e}	197.87	282.81	5.96
Zarinshahr	47.20 ± 10.42^{b}	41.41	53.65	1.2
Dastgerd	168.06 ± 39.42^{d}	133.12	204.24	4.29
Khomeinishahr	209.37 ± 33.48^{e}	174.11	254.75	5.35
Astaneh	69.83 ± 13.84^{c}	25.95	187.84	1.78
Vitamin C	39.14 ± 0.35^{a}	30.65	52.08	-

[a-e] Mean values with different letters are statistically significant (P<0.001).
Vit-C was used as positive control

The power of antioxidants (IC_{50}) which is defined as the concentration at which a component can inhibit 50 % free radicals and therefore free radical chain reactions will be ended [44]. In our study the ration of IC_{50} value of carrot samples to Vit- C value were calculated for each region as Zarinshahr (1.20), Astaneh (1.78), Dezful (3.95), Dastgerd (4.29), Khomeinishahr (5.35), Shoush (5.96) and Andimeshk (6.74)(Table 1). Based on this data it seems that carrots from Zarinshahr which followed by Astaneh are considered the most potent of free radicals terminator among Iranian cultivated carrots. Besides, in some scientific researches carried out on antioxidant activity of carrot have been shown; there are various phenolic compounds such as hydroxy cinamic acid and its derivatives

can significantly affect carrot antioxidant properties[14, 15, 45]. It is obvious that the formation, biosynthesis and the amounts of these types of compound in carrots can be structurally differed [46] and accordingly we expect that the levels of total phenols in carrots cultivated in Iran can be differed.

The association between scavenging activity and four predominant meteorological parameters

The results obtained from these associations have been shown in Table 2. As shown in this table ; the relationship between scavenging activity with meteorological parameters including Temperature (T) ; Relative humidity (RH) ; Rainfall (RF) and Altitude (A) were respectively as (R^2 of 0.36 , 0.058 , 0.130 and 0.024) in the whole of under studied regions .

Table 2. *Scavenging activity correlation with meteorological parameters

	(R^2)			
	T	**RH**	**RF**	**A**
The entire of regions of study	0.36	0.058	0.13	0.024

** Data resulted from mean of 210 carrot samples for seven regions of Iran*

Although some meteorological parameters in some studies have shown that they can have effect on caretenoids levels [32] we here showed that there is not any significant differences between some pivotal meteorologically parameters such as temperature, rainfall, relative humidity and altitude with scavenging activity of free radical of DPPH by carrot and we can not consider a significant relationship between antioxidant activity of carrots and climatic parameters. Further and complementary studies needed to make known these associations.

The results obtained from correlation between scavenging activity and carotenoids levels in whole regions of under study.

The results of this association were shown in Fig 3. As seen in this figure; there is not any significant correlation between carotenoids levels and free radical scavenging in whole regions of under study (R^2 of 0.314).

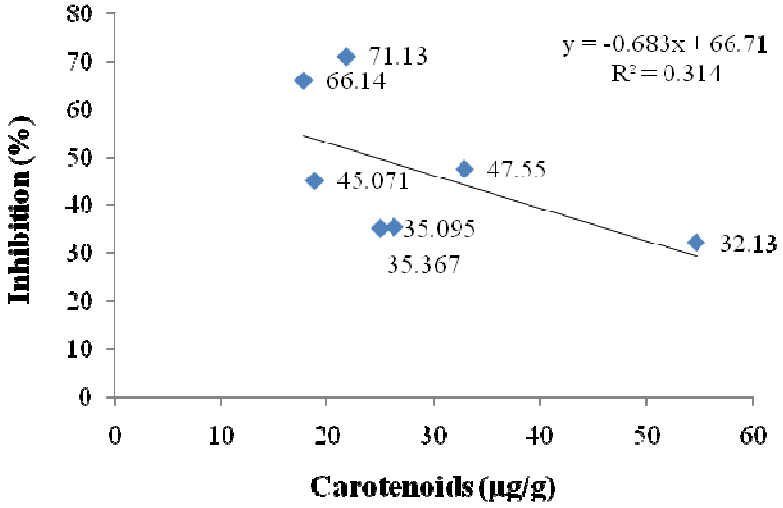

Fig 3. Correlation between scavenging activity of DPPH radical and the levels of carotenoids in Carrot samples

Our before studies on carotenoids levels demonstrated the carotenoids levels in some regions of Iran is not the same and there is significant differences in carotenoids levels in carrot taken from Khuzestan and Isfahan provinces [32]. Carotenoids containing compound may have effect on free radical scavenging and its antioxidant level [15, 23]. Contrary to this finding our results showed that there is not any relationship between carotenoids levels and antioxidant properties in carrots collected from different parts of Iran. It seems that some of the other factors including the capacity of total phenolic compounds, and the presence of some valuable compounds like xanthophylls may have effects in this difference [46].

CONCLUSION

Our results on antioxidant activity of carrots are consistent with some studies in which shown the major antioxidant properties of carrots was mostly associated to phenolic compounds rather than vitamin E , C and β –Carotene levels [47]. It has also been reported that the inhibitory potential of the free radicals by phenolic compounds such as chlorogenic acid is higher than Vitamin E and carotenoids` [46]. However the brightness and the colour of carrot can impact the free radical scavenging [13]. With regard to what mentioned above; it can be concluded that the Iranian carrots have in vitro the potential for inhibition of free radicals and in this direction some of them especially the carrots those were cultivated in central and northern areas of Iran including Zarinshahr and Astaneh are considered as highly inhibitor of DPPH free radical when compared to known inhibitor compounds like Vitamin C. In addition to this finding this study demonstrated that the antioxidant properties of Iranian carrots are independent of their carotenoids levels.

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