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The antioxidant and Anticancer Activities of Swiss Chard and Red Beetroot Leaves

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ABSTRACT

Plants have been used for many years as a source of traditional medicine to treat various diseases and conditions. Many of these medicinal plants are also excellent sources for phytochemicals, which contain potent antioxidant and anticancer activities. Swiss chard *(Beta vulgaris, Cicla, BVc)* and beetroot *(Beta vulgaris Rubra, BVr)* are vegetables belong to the Chenopodiaceae family, widely consumed in traditional western cooking. These vegetables represent a highly renewable and cheap source of nutrients. In Eygpt, beetroot leaves (*Beta vulgaris* L.) are commonly cut off and discarded before using its bulb due to lack of knowledge of how to use them. Aiming to use these leaves, in the present study, in fresh swiss chard and beetroot leaves were chemically characterized in terms of total phenolic compounds, flavonoids, antioxidant activity by DPPH, Chlorophyll A, B and carotenoids. The beetroot leaves showed the highest levels compared with swiss chard leaves. In addition, the anticancer activity of chard and beetroot leaves were examined. The major phenolic acids in water extract of chard leaves were e- vanillic, caffeine, ellagic acid and pyrogallol while the methanolic extract of chard leaves was enriched with e- vanillic, salycillic, protocatchoic acid and catechol. On contrary, the water and methanolic extracts of beetroot leaves were enriched with e- vanillic, pyrogallol, ellagic acid and protocatchoic acid. The water extract of chard leaves was enriched with hesperidin and rosmarinic, while the methanolic extracts of chard and beetroot leaves were enriched with vitexin and hesperidin, the water and methanolic extracts of beetroot leaves were enriched with rosmarinic. Dried powder of chard and beetroot leaves were added to mango and orange juices as an application to make use of it, all samples had significant different compared to the pure juices but generally they were acceptable by the sensory evaluation. Overall, the results suggested that chard and red beetroot leaves may provide a natural sources of antioxidant and anticancer activities and it can be added to juices as they raise the nutritional value of the juices and does not noticeably impact on the sensory properties of them.

Key words: Phytochemicals, antioxidant , anticancer, Swiss Chard, Beetroot Leaves

Introduction

From ancient time till now and tomorrow, the use of natural resources especially plants increases day by day for the discovery of new therapeutic agents. Natural products from some of these natural resources continue to be used in pharmaceutical preparations either as crude extracts, fractions, pure compounds or analogous compounds from highly active isolated compounds (Abdel-Hameed *et al*., 2012).

Vegetables constitute a food source for carbohydrates, vitamins, minerals and fibers with low fats and protein contents. Leafy vegetables, in particular, are a rich source of beta-carotene, ascorbic acid, minerals and fibers (Negi and Roy, 2001). Fruits and vegetables in the diet have been found in epidemiological studies to be a protective agent against several chronic diseases associated with aging such as cancer, cardiovascular disease, cataracts, and brain and immune dysfunction (Vinson and hontz, 1995). These natural protective effects have been attributed to various components, such as carotenoids, vitamin C, E, and phenolic and thiol (SH) compounds (Paganga *et al*., 1999). Many studies have focused on the biological activities of phenolics which are potent antioxidants and free radical scavengers (Marja *et al*., 1999). The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers (Ramarathnam *et al*., 1997). In addition, they have a metal chelation potential (Kuo *et al*., 1998).

Red beetroot (*Beta vulgaris Rubra*, BVr) and Swiss chard (*Beta vulgaris Cicla*, BVc) are members of the Chenopodiaceae. This family contains important food crops, such as *Spinacia oleracea* (spinach), which is the most consumed Chenopodiaceae leafy vegetable in Europe. BVc and BVr have been used for food since 1000 B.C. by all populations of the Mediterranean basin. The Romans utilized the BVc and BVr leaves for food,

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while the roots were used for medicinal applications. BVc and BVr seeds, leaves and roots are rich in phenolic compounds, whose concentration is dependent on the stage of plant development (Vali *et al.*, 2007).

Swiss chard (*Beta vulgaris*) is a herbaceous biennial leafy vegetable cultivated in many parts of the world, low cost and wide use in many traditional dishes (Gao *et al*., 2009). The leaves can be used in salads or cooked like spinach, and the stems are usually chopped and cooked like celery. The plant is more robust and easier to grow than spinach and celery. The leaves of chard contain nutritionally significant concentrations of Vitamins A, C and B, calcium, iron and phosphorus (Pyo *et al*., 2004). *Beta vulgaris L.* species are used as a popular folk remedy for liver and kidney diseases, for stimulation of the immune and hematopoietic systems, and as a special diet in the treatment of cancer (Kanner *et al*., 2001). Phytochemical screenings of chard have revealed the presence of some fatty acids (palmetic, stearic, oleic, linoleic and linolenic acids), phospholipids, glycolipids, polysaccharides, ascorbic acid, folic acid, pectin, saponins, flavonoids, phenolic acids (Bolkent *et al*., 2000), betalains (Kugler *et al*., 2004) and apigenin (Gao *et al*.,2009).

Swiss chard (Chenopodiaceae; *Beta vulgaris* subsp. *Cycla*) has been indicated in folk medicine as a hypoglycemic (Geziginci-Oktayoglu *et al*., 2014), anti-inflammatory and hemostatic herb (Kim *et al*., 2003). It has been proposed that another cultivar of *Beta vulgaris*, chard contains apigenin flavonoids, namely vitexin, vitexin-2-O-rhamnoside and vitexin-2-O-xyloside, which show antiproliferative activity on cancer cell lines (Nifali and Angelino , 2013). The beet root (*Beta vulgaris Rapacea*) has an anticancer activity. The cancer chemopreventive potential of the beet root is thought to be due to the betalains (Kujala *et al*. 2002), which are composed of two main groups: the red betacyanins and the yellow betaxanthins. Both are used as natural additives for food and are powerful radical scavengers (Pavlov *et al*., 2002). The betacyanins have been shown to inhibit the proliferation of tumor cells *in vitro* (Reddy *et al*., 2005). The betalains are also present in swiss chard (Kugler *et al*., 2004).

Chard and beetroot leaves were represent a highly renewable and cheap source of nutrients, so the present study aimed to investigate the antioxidant and anticancer activities of both and estimate the appreciated application to use.

Materials and Methods

Materials:

Plant material:

Chard and beetroot leaves were purchased from local market at down town. The samples were well preserved and identified by Botany Department, Faculty of Agriculture, Cairo University. They were carefully washed with tap water. Stems were removed and the green tissue was sliced into small pieces, crushed and then used freshly.

Orange fruits:

(*Citrus sinensis*) a balady orange variety at full ripe stage of maturity and mango fruits (Sucary species) were brought from the local whole sale market at Giza. Mango fruits (Sucary species) were sorted, washed, Peeled and the juice was extracted by special Brown blender machine, while orange fruits were sorted, washed, cut into halves and the juice was extracted by Brown hand reamer.The extracted juices were strained through stainless steel screen then directly freshly used.

Chemicals:

All Chemicals were obtained from Sigma Chemical Co. (St. Louis, MO, USA). The stock standard solutions were prepared by dissolving the standard phenolic compounds and flavonoids in the appropriate volume of 50% aqueous methanol to produce a final concentration of 1 mg/ml. Stock/working solutions of the standards were stored in the dark at -18º C.

Methods:

Extraction:

Methanol extracts:

Each sample of fresh ground chard and beetroot leaves (25 g) was extracted by mixing (using a magnetic stirrer) with 150 ml of methanol and centrifuged at 10000 rpm for 15 min and the supernatant was filtered.

Aqueous extracts:

Aqueous extracts were obtained by mixing fresh ground plant materials using a magnetic stirrer with 100 ml of distilled water for 15 min, followed by filtration.

Chemical analysis:

Total phenols and flavonoids contents:

Total phenols and flavonoids contents in each plant material extracts were determined by folin-ciocalteu reagent as described by Arnous *et al*., (2001) and Joyeux *et al*., (1995), respectively.

Determination of antioxidant activity:

Antioxidant activity of chard and beetroot leave extracts was determined by the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) method according to Brand-Williams *et al.*(1995*)*.

Determination of chlorophyll (A and B) and carotenoids:

Chlorophyll (A and B) and carotenoids were determined as described by Arnon (1949) and Wettestein (1957), respectively.

HPLC analysis of Polyphenols and flavonoids:

Polyphenols and flavonoids contents in the extracts of chard and beetroot leaves were determined using HPLC (Agilent series 1200).

Colum temperature was maintained at 35˚C gradient separation was carried Out with methanol and acetonitrile as a mobile phase at flow rate of 1 ml/min..This method was the modified of methods Goupy *et al*., (1999) and Mattilla *et al*., (2000) for fractionate the polyphenols and flavonoids, respectively.

Determination of anticancer activity:

Effect of chard and beetroot leaves as anticancer assays of potential cytotoxicity activity against the breast carcinoma cell line (MCF7) and liver carcinoma cell line (HEPG2) were tested by the sulforhodamine B (SRB) assay using the method of Skehane *et al*., (1990). This experiment was conducted at Egyptian Cancer Institute, Cairo, Egypt.

Sensory evaluation:

The organoleptic properties of the prepared juices were evaluated by nine well trained panelists from FTRI researcher staff. The investigated sensory attributes were color, odor, taste, appearance and total acceptability. All investigated attributes were scored of 10 degree (Khan *et al*., 2001).

Fortified juices preparation:

The following of fortified juices, with different levels of chard and beetroot leaves powder were prepared.

- M.J. fortified with 0.2g chard leaves /L: Add 0.2 gram powder of chard leaves to mango juice (one liter) and mix well by the blender.
- M.J. fortified with 0.3g chard leaves/L: Add 0.3 gram powder of chard leaves to mango juice (one liter) and mix well by the blender.
- M.J. fortified with 0.4g chard leaves/L: Add 0.4 gram powder of chard leaves to mango juice (one liter) and mix well by the blender.
- M.J. fortified with 0.3g beet leaves/L: Add 0.3 gram powder of beet leaves to mango juice (one liter) and mix well by the blender.
- M.J. fortified with 0.4g beet leaves/L: Add 0.4 gram powder of beet leaves to mango juice (one liter) and mix well by the blender.
- M.J. fortified with 0.5g beet leaves/L: Add 0.5 gram powder of beet leaves to mango juice (one liter) and mix well by the blender.
- O.J. fortified with 0.2g chard leaves/L: Add 0.2 gram powder of chard leaves to orange juice (one liter) and mix well by the blender .
- O.J. fortified with 0.3g chard leaves/L: Add 0.3 gram powder of chard leaves to orange juice (one liter) and mix well by the blender.
- O.J. fortified with 0.4g chard leaves/L: Add 0.4 gram powder of chard leaves to orange juice (one liter) and mix well by the blender.
- O.J. fortified with 0.3g beet leaves/L: Add 0.3 gram powder of beet leaves to orange juice (one liter) and mix well by the blender.
- O.J. fortified with 0.4g beet leaves/L: Add 0.4 gram powder of beet leaves to orange juice (one liter) and mix well by the blender.
- O.J. fortified with 0.5g beet leaves/L: Add 0.5 gram powder of beet leaves to orange juice (one liter) and mix well by the blender.

Statistical analysis:

The obtained data were exposed analysis of variance. Duncan's Multiple range tests at ($p \leq 0.05$) level was used to compare between means. The analysis was carried out using the PRO-ANOVA procedure of Statistical Analysis System (SAS, 1996).

Results and Discussion

As illustrated in Table (1), the antioxidant activity of fresh chard and beetroot leaves in water extract and methanolic extract. It is noticed that beetroot leave extracts had higher contents of total phenols and flavonoids (and consequently its antioxidant activity) than chard leave extracts. The current study was, confirmed by Oki *et al*., (2002) who concluded that a good linear correlation (R=0.943) between the total phenols content and the scavenging of DPPH radical in chard extracts. The water extract of chard had noticeably high of total flavonoids in comparison with the methanolic extract. The total phenols in water extract of beetroot leaves were higher than to that found in the methanolic extract. This may be due to that the nature of the phenolic and flavonoid compounds and the polarity of the solvent used in the extraction, as well as the polar phenolic or flavonoid compounds dissolved in water (Cai *et al.,* 2004 and Nickavar and Abolhsani, 2009) . The current study also was, in agreement with Pyo *et al.*, (2004) who revealed that the total concentration of phenolics in white swiss 124.7 mg/100 g (as fresh weight) and the flavonoid content in white chard was 13.5 mg/100 g FW. The concentration of phenolics in beet root leaves was 2.4 mg/g FW as found by Biondo *et al*., (2014).

Table 1: Antioxidant compounds and their activity in fresh chard and beetroot leaves extracts.

Antioxidant activity	Chard leaves		Beetroot leaves	
	Water extract	Methanol extract	Water extract	methanol extract
Total phenols (mg gallic acid equivalents /g fresh weight basis)	1.26	1.92	7.9	2.6
Total flavonoids (mg/g fresh weight basis)	0.29	0.19	0.44	0.43
Antioxidant activity (%)	73.3	80.37	86.6	82.4

As shown in Table (2), chlorophyll A, B and total carotenoids in fresh chard and beetroot leaves were higher in methanolic extracts than in water extracts. It could be, also noticed that chlorophyll A, B and carotenoids were higher in beetroot leaves than chard leaves in both the water and methanolic extracts. The present data are consistent with that found by Moreira *et al.,* (2003) who revealed that the initial chlorophyll contents of conventionally produced chard was 298.6 mg/100 g of wet sample. The current data was, also, confirmed by a previous studies Reif *et al.,* (2013) who found a strong correlation between chlorophyll A and B contents in green leafy vegetables. Overall, a ratio (w/w) of approximately 1:12 between the sum of carotenoids and the sum of chlorophylls was found.

Table 2: Chlorophyll A, B and total carotenoids (% on fresh weight basis) in chard and beetroot leaves extracts.

	Chard leaves		Beetroot leaves	
	Water extract	Methanol extract	Water extract	Methanol extract
Chlorophyll A	0.05	0.18	0.09	0.57
Chlorophyll B	0.02	0.14	0.10	0.25
Total carotenoids	0.018	0.033	0.027	0.064

As shown in Table (3), there are variations in phenolic compounds concentration among the chard and beetroot leaves in water and methanolic extracts. The water extract of chard leaves was higher in e- vanillic, caffeine, ellagic acid and pyrogallol (85.91, 28.45, 12.58 and 11.76 ppm) , respectively, while the methanolic extract of chard leaves was higher in e- vanillic, salycillic, protocatchoic and catechol (103.32, 62.32, 22.6 and 12.72 ppm) , respectively. On contrast, the water and methanolic extracts of beetroot leaves was higher in e-vanillic, pyrogallol, ellagic acid and protocatchoic.

Data presented in Table (4) showed the concentration of flavonoid compounds in water and methanol extracts of fresh chard and beetroot leaves. The water extract of chard leaves was enriched with hesperidin and rosmarinic (38.31 and 25.3ppm), while the methanolic extracts of chard and beetroot leaves were highly enriched with vitexin (375.55 and 523.6 ppm) , respectively. Also , the water and methanolic extracts of beetroot leaves were enriched with rosmarinic (305.8 and 782.2 ppm) , respectively .The current result was confirmed by Nifali and Angelino (2013) who reported that chard contains apigenin flavonoids, namely vitexin,vitexin-2-O-rhamnoside and vitexin-2-O-xyloside, which show antiproliferative activity on cancer cell lines.

Efficiency of chard and beetroot leaves as anticancer agent:

Cancer is a major public health burden in both developed and developing countries. It was estimated that 12.7 million became new cancer cases and 7.6 million cancer deaths occur in 2008 (Ferlay *et al*., 2010). The

environmental, chemical, physical, metabolic and genetic factors play a direct and/or indirect role in the induction and deterioration of cancers. The limited success of clinical therapies including radiation, chemotherapy, immunomodulation and surgery in treating cancer, as evident by the high morbidity and mortality rates, indicates that there is an imperative need of new cancer management (Dai and Mumper, 2010).

Drug discovery from medicinal plants has played an important role in the treatment of cancer and, indeed, most new clinical applications of plant secondary metabolites and their derivatives over the last half century have been applied towards combating cancer (Balunas and Kinghorn, 2005). The National Cancer Institute collected about 35,000 plant samples from 20 countries and has screened around 114,000 extracts for anticancer activity. It was estimated that 14 cancer drugs of the top 35 drugs in year 2000 based on worldwide sales were natural products and natural product derivatives (Shoeb, 2006). Thus, it is urgent to find more and more safe new compounds that kill cancer cells.

Table 3: The phenolic compounds fraction amount (as fresh weight basis) in fresh chard and beetroot leaves extracts.

Phenolic compounds	Concentration (ppm) as fresh weight basis			
	Chard leaves		Beetroot leaves	
	Water Extract	Methanol extract	Water extract	Methanol extract
Gallic	4.92^{a}	5.9^{a}	2.43^{b}	2.27^{b}
Pyrogallol	12.58^{c}	7.79^{d}	64.09^{a}	23.48^{b}
3- Hydroxy tyrosol	4.39^{a}	3.79^{a}	4.71^{a}	4.38^{a}
Caffeic acid	4.92^{c}	5.89^{c}	17.81^{a}	14.95^{b}
Protocatchoic	6.13^{d}	22.6^{c}	45.35^{b}	66.93^{a}
Chlorogenic	6.42^{c}	2.6^{d}	21.77^{a}	13.07^{b}
Epi-catechin	3.77^{c}	8.98^{b}	9.32^{b}	11.56^{a}
Catechin	1.94^{b}	4.63^{a}	4.81^{a}	5.96^{a}
Caffeine	28.45^{a}	4.65^{d}	10.19^{b}	6.97^{c}
4- amino Benzoic acid	0.69^{c}	2.63^{b}	4.51^{a}	5.45^{a}
p-OH.Benzoic	9.66^{b}	6.49^{c}	10.66^{b}	22^{a}
Vanillic	1.01^{c}	9.37^{a}	3^{b}	10.54^{a}
Ferulic	2.11^{d}	11.22^{b}	17.24^{a}	5.5^{c}
Iso-ferulic	1.33^{a}	2.24^{a}	1.36^{a}	1.89^{a}
Catechol	6.08^{d}	12.72^{c}	21.19^{b}	27.1^{a}
Reversetrol	2.33^{d}	8.57^{b}	7.69^{c}	18.2^{a}
E- vanillic	85.91^{c}	103.32 ^{b}	66.39^{d}	181.88^{a}
Ellagic acid	11.76^{c}	8.99^{d}	34.35^{a}	28.3^{b}
Alpha- coumaric	4.43^{c}	9.71^{a}	7.27^{b}	4.49^{c}
Benzoic	8.81^{d}	12^{b}	21.9^{a}	10.5^{c}
Salycillic	4.93^{d}	62.32 ^{a}	25.62^{c}	44.74^{b}
3,4,5 Methoxy cinnamic	1.85^{c}	1.9^{c}	6.68^{a}	3.33^{b}
Coumarin	0.41^{b}	1.28^{b}	0.74^{b}	2.95^{a}
p- Coumaric	2.91^{d}	10.78^{b}	6.2^{c}	16.3^{a}
Cinnamic	0.49^{a}	1.19^{a}	0.45^{a}	1.34^{a}

Means followed by the same superscript letter in the same rows are not significantly different ($p < 0.05$).

Table 4: The concentration of flavonoid compounds found in fresh chard and beetroot leaves extracts.

Flavonoids	Concentration (ppm) as fresh weight basis			
	Chard leaves		Beetroot leaves	
	Water extract	Methanol extract	Water extract	Methanol extract
Vitexin	9.29^{d}	375.55^{b}	135^{c}	523.6^{a}
Iuteolin	12.25^{d}	72.12^{b}	95.29^{a}	18.53^{c}
Naringin	12.3^{d}	13.17^{c}	24.71^{b}	26.03^{a}
Rutin	4.21^{c}	10.89^{b}	9.91^{b}	15.01^{a}
Hesperidin	38.31^{c}	35.9^{d}	90.2^{a}	70.72^{b}
Rosmarinic	25.3^{c}	1.77^{d}	305.8^{b}	782.2^{a}
Quercetrin	5.37^{c}	2.48^{d}	17.1^{b}	25.24^{a}
Quercetin	1.64^{b}	0.23^{b}	1.98^{b}	21.62^{a}
Kaempferal	0.82^{a}	0.62^{a}	0.93^{a}	2.25^{a}
Hespertin	2.44^{c}	6.87^{b}	1.42^{c}	17.36^{a}
Apegenin	0.33^{a}	0.07^{b}	1.13^{a}	1.49^{a}
Hydroxyl-flavone	7.72^{b}	0.26^{c}	1.1^{c}	50.8^{a}

Means followed by the same superscript letter in the same rows are not significantly different ($p < 0.05$).

Results in Table (5) showed the cytotoxic activity of chard and beetroot leaves as anticancer agent (towards to breast and liver) and IC50 dose. As illustrated in Table (5), the most efficient cytotoxic activity of chard leaves was against breast cancer (MCF7) (IC50=19.3) , while it has no efficient cytotoxic activity against liver cancer (HEPG2). It could be , also noticed that the most efficient cytotoxic activity of beetroot leaves was against (breast cancer) MCF7 (IC50=22.6), then against (liver cancer) HEPG2 (IC50=30.4). The current result was confirmed by Ninfali *et al*., (2007) who reported that the cytotoxic activity of the vitexin in swiss chard was

against breast cancer (MCF7). Also the present data are in the line with Ninfali and Angelino (2013) who observed that the administration of beetroot extract, rich in polyphenols and betalains, was able to decrease the carcinoma cell numbers.

Table 5: Cytotoxic activity of the chard and beetroot leaves.

Concentration $\mu g/ml$	Chard leaves		Beetroot leaves	
	MCF7 (breast)	HEPG2 (liver)	MCF7 (breast)	HEPG2 (liver)
0.0	1.000	1.000	1.000	1.000
5.0	0.962	0.927	0.870	0.853
12.0	0.735	0.817	0.821	0.787
25.0	0.297	0.712	0.429	0.558
50.0	0.257	0.537	0.266	0.310
IC 50	19.3	0.0	22.6	30.4

MCF7 (breast carcinoma cell line), HEPG2 (liver carcinoma cell line),IC 50 (dose of the tested compound which reduces survival to 50%)

From the Table (6), it could be concluded that the addition of both the chard and beetroot leaves to mango and orange juices do not affect the juices odor. Respect to the taste, the mango juice fortified with 0.2 and 0.3 g chard leaves /L and mango juice fortified with 0.3 g beet leaves /L had not significant different compared to the pure mango, but the fortified orange juices had significant different compared to the pure orange, the best taste was in orange juice fortified with 0.2g chard leaves /L and orange juice fortified with 0.3 g beetroot leaves /L. Respect to total acceptability, all samples had significant different compared to the pure juices but generally, all samples were acceptable by the sensory evaluation.

Table 6: Sensory evaluation of fortified juices with chard and beetroot leaves powder

Juices	Color	Odor	Taste	Appearance	Total acceptability
Mango Juice(M.J.)	9.8^{a}	10	9.7^{a}	9.8^{a}	9.5^{a}
Orange Juice(O.J.)	9.8^{a}	10	9.7^{a}	9.5^{a}	9.5^{a}
M.J. fortified with 0.2g chard leaves /L	8.5^{ab}	10	9.0^{ab}	7.5^{b}	8.0^{b}
M.J. fortified with 0.3g chard leaves/L	8.5^{ab}	10	9.0^{ab}	7.5^{b}	8.0^{b}
M.J. fortified with 0.4g chard leaves/L	8.0^{b}	10	8.3^{bcd}	7.3^{b}	7.5^{b}
M.J. fortified with 0.3g beet leaves/L	8.3^{ab}	10	8.7^{abc}	7.5^{b}	8.0^{b}
M.J. fortified with 0.4g beet leaves/L	7.8^{ab}	10	8.8^{bcd}	7.3^{b}	8.1^{b}
M.J. fortified with 0.5g beet leaves/L	7.5^{ab}	10	7.7^{dc}	7.3^{b}	7.9^{b}
O.J. fortified with 0.2g chard leaves/L	8.2^{ab}	10	8.0^{bcd}	6.8^{b}	8.0^{b}
O.J. fortified with 0.3g chard leaves/L	8.0^{ab}	10	7.7^{dc}	6.8^{b}	7.8^{b}
O.J. fortified with 0.4g chard leaves/L	7.8^{ab}	10	7.7^{dc}	6.5^{b}	7.5^{b}
O.J. fortified with 0.3g beet leaves/L	8.5^{ab}	10	8.3^{bcd}	7.0^{b}	7.8^{b}
O.J. fortified with 0.4g beet leaves/L	8.3^{ab}	10	8.0^{bcd}	7.0^{b}	7.5^{b}
O.J. fortified with 0.5g beet leaves/L	8.0^{ab}	10	7.3^{d}	6.8^{b}	7.3^{b}

Means followed by the same superscript letter in the same column are not significantly different ($p < 0.05$).

Conclusions

Chard and red beetroot leaves are popular vegetables in the Mediterranean diet, which represent a plentiful and inexpensive sources of natural antioxidant and anticancer nutrients and it can be added to juices as they raise the nutritional value of the juices and does not noticeably impact on the sensory properties of them.

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