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Antioxidant Activity and Phenolic Content of Eight Mediterranean Fruit Juices

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ABSTRACT

Antioxidants play an important role in health and disease by reducing oxidative damage. In this study, eight local Mediterranean fruit juices (orange, grapefruit, blackberry, black mulberry, black sweet cherry, strawberry, pomegranate and red grape) were assessed for their total phenolic content and antioxidant activity. These fruits are known for their health effects and antioxidant properties which can be attributed to their phenolic compounds. Total phenolic content was determined according to Folin-Ciocalteu method. The highest in content was black mulberry juice with amounts from 4.24 g_{GAE}/L_{juice} to 11.03 g_{GAE}/L_{juice}, while the lowest was red grape juice with amounts from 0.35 g_{GAE}/L_{juice} to 0.64 g_{GAE}/L_{juice}. Antioxidant activity was subsequently determined using Ferric Reducing Antioxidant Power (FRAP) assay and reducing power assay. Black mulberry juice had the highest antioxidant activity, while red grape juice had the lowest antioxidant activity. The study indicates that black mulberry juice is the richest source of phenolic compounds and provides the highest antioxidant potential compared with other fruit juices in this study.

KEYWORDS: Phenolic compounds; antioxidant activity; FRAP; oxidative damage.

Introduction

Phenolic compounds comprise a large group of plant secondary metabolites widely studied due to their beneficial effects on human health. These effects include protection against many age-related diseases such as cancer, cardiovascular diseases and neurodegenerative diseases like Alzheimer's disease and dementia (Kaur and Kapoor, 2001; Silva et al, 2007; Fabris et al, 2008; Loo et al, 2008; Koley et al, 2011). Nutrition therapy has become an interest for consumers especially after the growing popularity of functional food. Thus a diet rich in fruits and vegetables is highly encouraged because they are a major source of phenolic compounds. Due to their structure, rich in hydroxyl groups, phenolic compounds act as antioxidants, scavenging free radicals which have harmful effects on the body (Cirico and Omaye, 2006; Mezadri et al., 2008; Pang et al 2012). Phenolic compounds are also potential substitutes of synthetic antioxidants (Dykes et al., 2003) such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) in food products especially after the growing evidence of the potential toxic and carcinogenic effects of BHA and BHT (Kaur and Kapoor, 2001; Sun et al. 2007; Loo et al.,2008).

Due to the abundance of fresh and non-processed beverages and plant foods, the Syrian Mediterranean diet is rich in fruits consumed directly or as juice. Juice has been used for decades to remain healthy because it provides an easy way to obtain water, energy and nutrients as well as antioxidants namely, phenolic compounds (Saura-Calixto and Goni, 2006). For example, chokeberry juice showed the ability to reduce oxidative damage, while pomegranate juice can protect against cardiovascular diseases and cancer (Espin et al, 2007). Another example is grape juice which due to its content of phenolic compounds can inhibit low density lipoprotein (LDL) oxidation and reduce native plasma protein oxidation (Davalos et al., 2005).

The aim of the present study was to determine the total phenolic content and the antioxidant activity of frequently consumed fruit juices in Syria due to the lack of research aimed at comparing the total phenolic content in different types of fruit juices.

Materials and Methods

Chemicals and Apparatus

Di Sodium Phosphate was purchased from Merck, Germany, Ferric Chloride anhydrous was purchased from Qualikems, India, Ferrous sulfate was purchased from BDH, England, Folin-Denis' reagent and 2,4,6-Tris(2-pyridyl)-s-triazine were purchased from Fluka (Sigma-Aldrich), Gallic Acid was purchased from Biotech LTD, Potassium ferricyanide was purchased from May & Baker LTD, England, Sodium Acetate was purchased from BDH, England, Sodium Carbonate was purchased from BDH, England, Sodium Phosphate was purchased from Riedel-De Haen AG, Germany, Trichloroacetic acid was purchased from Riedel-De Haen AG, Germany, Analytical balance (RADWAG, AS 220/C/2), Micropipette was purchased from Labkit (Chemelex, S.A., Spain), Spectrophotometer (Jasco V-530 UV Waterbath), Water bath (K & H Industries), Oven (CARBOLITE).

Samples

Three samples of each of the following fruits were purchased from local markets: orange, grapefruit, blackberry, black mulberry, black sweet cherry, strawberry, pomegranate and red grape. Juice samples were prepared according to Mezadri *et al.* (2008). Each type of fruit was washed then squeezed using home blender. The obtained juice was filtered and kept in the freezer (– 20°C) until analysis. All samples were diluted properly in distilled water prior to analysis.

Total Phenolic Content

Total phenolic content in each juice was determined according to the Folin-Ciocalteu method described by Vermerris and Nicholson (2006). Two mL of freshly prepared 2% (w/v) sodium carbonate (anhydrous) solution were added to 0.1mL of the properly diluted sample, mixed and then allowed to stand for 5 min. Then 0.1 mL of 1:1 diluted Folin-Denis' reagent were added to the mixture and left to stand for 30 min. Absorbance was then read using a spectrophotometer at 750 nm. A blank solution was prepared using 0.1mL of distilled water instead of the sample. A standard curve was obtained using gallic acid solutions in concentrations between (0.08-0.6 g/L). The total phenolic content was expressed as grams of gallic acid equivalents (GAE) per liter or kilogram of juice.

Ferric Reducing Antioxidant Power

The Ferric Reducing Antioxidant Power (FRAP) was measured spectrophotometrically as described by George et al., (2004). FRAP reagent was prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) solution in 40 mM HCl and 20 mM ferric chloride solution at a ratio of 10:1:1 (v/v/v) respectively. 1 mL of FRAP reagent was added to 100 µL of sample. The mixture was then incubated for 4 min at room temperature. Absorbance was read at 593 nm and the results were calculated according to a calibration curve obtained using $FeSO_4$ solutions in concentrations between (100-600 µM). The results were expressed as mmol Fe^{2+}/L juice.

Reducing Power

The reducing power of the diluted sample was determined by a method described by Chua *et al.* (2008). 0.5 mL of the diluted sample were mixed with 0.5 mL of phosphate buffer 0.2 M, pH 6.6 and 0.5 mL of 1% potassium ferricyanide solution. The mixture was incubated at 50 ºC for 20 min in a water bath then 0.5 mL of 10% (w/v) trichloroacetic acid were added to the mixture. Finally, 1 mL of the mixture was mixed with 1 mL of distilled water and 0.2 mL of 0.1% ferric chloride solution. Absorbance was read at 700 nm. Increased absorbance indicated increased reducing power. Since each juice had a different dilution ratio, it was not possible to compare the absorbance directly. Due to the similarity between this assay and FRAP assay, a calibration curve was prepared using $FeSO_4$ solutions in concentrations between (100-1000 µM). The results were expressed as mmol Fe^{2+}/L juice.

Statistical Analysis

All the results were presented as means ± standard deviations. The differences between treatments were determined by applying the Student's t-test.

Results and Discussion

Total Phenolic Content

The total phenolic content was calculated using the equation obtained from gallic acid calibration curve (Fig. 1). The results expressed as g_{GAE}/L_{juice} and g_{GAE}/Kg_{juice} are shown in Table 1.

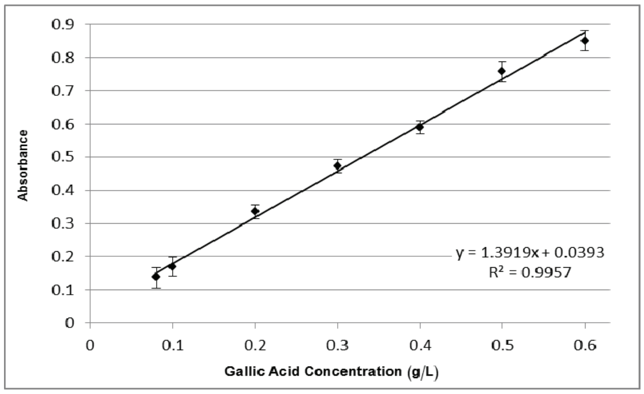

Fig. 1. Gallic acid calibration curve used to calculate total phenolic content in each juice.

TABLE 1

Total phenolic content of different types of fruit juices.

Type of Juice	Sample No.	Total Phenolic Content g_{GAE}/L_{juice} ± SD	Total Phenolic Content g_{GAE}/Kg_{juice} ± SD
Black mulberry	1	8.47 ± 0.73	7.9 ± 0.73
	2	11.03 ± 0.63	10.31 ± 0.63
	3	4.24 ± 0.05	4.16 ± 0.05
Blackberry	1	3.13 ± 0.07	3.11± 0.07
	2	3.89 ± 0.17	3.69 ± 0.17
	3	3.26 ± 0.04	3.16 ± 0.04
Sweet Black Cherry	1	3.58 ± 0.33	3.36 ± 0.33
	2	3.73 ± 0.25	3.5 ± 0.25
	3	2.4 ± 0.11	2.27 ± 0.11
Strawberry	1	4.26 ± 0.37	4.14 ± 0.37
	2	3.57 ± 0.11	3.51 ± 0.11
	3	2.16 ± 0.05	2.12 ± 0.05
Pomegranate	1	4.03 ± 0.05	3.8 ± 0.05
	2	2.6 ± 0.06	2.46 ± 0.06
	3	3.9 ±0.15	3.72 ± 0.15
Grapefruit	1	1.04 ± 0.06	0.997 ± 0.06
	2	1.7 ± 0.05	1.64 ± 0.05
	3	0.57 ± 0.01	0.55 ± 0.01
Orange	1	1.34 ± 0.13	1.25 ± 0.13
	2	0.93 ± 0.04	0.88 ± 0.04
	3	0.78 ± 0.02	0.75 ± 0.02

TABLE 1 *Contd...*

Type of Juice	Sample No.	Total Phenolic Content g_{GAE}/L_{juice} ± SD	Total Phenolic Content g_{GAE}/Kg_{juice} ± SD
Red Grape	1	0.64 ± 0.08	0.72 ± 0.03
	2	0.35 ± 0.02	0.667 ± 0.02
	3	0.61 ± 0.02	0.59 ± 0.02

Black mulberry juice had the highest phenolic content among all studied juices with a concentration range between (4.19 – 10.3 g_{GAE}/Kg_{juice}). Black mulberry juice is particularly rich in anthocyanidins (Bae and Suh, 2007). The obtained results were higher than those of Khalid *et al*. (2011) which were (2.05 g_{GAE}/Kg_{juice}).

Blackberry juice is rich in ellagic acid, gallic acid, anthocyanidins, cyanidins and quercetin (Ivanovic et al., 2014), which led to a total phenolic content ranging between (3.13 – 3.89 g_{GAE}/L_{juice}). These results were higher than multiple results indicating that total phenolic content of blackberry juice is (0.52 g_{GAE}/L_{juice}) (Kopjar et al., 2011), (1.83 g_{GAE}/L_{juice}) (Jakobek et al., 2007) and (0.64 g_{GAE}/L_{juice}) (Guerrero et al., 2010). The difference in results among studies could be due to climate change and the stage of ripening when fruits were harvested (Reyes-Carmona et al., 2005; Mahmood et al., 2012).

Cherry contains multiple phenolic compounds like cyanidin, catechin and quercetin. These compounds play a role in promoting apoptosis and cell differentiation. They also inhibit cyclooxegenase II, reducing the inflammatory response (Ferretti et al., 2010). Phenolic content of black sweet cherry juice ranged between (2.4 – 3.73 g_{GAE}/L_{juice}), while Jakobek et al. (2007) reported that phenolic content of sweet cherry is (1.57 g_{GAE}/L_{juice}). Environmental and genetic factors affect the concentration of phenolic compounds which may explain the difference in results (Ferretti et al., 2010).

The total phenolic content of strawberry juice ranged between (2.16 – 4.26 g_{GAE}/L_{juice}). Strawberry is a rich source of ellagic acid (Guerrero et al., 2010), p-coumaric acid, flavonoids and anthocyanidins (Panico et al., 2009). Other studies revealed that content of phenolic compounds in strawberry juice is (0.54 g_{GAE}/L_{juice}) (Guerrero et al., 2010), and (1.27 g_{GAE}/L_{juice}) (Jakobek et al., 2007). Panico et al. (2009) indicated the role of temperature and light exposure on the accumulation of anthocyanidins in strawberry fruits which explain the difference in results.

Pomegranate juice is rich in anthocyanidins and ellagitannins (Seeram et al., 2008), as well as gallic acid, chlorogenic acid (Arjmand, 2011), cyaniding and pelargonidin (Gil et al., 2000). Pomegranate juice content of phenolic compounds ranged between (2.6 – 4.03 g_{GAE}/L_{juice}). Several studies obtained similar results such as (2.05 – 2.91 g_{GAE}/L_{juice}) (Alighourchi et al., 2013), (2.46 – 2.55 g_{GAE}/L_{juice}) (Arjmand, 2011) and (2.12 g_{GAE}/L_{juice}) (Gil et al., 2000). Some other studies obtained notably lower results with a total phenolic content of (0.12 g_{GAE}/L_{juice}) (Keskin-Šašić et al., 2012) and (0.73 g_{GAE}/L_{juice}) (Moreno-Montoro et al., 2015).

The study showed that grape fruit juice content of phenolic compounds ranged between (0.57 – 1.7 g_{GAE}/L_{juice}), while total phenolic content in orange juice was between (0.78 – 1.34 g_{GAE}/L_{juice}). A study by Moreno-Montoro et al. (2015) showed that total phenolic content was (0.54 – 0.65 g_{GAE}/L_{juice}) in grapefruit juice and (0.54 – 0.76 g_{GAE}/L_{juice}) in orange juice.

Grape juice is a rich source of flavonoids (Davalos et al., 2005), alongside with quercertin and resveratrol which have anti-tumor properties (de Freitas et al., 2013). Grape juice may reduce the risk of cancer, ulcer, inflammation and atherosclerosis (Aliakbarlu et al., 2014). Red grape juice showed the lowest phenolic content among all studied juices with concentrations between (0.35 – 0.64 g_{GAE}/L_{juice}). Moreno-Montoro et al. (2015) reported that total phenolic content in red grape juice ranges between (0.28 – 1.73 g_{GAE}/L_{juice}), while the results obtained by Davalos et al. (2005) showed a range between (0.7 – 1.2 g_{GAE}/L_{juice}), another study showed that total phenolic content in red grape juice could reach concentrations as high as (1.6 g_{GAE}/L_{juice}) (Owczarek et al., 2004). It is important to note that phenolic compounds in grape are mainly found in seeds followed by peel then leaves. Grape juice has the lowest content compared to other parts of the plant (Xia et al., 2010; Moreno-Montoro et al., 2015).

Difference in results among the three samples of the same juice could be attributed to the different conditions these fruits underwent. The fruits were obtained from different shops, which means they could have been grown in different geographic areas with different climate and soil, the time in which fruits were harvested and the storing conditions are also unknown (Gorinstein et al., 2006), chemical composition of the fruit also depends on factors such as genetics and fruit maturity (Borguini et al., 2013).

Ferric Reducing Antioxidant Power

Phenolic compounds reduce TPTZ/Fe^{+3} complex to TPTZ/Fe^{+2} blue complex. Results were calculated according to the equation of the calibration curve prepared by using different concentrations of ferrous sulfate (Fig. 2). Table 1 shows antioxidant activity of different types of fruits juices according to FRAP method. Results were expressed as (mM Fe^{+2}/L_{juice}).

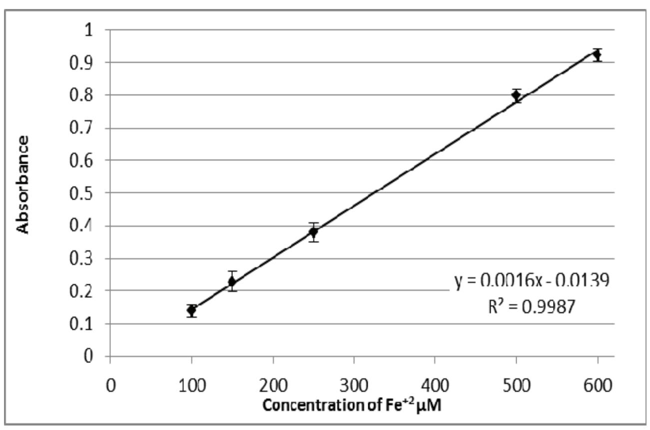

Fig. 2. Ferrous sulfate calibration curve used to calculate antioxidant activity in each juice according to FRAP method.

TABLE 2

Antioxidant activity of different types of fruit juices according to FRAP method.

Type of Juice	Sample No.	Antioxidant activity mM Fe^{+2}/L_{juice} ± SD
Black mulberry	1	65.98 ± 0.4
	2	108.3 ± 0.7
	3	60.2 ± 0.3
Blackberry	1	24.5 ± 0.2
	2	36.1 ± 0.4
	3	33.7 ± 0.5
Sweet Black Cherry	1	19.9 ± 0.4
	2	22.6 ± 0.2
	3	15.8 ± 0.1
Strawberry	1	26.3 ± 0.3
	2	21.9 ± 0.2
	3	19.7 ± 0.3
Pomegranate	1	35.5 ± 0.4
	2	24.3 ± 0.4
	3	24.8 ± 0.3
Grapefruit	1	9.6 ± 0.1
	2	13.2 ± 0.2
	3	8.6 ± 0.1
Orange	1	20.6 ± 0.4
	2	13.3 ± 0.1
	3	7.9 ± 0.2
Red Grape	1	2 ± 0.2
	2	1.5 ± 0.1
	3	1.9 ± 0.1

Black mulberry juice possessed the highest antioxidant activity among all juices studied ranging between (60.2 – 108.3 mM Fe^{+2}/L_{juice}). These high results could be attributed to its high content in cyanidin and pelargonidin compounds (Khalid et al., 2011; Kutlu et al., 2011).

The antioxidant activity of blackberry juice ranged between (24.5 – 36.1 mM Fe^{+2}/L_{juice}). These results were higher than those obtained by Ivanovic et al. (2014), which were (0.19 mM Fe^{+2}/L) of blackberry extract. The low results could be due to the poor yield of extraction method used in their study.

Black sweet cherry juice contains cyanidins and hydroxycinnamate compounds which have higher antioxidant activity than those isolated from blackberry or strawberry (Ferretti et al., 2010). Nevertheless, Black sweet cherry juice showed antioxidant activity ranging between (15.8 – 22.6 mM Fe^{+2}/L_{juice}), while strawberry juice possessed a higher antioxidant activity (19.7 – 26.3 mM Fe^{+2}/L_{juice}). Anthocyanidins present in strawberry are considered high in antioxidant activity due to the oxonium ion on the C ring.

Pomegranate juice can protect LDL from oxidation, it also reduces the risk of Alzheimer' disease and colon cancer (Arjmand, 2011). The antioxidant activity of pomegranate juice and its content of punicalagin –a phenolic compound which is characteristic of pomegranate fruit – are responsible for its previously mentioned health benefits (Espin et al., 2007). Antioxidant activity of pomegranate juice ranged between (24.3 – 35.5 mM Fe^{+2}/L_{juice}). Moreno-Montoro et al. (2015) mentioned that antioxidant activity of pomegranate juice ranges between (8.6 – 10 mM Fe^{+2}/L_{juice}).

Grapefruit juice showed antioxidant activity relatively lower than orange juice. The antioxidant activity ranged between (8.6 – 13.2 mM Fe^{+2}/L_{juice}) for grapefruit juice, and (7.9 – 20.6 mM Fe^{+2}/L_{juice}) for orange juice. Although the phenolic content of both juices were close, and relatively higher in grapefruit juice, the antioxidant activity was in favor of orange juice. In fact, grapefruit is rich in naringin, while orange is rich in hesperidin. Even though these two compounds have similar structures, the antioxidant activity of hesperidin is much higher than the antioxidant activity of naringin (Gorinstein et al., 2006). Gorinstein et al. (2006) arrived at similar results, stating that the antioxidant activity of orange reached values as high as (20.5 mM Fe^{+2}/Kg_{juice}), while it only reached (8.3 mM Fe^{+2}/Kg_{juice}) in grapefruit.

Red grape juice can reduce oxidative stress, it can also inhibits DNA synthesis in breast cancer cells (Xia et al., 2010; Burin et al., 2010). Red grape juice showed the lowest antioxidant activity among all studied juices with results ranging between (1.5 – 2.04 mM Fe^{+2}/L_{juice}). Xia et al. (2010) reported that the antioxidant activity of grape juice was (32 mM Fe^{+2}/L_{juice}) which is significantly higher than our results. The type of the grape was not specified in that study which could explain the difference.

Reducing Power

Due to the similarity between FRAP method and reducing power method, a calibration curve using ferrous sulfate solutions was prepared (Fig. 3). Calibration curve allows the comparison of the studied juices since each juice was diluted differently which eliminated the possibility of comparing absorbance directly. Absorbance was plotted against the concentration of ferrous sulfate. Good linearity was observed (R^2 = 0.9955), thus the equation was used to calculate the antioxidant activity of each juice expressed as mM Fe^{+2}/L_{juice} (Table 3).

However, Student's test showed significant difference between the two methods which indicates that one method cannot replace the other since they give significantly different results.

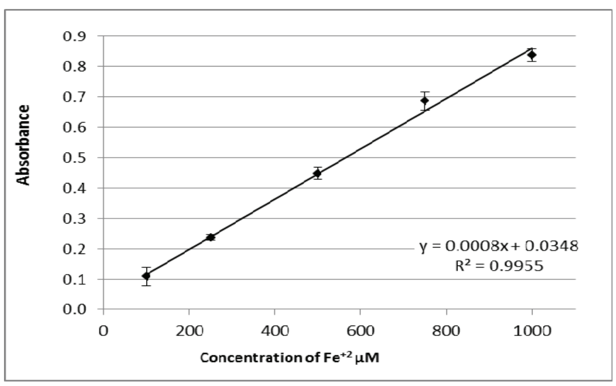

Fig. 3. Ferrous sulfate calibration curve used to calculate antioxidant activity in each juice according to reducing power method.

TABLE 3

Antioxidant activity of different types of fruit juices according to reducing power method.

Type of Juice	Sample No.	Antioxidant activity mM Fe^{+2}/L_{juice} ± SD
Black mulberry	1	75.5 ± 0.9
	2	100.5 ± 6.5
	3	35.2 ± 0.3
Blackberry	1	29.8 ± 0.2
	2	40.1 ± 0.1
	3	36.5 ± 0.5
Sweet Black Cherry	1	29.6 ± 0.2
	2	30.9 ± 0.6
	3	13.6 ± 0.2
Strawberry	1	26.3 ± 0.3
	2	23.2 ± 0.3
	3	19.2 ± 0.2
Pomegranate	1	43.7 ± 0.4
	2	40.3 ± 0.3
	3	41 ± 0.2
Grapefruit	1	4.2 ± 0.02
	2	7.6 ± 0.2
	3	3 ± 0.03
Orange	1	11.5 ± 0.1
	2	5.8 ± 0.03
	3	3.8 ± 0.03
Red Grape	1	2.4 ± 0.03
	2	0.7 ± 0.01
	3	1.4 ± 0.01

Conclusions

In the present study mulberry juice possessed the highest antioxidant activity among other juices under investigation, which indicates that this high antioxidant activity could be attributed to its high phenolic content. The present study also indicates that the reducing power assay cannot replace FRAP assay.

Acknowledgements

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