Relationship between antioxidant activity and total phenolics in selected vegetables, fruits, herbs and spices commonly consumed in Egypt

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Abstract: Antioxidants are compounds that inhibit or delay the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reaction. Several researchers have investigated the ability of some phenolic compounds to act as antioxidants and can play a major role in the antioxidant activity of plant materials. In this work the antioxidant activities and total phenolics of 31 plant products, including vegetables, fruits, herbs and spices in commonly consumed in Egypt were determined. The total phenolic content of selected products varied from 89 to 3701 mg.100 g^{-1}. Antioxidant activity of methanolic extract was determined and calculated in four different ways Antioxidant value (AOX, A/h), Antioxidant activity (AA,%), Oxidation rate ratio (ORR) and Antioxidant activity coefficient (AAC) ranged 0.48 to 0.349, 31.56 to 92.63, 0.039 to 0.646 and 87.12 to 1143.65, respectively. Statistically analysis, correlation and/or regression coefficient between total phenolics and antioxidative activities was significant in some plant products and others was non significant, which indicates that factors beside the total phenolics can play a principle role in the antioxidant activity of that plant materials. Therefore, further work is needed to elucidate the identity of compounds responsible for their antioxidant activities.
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1. Introduction

Current life style causes over production of free radicals and reactive oxygen species (ROS). In the body, oxidation products and ROS may lead to a number of diseases and tissue injuries such as those of the lungs, heart, kidney, liver, gastrointestinal tract, blood, eye, skin, muscle, and brain, as well as the aging process (Meskin *et al.,* 2002). In healthy individuals, ROS are neutralized by the action of antioxidant defense system including biological products and antioxidant enzymes. However, when the action of the antioxidant defense system is inadequate because of illness and during infancy or due to aging, the oxidation process is not controlled naturally, and augmentation may provide the necessary means to combat degenerative diseases and other ailments caused by ROS. Antioxidants scavenge ROS and are associated with reduced risk of related diseases associated diseases therefore play an important role in health care (Lopez *et al.,* 2007 and Prakash *et al.,* 2007). The manner in which antioxidants intervene is by their effect in a multistage process and may involve prevention of lipid oxidation, protein cross-linking, and DNA mutation, among others (Shahidi and Naczk, 1995).

In general, there are two basic categories of antioxidants, namely, synthetic and natural. Synthetic antioxidants are compounds with phenolic structures of various degrees of alkyl substitution, whereas natural antioxidants can be vitamins (C and E), minerals (selenium and copper) and phytonutrients (phenolic compounds, nitrogen compounds and carotenoids) (Jacob and Burri, 1996 and Hall and Cuppett. 1997). Science the beginning of 20th century, synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have been used as antioxidants. Restrictions on the use of these compounds, however, are being imposed because of their carcinogenicity (Branen, 1975 and Ito *et al.,*1983). Thus, the interest in natural antioxidants has increased considerably.

The ability of phenolic compounds (tocopherols, flavonoids, and phenolic acids) to act as antioxidants has been demonstrated in the literature (Foti *et al.,* 1996; Elhassaneen and Sanad, 2006; Prakash *et al.,* 2007 and Reddy *et al.,* 2010). Phenolic compounds are the most widely distributed secondary metabolites in plants, and constitute several thousands of compounds. Phenolics in plants are primarily responsible for their protection from free radical stress under photosynthetic conditions and ultraviolet light, and act against herbivores and pathogens (Shahidi, 2000). They also contribute to the variety of color and taste of foods containing them (Shahidi and Naczk, 1995). In addition, they serve as wound-healing agents in plants as a result of their oxidation and subsequent condensation with free amino acids and proteins.

Plant foods are the primary source of natural antioxidants for human being. Fruits, vegetables, herbs and spices are important dietary sources of antioxidant phenolics to humans (Vayalil, 2002; Ghaly, 2004 and Hanson *et al.,* 2006). Large epidemiological studies have shown that increased consumption of these plant–rich phenolics is associated with a lower risk of degenerative diseases (Ames *et al.,* 1993; Meskin *et al.,* 2002 and Sambanthamurthi, 2011). Different classes of phenolics especially flavonoids exhibit a wide range of biological effects, including antibacterial, antiviral, anti-inflammatory, antiallergic, anti-thrombotic, and vasodilatory actions (Meskin *et al.,* 2002). Antioxidant activity is a fundamental property important for life. Many of the biological functions, such as antimutagenicity, anticarcino-genicity, and antiaging, among others, originate from this property (Huang *et al.,* 1992; Cook and Samman, 1996 and Sanjib, 2012). In Egypt, there is a dearth of information regarding the antioxidant activity and phenolic content of plant foods commonly consumed by the populations, which have received less attention as functional foods so far. Therefore, the objective of this study was to determine the contents of total phenolics in selected vegetables, fruits, herbs and spices most commonly consumed in Egypt and relate it to their antioxidant activity.

2. Materials and Methods

Plant material

Thirty-one plant products, ten vegetables (Grean beans, *Phaseolus vulgaris*; Pea, *Pisum savitum*; Beans, *Vicia faba*; Horseradish root, *Armoracia rusticana*; Carrot, *Daucus carota*; Tomato, *Lycopersicum esculentum*; Pepper, *Capsicum annuum*; Eggplant, *Solanum melongena*; Cabbage, *Brassica oleracea* and Potatoes, *Solanum tuberosum*), eight fruits (Strawberry, *Fragaria ananassa*; Red and green apple, *Malus pumila* and Red, green and black currant, *Ribes rubrum*, Guava, *Psidium guajava* and Pomegranata, *Punica granatum*) and thirteen herbs and spices (Camomile flower, *Matricaria chamomilla*; Caraway seeds, *Carum carvi*; Hop, *Humulus lupulus*; Thyme, *Thymus vulgaris*; Flax seeds, *Linum usitatissimum*; Coriander, *Coriandrum sativum*; Dill, *Anethum grave*olenst; Cumin seeds, *Cuminum cyminium*; carnation, *Dianthus caryophyllus* ; Mint leaves, *Mentha arvensis*; Rosemary, *Rosmarinus officinalis*, Basil, *Ocimum basilicum* and marjoram, *Origanum majorana*) were assayed for antioxidant activity and phenolic content. For vegetable s and fruits samples, four samples of each variety of fresh plant parts were purchased from each of the three local markets (Minoufiya, Dakhlia and Tanta Governorates), pooled and considered as a single sample of that market. Total quantity of each pooled sample was 400-500 g. Care was taken to avoid unripe, damaged, overripe and defected plant parts including fruits, grains and roots. Plant parts purchased from the market were cleaned, edible portions were cut into small pieces and store at -20 ^{0}C until analysis. Herbs and spices in a powder case were purchased from three famous merchandizes, Bab El-Khlek, Cairo, Egypt. All of the obtained plant parts were subjected to botanical examination in Agriculture Research Center, Giza, Egypt for confirming the corrected varieties. Total quantity of each pooled sample was 100-200 g. Plant parts were cleaned, sieving and stored at -20 ^{0}C until analysis. All samples were either ground or homogenized before they were freeze-dried to ensure equal moisture content.

Extraction

Ground samples of dehydrated plant parts (100 g) were extracted with 80% aqueous methanol (750 ml) on an orbital shaker for 120 min at 70 °C. For the anthocyanin-containing samples-strawberry, red apple, red and black currants and pomegranate -extraction temperature was 25 °C. The lower extraction temperature for the anthocyanin-containing samples was used to prevent any thermal breakdown of the anthocyanin pigments. The mixture was subsequently filtered (Whatman No.5) on a Buchner funnel, and the filtrate was assayed for antioxidant activity.

Determination of antioxidant activity and total phenolics

Antioxidant activity

Antioxidant activity of plant extract and standards (α-tocopherol, BHA, and BHT; Sigma Chemical Co., St. Louis, Mo) was determined according to the β-carotene bleaching method following a modification of the procedure described by Marco (1968). For a typical assay, 1mL of β-carotene (Sigma) solution, 0.2 mg/mL in chloroform, was added to round-bottom flasks (50 mL) containing 0.02 mL of linoleic acid (J.T. Baker Chemical Co., Phillipsburg, NJ) and 0.2 mL of Tween 20 (BDH Chemical Co., Toronto, On). Each mixture was then dosed with 0.2 mL of 80% MeOH (as control) or corresponding plant extract or standard. After evaporation to dryness under vacuum at room temperature, oxygenated distilled water (50 ml) was added and the mixture was shaken to form a liposome solution. The samples were then subjected to thermal autooxidation at 50 ^{0}C for 2 h. The absorbance of the solution at 470 nm was monitored on a spectrophotometer (beckman DU-50) by taking measurements at 10 min intervals, and the rate of bleaching of β-carotene was calculated by fitting linear regression to data over time. All samples were assayed in triplicate. Various concentrations of BHT, BHA, and α-tocopherol in 80% methanol was used as the control.

Antioxidant activity was calculated in four different ways. In the first, absorbance was plotted against time, as a kint curve, and the absolute value of slope was expressed as antioxidant value (AOX). Antioxidant activity (AA) was all calculated as percent

inhibition relative to control using the following equation (Al-Saikhan *et al.,* 1995).

$AA= (R_{control} - R_{sample}) / R_{control} \times 100$

Where: $R_{control}$ and R_{sample} were the bleaching rates of beta-carotene in reactant mixture without antioxidant and with plant extract, respectively.

The third method of expression based on the oxidation rate ratio (ORR) was calculated according to the method of Marinova *et al., (*1994) using the equation:

$ORR = R_{sample} / R_{control}$

Where: $R_{control}$ and R_{sample} are the same in the previous equation.

In the fourth method, the antioxidant activity coefficient (AAC) was calculated as described by Mallet *et al., (*1994).

$(AAC) = (Abs_{S\,120} - Abs_{C\,120}) / Abs_{C\,0} - Abs_{C\,120}) \times 1000$

Where: $Abs_{S\,120}$ was the absorbance of the antioxidant mixture at time 120 min, $Abs_{C\,120}$ was the absorbance of the control at time 120 min, $Abs_{C\,0}$ was the absorbance of the control at zero time.

Total phenolics

Total phenolics in plant parts extracts were determined using Folin-Ciocalteu reagent (Singleton and Rossi, 1965). Two hundred milligrams of sample was extracted for 2 h with 2 ml of 80% MeOH containing 1% hydrochloric acid at room temperature on an orbital shaker set at 200 rpm. The mixture was centrifuged at 1000g for 15 min and the supernatant decanted into 4 ml vials. The pellets were combined and used for total phenolics assay. One hundred microliters of extract was mixed with 0.75 ml of Folin-Ciocalteu reagent (previously diluted 10-fold with distilled water) and allowed to stand at 22 ^{0}C for 5 min; 0.75 ml of sodium bicarbonate (60 g/l) solution was added to the mixture after 90 min at 22 ^{0}C, absorbance was measured at 725 nm. Results are expressed as ferulic and equivalents.

Statistical analysis

The correlation studies were performed by using MINITAB-12 computer program (Minitab Inc., State College, PA).

3. Results and Discussion

Antioxidant activities and total phenolics in selected plant parts

The antioxidant activities and total phenolics of 31 plant products, including vegetables, fruits, herbs and spices are shown in Table (1). The decrease in absorbance of β-carotene in the presence of different methanolic plant extracts (and well-known antioxidants used as standards) with the oxidation of β-carotene and linoleic acid is shown in Figures (1-3). From such data it could be noticed that the dehydrated vegetables extracts showed considerable differences in antioxidant activity (AA = 31.56 – 76.00 %) when it was calculated by the four different methods used in this study. Potato extracts showed strong activity because of its high phenolic content (391 mg/100 g). Comparative studies indicated that antioxidant activity of the selected vegetables is smaller than the standard synthetic antioxidant, BHT and α-tocopherol which recorded 86.15-98.77%. The present data are going well with the study of Ghoneem, (2005) who determined the antioxidant activities and total phenolics of six vegetable products , including red pepper, potatoes, horseradish root, tomato, cabbage and carrot and found that the total phenolic content of the selected vegetable extracts varied from 21.13 to 301.19 mg.100 g^{-1} of dry product. Also, Kumar *et al*., (1991) and

Onyeneho and Hettiarachchy (1993) reported that big differentiations have been recorded amongst different vegetables as well as different species of the same genus regarding antioxidant activity and phenolics content.

For selected tested fruits, the anthocyanin-rich samples, strawberry, red apple, red and currant and pomegranate generally showed very strong activities (Figure 2) as well as high phenolic contents (Table 1). The antioxidant activity of these samples was ranged 89.15 to 92.63% and the total phenolics were 1155 to 3701 mg GAE.100g^{-1}. Comparative studies indicated that antioxidant activity of the selected fruits is higher than the standard synthetic antioxidant, BHT (50 mg.l^{-1}) and close to the BHT (200 mg.l^{-1} as well as α-tocopherol (50 mg.l^{-1}). The results confirm that anthocyanins possess strong antioxidant activities (Wang *et al.,* 1997 and El-Mokadem, 2010). Anthocyanins are probably the largest group of phenolic compounds in the human diet and their strong antioxidant activities suggest their importance in maintaining health**.** The rest of tested fruits, the anthocyanin-non rich samples including green apple, green currant and guava also showed high antioxidant activity ranged 81.15 to 84.65. All of these samples also contained above average levels of phenolic compounds, 695 to 989 mg GAE.100g^{-1}. This is confirming that all of the tested fruits in the present study could be constituted a good source of antioxidant compounds which the phenolics represent the major part.

Regarding the medical plant parts, herbs and spices, some of the tested samples showed very strong antioxidant activities including thyme and hop (AA = 91.98 and 90.54, respectively) following by plants, chamomile flowers, coriander fruits, marjoram and rosemary (AA= 87.54, 86.29, 86.32 and 80.35%, respectively) (Table 1 and Figure 3). All of these samples also contained above average levels of phenolic compounds ranged 985 to 2019 mg GAE.100g-1. Comparative studies indicated that antioxidant activity of these samples except rosemary

is higher than the standard synthetic antioxidant, BHT (50 mg.l $^{-1}$). Other plants were generally less potent, with AA value from 58.41% for dill seeds to 60.89% for flax seeds.

Table 1. Antioxidant activity and total phenolics of methanolic extracts of selected plant products

Sample name	Antioxidant value[a] AOX (A/h)	Antioxidant activity[b] AA (%)	Oxidation rate ratio[c] (ORR)	Antioxidant activity coefficient[d] (AAC)	Total phenolics (mg GAE/100 g)
Vegetables:					
Green beans	0.315[e]	40.54	0.556	243.23	159
Pea	0.334	37.13	0.590	183.95	162
Beans	0.300	43.12	0.530	288.09	191
Horseradish root	0.256	51.02	0.452	425.42	293
Carrot, flesh	0.366	31.56	0.646	87.12	89
Tomato	0.170	66.24	0.300	690.02	272
Fresh pepper	0.138	75.62	0.243	486.93	264
Eggplant	0.164	71.00	0.289	406.62	324
Cabbage	0.349	38.17	0.617	137.42	153
Potatoes	0.136	76.00	0.239	493.54	391
Fruits:					
Strawberry	0.048	91.51	0.084	1064.71	1299
Red apple	0.061	89.15	0.108	1023.68	1155
Green apple	0.087	84.65	0.153	945.45	989
Red currant	0.052	90.86	0.091	1053.41	1421
Black currant	0.042	92.63	0.073	1084.18	2317
Green currant	0.107	81.15	0.188	884.61	873
Guava	0.088	84.34	0.156	940.06	695
Pomegranate	0.053	90.65	0.093	748.22	3701
Herbs and spices:					
Chamomile, flower	0.047	87.54	0.083	1066.46	1312
Caraway, seed	0.115	75.63	0.202	859.41	788
Hop	0.030	90.54	0.054	1118.62	2109
Thyme	0.022	91.98	0.039	1143.65	1522
Flax, seed	0.198	60.89	0.350	603.16	492
Coriander	0.077	86.29	0.137	672.43	1028.64
Dill seed	0.235	58.41	0.415	187.74	291.30
Cumin seed	0.201	64.38	0.355	291.53	213.65
Carnation	0.171	69.70	0.302	384.02	421.82
Mint leaves	0.160	71.60	0.283	417.05	698.45
Rosemary	0.111	80.35	0.196	569.16	985.32
Basil	0.122	78.48	0.215	536.65	268.34
Marjoram	0.077	86.32	0.136	672.95	1024
Control	0.565	0.00	0.998	0.00	
BHT, 50 mg/L	0.078	86.15	0.138	971.53	
BHT, 200 mg/L	0.017	96.99	0.030	1159.98	
α-tocopherol, 50 mg/L	0.007	98.77	0.012	1190.92	

[a] Antioxidant value (AOX, A/h) = The absolute value of slope (Abs was plotted against time).

[b] Antioxidant activity (AA, %) = (R control - R sample) / R control x 100 where: R control and R sample were the bleaching rates of beta-carotene in reactant mixture without antioxidant and with plant extract, respectively

[c] Oxidation rate ratio (ORR) = R sample / R control.

[d] Antioxidant activity coefficient (AAC) = (Abs S120- Abs C120) / Abs C0- Abs C120) x 1000 where: .Abs S 120 was the absorbance of the antioxidant mixture at time 120 min, Abs C 120 was the absorbance of the control at time 120 min, Abs C_0 was the absorbance of the control at zero time.

[e] Each value represents mean of three replicates.

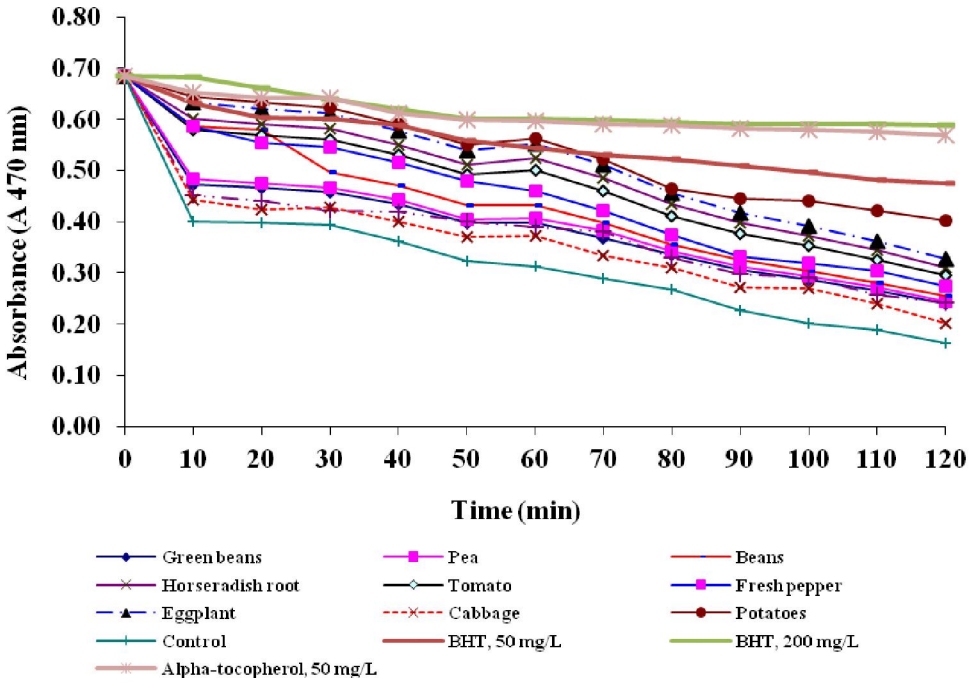

Figure 1. Antioxidant activity of methanolic extracts of vegetable parts assayed by the β-carotene bleaching methods (BHT and α-tocopherol were used as references).

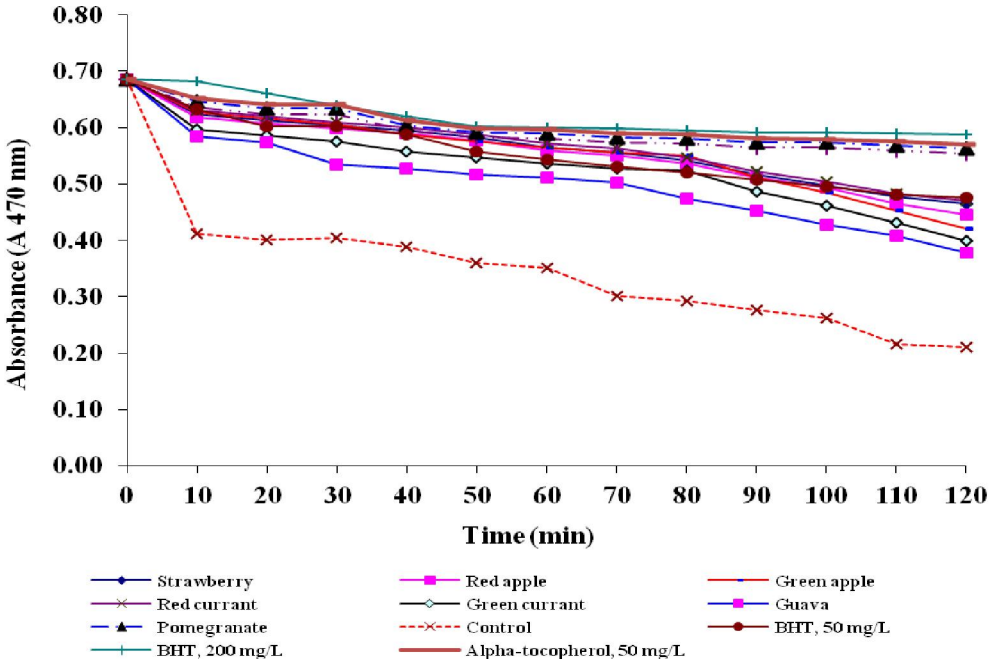

Figure 2. Antioxidant activity of methanolic extracts of fruits parts assayed by the β-carotene bleaching methods (BHT and α-tocopherol were used as references)

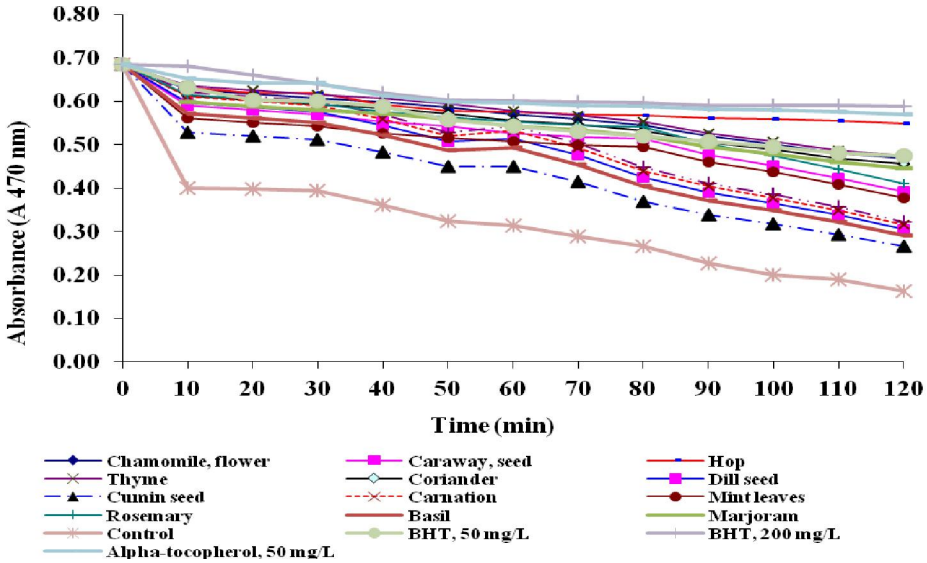

Figure 3. Antioxidant activity of methanolic extracts of herbs and spices plant parts assayed by the β-carotene bleaching methods (BHT and α-tocopherol were used as references)

Relationship between phenolics content and antioxidant activity

The total phenolic content of the plant materials investigated in this study varied from 89 to 2317 mgGAE.100 g^{-1} (see Table 1). The relationship between total phenolic content and antioxidant activity of plant material is shown in Table (2) and Figure (4). Statistically significant strong relationships were observed between total phenolics and antioxidant activity of vegetable materials (R^2= 0.801; $p < 0.001$). In similar study, Ghoneem, (2005) found that when six vegetables products , including red pepper, potatoes, horseradish root, tomato, cabbage and carrot included in the statistical analysis, there was a positive and highly significant ($p< 0.001$) relationship between total phenolics and antioxidant activity. This indicates that phenolics can play a major role in the antioxidant activity of selected spices. Also, Elhassaneen and Sanad (2009) reported that methanolic extract of Egyptian red onion showed relatively higher antioxidant activity than the white one which correlated well with the total phenolics of each extract. However, the relationship between phenolics and antioxidant activity for the anthocyanin-rich materials (R^2 = 0.633) and for the medical plants (herbas and spices, R^2 = 0.687) was low significant ($p < 0.05$). The lack of a significant correlation between total phenolics and AA of these samples reflects the exceptionally high antioxidant activity of fruits and medical plants despite their very different contents of total phenolics (Table 1). The composition of the phenolics in vegetables samples, primarily phenolics acids (ElSadany, 2001), is very different from the phenolics of almost colored fruits, which are primarily anthocyanins (http://lpi.oregonstate. edu/ss01/anthocyanin.html). The lack of correlation between the phenolics and AA of some plant samples may be due of the carotenoid-rich carrot, vitamin C-rich cabbage and guava (Ajit and Dave, 2013 and http://www. wafarmtoschool. org/ToolKit/15/Cabbage/Facts). When the results for fruits and medical plants were added to the regression analysis of vegetable samples, the relationship between the phenolic contents and antioxidant activities was relatively weak significant ($p<0.05$). This indicates that factors other than total phenolics can play a principal role in the antioxidant activity of plant materials such as fruits and medical plants. In similar study, Velioglu *et al*., (1998) reported that the correlation coefficient between total phenolics and antioxidative activities of 28 plant products, including sunflower seeds, flaxseeds, wheat germ, buckwheat, several fruits, vegetables, and medicinal plants was statistically significant. Also, Ibrahim (2008) reported that methanolic extract of spearmint showed strong activity probably due to its high phenolic content (3034 mg.100 g^{-1}). Additionally, El-Safty (2008) found that methanolic extract (MeOH) of marjoram showed strong activity probably due to its high phenolic content (798 mg.100 g^{-1}) while aqueous extract showed relatively low antioxidant activity (AA= 48.56 %) and low concentration of total phenolics (476 mg.100 g^{-1}).

In general, the data of this study with the others proved the importance of using selected plant parts extract as natural antioxidants in nutritional therapy. For examples, Majid *et al*., (1991) found feeding of phenolic acid (ellagic) significantly increased the levels of reduced glutathione and glutathione reductase in liver and lungs of male and female mice as well as increase in inhibition of NADPH-dependent lipid peroxidation. In the case of heart disease, inhibition of

LDL cholesterol oxidation helps in prevention of foam cells formation and lipid streaks development. Oxidized LDL cholesterol is more atherogenic than native LDL and is also known to affect tissue factor expression. Several recent studies provide compelling evidence that dietary intake of antioxidants including phenolic compounds can lower the production of atherogenic oxidized LDL cholesterol and thus may decrease the risk of cardiovascular disease (Steinberg *et al.,* 1989; Niki, 1991 and Laranjinha *et al*., 1994).

Table 2. Relationship between antioxidant activities (AA) and total phenolic contents of selected plant products

	Relationship with total phenolics	R^2
All plant products (n=31)	Total phenolics (mg GAE.100 g^{-1}) = 29.064 (Antioxidant activity, %) + 1262.2	0.4732 *
Vegetable products (n=10)	Total phenolics (mg GAE.100 g^{-1}) = 4.8052 (Antioxidant activity, %) + 25.069	0.801**
Fruits products (n=8)	Total phenolics (mg GAE.100 g^{-1}) = 170.7 (Antioxidant activity, %) + 13439	0.633*
Herbs and spices (n=13)	Total phenolics (mg GAE.100 g^{-1}) = 40.579 (Antioxidant activity, %) + 2270	0.6873*

* P‹0.05, ** P‹0.001

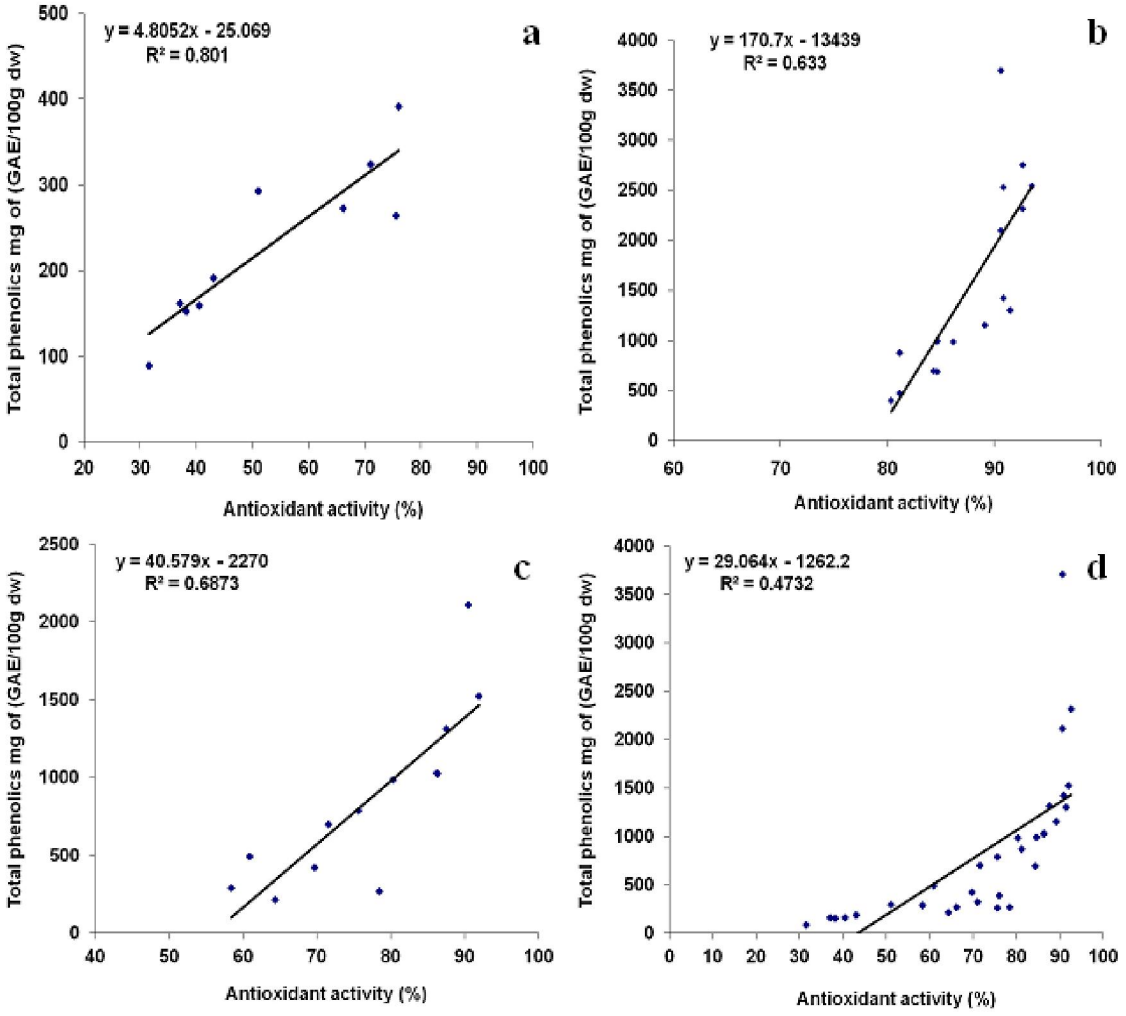

Figure 4. Relationship between total phenolic content and antioxidant activity (AA) of selected plant products. a) vegetable products, b) fruit products, c) herbs and spices products, and d) all plant products

In terms of neutralizing free radicals, phenolics are well known to protect cells and their components against cancer development. Simple phenolic acids and tocopherols have been shown to be potent inhibitors of formation of carcinogens such as *N*-nitroso compounds (Kuenzig *et al.,* 1984). Meanwhile, inhibition of benzo(*a*)pyrene induced neoplasia in the forestomach of mice fed various plant phenolics has been reported by Wattenberg *et al.,* (1980). Chromosomal aberrations induced by polycyclic aromatic hydrocarbons were inhibited by caffeic acid (Raj *et al.,* 1983), while chlorogenic acid blocked chemically induced carcinogens in the large intestine of hamsters (Mori *et al.,* 1986). Chang *et al.,* (1985) have demonstrated antitumor-promoting activity of ellagic acid and quercetin. Flavonoids, including catechins, were also found to reduce hyperlipedemia in animals (Choi *et al.,* 1991).

In conclusion, tested vegetables, fruits, herbs and spices showed significantly higher antioxidant activities and contained higher phenolics content. Factors beside phenolics can play a principal role in the antioxidant activity of these plant materials. Further work is needed in the future to elucidate the identity of the other compounds responsible for the antioxidant activity. Nevertheless, this is the first study from Egypt reporting total phenolics content and AA of commonly consumed plant parts. This study also demonstrates that the total phenolics in these plant parts could be a major contributor to their AA. The present data will be useful to consumers to plan antioxidant rich diets and to the nutritionists in estimating the daily intakes of phenolic antioxidants and their impact in health and disease.

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