Journal of Agricultural Science; Vol. 11, No. 3; 2019
ISSN 1916-9752 E-ISSN 1916-9760
Published by Canadian Center of Science and Education

Determination of Antioxidant Content and Capacity of Four Jordanian Fresh Citrus Fruits

H. F. Al-Sayyed[1], R. A. Al-Kurd[1], M. M. Mwalla[1] & S. M. Abdel Qader[1]

[1] Department of Nutrition, Faculty of Pharmacy and Medical Sciences, University of Petra, Amman, Jordan

Correspondence: H. F. Al-Sayyed, Department of Nutrition, Faculty of Pharmacy and Medical Sciences, University of Petra, Amman, Jordan. Tel: 962-795-489-252. E-mail: halsayyed@uop.edu.jo

Received: November 9, 2018 Accepted: December 23, 2018 Online Published: February 15, 2019

doi:10.5539/jas.v11n3p605 URL: https://doi.org/10.5539/jas.v11n3p605

The research was financed by the Deanship of Academic Research at the University of Petra, Amman, Jordan.

Abstract

Citrus fruits are considered one of the most grown crops worldwide including Jordan with high nutritive and non-nutritive value. The consumption of foods that contain natural antioxidants is thought to be an efficient way for reducing the risk for oxidative stress diseases. Determination of antioxidant content and capacity allows the screening of plants that are probably involved in the prevention and/or treatment of oxidative stress diseases. This study aimed at comparing four fresh Jordanian citrus fruits (namely: orange, lemon, pomelo, and mandarin) for their antioxidant content (using two methods namely: Folin-Ciocaltaeu method and total flavonoids method) and capacity (using two methods namely: 2,2-diphenyl-picrylhydrazyl (DPPH) and cupric antioxidant reducing capacity (CUPRAC) assays). Three solvents were used for the fruit extraction (ethanol, methanol, and water). There were significant ($P < 0.05$) differences between the fruits in terms of antioxidant content and capacity. Regardless of the extraction solvent, the fruit content of total polyphenols (M catechin/100 g) was as follows: lemon > mandarin > pomelo > orange. The total flavonoid content (mM rutin/100 g) of the fruits was: orange > mandarin > lemon > pomelo. On the other hand, the antioxidant capacity (M trolox/100 g) of the fruits was: lemon > mandarin > orange > pomelo. The antioxidant capacity (measured as mg vitamin C/ml extract) of the fruits was: mandarin > orange > pomelo > lemon. Different extracts of different fruits showed significantly ($P < 0.05$) different antioxidant contents and capacities. No correlation between antioxidant content and antioxidant capacity of the studied fruits has been found.

Keywords: polyphenols, flavonoids, free radicals, orange, lemon, pomelo, mandarin

1. Introduction

Fruits and vegetables are considered of the most important natural sources of antioxidants (Apak et al., 2007). Citrus fruits are considered one of the most grown crops worldwide, including Jordan, with high nutritive and health values. The health benefits of citrus fruits have been attributed mainly to the presence of antioxidants such as phenolics and ascorbic acid (Kumar, Lamers, Singh, Ladaniya, & Sthapit, 2015). Jordan environment is rich and there is diversity in the Jordanian crop production (Qura'n, 2010). The Jordanians' consumption of citrus fruit is estimated to be 7.64 Kg/*caput*/year (FAO, 2018).

Free radicals are highly reactive chemical species that are able to affect the unstable atoms or molecules leading to a subsequent cascade of free radical oxidative reactions. Oxidative reactions are considered important because they might lead to imbalanced oxidative status of a living cell and to oxidative stress diseases accordingly. Many chronic diseases are thought to have oxidative stress background in their pathogenesis. Diabetes mellitus, cardiovascular diseases, and some types of cancers are considered nowadays as oxidative stress diseases. Antioxidants are chemical substances that are present naturally or synthetically capable for stopping a free radical chain reaction either by scavenging free radicals or by neutralizing the effects of free radicals. The consumption of antioxidants is thought to reduce the risk for oxidative stress diseases (Halliwel & Gutteridge, 1995).

There were many attempts to find the antioxidant content, capacity, and specific antioxidant compounds in citrus fruits. Nonetheless, there is no figures regarding the antioxidant content and capacity for fresh citrus fruit

produced in Jordan using different extraction solvents. Thus, the purpose of this study is to screen four Jordanian fresh citrus fruits that are consumed regularly in Jordan (namely: orange, lemon, pomelo, and mandarin) for their antioxidant content and capacity. Antioxidant content was determined by two methods namely: Folin-Ciocultae method and total flavonoids method. Antioxidant capacity was determined by two methods namely: 2,2-diphenyl-picrylhydrazyl (DPPH) and cupric antioxidant reducing capacity (CUPRAC) assays. This study aimed also to find (if any) a correlation between the antioxidant content and antioxidant capacity of the selected fruits. It is probably the first study that evaluated the antioxidant content and capacity of locally produced fresh Jordanian citrus fruits extracted by three solvents (*i.e.* ethanol, methanol, and water). We expect to add a value to the scientific antioxidant database.

Based on the previous research reviewed by the researchers, the null hypotheses (H_0) of this research state that: (a) there is a significant difference between the antioxidant content and capacity values of four fresh Jordanian citrus fruits namely: orange, lemon, pomelo, and mandarin extracted by three solvents (ethanol, methanol, and water) and (b) there is a correlation between the antioxidant content and capacity values of the fruits (the antioxidant capacity of the fruits is related to the fruit content of the extracted antioxidants). The alternative hypotheses (H_1) of this research are: (a) there is no significant difference between the antioxidant content and capacity values of four fresh Jordanian citrus fruits namely: orange, lemon, pomelo, and mandarin extracted by three solvents (ethanol, methanol, and water) and (2) there is no correlation between the antioxidant content and capacity values of the fruits.

2. Materials and Methods

2.1 The Material Studied

Fresh citrus fruits that were locally produced in Jordan were studied. As reported by the seller, fruits were collected in the same day of purchasing (morning time), neither stored nor treated.

2.2 Sample Preparation

The fruits were purchased from local (Jordanian) market and analyzed freshly. Fruits were prepared by peeling, chopping finely by knife or food chopper (Ariete®, China). Representative samples (1-3 g) were extracted conventionally by 10 ml of each of the three extraction solvents (methanol, ethanol, and water) at 50 °C, 50 °C, and 90 °C respectively for 2 hours with intermittent shaking. The extracts were centrifuged at 3000 rpm for 10-15 minutes (HuMax®, Germany) and filtered (Wattman filter paper No.4), purged with liquid nitrogen (Apak et al., 2007), and stored at -20 °C (for not more than two months) until analyzed. Deionized water was used for the preparation of all standard solutions and to complete the reactions (Apak et al., 2007).

2.3 Analyses

Chemicals were purchased from GCC® (UK), Fischer® (China), Labscan® (Thaihland), LabChem® (USA) and Sigma® (China). Standard curves were prepared to have r^2 value of 0.96-0.99. Samples were analyzed in duplicate with an accuracy of not less than 95% (Luterotti, Bicanic, & Pozgaj, 2007) and coefficient of variation not more than 15%. Samples were analyzed in duplicates. Absorbance values were measured using UV-visible spectrophotometer (SCO Tech, Model SPUV®) at the specified wavelength values against standard concentrations of certain antioxidants and blank solutions.

2.3.1 Determination of Antioxidant Content

(1) Folin-Ciocultae Method

Folin-Ciocaltae method was used for the determination of antioxidant content according to Agbor, Vinson, and Donnelly (2014). Ten to 100 µl sample was completed to the volume of 1000 µl by 10x freshly prepared Folin-Ciocultae reagent. The reaction was completed within 15- minutes. Sample concentration for antioxidants was measured against freshly prepared catechin standard (catechin standard was dissolved in methanol) at 750 nm wavelength.

(2) Total Flavonoid Method

Total flavonoids were analyzed by the method of Pękal and Pyrzynka (2014). Half milliliter of the methanolic solution (2% w/v) of $AlCl_3$ was added to 1 ml sample. Then, 0.5 ml of deionized water and 0.5 ml of 1M HCl were added respectively, the mixture was shaken vigorously, and the reaction was completed within 10 minutes. The absorbance was measured at 400 nm wavelength against different concentrations of rutin standard solutions (rutin was dissolved either in ethanol or in methanol). The absorbance was measured at 400 nm wavelength against different concentrations of rutin standard solutions (rutin was dissolved either in ethanol or in methanol).

2.3.2 Determination of Antioxidant Capacity

(1) CUPRAC Assay

Concentrated (36%) hydrochloric acid (10.21 ml) was added to a suitable amount of sample extracts (0.5-5 ml), the volume was then completed to 100 ml by 50% methanol and refluxed at 80 °C for 2 hours and cooled down to room temperature. Sample mixture was then neutralized to pH 7 by 1M NaOH. Then, 1 ml $CuCl_2$, 1 ml neucoprine, and 1 ml acetate buffer, and suitable sample volume (500-1100 µl) were added respectively to complete the reaction volume to 4.1 ml. The reaction mixture was then incubated at 50 °C for 20 minutes, cooled to room temperature and centrifuged at 3000 rpm for about 7 minutes. Sample absorbance was measured using a spectrophotometer at 450 nm (Apak et al., 2007) against different concentrations of trolox standard solutions (trolox was dissolved either in ethanol or methanol).

(2) DPPH Assay

The DPPH assay procedure was performed according to Molyneux (2003). The free radical 2,2-diphenyl-picrylhydrazyl (DPPH) (2.95 ml of 0.1 mM, prepared in 80% ethanol) was added to 50 µl sample. The mixture was incubated at room temperature for 30 minutes in dark place. The absorbance was measured at 517 nm wavelength against ascorbic acid as a standard to detect DPPH radical scavenging percentage. The scavenging percentage was calculated according to the following equation:

$$\text{Scavenging effect } (\%) = (A_0\text{-}A_1)/A_0*100\% \quad (1)$$

Where, A_0: is the absorbance of the control; A_1: is the absorbance of the sample.

3. Results

3.1 Statistics and Data Analysis

The statistical analysis of data was performed using the software package for social sciences (SPSS, version 23). To detect the differences between the 4 different fruits as well as the extraction solvent, data were analyzed by factorial mixed (effect of type of plant and extract type) analysis of variance (ANOVA) (Laerd Statistics, 2018). Significant differences were considered at $P < 0.05$. Data are expressed in the tables as mean±standard deviation. Pearson's correlation coefficients were calculated and considered significant at $P < 0.05$.

3.2 Antioxidant Content

3.2.1 Total Polyphenol Content of the Fruits

Table 1 shows the antioxidant content (M catechin/100 g) of the fruit extracts determined by Folin-Ciocaltae method. The descending order of total polyphenol content of the fruits (regardless of the extraction solvent) is: lemon, mandarin, pomelo, orange. Nonetheless, there were no significant ($P > 0.05$) differences between the following pairs of fruits in terms of total polyphenol content: lemon-mandarin, mandarin-orange, and pomelo-orange. Within the same context, different extraction solvents exhibited different ($P < 0.001^{**}$) extraction efficacies. Water had extracted the highest ($P < 0.001^{**}$) amounts of antioxidants from orange, lemon, mandarin, and orange. Methanol had extracted the highest amounts of antioxidants from pomelo. On the other hand, methanol extracted the lowest ($P < 0.001^{**}$) amounts of antioxidants from all of the fruits.

Table 1. The antioxidant content (M catechin/100 g) of the methanolic, ethanolic, and water extracts of the fruits determined by Folin-Ciocaltaeu method[1,2]

Fruit	Antioxidant content (M catechin/100 g) as determined by Folin-Ciocaltaeu method			P-value
	Extract			
	Ethanol	Methanol	Water	
Orange	0.8072±0.0930	0.7936±0.0917	1.2355±0.0187	0.001^{**}
Lemon	1.1405±0.0171	1.1372±0.0751	1.3751±0.1394	
Pomelo	0.9917±0.0200	1.0671±0.0141	0.9244±0.0155	
Mandarin	0.8080±0.0483	1.0672±0.0654	1.3432±0.0582	

Note. [1] Values of the tables are average of duplicates ± SD with c.v. of not more than 15%.

[2] P values are used to express significant differences between different fruit extracts at $P < 0.05$.

3.2.2 Total Flavonoid Content of the Fruits

Table 2 shows the antioxidant content (mM rutin/100 g) of the fruit extracts determined by total flavonoid method. The descending order of the total flavonoid content (regardless of the extraction solvent) in the fruits is: orange, mandarin, lemon, pomelo. Nonetheless, there were no significant ($P > 0.05$) differences among orange, mandarin, and lemon. Within the same context, different extraction solvents exhibited different ($P < 0.001^{**}$) extraction efficacies. Water had extracted the highest ($P < 0.001^{**}$) amounts of antioxidants from all of the fruits. Ethanol had extracted more antioxidants than methanol from orange, mandarin, and lemon. On the other hand, methanol had extracted more antioxidants than ethanol from pomelo.

3.3 Antioxidant Capacity of the Fruits

3.3.1 CUPRAC Assay

Table 3 shows the antioxidant capacity (M trolox/100 g) of the fruit extracts determined by CUPRAC assay. The descending order of antioxidant capacity for the fruits (regardless of the extraction solvent) is: lemon, mandarin, orange, pomelo. In the same context, different extraction solvents exhibited different ($P < 0.001^{**}$) extraction efficacies. The highest value for antioxidant capacity expressed by CUPRAC assay for lemon was for methanol despite the fact that methanol extracts of lemon contained the least ($P < 0.05$) amounts of antioxidants (Tables 1 and 2). Methanol had the highest ($P < 0.001^{**}$) extraction efficiency from lemon and mandarin. Water had the highest ($P < 0.001^{**}$) extraction capacity from orange and pomelo. On the other hand, ethanol had the lowest ($P < 0.001^{**}$) extraction efficiency from all of the fruits.

Table 2. The antioxidant content (mM rutin/100 g) of the methanolic, ethanolic, and water fruit extracts determined by total flavonoid method[1,2]

Fruit	Antioxidant content (mM rutin/100 g) as determined by total flavonoids method			P-value
	Extract			
	Ethanol	Methanol	Water	
Orange	2.4704±0.3074	0.5987±0.0253	12.339±1.3493	$<0.001^{**}$
Lemon	3.2751±0.3637	0.8752±0.0796	11.1156±0.0230	
Pomelo	0.8613±0.0759	1.0657±0.0575	5.6370±0.8113	
Mandarin	1.2162±0.1072	0.5692±0.0738	13.5046±0.1724	

Note. [1] Values of the tables are average of duplicates ± SD with c.v. of not more than 15%.

[2] P values are used to express significant differences between different fruit extracts at $P < 0.05$.

3.3.2 DPPH Assay

Table 4 shows the antioxidant capacity (expressed as % of DPPH radical scavenging and mg vitamin C/ml) of the fruit extracts determined by DPPH assay. The descending order ($P < 0.01^{**}$) of antioxidant capacity (as DPPH radical scavenging percentage) of the fruits (regardless of the extraction solvent) is as follows: orange, mandarin, pomelo, lemon. Within the same context, different extraction solvents exhibited different ($P < 0.001^{**}$) extraction efficacies. The descending ($P < 0.01$**) order for the fruits in terms of antioxidant capacity (measured as mg vitamin C/ml) (regardless of the extraction solvent) is: mandarin, orange, pomelo, lemon. Nonetheless, there were no significant ($P > 0.05$) differences between the following pairs of fruits: pomelo-lemon and mandarin-orange. Within the same context, different extraction solvents exhibited different ($P < 0.001^{**}$) extraction capacities. Water was the most ($P < 0.001^{**}$) efficient extraction solvent from all of the fruits when the result was expressed as % DPPH scavenging. Methanol was the least ($P < 0.001^{**}$) efficient extraction solvent from orange and pomelo. Ethanol was the least efficient extraction solvent from lemon and mandarin. When the results were expressed as (mg vitamin C/ml extract), water was the most ($P < 0.001^{**}$) efficient extraction solvent from orange and pomelo. Methanol was the most ($P < 0.001^{**}$) efficient extraction solvent from lemon and pomelo. On the other hand, ethanol was the least efficient ($P < 0.001^{**}$) extraction solvent from all of the studied fruits. No correlation has been found between antioxidant content and capacity of the studied fruits.

4. Discussion

The first null hypothesis of the research has been accepted as there were significant differences among the studied fruits in terms of antioxidant contents and capacities. However, the second alternative hypothesis has

been rejected since no correlation between the antioxidant content and capacity of the studied fruits has been found.

Table 3. The antioxidant capacity (M trolox/100 g) of the methanolic, ethanolic, and water fruit extracts determined by CUPRAC assay[1,2]

Fruit	Antioxidant capacity (M trolox/100 g) as determined by total CUPRAC assay			P-value
	Extract			
	Ethanol	Methanol	Water	
Orange	0.9307±0.0968	0.7345±0.0966	1.7755±0.0031	<0.001**
Lemon	2.0157±0.0033	5.4847±1.9391	1.0904±0.0546	
Pomelo	0.6687±0.0367	0.4614±0.0072	0.8835±0.0360	
Mandarin	1.0714±0.0453	1.9472±0.2211	0.7751±0.0333	

Note. [1] Values of the tables are average of duplicates ± SD with c.v. of not more than 15%.

[2] P values are used to express significant differences between different fruit extracts at $P < 0.05$.

Table 4. The antioxidant capacity of the methanolic, ethanolic, and water fruit extracts determined by DPPH assay[1,2]

Fruit	Antioxidant capacity (expressed as % of DPPH radical scavenging and as mg vitamin C/ml)			P-value
	Extract			
	Ethanol	Methanol	Water	
Orange (expressed as % of DPPH radical scavenging)	38.8308±1.4116	24.0945±2.0044	47.5872±0.9479	<0.001**
Orange (expressed as mg vitamin C/ml)	38.8308±1.4115	160.9585±6.5036	275.3612±5.3734	
Lemon (expressed as % of DPPH radical scavenging)	10.2232±0.0000	33.3463±0.3843	33.7572±2.3783	
Lemon (expressed as mg vitamin C/ml)	10.2232±0.0000	217.6533±0.7125	196.9602±13.4824	
Pomelo (expressed as % of DPPH radical scavenging)	30.0573±0.5165	26.8574±0.0525	30.5821±0.9830	
Pomelo (expressed as mg vitamin C/ml)	30.0587±0.5184	218.4194±0.3731	178.9609±5.5723	
Mandarin (expressed as % of DPPH radical scavenging)	20.0504±0.8654	34.0450±0.2750	46.4723±2.1463	
Mandarin (expressed as mg vitamin C/ml)	20.2484±0.5854	221.5580±0.8906	269.0426±12.1670	

Note. [1] Values of the tables are average of duplicates ± SEM with c.v. of not more than 15%.

[2] P values are used to express significant differences between different fruit extracts (columns) at $P < 0.05$.

Table 5. Comparison between values of antioxidant content and capacity of this study and of other researches

Variable	Fruit	Value found in this research	Value found in other reports	Reference
Antioxidant content: total polyphenols	Lemon	corresponds to 0.2018 g catechin/kg fresh lemon	2.16 g catechin/kg fresh lemon	Park, Lee, & Park, 2014
	Mandarin	4.7701 mg catechin equivalent/100 ml extract; average of our three extracts	2.5 mg catechin equivalent/100 ml	Barros et al., 2012
	Pomelo	~29 g catechin/ kg fresh pomelo	2.1 g catechin/kg fresh pomelo	Park et al., 2015
Antioxidant content: total flavonoids	Orange	5.1360 mg rutin/100 g orange, average of the three extracts	0.06 mg rutin/g orange pulp.	Ortutu & Aremu, 2016
	Mandarin	corresponds to 103.7213 mg rutin equivalent/g fresh sample. Assuming that mandarin contains about 87% moisture (Barros, Ferreira, & Genovese 2012); our value correspond to 81.6703 mg rutin equivalent/ g dry mandarin.	38.97 mg rutin equivalent/g dry weight (average of 19 mandarin varieties available in Chinese markets)	Wang et al., 2018
	Lemon	10.3557 mg rutin equivalent/g fresh lemon (average of the three extracts)	4.41 mg rutin equivalent/ g fresh lemon (average value for 5 cultivars of Chinese lemon)	Xi, Lu, Qun, & Jiao, 2017
	Pomelo	Our values ranged between 0.8613 and 5.6370 M rutin/100 g fresh pumelo for the three extracts. Assuming 89.10% moisture content (United States Department of Agriculture [USDA], 2018), our values correspond to 47.07 mg rutin equivalent/g dry pomelo (average of the three extracts).	5.10 and11.76 mg rutin equivalent/g dry pumelo flavedo and albedo in 10 different Chinese verities.	Zefang et al., 2016
Antioxidant capacity: CUPRAC assay	Lemon	2.8636 µmol/g fresh lemon; average of the three extracts	1.3 µmol trolox/g fresh lemon	Bayili et al., 2011
	Mandarin	1.2646 M trolox/100 g fresh mandarin (average of our three extracts)		
	Mandarin	corresponds to 46.1068 mg trolox equivalent/ g dry weight if we assume 87% moisture in mandarin (Barros et al., 2012)	48.36 mg trolox equivalent/g dry weight (an average value of 19 mandarin varieties available in Chinese market)	Wang et al., 2017
	Orange	corresponds to 1164.9 µmol trolox/100 g fresh orange	722 µmol trolox/100 g fresh orange	Gorinstein et al., 2004
			849 µmol trolox/100 g fresh orange	Proteggente et al., 2002
			3740 µmol trolox/100 g fresh orange	Nilsson et al., 2006
			3740 µmol trolox/100 g fresh orange	Nilsson et al., 2003
	Pomelo	corresponds to 6.712 mmol trolox equivalent/100 g fresh pomelo (average value for the three extracts)	4.98 µmol trolox equivalent/ g fresh pomelo	(Park et al., 2014)
Antioxidant capacity: DPPH scavenging%	Orange	36.8375 (average of the three extracts)	80.14% (for ripe Nigerian orange)	Ortutu & Aremu, 2016
		20.0504%, 34.0450%, and 46.4723% for ethanol, methanol, and water extracts respectively	56.19% (for mandarin juice)	Al-Juhaimi & Ghafoor, 2013
	Pomelo	29.1656% (average of DPPH% value for the three extracts)	40.65% (in 7 pumelo varieties in Thailand)	Pichaiyongvongdee , Rattanapun, & Haruenkit, 2014
	Pomelo		33.5%	Park et al., 2014
	Lemon	33.7572% and 33.3463% for water and methanol extracts respectively	35.1%	Park et al., 2014
Antioxidant capacity DPPH scavenging: expression related to ascorbic acid	Mandarin	Supposing 87% moisture content (Barros et al., 2012), thus our value corresponds to 104.6169 m equivalent vitamin C/g dry mandarin.	21.92 mg/g dry mandarin (average value of 19 mandarin varieties available in Chinese market)	Gorinstein et al., 2004
	Pomelo	142.4797 mg vitamin C/ml extract (average of the three extracts)	9.93 mg vitamin C/g dried pomelo extract (average of six varieties in Thailand)	Mäkynen et al., 2013
	Pomelo	354.879 mg vitamin C equivalent /100 g fresh pomelo (the average of our three extracts)	383.5 mg vitamin C equivalent /100 g fresh blueberry	Floegel, Kim, Chung, Koo, & Chun, 2011
	Lemon	correspond to 265.7, 597.1, and 570.3 mg vitamin C equivalent/100 g lemon in the ethanol, methanol, and water extracts of fresh lemon respectively.	101.2±2 mg vitamin C equivalent/100 g lemon.	Floegel et al., 2011

Table 5 shows a comparison between the values found in this research and the values found in other reports for all of the studied parameters. Some of the values for total polyphenols found in this research are near to those found by other researchers, other are either higher or lower. The highest value for antioxidant capacity expressed by CUPRAC assay for lemon was for methanol despite the fact that methanol extracts of lemon contained the least ($P < 0.05$) amounts of antioxidants (Tables 1 and 2). This probably implies that there are methanol-soluble antioxidants that can be analyzed by other assay methods that might have contributed to the antioxidant capacity of the methanolic extracts (Table 3).

The highest antioxidant capacity expressed by DPPH% scavenging was for water extract of lemon. A result that is expected since the highest antioxidant content (measured by Folin-Cioculteau and total flavonoid methods) were exhibited for water extracts of lemon. The values of DPPH % scavenging were reflected as mg vitamin C/ml in Table 4.

Most of the antioxidants of mandarin analyzed in this research (total polyphenols and total flavonoids) were extracted by water ($P < 0.05$). This was reflected by the highest ($P < 0.05$) antioxidant capacity (measured by DPPH% and vitamin C equivalent) for water extracts among all of the extraction solvents of mandarin. Nonetheless, the antioxidant capacity measured by CUPRAC assay of the methanol extract of mandarin was the highest among the extraction solvents. This probably reflects the presence of certain methanol-soluble/water-insoluble (or having low water solubility) antioxidants that might have contributed to the highest antioxidant capacity (quantified by CUPRAC assay) of mandarin methanol extract. Due to the result that water had extracted the highest ($P < 0.05$) concentration of polyphenolic compounds from orange, it seems that orange contains water soluble antioxidants higher than those that are soluble in ethanol and/or methanol. Similar to total polyphenols, it seems that the flavonoids that had been extracted from orange were water soluble. This was reflected by the highest values (for orange) of antioxidant content and capacity in water extracts. Table 5 shows a comparison between values of antioxidant content and capacity found in this study and other researches. The differences between the antioxidant content and capacity values found in this study and values found in the previous literature are probably due to the analyzed fruit variety, fruit growing conditions, and experimental procedures. The difference in extraction solvent polarity allows the extraction and quantification of different antioxidant content and capacity of the fruits (Zefang, Zhao, Hongmei, Zhiqin, & Jie, 2016; Wang, Yang, & Zhou, 2018). No correlation has been found between any of the studied parameters. This probably implies that there are antioxidants other than those analyzed by the researchers that might have contributed to the antioxidant capacity values shown in Tables 3 and 4.

This study is limited by the type of extraction solvent (in terms of polarity), method of extraction (conventional extraction), and the method of analysis (spectrophotometric determination). However, results of this research will probably start a database for the antioxidant content and capacity for Jordanian fruits and vegetables that is recommended to be completed by other researchers.

5. Conclusions

It has been concluded that the studied citrus fruits contained polyphenols in descending order of: lemon, mandarin, pomelo, orange. On the other hand, the fruits contained total flavonoids in descending order of: orange, mandarin, lemon, pomelo. Water has extracted the highest amounts of antioxidants. In terms of antioxidant capacity, the fruits exhibited the antioxidant capacity (expressed as M trolox/100 g, analyzed by CUPRAC assay) in descending order of: lemon, mandarin, orange, pomelo. On the other hand, the fruits efficiency in scavenging the DPPH radical was in descending order of: mandarin, orange, pomelo, lemon. Different solvents exhibited different efficacies of extraction. No correlation has been found between any of the studied parameters. This probably implies that there are antioxidants other than those analyzed by the researchers that might have contributed to the antioxidant capacity values.

Acknowledgements

The authors would like to thank the Deanship of Academic Research at the University of Petra for financially supporting this research (Grant 5/4/2015).

References

Agbor, G., Vinson, J., & Donnelly, P. (2014). Folin-Ciocalteau reagent for polyphenolic assay. *IJFS, Nutrition and Dietetics, 3*(8), 147-156. https://doi.org/10.19070/2326-3350-1400028

Al-Juhaimi, F., & Ghafoor, K. (2013). Bioactive compounds, antioxidant and physico-chemical properties of juice from lemon, mandarin and orange fruits cultivated in Saudi Arabia. *Pak J Bot, 4*(4), 1193-1196.

Apak, R., Güçlü, K., Demirata, B., Özyürek, M., Çelik, S., Bektaşoğlu, B., Berker, K., & Özyurt, D. (2007). Comparative evaluation of various total antioxidant capacity assays applied to phenolic compounds with the CUPRAC assay. *Mol, 12*(7), 1496-1547. https://doi.org/10.3390/12071496

Barros, H., Ferreira, T., & Genovese, M. (2012). Antioxidant capacity and mineral content of pulp and peel from commercial cultivars of citrus from Brazil. *Food Chem, 134*(4), 1892-1898. https://doi.org/10.1016/j.foodchem.2012.03.090

Bayili, R., Abdoul-Latif, F., Kone, O., Diao, M., Bassole, I., & Dicko, M. (2011). Phenolic compounds and antioxidant activities in some fruits and vegetables from Burkina Faso. *AJB, 10*(62), 13543-13547.

Floegel, A., Kim, D. O., Chung, S. J., Koo, S., & Chun, O. (2011). Comparison of ABTS/DPPH assays to measure antioxidant capacity in popular antioxidant-rich US foods. *J. Food Compos. Anal., 24*(7), 1043-1048. https://doi.org/10.1016/j.jfca.2011.01.008

Food and Agricultural Organization. (2018). *FAOSTAT*. Retrieved from http://www.fao.org/faostat/en/#compare

Gorinstein, S., Huang, D., Leontowicz, H., Leontowicz, M., Yamamoto, K., Soliva-Fortuny, R., … Trakhtenberg, S. (2004). Determination of naringin and hesperidin in citrus fruit by high-performance liquid chromatography: The antioxidant potential of citrus fruit. *Acta Chromatographica, 17*(7), 108-124.

Halliwell, B., & Gutteridge, J. C. (1995). The definition and measurement of antioxidants in biological systems. *Free Radic Biol Med, 18*(1), 125-6. https://doi.org/10.1016/0891-5849(95)91457-3

Kumar, D., Lamers, H., Singh, I. P., Ladaniya, M. S., & Sthapit, B. (2015). Phytochemical evaluation of pummelo fruits (*Citrus grandis*) in India for enhancing marketing opportunities. *Indian J Plant Genet Resour, 28*(1), 50-54. https://doi.org/10.5958/0976-1926.2015.00007.8

Laerd Statistics. (2018). *Statistical tutorials and software guides*. Retrieved from https://statistics.laerd.com

Luterotti, S., Bicanic, D., & Pozgaj, R. (2006). New simple spectrophotometric assay of total carotenes in margarines. *Analytica Chimica Acta, 573-574*, 466-473. https://doi.org/10.1016/j.aca.2006.04.017

Mäkynen, K., Jitsaardkul, S., Tachasamran, P., Sakai, N., Puranachoti, S., Nirojsinlapachai, N., … Adisakwattana, S. (2013). Cultivar variations in antioxidant and antihyperlipidemic properties of pomelo pulp (*Citrus grandis* [L.] Osbeck) in Thailand. *Food Chem, 139*(1-4), 735-743. https://doi.org/10.1016/j.foodchem.2013.02.017

Molyneux, P. (2003). The use of the stable radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. *SJST, 26*(2), 211-219.

Nilsson, J., Pillai, D., Oenning, G., Persson, Ch., Nilsson, A., & Akesson, B. (2003). Total antioxidant capacity of plant foods, beverages and oils consumed in Italy assessed by three different *in vitro* assays. *J Nutr, 133*(9), 2812. https://doi.org/10.1093/jn/133.9.2812

Nilsson, J., Pillai, D., Oenning, G., Persson, Ch., Nilsson, A., & Akesson, B. (2006). Determination of naringin and hesperidin in citrus fruit by high-performance liquid chromatography. the antioxidant potential of citrus fruit. *Mol Nutr Food Res, 49*, 239. https://doi.org/10.1002/mnfr.200400083

Ortutu, S., & Aremu, M. (2016). Antioxidant capacity and free radical scavenging effects on nigerian pineapple (*Ananas cormosus*) and orange (*Citrus sinensis*) pulp extracts at different levels of ripeness. *Open Pharm Sci J, 3*, 25-30. https://doi.org/10.2174/1874844901603010025

Park, J. H., Lee, M., & Park, M. (2014). Antioxidant activity of orange flesh and peel extracted with various solvents. *PNF, 19*(4), 291-298. https://doi.org/10.1016/j.lwt.2015.03.057

Park, Y. S., Im, M., Ham, K. S., Kang, S. G., Park, Y. K., Namiesnik, J., … Gorinstein, S. (2015). Quantitative assessment of the main antioxidant compounds, antioxidant activities and FTIR spectra from commonly consumed fruits, compared to standard kiwi fruit. *LWT-Food Sci Technol, 63*, 346-352. https://doi.org/10.1016/j.lwt.2015.03.057

Pękal, A., & Pyrzynska, K. (2014). Evaluation of aluminium complexation reaction for flavonoid content assay. *Food Anal Methods, 7*, 1776-1782. https://doi.org/10.1007/s12161-014-9814-x

Pichaiyongvongdee, S., Rattanapun, B., & Haruenkit, R. (2014). Total polyphenol content and antioxidant properties in different tissues of seven pomelo (*Citrus grandis* (L.) Osbeck) cultivars. *Kasetsart J. (Nat. Sci.), 48*, 989- 996.

Proteggente, A., Pannala, A., Paganga, G., Van Buren, L., Wagner, E., Wiseman, S., … Rice-Evans, C. (2002). The antioxidant activity of regularly consumed fruit and vegetables reflects their phenolic and vitamin C composition. *Free Radical Res, 36*(2), 217-233. https://doi.org/10.1080/10715760290006484

Qura'n, S. (2010). Ethnobotanical and ecological studies of wild edible plants in Jordan. *Libyan Agriculture Research Center Journal International, 1*(4), 231-243.

USDA (United States Department of Agriculture). (2018). Retrieved from http: //www.usda.gov

Wang, H., Yang, Y., & Zhou, Z. (2018). Phenolic and flavonoid contents of mandarin (*Citrus reticulate* Blanco) fruit tissues and their antioxidant capacity as evaluated by DPPH and ABTS methods. *JIA, 17*(1), 256-263.

Wang, Y., Qian, J., Cao, J., Wang, D., Liu, C., Yang, R., Li, X., & Sun, C. (2017). Antioxidant capacity, anticancer ability and flavonoids composition of 35 citrus (*Citrus reticulata* Blanco) varieties. *Mol, 22*(7), 1114-1134. https://doi.org/10.3390/molecules22071114

Xi, W., Lu, J., Qun, J., & Jiao, B. (2017). Characterization of phenolic profile and antioxidant capacity of different fruit part from lemon (*Citrus limon* Burm.) cultivars. *J Food Sci Technol, 54*(5), 1108-1118. https://doi.org/10.1007/s13197-017-2544-5

Zefang, L., Zhao, Z., Hongmei, W., Zhiqin, Z., & Jie, Y. (2016). Phenolic composition and antioxidant capacities of Chinese local pummelo cultivars' Peel. *HPJ, 2*(3), 133-140.