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Quantification of Total Phenolic and Total Flavonoid Contents in Extracts of Some Egyptian Green leaves and Estimation of Antioxidant Activity.

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ABSTRACT

This study was conducted to determine the total poly phenols and flavonoids, estimation of antioxidant activity *in vitro* by two methods (DPPH radical scavenging activity and -carotene) for 16 plant leaves. Radish (white, red) , attract, cabbage (white, red), dill, boil, carrots (red, yellow), red beet, sugar beet, cauliflower, coriander, parsley, spinach and lettuce extracted by cold and boiled water for 10, 30 and 60 minutes. Total phenolic and flavonoid contents in the extracts were determined using Folin-Ciocalteu reagent and $AlCl_3$ method and their amount calculated as gallic acid/100g and rutin/100g fresh weight respectively.

Key words: Plant Leaves- Antioxidant activity-Total Polyphenols- Flavonoids- Gallic acid- Rutin.

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INTRODUCTION

Plants are the good sources for the discovery of pharmaceutical compounds and medicines. Natural products could be potential drugs for humans or live stock species and also these products and their analogues can act as intermediates for synthesis of useful drugs (Makkar *et al*, 2009). Plants possess many phytochemicals with various bioactivities including, carotenodis, ascorbic acid, α- tocopherol and polyphenols (Salah *et al*., 1995, Edge *et al*., 1997 and Papas 2002). Natural antioxidants haven't cause health problems that may arise from the use of synthetic antioxidants which have side effects (Arouma *et al*., 1992).

Free radical reaction occur in the human body and food systems. Free radicals, in the form of reactive oxygen and nitrogen species, are an integral part of normal physiology. Over production of these reactive species can occur due to oxidative stress brought about by an imbalance of the bodily antioxidant defense system or free radical formation. These reactive species can react with biomolecules, causing injury and death (Halliweel, 2008).

Antioxidant substances block the action of free radicals which have been implicated in the pathogenesis of many diseases including atherosclerosis, ischemic heart disease, cancer, Alheimer's disease and in the aging process (Aruoma, 1998). Antioxidant are also used to preserve food quality mainly because they arrest oxidative deterioration of lipids.

The natural antioxidants in wine, fruits and vegetables have been studied widely due to their health benefits and commercial values. Besides fruits, other parts of plants such as bark, leaves, fruit peels and roots are also being exploited extensively for their antioxidant properties.

For instance, antioxidant studies were conducted in green leafy vegetables such as amaranth, spinach, back choi and kang kong as well as in leaves of guava leaves, blackberry leaves, red raspberry leaves, strawberry leaves, cabbage broccoli, cauliflower and Brussels (Wng and Lin, 2000, Yang *et al*., 2005 and Soengas *et al*., 2011).

In this study, 16 plant leaves in Egypt, radish (White, red), attrach, cabbage (White, red), dill, boil, carrots (red, Yellos), red beet, sugar beet, cauliflower, coriander, parsley, spinach, and lettuce) were selected from local market.

The objective of this study was to investigate the effect of extracting time with cold and boiled water (100°C for 10, 30, 60min) on the yield of total polyphenol, total flavonoid contents and antioxidant activity. The leaf with the highest antioxidant property can be used as a suitable source for natural antioxidant to substitute the usage of synthetic antioxidant.

MATERIAL AND METHODS

Sample collection

Fresh leaves from radish (white, red), attract, cabbage (white, red) dill, boil, carrots (red, yellow), red beet, sugar beet, cauliflower, coriander, parsley, spinach, lettuce were collected from local market in Egypt.

Preparation of crude plant extract

Ten grams of each leaves were extracted with cold water and boiled water (100°C in 100ml) for 10, 30 and 60 minutes. both extractions were then filtered (Whatman No. 1).

The filtrates were analyze for total poly phenol and flavonoid contents and antioxidant activity by DPPH free radical scavenging assay and β-carotene Linoleic assay.

Determination of total Phenolic compounds

The concentration of phenolics in leaves extracts was determined using spectrophotometric method (Sing Leton *et al*., 1999).

The reaction mixture was prepared by mixing 0.5ml of aqueous solution of extract, 2.5ml of 10% Folin- Ciocalteu's reagent dissolved in water and 2.5ml of 7.5% of $NaHCo_3$.

The samples were there after incubated in thermostat at 45°C for 45min. the absorbance was determined using spectrophotometric at λmax = 765nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of gallic acid and the calibration line was construed. Based on the measured absorbance, the concentration of phenolics was read (mg/ml) from the calibration line, then the content of phenolics in extracts was expressed in terms of gallic acid equivalent (mg of GA/ g of extract).

Determination of flavonoid content

The content of flavonoids in the examined leaves extracts was determined using spectropboiledometric method (Quettier *et al*., 2000). The sample contained 1ml of aqueous solution of the extract in the concentration of 1mg/ml and 1ml of 2% $AlCl_3$ solution dissolved in methanol. The samples were incubated for an hour at room temperature. The absorbance was determined using spectrophotometric at λ_{max}= 415nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of rutin and the calibration line was construed. Based on the measured absorbance, the concentration of flavonoids was read (mg/ml) on the calibration line; then, the content of flavonoids in extracts was expressed in terms of rutin equivalent (mg of Ru/g of extract).

Evaluation of antioxidant activity

DPPH radical scavenging activity

The free radical – scavenging activity of each extract was determined as described by Braca *et al*., (2001).

Plant leaves extracts were added to 3ml of a0.004% methanol solution of DPPH. Absorbance at 517nm was measured under constant mixing at room temperature after 30 min and percent inhibitory activity was calculated from:

$$\text{Inhibition}\,(\%)=\frac{(\text{control}-\text{test})}{\text{control}}\times 100$$

β-carotene – linoleic acid assay

β-carotene bleaching assay was carried out according to the method developed by Wettashinghe and Shahidi (1999). One milliter of β-carotene solution (0.2 mg/ ml chloroform) was pipetted into a round – bottom flask (50ml) containing 0.02 ml of linoleic acid and 0.2 ml of 100% Tween 20. the mixture was then evaporated at 40°C for 10 min using arotary evaporator to remove chloroform. After evaporation, the mixture was immediately diluted with 100ml of distilled water.

The distilled water was added slowly to the mixture with vigorous agitation to form an emulsion. Five ml aliquots of the emulsion were transferred into different test tubes containing 0.2ml of samples in 80% methanol at 1mg/ ml. The mixture was then gently mixed and placed in a water bath at 50°C for 2h. Absorbance of the sample was measured every 30 min for 2h at 470nm using a spectrophotometric. Blank solution was prepared, containing the same concentration of sample of sample without β- Carotene. All determinations were performed in triplicate. The total antioxidant activity was calculated based on the following equation.

$$AA=[1-\frac{A_o-At}{A_o^o-A_t^o}]\,x100$$

Where AA is antioxidant activity. A_o and A_o^o are the absorbance values measured at the initial incubation time for samples and control, respectively. While A_t and A_t^o are the absorbance values measured in the samples or standards and control at t= 120 min.

RESULTS AND DISCUSSION

Plant tissues contain arrange of components that may be broadly classed as antioxidants. The relative activity of each component may differ depending upon the type of system (Lipidic or aqueous) used, and several methods have been developed to evaluate the antioxidant activity in plant tissues (Velioglue *et al*., 1998).

In this study the total phenolic, total flavonoid content and antioxidant activity were determined by two complementary tests: scavenging of DPPH free radicals and the β-carotene bleaching test for 16 plant leaves radish (white, red), attract, cabbage (white, red), dill, boil, carrots (red, yellow), red beet , sugar beet, cauliflower, coriander, parsley, spinach and lettuce, were extracted with cold water or boiled water (100°C for 10, 30, 60 min).

The moisture content of 16 plant leaves were determined and were ranged from (56.05- 86.29%) (Table 1). The amount of total phenol was determined with the Folin – Ciocalteu reagent. Gallic acid was used a standard compound and the total phenols were expressed as (mg/ 100g fresh weight). The total polypherol content in 16 plant leaves extracts with cold water analyzed was in the range of 6.31-61.75mg GALLIIC ACID/100g fresh weight. (Table 1). The total polyphenols content in cauliflower cold water extract was the highest one (61.75mg/ 100g FW) and the lowest one was white cabbage (6.31 mg/100g FW) when expressed against the total polyphenol content in leaves extracts with boiled water (100°C for 60 min) for radish (red), attract, cabbage (red, white), dill, boil, carrots (red, yellow), red beet, sugar beet, parsley and lettuce were the highest than the extracts with coldwater or with boiled water 100°C for (10 min, 30 min). But the highest polyphenol content in white radish, cauliflower, coriander and spinach in cold water extracts, followed by the boiled water extracts (100°C for 10, 30, 60min) compared to other samples.

The results of the present study are generally in agreement with other reports. It was previously reported that total phenols content for red cabbage grown in the United States was 254 mg per 100g FW (Wu *et al*., 2004), which is higher than that obtained in this study. Bahorun *et al*., (2004) reported that white cabbage from Mauritius had 15.3mg total phenols per 100g FW, that is, lower as compared with white cabbage cultivated in Egypt (21.78 mg/100g FW extracted with boiled water 100°C for 60 min).

Milan Ciz *et al*., (2010) found higher levels of polyphenols in parsley leaves, radish, dill, carrot root and red beet extracts (599.7, 89.9, 150.4, 35.2 and 81.5 mg gallic acid/ 100g FW) respectively. The antioxidant property of flavonoids which contributed to good health in human has been studies intesiviely. Flavonoids are included anthocyanisn, proanthocyanidins, flavonols and catenchins (Mervat and Hanan 2009). Flavonoids generally work by scavenging or chelating process (Cook and Samman 1996). The total flavonoids amount in the extracts were calculated based on catechin equivalent. The flavonoids contents of 16 edible plant leaves extracts with cold water ranged between 20.0mg/ 100g FW and 299.0 mg/ 100g FW (Tab 2). Cauliflower has the highest flavonoids content at 299.0mg/100g FW followed by spinach leaves (292.1mg/100g FW).

The total flavonoid content in 16 plant leaves extracts with boiled water (100°C for 60min) for red radish, attract, red and white cabbage, dill, boil, red and yellow carrots, red beet, sugar beet, coriander, parsley and lettuce were highest than the extracts with cold water or boiled water for (10min, 30min). The highest flavonoid content in cauliflower spinach and white radish leaves extracts found in cold water extracts (299.0, 292.1m 138.0mg/100g Fw) respectively.

Comparatively, a study conducted by Koo and Mohamed, (2001) concluded that highest content of total flavonoids is found in leaves of onion (1497.5mg/ Kg quercetin) followed by black tea (1491.0mg/Kg) and papaya shoots (1264.0 mg/ Kg). on the other hand, commonly consumed vegetables such as soybean sprout (78.5 g/Kg) red spinach (29.5 mg/kg) and kalian (14.5 mg/Kg) showed lower flavonoids contents compared to

the leaves extracts in this study. Hence, this results can conclude that leaves of 16 plants in this study have the potential to be a suitable source of flavonoids.

The importance of the antioxidants in plant materials to the maintenance of health and protection from coronary heart disease and cancer is also raising interest among scientists, food manufactures and consumers (Loliger, 1991). Tanaka *et al*. (1988) reported that polyphenol compounds had inhibitory effects on mutagenesis and carcinogenesis in humans, when up to 1.0g was ingested daily from a diet rich in fruits and vegetables.

Table 1: Effect of temperature and time of extraction on the total poly phenols in some plant leaves.

Plant leaves	Moisture %	Total poly phenols (mg/100g fresh weight)			
		Cold water	Boiled water (100°C)		
			10 min	30 min	60 min
White radish	75.20	20.80	20.80	18.20	16.25
Red radish	67.40	29.51	37.70	42.25	48.75
Attract	59.62	42.64	67.63	81.25	94.25
Red cabbage	65.90	35.75	65.33	80.93	97.50
White cabbage	79.34	6.31	11.70	16.25	21.78
Dill	56.05	19.37	21.45	52.50	42.25
Boil	72.55	10.73	18.14	22.75	28.93
Red carrots	78.75	19.70	38.19	48.75	57.53
Yellow carrots	75.18	11.28	25.51	32.50	39.00
Red beet	77.47	18.10	30.88	42.25	48.75
Sugar beet	66.55	16.02	24.60	29.25	35.75
Cauliflower	65.29	61.75	32.50	32.50	29.25
Coriander	81.16	48.75	48.75	42.25	39.65
Parsley	76.94	29.90	32.50	42.25	52.00
Spinach	76.16	44.53	33.70	32.50	32.18
Lettuce	86.29	10.89	10.92	16.35	17.88

Table 2: Effect of temperature and time of extraction on the total flavonoid in some plant leaves.

Plant leaves	Total poly phenols (mg/100g fresh weight)			
	Cold water	Boiled water (100°C)		
		10 min	30 min	60 min
White radish	138.00	135.00	115.00	92.00
Red radish	204.93	230.00	276.00	319.70
Attract	228.90	261.30	299.00	391.00
Red cabbage	132.70	207.00	276.00	368.00
White cabbage	20.00	50.60	92.00	135.70
Dill	170.20	176.18	202.4	230.00
Boil	187.68	232.53	299.00	345.00
Red carrots	209.30	230.00	276.00	345.00
Yellow carrots	144.90	211.83	322.00	368.00
Red beet	236.21	245.64	276.00	333.50
Sugar beet	178.94	265.65	345.00	381.80
Cauliflower	299.00	219.19	207.00	195.50
Coriander	226.78	257.60	322.00	402.50
Parsley	163.30	207.00	276.00	276.00
Spinach	292.10	253.00	230.00	217.12
Lettuce	22.31	23.00	29.90	51.52

DPPH radicals are widely used in the model system to investigated the scavenging activities of several natural compounds. When DPPH radical is scavenged, the color of the reaction mixture changes from purple to yellow with decreasing of absorbance at wavelength 517nm. Table (3) shows the scavenging activity against DPPH radicals of cold water, boiled water 100°C for (10. 30, 60 min) extracts of 16 plant leaves, the examination of antioxidant activities of plant extracts showed different values. The obtained values varied

from (1.57 to 31.5%) for cold water extracts. The largest capacity to neutralize DPPH radicals was found for cauliflower leaves and the low activity for sugar beet leaves.

Table 3: Antoxidant activity of some plant leaves extract with DPPH method.

Plant leaves	DPPH free radical scavenging aetivity%			
	Cold water	Boiled water (100°C)		
		10 min	30 min	60 min
White radish	20.50	70.65	56.40	40.0
Red radish	19.50	28.38	28.55	30.10
Attract	10.6	45.33	31.89	20.0
Red cabbage	24.57	26.90	64.28	65.04
White cabbage	8.00	8.50	6.0	2.20
Dill	18.00	40.50	50.60	65.84
Boil	10.5	20.20	32.50	35.00
Red carrots	19.2	21.50	19.50	18.50
Yellow carrots	22.50	28.5	30.20	15.20
Red beet	11.57	18.52	10.65	9.20
Sugar beet	1.57	8.52	0.65	0.20
Cauliflower	31.5	30.2	29.75	25.0
Coriander	30.25	30.46	20.10	18.50
Parsley	3.30	2.79	2.50	2.00
Spinach	16.65	16.0	14.2	13.0
Lettuce	10.8	18.50	15.20	15.0

Table 4: Antoxidant activity of some plant leaves extract with β-carotene bleaching method.

Plant leaves	β-carotene bleaching AAC			
	Cold water	Boiled water (100°C)		
		10 min	30 min	60 min
White radish	31.82	36.2	57.45	10.0
Red radish	19.70	44.40	70.20	36.20
Attract	13.64	45.45	85.00	30.0
Red cabbage	27.27	45.45	80.90	85.60
White cabbage	18.18	47.72	36.2	36.2
Dill	19.70	36.20	40.4	57.4
Boil	28.18	34.85	57.4	20.50
Red carrots	26.06	39.40	36.2	10.0
Yellow carrots	27.27	31.82	57.40	30.5
Red beet	34.85	34.85	57.4	30.4
Sugar beet	34.85	37.88	70.20	20.0
Cauliflower	32.27	33.33	78.7	0.0
Coriander	42.42	30.30	66.0	0.0
Parsley	32.88	40.90	70.00	0.0
Spinach	29.40	39.40	68.1	0.0
Lettuce	42.80	33.33	21.3	5.0

ACC the antioxidant activity coefficient calculated (as described in experimental part).

The highest antioxidant activity (DPPH) for 16plant leaves extracts by boiled water (10,30,60 min) were white radish (70.65%), red cabbage (64.28%) and dill (65.84%) respectively. These results are consistent with those of Holasove et al., (2002), who found that total phenol content increased with antioxidative activity. Gheldof and Engesth (2002) reported a linear correlation between phenolic content and antioxidative activity. Phenolic compounds are commonly found in both edible and in edible plants and have been reported to have multiple biological effects including antioxidative activity. Crude extracts of fruits, herbs, vegetables, cereals, nuts and other plant materials rich in phenolic compounds are increasingly attracting interest in the food industry.

The β-carotene bleaching method is usually used to evaluate the antioxidant activity of compounds in emulsions accompanied with the coupled oxidation of β-carotene and linoleic acid. Table (4) shows the

antioxidant activity coefficients (AAC) of 16 plant leaves extracts by coldwater and boiled water (100°C) for 10 min or 30 min or 60 min. The highest one for plant leaves of cold extracts was lettuce leaves (42.88) and for boiled water extracts 100°C for 10 min (white cabbage leaves 47.72), 30 min (attract leaves 85.0) and 60 min (red cabbage leaves 85.6).

In spite that polar compound like ascorbic acid are well known antioxidants, the β-carotene bleaching test did not show antioxidative properties. This interesting phenomenon has been described as the polar paradox (Kulisic *et al*., 2004). Polar antioxidants remaining in the aqueous phase of the emulsion are more diluted in the lipid phase and are thus less effective in protecting linoleic acid β-carotene undergoes rapid depolarization in the absence of an antioxidant. The presence of different antioxidants can hinder the extent of β-carotene bleaching by neutralizing linoleate free radicals and other radicals formed in the system (Siramon and Ohtani, 2007).

CONCLUSION

The result of experiments including total polyphenol, flavonoid content, DPPH radical and β-Carotene bleaching activity demonstrated that the antioxidant activity was directly related to the total amount of phenolics and flavonoids found in 16 plant leaves extracts. Hence the plant leaves used in this study can be used as an easy and safe accessible source of natural antioxidants, as food supplements, or in the pharmaceutical and medical industries.

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