

PHENOLIC COMPOUNDS CONTENT, ANTIOXIDANT AND ANTIDIABETIC POTENTIALS OF SEVEN EDIBLE LEAVES

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ABSTRACT

The objectives of this study were to investigate antioxidant and enzyme inhibitory activity of ethanolic and aqueous extracts of seven leaves. The antioxidant activity of the leaves was determined by two different methods: DPPH and β -carotene-bleaching test (β C). The enzyme inhibitory activity was evaluated against α -glucosidase. Antioxidant (EC_{50}) and α -glucosidase (IC_{50}) activity were determined as 8.29-238.8 g of leaf/g of DPPH and 35.59-620.29 μ g/mL respectively. Among seven leaves studied, leaf of grape (*Vitis vinifera*), quince (*Cydonia oblonga*) and nettle (*Urtica dioica*) exhibited higher antioxidant and α -glucosidase inhibition activity than the rest of the leaves. Total phenolic content (TPC), total flavonoid content (TFC) and total hydrolyzable tannin content (TTC) of the leaves were between 0.22-16.37 mg gallic acid equivalents (GAE), 0.07-9.32 mg catechin equivalents (CE) and 0.49-16.38 tannic acid equivalent (TAE) per g of leaf, respectively.

Keywords: Antioxidant activity, edible leaves, enzyme inhibitory activity, α -glucosidase activity

YEDİ YENİLEBİLİR YAPRAĞIN FENOLİK BİLEŞİK İÇERİĞİ, ANTİOKSİDAN VE ANTİDİYABETİK POTANSİYELİ

ÖZ

Bu çalışmanın amacı seçilmiş yedi tane yenilebilir yaprağın etanolik ve sulu ekstraktlarının antioksidan ve enzim inhibitör aktivitelerinin belirlenmesidir. Yaprakların antioksidan kapasiteleri DPPH ve β -Karoten ağartma yöntemleri kullanılarak analiz edilmiştir. Enzim inhibitör aktivite analizinde ise yaprakların α -glukozidaz enzimini inhibe etme özelliği tespit edilmiştir. Yaprakların antioksidan (EC_{50}) ve α -glukozidaz (IC_{50}) aktiviteleri sırasıyla 8.29-238.8 g örnek/g DPPH ve 35.59-620.29 μ g/mL arasında bulunmuştur. İncelenen yapraklar arasında, üzüm yaprağı (*Vitis vinifera*), ayva (*Cydonia oblonga*) yaprağı ve ısırgan otunun (*Urtica dioica*) diğer yapraklardan daha yüksek antioksidan ve α -glukozidaz inhibisyon aktivite gösterdiği belirlenmiştir. Yaprakların toplam fenolik madde miktarı (TPC), toplam flavonoid madde miktarı (TFC) ve toplam hidrolize edilebilir tanin miktarı (TTC) sırasıyla 0.22-16.37 mg gallik asit eşdeğeri (GAE), 0.07-9.32 mg kateşin eşdeğeri (CE) ve 0.49-16.38 tannik asit eşdeğeri (TAE)/g örnek arasında olduğu tespit edilmiştir. Bu çalışma, seçilmiş yedi tane yenilebilir yaprağın biyoaktif bileşenleri ile ilgili doğrudan karşılaştırmalı veriler sunmaktadır.

Anahtar kelimeler: Antioksidan aktivite, yenilebilir yapraklar, enzim inhibitör aktivitesi, α -glukozidaz aktivitesi

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INTRODUCTION

Diabetes mellitus which is defined by metabolic disorders is seen commonly all over the world (Kitabchi et al., 2009). It is important to balance the glucose level after meal in diabetic patients. α -glucosidase secreted from intestinal chorionic epithelium plays an important role in the conversion of carbohydrates into glucose. By inhibiting α -glucosidase, glucose levels in the blood can be balanced. α -glucosidase inhibitors such as acarbose, miglitol, and voglibose are used as therapeutic agents for the treatment of diabetes mellitus because they help to control blood glucose levels after food uptake in diabetic patients for long time (Kumar et al., 2011). However, these drugs can also cause some problems such as diarrhoea, abdominal cramping, and flatulence (Ma et al., 2015).

Antioxidants have been commonly used to provide protection against oxidative degradation of foods by free radicals. Free radicals are known as Reactive Oxygen Species and play an important role in human body as they cause oxidative damage to the human body, eventually leading to cancer, aging, atherosclerosis, hypertension, heart attacks and other chronic diseases (Ani et al., 2006; Basma et al., 2011; Meziti et al., 2012). Human body copes with free radicals because of its antioxidant defense system. However, the balance between antioxidant defense system and free radicals could be changed in time. Therefore, intake of antioxidants is important issue to reduce oxidative damage in human body (Meziti et al., 2012). Synthetic antioxidants such as butylated hydroxytoluene and butylated hydroxyanisole gave rise to health problems are used more than natural antioxidants in food industry (Turkoglu et al., 2007).

There is also an important relationship between diabetes mellitus and Reactive Oxygen Species as oxidation caused by Reactive Oxygen Species are a harmful factor contributing to diabetes mellitus (Ceriello and Motz, 2004). Furthermore, previous studies indicated that Reactive Oxygen Species are increased under diabetic conditions (Noh and Ha, 2011).

Numerous studies suggests that consumption of natural sources decreases the risks of metabolic disorders such as diabetes mellitus and helps to cope with free radicals. Therefore, with rising consciousness level and research opportunities led the science to deeper study on usage of natural sources for the treatment of various human diseases. Edible leaves which are one of the important natural sources have also become a prominence issue and numerous researches have been made regarding their effect on human health and food quality in recent years as they are a valuable source of different bioactive compounds including secondary metabolites and macromolecules (Ju et al., 2013; Jain et al., 2017). However, to the best of our knowledge, no studies have been conducted direct comparative data on antioxidant and α -glucosidase inhibition activities of seven edible leaves investigated in our study.

Due to all these reasons, reliable and natural resources have gained attention all over the world in these days (Braithwaite et al., 2014; Klaus et al., 2015). In this sense, spices, plants and their parts, especially leaves are the potential reliable and natural sources of bioactive compounds (Yanishlieva et al., 2006). Therefore, we have studied some analytical properties of seven edible leaves, namely grape (*Vitis vinifera*), quince (*Cydonia oblonga*), mulberry (*Morus*), beans (*Phaseolus vulgaris*), cherry (*Prunus avium*), nettle (*Urtica dioica*), chard (*Beta vulgaris vulgaris*) which are important parts of Turkish cuisine in the present study. The leaves were investigated for their antioxidant, α -glucosidase inhibition activity, total phenolic, total flavonoid and total hydrolyzable tannin contents.

MATERIAL AND METHOD

Plant materials and chemicals

Leaf of grape (*V. vinifera*), quince (*C. oblonga*), mulberry (*Morus*), bean (*P. vulgaris*) and cherry (*P. avium*) was collected from the plants in August in Kayseri city (Turkey). Leaf of chard (*B. vulgaris vulgaris*) and nettle (*U. dioica*) was supplied from markets in January. All leaves were stored at -25°C until analysis. Chemicals were obtained

either from Sigma or Merck unless otherwise stated.

Optimization of solvent composition

Simplex-Lattice design was used to determine the optimum solvent concentrations. Total phenolic content in the extracts was used as response. The ratio of ethanol to water was selected as independent variable. Experiments were conducted based on following conditions: 10 g of milled leaf was weighed into a vial, and 150 mL of

ethanol-water combination was added into the vial. This mixture was kept in a shaking water bath (Heat Tech 26 L, Thermo Scientific) at 40 °C for 30 min and then centrifuged (Model NF800R, Nüve, Ankara, Turkey) at 4000 rpm for 5 min. The resulting supernatant was collected and stored at -18 °C until analyzed. Simplex-Lattice design points, the ratio of ethanol to water, were given in Table 1. All the leaves were subjected to extraction experiments as indicated in Table 1.

Table 1. Simplex-Lattice design points for solvent optimization

Experiment number	Water (%)	Ethanol (%)
1	50	50
2	0	100
3	100	0
4	100	0
5	25	75
6	0	100
7	75	25
8	50	50

Total phenolic content

Total phenolic content (TPC) of leaves was determined using Folin-Ciocalteu (Singleton and Rossi, 1965) with some modification using gallic acid as a standard. 0.4 mL of diluted extract solution was mixed with 2 mL of Folin-Ciocalteu reagent (the reagent was pre-diluted, 10 times, with distilled water) and 1.6 mL of sodium carbonate (7.5% w/v). After 60 min of incubation at room temperature, absorbance was measured at 765 nm using UV-Vis spectrophotometer (Agilent 8453, USA) versus prepared blank.

Total flavonoid content

Total flavonoid content (TFC) was determined by the method of Zhishen et al. (1999). At time zero, 1 mL of extract was mixed with 4 mL of water and 0.3 mL of 5% NaNO₂ into a 10 mL volumetric flask. After 5 min, 0.3 mL of 10% AlCl₃ was added into the flask. At 6th min, 2 mL of 1 M NaOH was added to the mixture. Immediately, 2.4 mL of distilled water was added to the mixture in order to reach a final volume and thoroughly mixed. The absorbance of the resulting pink colored solution was read at 510 nm

versus the prepared blank. The results were expressed as mg catechin equivalents per g of leaf.

Total hydrolysable tannin content

Total hydrolyzable tannin content (TTC) of leaves was determined by the method of Willis and Allen (1998). 1 mL of diluted extract solution was mixed with 5 mL of 2.5% KIO₃. The absorbance of the resulting red colored solution was measurement at 550 nm versus the prepared blank. Final results were expressed as mg tannic acid equivalents per g of leaf.

Antioxidant activity

Two different antioxidant activity methods (Beta-carotene bleaching and DPPH assays) were used to determine antioxidant activities of the leaves.

DPPH assay was determined according to method of Singh et al. (2002). Briefly, 0.1 mL of diluted extract solution was mixed with 3.9 mL of a 25 mg/L methanolic solution of DPPH and this mixture was vortexed for 10 s. After 30 min of incubation at room temperature, absorbance was measured at 515 nm using UV-Vis spectrophotometer (Agilent 8453, USA) versus

prepared blank. Final results were expressed as EC₅₀ value which is defined as amount of sample necessary to decrease initial DPPH concentration by 50%. EC₅₀ was expressed as gram of leaf to gram of DPPH.

β-carotene bleaching assay (βC) was determined according to method of Singh et al. (2002). 0.2 mg of β-carotene, 20 mg of linoleic acid and 200 mg of tween-40 were dissolved in 0.2 mL chloroform. After removing chloroform under nitrogen flush for 5 min, 10 mL demineralized water was added with vigorous stirring to form an emulsion. Four milliliter of this solution was added to each tube containing 0.2 mL of diluted samples. The control samples were prepared by adding 0.2 mL of distilled water instead of samples. Absorbance was measured at 470 nm using UV-Vis spectrophotometer (Agilent 8453, USA) versus prepared blank which was prepared as b-carotene emulsion but without adding b-carotene. Mixtures were incubated at 50 °C in a water bath until the control sample was bleached (90 min). Tubes were cooled to room temperature and absorbance was re-measured. Final results were expressed as percent inhibition in B-carotene

assay. β-carotene- bleaching assay (βC) was using the following equation:

$$\beta C (\%) = \left[1 - \left(\frac{A_{s:0} - A_{s:90}}{A_{c:0} - A_{c:90}} \right) \right] * 100$$

Where $A_{s:0}$ is the absorbance of sample at 0th min, $A_{s:90}$ is the absorbance of sample at 90th min, $A_{c:0}$ is the absorbance of control at 0th min, $A_{c:90}$ is the absorbance of control at 90th min.

α-glucosidase inhibition activity

Alpha glucosidase inhibition activity of leaves was determined by employing the methods given in the literature (Mcdougall et al., 2005). Briefly, 50 μL of diluted extract was mixed with 1250 μl phosphate buffer (pH: 6.8) and then, 50 μL of glutathione, 50 μL of α-glycosidase enzyme and 125 μL of substrate were added respectively to the mixture and incubated at 37 °C for 20 min. The reaction was stopped by adding 2 mL sodium carbonate. The absorbance of the reaction mixture was measured at 400 nm. Resulting α-glucosidase inhibitory activities were calculated as inhibition (%) by following equation:

$$\text{Inhibition (\%)} = \frac{(\text{Absorbance of control} - \text{Absorbance of extract})}{\text{Absorbance of control}} * 100$$

The concentration of the extract required to inhibit 50% of α-glucosidase activity under the assay conditions was defined as the IC₅₀ value. IC₅₀ was expressed as microgram ratio of leaf to milliliter.

Statistical analysis

A statistical software (Design Expert 7.0.0, Stat-Ease Inc., Minneapolis, MN) was used for designing and randomizing the experimental points in Simplex-Lattice design. Quadratic models were constructed from the experimental results for each leaves. Analysis of variance, and fitting of quadratic models, and Tukey's multiple comparison tests were performed using the SPSS 22.0 statistical package for Windows (SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSION

Optimization of solvent concentration

Response surface methodology is commonly used in analyzing experimental data and the optimization of different parameters (Madamba, 1997). Simplex-Lattice design was used to determine the optimum ratio of ethanol to water for the extraction of bioactive components from the leaves. The results obtained from Simplex-Lattice design were presented in Table 2. The best equations (Linear or quadratic) giving the highest TPC of the leaves were constructed for each leaves. The leaves were subjected to extraction at optimum solvent concentrations, and then analyzed for the determination of bioactive properties.

Table 2. Optimum solvent concentrations and regression equations for each leaves

Leaves	Water-Ethanol (%,%)	Regression equation for TPC (mg/g)
Grape	44-56	TPC=13.47A+16.16B+17.54AB
Quince	78-22	TPC=8.46A+4.44B+22.89AB
Mulberry	100-0	TPC=2.58A+1.11B
Bean	100-0	TPC=1.79+0.52B
Nettle	75-25	TPC=10.04A+0.69B+21.08AB
Cherry	76-24	TPC=3.76A+2.60B
Chard	100-0	TPC=0.67A+0.51B

A and B for water and ethanol concentrations, respectively

Total phenolic content

TPC of all leaves are expressed in Table 3. The TPC of the leaves ranged from 1.17 to 16.37 mg GAE/g. There was a significant difference ($P < 0.05$) between the TPC of some leaves. Among seven leaves, the highest TPC was found in grape (*V. vinifera*) leaf. On the other hand, chard (*B. vulgaris vulgaris*) showed the lowest TPC which was ~14 fold lower than grape (*V. vinifera*) leaf. Different phenolic contents are evaluated for different and the same leaves in the literature (Kubola and Siriamornpun, 2008; Uysal et al., 2016; Lima et al., 2016). TPC of seven leaves were more than those of other leaves such as coriander leaf (ranged from 0.36 to 5.45 mg GAE/100g) (Wangensteen et al., 2004). TPC of seven leaves were less than those of other leaves such as carob leaf (130 mg GAE/g) (Hsouna et al., 2011), *Pimpinella affinis* leaf (ranged from 31.0 to 155.5 mg GAE/g), *Parrotia persica* leaf (ranged from 37.7 to 506.5 mg GAE/g), *Smilax excelsa* leaf (ranged from 19.3 to 239 mg GAE/g) (Dehghan et al., 2016).

Total flavonoid content

TFC of the leaves ranged from 0.43 to 9.32 mg CE/g. The changes in flavonoid content of some leaves were statistically significant ($P < 0.05$). Grape (*V. vinifera*) leaf had the highest flavonoid content while the lowest was in chard (*B. vulgaris vulgaris*) (Table 3). Quince (*C. oblonga*) leaf and nettle (*U. dioica*) also had high levels of TFC compared to other leaves. As shown in Table 3, linear relations were evaluated between TFC and TPC of leaves. The TFC and TPC of grape (*V. vinifera*), nettle (*U. dioica*) and quince (*C. oblonga*) were 9.32, 6.90, 3.27 mg CE/g, 16.37, 10.95, 8.75

mg GAE/g respectively. This trend is not a surprise because of the fact that flavonoids are one of the major phenolic compounds. However, TFC of grape (*V. vinifera*) leaf determined in our study was lower than those reported by Uysal et al. (2016) who reported that TFC of methanol and aqueous extracts of grape (*V. vinifera*) leaf was 19.61 and 44.92 mg rutin equivalent/g respectively. TFC of the leaves were less than those of other leaves such as *Mellilotus arvensis* (57.0 mg quercetin equivalent/g) and *Epilobium hirsutum* leaves (58.45 mg quercetin equivalent/g) (Ebrahimzadeh et al., 2008). The differences between the results might be the reference materials that were catechin in our study to express the final flavonoid concentrations.

Total hydrolyzable tannin content

TTC of leaves are given in Table 3. The TTC of leaves in our study contained from 1.17 to 16.19 mg TAE/g. The richest source of these compounds was found by mulberry (*Morus*) leaf (16.18 mg TAE/g) followed by nettle (*U. dioica*) (9.61 mg TAE/g), chard (*B. vulgaris vulgaris*) (5.69 mg TAE/g), quince (*C. oblonga*) leaf (3.93 mg TAE/g), cherry (*P. avium*) leaf (3.64 mg TAE/g), bean (*P. vulgaris*) leaf (2.30 mg TAE/g) and grape (*V. vinifera*) leaf (1.17 mg TAE/g). Previous data regarding the tannin content of leaves such as aqueous extract of pomegranate leaf (64.40 mg TAE/g), methanol extract of pomegranate leaf (128.02 mg TAE/g) (Elfalleh et al., 2012), aqueous extract of grape leaf (33.27 mg rutin equivalent/g) and methanol extract of grape leaf (36.37 mg rutin equivalent/g) (Devi and Singh, 2017) were reported.

Antioxidant activity

The results of antioxidant activity of leaves were expressed using the term EC_{50} (Table 3). The lower the EC_{50} the higher the antioxidant activity. Statistically significant differences ($P < 0.05$) were observed among some leaves in terms of antioxidant activity. Among seven edible leaves studied, nettle (*U. dioica*) leaf (8.29 g of leaf/g of DPPH), grape (*V. vinifera*) leaf (8.34 g of leaf/g of DPPH) and quince (*C. oblonga*) leaf (25.43 g of leaf/g of DPPH) exhibited the highest values of antioxidant activity while the lowest was observed in cherry (*P. avium*), mulberry (*Morus*) and bean (*P. vulgaris*) leaf (190.40, 238.80 and 288.90 g of leaf/g of DPPH respectively). As seen in Table 3, β -carotene bleaching method (β C), ranking of the samples with respect to their antioxidant activity was the same as that observed in DPPH assay. The β C of the leaves ranged from 18.96 to 78.36%. The grape leaf (*V. vinifera*) performed the best in this assay with 78.36%, followed nettle (*U. dioica*) leaf > quince (*C. oblonga*) leaf > bean (*P. vulgaris*) leaf > chard (*B. vulgaris vulgaris*). Previous studies on antioxidant activity of leaf such as red cabbage (38.6 μ g/mL) (Hassimotto et al., 2005), white grape (0.175 mg/mL) and red grape leaf (0.213 mg/mL) (Lima et al., 2016) were also reported. Antioxidant activities of the leaves determined in our study were different than those reported by these authors. The differences are due to different parameters such as cultivars, agricultural practices. Furthermore, the extraction solvents affect strongly antioxidant activity (Jang et al., 2007). For example, Elfalleh et al. (2012) indicated that antioxidant activities of aqueous and methanol extracts of pomegranate leaf were 26.65 and 11.44 μ g/mL respectively.

α -glucosidase inhibition activity

Activities and properties of α -glucosidase inhibitors is very important because α -glucosidase can release glucose as a results of some reactions such as hydrolysis of linear and branched isomaltose oligosaccharides, resulting in postprandial hyperglycemia (Casirola et al., 2006; Zhang et al., 2011). As mentioned above, commercial α -glucosidase inhibitors have been used to treat diabetes. However, they have side effects. Therefore, consumers have turned to

alternative and reliable sources possessed biological activities for treatment of diabetes mellitus, recently. We also investigated the enzyme inhibitory activity against α -glucosidase of edible leaves as alternative and reliable sources in this study. The α -glucosidase inhibition activities of the leaves are shown in Table 3. Five out of seven leaves exhibited the enzyme inhibitory activity against α -glucosidase. The antidiabetic activity of leaves as IC_{50} was between 35.59 and 620.29 μ g/mL. The lower IC_{50} has higher antidiabetic activity. Grape (*V. vinifera*) leaf (35.59 μ g/mL) exhibited the highest values of α -glucosidase inhibition activity, followed by quince (*C. oblonga*) leaf (71.75 μ g/mL) > nettle (*U. dioica*) (97.09 μ g/mL) > bean (*P. vulgaris*) leaf (313.72 μ g/mL) > cherry (*P. avium*) leaf (620.29 μ g/mL) (Table 3). These results show that plants and their parts are so important source to threat metabolic diseases including diabetes (Mukherjee et al., 2006). Five leaves, especially grape (*V. vinifera*), quince (*C. oblonga*) and nettle (*U. dioica*) leaf are potential sources for α -glucosidase inhibitor active compounds. Previous data regarding the antidiabetic activity of vine leaf (Sendogdu et al., 2006), guince leaf (Aslan et al., 2010), mulberry leaf (Kim et al., 2013) was reported by in vivo studies. Different studies also evaluated different plants or their leaves such as spinach (6.03 μ g/mL) (Vyas, 2017) *Asystasia gangetica* (325 μ g/mL) (Reddy et al., 2010), *Parrotia persica* leaf (6.9 μ g/mL), *Primula heterochroma* leaf (8.1 μ g/mL), *Pyrus boissieriana* leaf (4.7 μ g/mL) (Dehghan, 2016) *Neptuniaoleracea* leaf (19.09 μ g/mL) (Lee et al., 2014). The results show that the α -glucosidase inhibition varied significantly from one leaf to another.

Correlation

Pearson's correlation coefficients between the means of each variable were computed. Statistically significant ($p < 0.01$) correlation coefficient were observed among several bioactive compounds obtained in our study (Table 4). As expected, the highest correlation coefficient were found between the phenolic and flavonoid contents values, for which $R^2 = 0.978$. The correlation coefficient between phenolic compounds and antioxidant (EC_{50}) and α -

glucosidase inhibition (IC_{50}) activity was found to be $R^2 = -0.903$ and -0.765 respectively. This means that the phenolic compounds of the leaves extracts contributed by 90.3% and 76.5% to their antioxidant and α -glucosidase inhibition activities respectively. The positive correlations between phenolic compounds and antioxidant activities in our study are similar to that demonstrated in previous studies (Barreira et al., 2008; Zhang et al., 2010). Moreover, correlation coefficients between total phenolic content and β -carotene-bleaching

activity ($R^2 = 0.969$) determined in our study is similar to that reported by Leontowicz et al. (2003) who indicated that correlation coefficients, $R^2 = 0.935$. Strong correlations were also observed between antioxidant activity and flavonoid compounds ($R^2 = -0.849$) and α -glucosidase inhibition activity ($R^2 = 0.82$). Due to the low correlation coefficients of TTC with EC_{50} and IC_{50} , we could say that TTC did not play an important role on antioxidant and α -glucosidase inhibition activities.

Table 3. Some analytical properties of leaves

Leaves	Analytical Properties					
	Total Phenolic (mg GAE/g)	Total Flavonoid (mg CE/g)	β -carotene bleaching assay (%)	Hydrolysable tannin (mg TAE/g)	Antioxidant Activity (g leaf/g DPPH)	α -glucosidase inhibition (μ g/mL)
Grape	16.37 \pm 1.08 ^a	9.32 \pm 0.57 ^a	78.36 \pm 0.33 ^a	1.17 \pm 0.13 ^c	8.34 \pm 0.71 ^c	35.96 \pm 13.1 ^c
Quince	8.75 \pm 0.36 ^c	3.27 \pm 0.16 ^c	30.72 \pm 0.70 ^c	3.93 \pm 0.55 ^d	25.43 \pm 1.12 ^c	71.70 \pm 26.55 ^c
Mulberry	3.12 \pm 0.53 ^{cd}	1.36 \pm 0.10 ^c	n.d	16.18 \pm 0.88 ^a	238.80 \pm 6.86 ^{ab}	n.d
Bean	2.12 \pm 0.13 ^{cd}	0.89 \pm 0.04 ^{cd}	21.09 \pm 0.38 ^d	2.30 \pm 0.70 ^{de}	288.90 \pm 26.02 ^a	313.70 \pm 26.50 ^b
Nettle	10.95 \pm 0.22 ^b	6.90 \pm 0.03 ^b	48.27 \pm 1.88 ^b	9.61 \pm 0.29 ^b	8.29 \pm 0.07 ^c	97.10 \pm 10.05 ^c
Cherry	3.59 \pm 0.19 ^d	1.29 \pm 0.08 ^{cd}	n.d	3.64 \pm 0.24 ^d	190.40 \pm 9.19 ^b	620.30 \pm 30.52 ^a
Chard	1.17 \pm 0.13 ^d	0.43 \pm 0.03 ^d	18.96 \pm 1.67 ^d	5.69 \pm 0.50 ^c	n.d	n.d

n.d: not determined

Values are means of duplicate analysis. Data expressed as means \pm standard deviation. Means within each column with different letters (a–e) differ significantly ($P < 0.05$).

Table 4. Correlation coefficient among the levels of analyzed parameters

	TPC	TFC	β C	TTC	EC_{50}	IC_{50}
TPC	1					
TFC	0.978 ^a	1				
β C	0.947 ^a	0.969 ^a	1			
TTC	-0.259	-0.195	-0.193	1		
EC_{50}	-0.903 ^a	-0.849 ^a	-0.664	0.298	1	
IC_{50}	-0.765 ^a	-0.726 ^b	-0.713 ^b	-0.143	0.821 ^a	1

a,b = correlation is significant at the 0.01 and 0.05 level respectively

TPC= total phenolic content; TFC= total flavonoid content; β C= β - carotene bleaching assay;

TTC= Total hydrolysable tannin; EC_{50} = antioxidant activity; IC_{50} = α -glucosidase inhibition

CONCLUSION

From the observed results, leaves of the present study were rich sources of some components such as flavonoids, tannins and phenolics. Furthermore, we indicated that some of them not only possess these components but also exhibited antioxidant activity and inhibitory potential against α -glucosidase in vitro.

Therefore, they could be consumed as alternative to synthetic materials and drugs for treatment some disease because of their high biological activity. On the other hand, in vitro studies are not enough for clinical studies. Further in vivo and in vitro studies are necessary to provide better knowledge about both these leaves and other plants' leaves.

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