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ANTIOXIDANT CAPACITY AND CHEMICAL COMPOSITION OF FIVE IRANIAN WILD STRAWBERRIES (*Fragaria vesca* L.)

SUMMARY

Strawberry is small fleshy fruit, with high values of biochemical contents. The essential effects of strawberry in human health have been approved due to significant amount of its vitamin C, antioxidant, phenolic compounds and anthocyanin. Wild strawberry (*Fragaria vesca* L.), from Rosaceae family, grows wildly in some north regions of Iran and have been traditionally used for food and medicinal purposes. In this study, five wild strawberry populations from Gilan province in the north of Iran, were used to investigate the value of chemical traits including; total soluble solid (TSS), titratable acidity (TA), vitamin C and bioactive content; antioxidant activity, total anthocyanin and total phenolic content. Among these five populations, Masoleh population showed the highest value of assessing factors while Ardeh population had the lowest. The total phenolic content was statistically correlated to the antioxidant activity ($r = + 0.99$; $p < 0.05$) and total anthocyanin content ($r = +0.93$; $p < 0.05$). Correlation matrix of biochemical contents showed that the climate condition (altitude, longitude, latitude) of collected fruits can be related to the biochemical quantity. According to the results, high concentration of the antioxidant activity was presented in the Iranian wild strawberries. Current study suggests that environmental factors can change the antioxidant capacity and biochemical compositions of wild strawberry. To sum it up, wild strawberry populations of Iran can be considered as a good source for breeding program, plus it may be introduced as a fruit with great value for human health.

Keywords: Biochemical compounds, Small fruits, Diversity, Cluster analysis, Correlation

INTRODUCTION

Strawberry, a high-value berry crop, is grown in many temperate and sub-tropical regions of the world. The strawberry is small fleshy fruits, which are commercially cultivated and commonly consumed in fresh and processed forms (Seeram et al., 2008).This tasty fruit classified in Fragaria genus. The Fragaria

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genus consists of at least 20 species and belongs to Rosaceae family (Hummer et al., 2011; Liston et al., 2014). Diploid wild strawberry (*Fragaria vesca* L.) grows naturally throughout Europe, North America and North Asia (Sillasoo, 2006; Ozcan and Haciseferogullari, 2007). Worth to mention, it grows wildly in north regions of Iran especially in Mazandaran and Gilan proveniences (Figure 1) and has been used as food and medicinal remedies in folk medicine in this region of Iran.

Figure 1. Wild Strawberry in the Masoleh region (Gilan province)

Biochemical contents such as vitamin C, are one of the most important nutritional quality factors in strawberries (Du et al., 2009). Strawberries as well as blackberries, and raspberries are rich in antioxidant, phenolic compounds and anthocyanin and the high amounts of these biochemical contents propound essential effects of strawberries in human health in many ways (Herrmann and Nagel, 1989; Macheix et al., 1990).

Several studies have been reported about the biochemical compounds in different species of strawberry. Biochemical compositions have been reported in Fragaria × ananassa (Kafkas et al., 2007; de Souza et al., 2014). Furthermore, the antioxidant capacity of strawberry has been reported in different strawberry species such as: *Fragaria* × *ananassa* (Wang and Lin 2000; de Souza et al., 2014) and *Fragaria vesca* L. (Dyduch-Siemińska et al., 2015).

As our knowledge, there is not sufficient information about wild diploid strawberry populations in Iran. This study is the first report which reveals

biochemical properties and bioactive compositions in Iranian wild strawberries. Therefore, we aimed to investigate the native origin variations in five *Fragaria vesca* L. populations of Gilan province in north of Iran. In current study, biochemical properties including; total soluble solid, titratable acidity, total anthocyanin, total phenolic content, vitamin C and antioxidant activity of Iranian wild strawberry populations were evaluated. Soil properties of collected populations in mentioned regions were analyzed in relation to the biochemical compositions of wild strawberry populations.

MATERIAL AND METHODS

Sample collection

Five wild strawberry populations (1kg for each population) were collected from different regions of Gilan province in North of Iran from June to July 2016. These strawberries populations are day neutral and collected in growing period.

All samples were collected from their native origin and there were no treatments on them. The names of populations come from sample collection (Sangdeh, Ardeh, Ponel, Zendaneh and Masoleh). The geographical properties of collected sample region including latitude, longitude and altitude were determined by the GPS and are shown in Table 1. Also some of climatic characteristics belonged to the nearest weather station to the studied regions are presented in Table 2.

Table 1. Location information of five regions that samples were collected

Region	Latitude (N)	Longitude (E)	Altitude (m)
Sangdeh	53°13'	36°40'	1273
Ardeh	48°49'	37°32'	1018
Ponel	49°60'	37°31'	1054
Zendaneh	48°44'	37°32'	1720
Masoleh	48°59'	37°90'	1289

Table 2. Climate condition parameters during in the months of growing to collected samples (2016)

	April	May	June	July
Max. Temp (°C)	29.8	29.2	32.1	33.2
Min. Temp (°C)	3	7.4	14	17.5
RH (%)	77	78	73	68
Precipitation (mm)	110.3	34.5	48.20	40.4
The average wind speed (m/s)	20	8	11	9
The average sunshine hours (h)	86.8	122.8	190.9	220.6
The average of temperature (°C)	13.4	18.2	22.2	25.5

Determination of Chemical fruit characteristics

Total soluble solid was measured by using a digital refractometer (Erma, Tokyo, Japan; calibrated using distilled water). Titratable acidity was measured by following the official method of AOAC (1984).

Determination of bioactive contents

Vitamin C content was determined by using 2, 6-dichlorophenol indophenol dye according to the method described by Ruck (1963). Briefly, a juice sample (30 g) was homogenized in 500 ml of 0.4% oxalic acid. After infiltration, 20 ml of the mixture was tittered with the dye and finally results were expressed as mg/100 g juice.

Total anthocyanin content was determined using the pH-differential method (Muanda et al., 2011). The pH values of the diluted pulp juice were 1.0 (in 0.025 M potassium chloride buffer) and 4.5 (in 0.4 M sodium acetate buffer), respectively. As a brief, in this method, 400 μL from extract solution was mixed separately 3.6 mL with each buffer. Using a spectrophotometer, absorbance was measured at 510 nm and at 700 nm. The amount of absorption is calculated as follows: $A = (A_{510} - A_{700})_{pH\ 1.0} - (A_{510} - A_{700})_{pH\ 4.5}$

Total phenolic contents were estimated as gallic acid equivalent by using the Folin-Ciocalteu colorimetric method described by Singleton and Rossi (1965). The extracted juice (1 mL) was mixed with 5 mL Folin-Ciocalteau reagent, and then 4 mL sodium carbonate (7.5% w/v) was added. The combination was diluted to 100 mL with distilled water and was kept in the dark at room temperature for 2 h. the absorbance of the mixture was measured at 765 nm with spectrophotometer. Gallic acid was used as standard and the concentration of total phenolic compounds in the extracts were expressed as galic acid equivalent g^{-1} extract.

Antioxidant activity was assessed using the DPPH method and have strong absorption over the wavelength 520 nm (Thaipong et al., 2006). In the present study, for measuring the antioxidant activity, 2 ml of 5, 10, 20, and 40 (ppm) plant juice concentrations was diluted by 2 ml of DPPH (100 μM methanol solution) and mixture was taken at room temperature for 30 minutes. The absorbance was measured at 520nmwith spectrophotometer.

Determination of soil properties

Determination of physical and chemical soil properties of studied regions were figured out by using the standard laboratory procedure and presented in Table 2. After transferring the samples to laboratory, they were air-dried and passed through a 2 mm sieve. Soil samples were analyzed to determine particle-size distribution (hydrometer method), pH (measure of acidity), EC (saturated extract at 25 $^{\circ}$C), Moisture (%)and SOC (%) (Soil organic carbon percent). Also, total nitrogen (N), phosphor (P), potassium (K) and Sodium (Na), in saturation extract were assessed (Miransari et al., 2008).

Data analysis

The experiment was a completely randomized design with three replications. The normality test was done using the Kolmogorov–Smirnov test

and data were analysed by the MSTATC software (version 1.2, Michigan State University, East Lansing, MI).

Comparison of means carried out by Duncan's multiple range test. Differences were considered significant at the level of$p \leq 0.05$. Pearson's correlation coefficients (r) were calculated using Microsoft Excel 2010 software.Cluster analysis using the Ward's method was also carried out using SPSS statistical software (version. 21.0 for Windows) to classify and group the population according to their biochemical properties.

RESULTS AND DISCUSSION

In Table 3, it can be observed the results of soil properties in studied regions. Results of analysis of variance indicated that there were highly significant differences (p<0.05) among samples for all parameters (Table 4). Also, some important biochemical characteristics of Iranian wild strawberry populations are summarized in Table 5.

Table 3. Physical and chemical properties of soil in studied regions

Region	Soil texture	N (%)	P Av. (mg/kg)	K Av. (mg/kg)	Moisture (%)	Organic carbon (%)	EC (dS/m)	pH
Sangdeh	Loam	0.15	12	180	42	1.4	3.3	6.1
Ardeh	Clay	0.14	13	190	51	1.3	1.6	6.5
Ponel	Loam	0.17	12	260	62	1.6	1.6	6.7
Zendaneh	Loam	0.17	12	160	67	2.12	2.2	6.3
Masoleh	Loam	0.18	12	250	71	2.24	1.3	6.2

Table 4. Analysis of variance for biochemical and bioactive composition of Iranian wild strawberry populations

S.O.V	df	Total soluble solid	Titratable acidity	Total phenolic content	Total anthocyanin content	Vitamin C	Antioxidant activity
Region	4	1.73**	0.26**	3905333.08**	0.49**	101.74**	34.33**
Error	10	0.03	0.007	0.55	0.005	0.07	0.53
CV (%)	-	2.26	6.05	0.01	2.21	0.92	1.77

S.O.V: Source of variation, d.f: degree of freedom, C.V: coefficient of variation and ** significant at 1% probability levels

Table 5. Means of biochemical and bioactive compositions of some Iranian wild strawberry populations

Origin	Total soluble solid (Brix)	Titratable acidity (%)	Total phenolic content (mg/100 g)	Total anthocyanin content (mg/100 g)	Vitamin C (mg/100 g)	Antioxidant activity (%)
Sangdeh	7.7^{b}	1.50^{b}	574.75^{b}	3.36^{b}	35.32^{a}	43.17^{b}
Ardeh	7.2^{c}	1.03^{d}	463.20^{e}	2.96^{d}	22.24^{d}	37.04^{e}
Ponel	7.3^{c}	1.50^{b}	512.78^{d}	3.13^{c}	26.46^{c}	38.82^{d}
Zendaneh	8.7^{a}	1.23^{c}	522.20^{c}	3.02^{cd}	28.12^{b}	40.32^{c}
Masoleh	8.7^{a}	1.80^{a}	757.51^{a}	3.95^{a}	35.66^{a}	45.50^{a}

Values in the columns followed by the same letter are not significantly different, $p \leq 0.05$, Duncan multiple range test.

Chemical properties

In present study, the amounts of total soluble solid (TSS) value varied between 7.2 °Brix (Ardeh population) to 8.8 °Brix (Zendaneh and Masoleh populations). Titratable acidity varied from 1.03 to 1.80% in Ardeh and Masoleh populations, respectively.

Vitamin C contents of present study were between 22.22 mg per 100 g (Ardeh population) to 35.66 mg per 100 g (Masoleh population). The vitamin C levels in this studywere lower than the value in strawberry species (*Fragaria* × *ananassa*), blackberry (*Rubus spp)*, red raspberry (*Rubus idaeus)* and blueberry (*Vaccinium corymbosum)* (deSouza et al., 2014; Celik et al., 2018). These differences may be related to their genetic sources, soil property, as well as growing condition.

In terms of total anthocyanin content, this parameter ranged between 2.96 mg per 100 g in Ardeh population to 3.95 mg per 100 g in Masoleh population. Total anthocyanin contents of wild Iranian strawberry populations were higher than other *Fragaria vesca* L. cultivars (Baron von Solemacher, Yellow Wonder and Regina) cultivars which reported by Dyduch-SiemiNska et al. (2015).

Total phenolic contents varied from 463.20 to 757.51 mg GAE per 100 g in Ardeh and Masoleh populations, respectively. Total phenolic contents in this study showed higher valuesrather than other *Fragaria* species; *Fragaria* × *ananassa* (Wang and Lin 2000; Hakala et al., 2003; Pantelidis et al., 2007; de Souza et al., 2014) and *Fragaria vesca* L. (Dyduch-Siemińska et al., 2015). Different climatic conditions and growth conditions can be affected by biochemical properties in strawberries species (Hakala et al., 2003).

Antioxidant activity value assessed as 37.04% in Ardeh population and 45.50% in Masoleh population. The result of this study was supported by other studies in *Fragaria* species (deSouza et al., 2014;Kafkas et al., 2007).

As a brief, the highest values of chemical compositions and antioxidant capacity were observed in Masoleh population, while the lowest rank was seen in Ardeh population (Table 2). The diploid species of the *Fragaria* genus have been adapted to their localized environments (Hancock and Luby, 1993). The chemical compositions and antioxidant capacity depends on genetic factor as well as environmental factors (Mohammadi and Asadi-Gharneh., 2018).

In comparison to the result of this study and the literature, the analysed fruits presented high concentrations of antioxidant activity (Table 6). Present study demonstrates Iranian wild strawberry population have enormous nutritional value and are considered important as a valuable germplasm. Also the high value of antioxidant capacity in the wild strawberries, as shown in previous studies in this field, may reduce the risk of developing cancer and heart diseases (Ascherio et al., 1992; Renaud and Lorgeril 1992; Wargovich, 2000).

Correlation analysis

Correlation coefficients among all measured biochemical traits in the wild diploid strawberry populations are given in Table 7.

In current study, vitamin C is clearly positive correlated with total anthocyanin content ($r = 0.97$; $p< 0.05$) as well as titratable acidity ($r = 0.83$; $p< 0.05$), total phenolic content($r = 0.83$; $p< 0.05$) and antioxidant capacity($r = 0.82$; $p< 0.05$). There is several reports about relationships between the antioxidant activity and the phenolic compounds and anthocyanin contents in strawberry (deSouza et al., 2014) as well as berries and cherries (Hassimotto et al., 2008; Koca and Karadeniz 2009; Wu et al., 2010). Current observation supported by the previous researches reported in berries and cherries fruits (deSouza et al., 2014).

Correlation matrix of biochemical contents showed that the climate condition (Altitude, longitude, latitude) of collected plants can be related to the biochemical contents. In this study altitude had the positive correlation with total soluble solid ($r = +0.83$; $p< 0.05$). Also longitude and latitude had negative ($r = -0.93$; $p< 0.05$) and positive ($r = +0.86$; $p< 0.05$) correlation with EC of the soil, respectively. Furthermore, moisture of soil had the positive ($r = +0.87$; $p< 0.05$) and negative ($r = -0.76$; $p< 0.05$) correlation with longitude and latitude, respectively. The effect of climate and altitude on physiochemical traits of different populations is the notable point (Khakdaman et al., 2007). Climatic conditions have considerable effects on biochemical properties of wild populations (Mohammadi and Asadi-Gharneh., 2018; Javanmard et al., 2018).

The total phenol content was positively correlated to the antioxidant activity($r = +0.99$; $p< 0.05$) and anthocyanin content($r = +0.93$; $p< 0.05$). Anthocyaninsclassified in phenolic compounds which are water-soluble pigments responsible for the orange, red and blue colour of many fruits (Mazza and Miniati 1993). Therefore, this strong correlation between the phenol contents and anthocyanins can be expected.

Table 6. Comparison ofchemical and bioactive components of strawberry species.

Reference	Species	Vitamin C (mg/100 g)	Total soluble solid (Brix)	Titratable acidity (%)	Antioxidant activity (%)	Total phenolic content (mg/100 g)	Total anthocyanin content (mg/100 g)
Kafkas et al., (2007).	*Fragaria annanasa*	-	6.33–10.86	0.60–1.31	-	-	
Wang and Lin, (2000)	*Fragaria annanasa*	-	-	-	12.2-17.4	95-152	2.3-4.5
Hakala et al., (2003)	*Fragaria annanasa*	32.4-84.7	-	-	-	-	-
Pantelidis et al., (2007)	*Fragaria annanasa*		-	-	-	-	-
de Souza et al., (2014)	*Fragaria annanasa*	90.1	10.5	0.9	37.8	621.92	1.6
Dyduch-SiemiNska et al. 2015	*Fragaria vesca*	-	-	-	11.2-15.3	164-284	0.9-1.6
Current study	*Fragaria vesca*	22.2 -35.6	7.2-8.7	1.0-1.8	37.0-45.5	463.2-757.5	2.9-3.9

Table 7. Correlation coefficients between all measured biochemical traits in the wild strawberry population

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1- Latitude	1.00															
2- Longitude	**-0.89**[a]	1.00														
3- Altitude	-0.11	0.02	1.00													
4- N	-0.34	0.61	0.42	1.00												
5- K	-0.18	0.52	-0.56	0.52	1.00											
6- P	-0.32	0.07	-0.51	-0.75	-0.23	1.00										
7- Moisture	**-0.76**	**0.87**	0.38	**0.87**	0.42	-0.36	1.00									
8- Organic carbon	-0.49	0.66	0.67	**0.88**	0.17	-0.57	**0.88**	1.00								
9- EC	**0.86**	**-0.93**	0.30	-0.40	-0.63	-0.28	-0.71	-0.36	1.00							
10- pH	-0.44	0.28	-0.52	-0.04	0.44	0.32	0.17	-0.30	-0.56	1.00						
11- Antioxidant activity	0.08	0.37	0.02	0.56	0.51	-0.45	0.35	0.55	-0.18	-0.54	1.00					
12- Total phenolic content	0.00	0.42	0.17	0.64	0.44	-0.50	0.45	0.66	-0.19	-0.57	0.99	1.00				
13- Total anthocyanin content	0.31	0.09	0.33	0.54	0.20	-0.65	0.23	0.57	0.18	-0.78	0.91	0.93	1.00			
14- Total soluble solid	-0.27	0.40	**0.83**	0.68	-0.15	-0.54	0.64	**0.92**	-0.04	-0.62	0.53	0.66	0.68	1.00		
15- Titratable acidity	0.24	0.23	0.01	0.70	0.65	-0.73	0.36	0.50	-0.08	-0.34	**0.90**	**0.88**	**0.85**	0.41	1.00	
16- Vitamin C	0.51	-0.14	0.31	0.43	0.13	-0.70	0.05	0.42	0.38	**-0.80**	**0.82**	**0.83**	**0.97**	0.57	**0.83**	1.00

[a] Bold digit showed the high positive and negative correlation.

Cluster analysis

Cluster analysis based on studied biochemical properties, grouped the wild strawberry population into two main groups with a respective distance of 10 out of 15 (Figure 2). Lambda statistic of Wilks was done for determining cutoff point in cluster analysis.

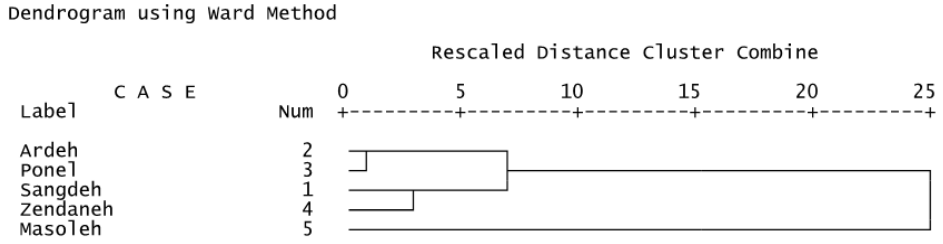

Figure 2. The cluster analysis of the wild strawberry populations according to the biochemical traits using the Ward's method

The first cluster containing 4 wild populations which are divided to two sub-cluster. Ardeh and Ponel populations were placed in one sub-cluster as well as Sangdeh and Zendaneh. In the first sub-cluster, the average of traits including total soluble solid, total phenolic content, vitamin C and antioxidant activity were less than other population and have the average levels of biochemical properties. The populations of Sangdeh and Zendaneh were in the second sub-cluster and in terms of studied traits; higher amounts were reported in comparison rather than other sub-cluster. Finally, Masoleh population was placed in a separate cluster that is defined with high level of studied traits. In current study; Ardeh and Masoleh population had the most distance. In general, some differences in biochemical characteristics affected the separation of wild population in to different clusters.

CONCLUSIONS

In the present study, we evaluated the antioxidant capacity and chemical composition of some wild Iranian strawberry populations. There are significant differences among the biochemical properties of wild strawberry populations. Masoleh population was assessed as the highest value of antioxidant activity while Ardeh population was in the lowest rank. This variation in wild populations can be exploited by breeders to expand of this plant as a source of natural bioactive compounds that could replace with the synthetic antioxidant. Concluding, this that environmental factors may have influence on antioxidant capacity and biochemical compositions of wild strawberry.

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