Total antioxidant capacity of commonly used fruits, vegetables, herbs and spices of Pakistan

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Abstract: The current study was aimed at investigating the total antioxidant activity (TAC) of various fruits, vegetables, herbs and spices habitat in Pakistan. The ferric reducing ability of plasma (FRAP) assay was used to measure the TAC of various extracts (aqueous, ethanolic and aqueous-ethanolic). Following is the potency order for fruits (guava >strawberry >Pomegranate >apple >kinnow >melon >lemon >banana), vegetables (spinach >Cabbage (Purple) >Jalapeno >Radish >Brinjal >Bell Pepper >Lettuce >Carrot >Cabbage (White) >Onion >Potato >Tomato >Cucumber) and herbs/spices (clove >Rosemary >Thyme >Oregano >Cinnamon >Cumin >Kalonji >Paprika >Neem (Flower) >Fennel >Black Cardamom >Turmeric >Coriander >Ginger >Garlic). In conclusion, the guava, spinach and clove provide the best natural dietary option for treatment / prevention of oxidative stress and thus could alleviate several associated ailments.

Keywords: Fruits, total antioxidant activity, FRAP assay.

INTRODUCTION

Reactive oxygen species (ROS) are generated within the body through various sources such as mitochondria, prostanoids metabolism, catecholamines, xanthine oxidase and NADPH (nicotinamide adenine dinucleotide phosphate-oxidase) oxidase (Sen *et al*., 2000). In order to detoxify these ROS, certain antioxidants are also present in the human body such as glutathione, superoxide dismutase, melatonin and lipoic acid (Halliwell, 1996). Oxidative stress is an imbalance between the production and detoxification of ROS by antioxidants, which underlies pathogenesis of various ailments such as hypertension, stroke, atherosclerosis, diabetes, Alzheimer's, Parkinson, glaucoma, pre-eclampsia and cancer (Valko *et al*., 2007). In such cases, antioxidant supplementation is often required in order to overcome the situation. However, nutrition can also play a vital role in managing the oxidative stress. In this regard, the commonly used fruits, vegetable, herbs and spices have been reported to possess antioxidant potential. The study compared the antioxidant capacity of commonly used fruits and found strawberry to be the most potent antioxidant (Wang *et al*., 1996). Another comparative study reported guava to be most effective antioxidant (Lim *et al*., 2007). The aforementioned literature is also suggestive of differences in antioxidant abilities of fruits of different origin. Similarly, the vegetables are also reported to be rich source of antioxidants (phenolic contents). A study report the following sequence of antioxidant potential of vegetables: shallots >spinach >swamp >cabbage >cabbage >kale (Ismail *et al*., 2004). Furthermore, the herbs are also rich source of phenolics possessing antioxidant ability. A study compared the antioxidant action of 32 herbs and found *E. hirsutum* and *H. perforatum* to be most potent (Wojdyło *et al*., 2007). Similarly, the protective role of spices against degenerative disorders has long been debated and primarily attributed to their antioxidant potential. These spices are commonly used in South East Asian countries and assumed to underlie the low prevalence of various diseases in this part of the world (Kannappan *et al*., 2011). For instance, the curcumin was reported to offer protection against amyloid beta induced toxicity (Ono *et al*., 2004) while cinnamon was found to be effective against tau pathology (Peterson *et al*., 2009). Furthermore, literature revealed a comparative study on the spices, which exhibited that clove possesses high antioxidant capacity (Shobana and Naidu, 2000). The aforementioned literature revealed that commonly used fruits, vegetables, herbs and spices can offer economical protection against oxidative stress. However, no comprehensive report was found on the literature regarding aforementioned substances from Pakistan, which incited us to undertook present study aimed at exploring the total antioxidant capacity of various commonly used fruits, vegetables, herbs and spices using ferric reducing antioxidant power (FRAP) assay.

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MATERIALS AND METHODS

Chemicals

The chemicals used were: Ferric chloride, glacial acetic acid, L-ascorbic acid and sodium acetate (BDH

Table 1: Effect of various fruit extracts on total antioxidant activity using FRAP assay

S. No.	Description	FRAP Value (μM/g)			
		Water	Ethanol	Water:EtOH	Average ± SD
1	Apple	272	416	202	297 ± 109
2	Banana	103	72	86	87 ± 16
3	Guava	1292	768	1124	1061 ± 268
4	Kinnow	187	343	156	229 ± 58
5	Lemon	103	106	118	109 ± 8
6	Melon	214	56	85	118 ± 84
7	Pomegranate	103	569	584	419 ± 273
8	Strawberry	735	1,131	1134	1000 ± 230

Table 2: Effect of various vegetable extracts on total antioxidant activity using FRAP assay

S. No.	Description	FRAP Value (μM/g)			
		Water	Ethanol	Water:EtOH	Average ± SD
1	Cucumber	18	12	18	16 ± 3
2	Tomato	31	39	40	37 ± 5
3	Potato	40	42	66	49 ± 14
4	Onion	71	139	85	98 ± 36
5	Cabbage (White)	85	147	90	107 ± 34
6	Carrot	98	148	147	131 ± 29
7	Lettuce	103	148	150	134 ± 27
8	Bell Pepper	107	283	196	195 ± 88
9	Brinjal	138	286	223	216 ± 74
10	Radish	147	302	243	231 ± 78
11	Jalapeno	245	516	244	335 ± 157
12	Cabbage (Purple)	312	617	264	398 ± 191
13	Spinach	798	630	822	750 ± 105

chemicals, USA). Hydrochloric acid and ferrous sulphate (RDH chemicals, Gernany). DL-α-tocopherol (Merck, Germany). TPTZ and ethanol (Fluka, USA).

Preparation of sample

The fruits (Apple (Slice with peel), Banana (Edible part slice), Guava (Slice with peel), Kinnow (pulp), Lemon (pulp), Melon (Edible part without peel), Pomegranate (Seeds with pulp) and strawberry (Slice)); vegetables (Bell Pepper (Slice without seeds), Brinjal (Slice with peel), Cabbage (White, Slice of leaf), Cabbage (Purple, Slice of leaf), Carrot (Slice), Cucumber (Slice with peel), Jalapeno (Slice without seeds), Lettuce (Part of leaf), Onion (Slice, outer hard peel removed), Potato (Slice), Radish (Slice), Spinac (Part of leaf) and Tomato (Slice without seeds)) and herbs / spices (Black Cardamom (Seeds and peel), Cinnamon (Bark), Clove (Whole), Coriander (Leaves), Cumin (Seeds), Fennel (Seeds), Garlic (Rhizome without peel), Ginger (Slice without peel), Kalonji (Seeds), Neem (Flower), Oregano (Powder), Paprika (Powder), Rosemary (Dried leaves), Thyme (Powder of Leaves) and Turmeric (Fresh Rhizome slice without peel)) were purchase from the local market. As described above, various parts of the fruits, vegetables and spices were weighed (1.25g) in triplicate and minced. Cold extraction (aqueous, ethanolic and aqueous ethanolic) was performed by mixing the minced substances in 25 ml of water, ethanol or aqueous/ ethanol (60:40) and shaken. After 30 minutes, centrifugation was performed followed by filtration of extract.

Ferric reducing ability of plasma (FRAP) Assay

The working FRAP reagent was prepared by mixing acetate buffer (300mM, pH= 3.6), TPTZ (2, 4, 6-tripyridyl-s-triazine, 10mM) in HCI and 20mM $FeCl_3.6H_20$ (20mM) in the ratio of 10: 1: 1 (Benzie and Strain, 1996). The reagent was mixed with 10 μl of test sample and absorbance (593 nm) was taken after 4 minutes using Hitachi 2800, Japan. The TAC activity was calculated from ferrous sulfate standard curve.

RESULTS

Effect of aqueous, ethanolic and aqueous ethanolic fruits extracts on total antioxidant capacity

Among various aqueous extracts, Guava exhibited most potent antioxidant activity with average FRAP value of 1292μM/g (table 1). The Banana, Lemon and Pomegranate showed lowest values of 103 μM/g. Among various ethanolic fruit extracts, the strawberry showed most potent antioxidant activity with FRAP values of 1131μM/g. The melon manifested lowest values of 56

Table 3: Effect of various herbs and spices on total antioxidant activity using FRAP assay

S. No.	Description	FRAP Value (μM/g)			
		Water	Ethanol	Water:EtOH	Average ± SD
1	Black Cardamom	1,359	877	1,623	1286 ± 378
2	Cinnamon	6,636	2,962	2,919	4172 ± 2133
3	Clove	633,315	291,922	86,240	337159 ± 276329
4	Coriander	468	813	848	710 ± 210
5	Cumin	2,224	1,954	2,626	2268 ± 338
6	Fennel	1,613	908	1,669	1397 ± 424
7	Garlic	80	27	115	74 ± 44
8	Ginger	428	630	699	586 ± 141
9	Kalonji	3,802	1,190	1,210	2067 ± 1502
10	Neem (Flower)	1,194	950	2,422	1522 ± 789
11	Oregano	4,350	1,690	10,505	5515 ± 4522
12	Paprika	1,243	1,801	1,724	1589 ± 302
13	Rosemary	41,342	56,212	60,390	52648 ± 10012
14	Thyme	36,653	24,429	70,696	43926 ± 23976
15	Turmeric	446	835	1,913	1065 ± 760

μM/g (table 2). Among various aqueous ethanolic fruits extracts, the strawberry exhibited most potent antioxidant activity with FRAP values of 1134μM/g (table 2). The melon showed lowest values of 103μM/g (table 1). The potency order for average total antioxidant activity (TAC) exhibited by various fruit extracts is guava >strawberry > Pomegranate >apple >kinnow >melon >lemon >banana. Among all extracts, the aqueous guava extract exhibited the most potent antioxidant activity (1292μM/g) while the melon ethanolic extract showed lowest TAC (56μM/g).

Effect of aqueous, ethanolic and aqueous ethanolic vegetables extracts on total antioxidant capacity

Among various aqueous extract the Spinach exhibited most potent antioxidant activity with average FRAP value of 798μM/g (table 2). The cucumber showed lowest values of 18μM/g. Among various ethanolic extracts, again the spinach showed most potent antioxidant activity with FRAP values of 630μM/g. The cucumber manifested lowest values of 12μM/g. Among various aqueous ethanolic extracts, the spinach exhibited most potent antioxidant activity with FRAP values of 822μM/g. The melon showed lowest values of 18μM/g. The potency order for average total antioxidant activity (TAC) exhibited by various vegetable extracts is spinach >Cabbage (Purple) >Jalapeno >Radish >Brinjal >Bell Pepper >Lettuce >Carrot >Cabbage (White) >Onion >Potato >Tomato >Cucumber. Among all extracts, the aqueous: ethanol spinach extract exhibited the most potent antioxidant activity (822μM/g) while the cucumber ethanolic extract showed lowest TAC (12μM/g).

Effect of aqueous, ethanolic and aqueous ethanolic herbs and spices on total antioxidant capacity

Among various aqueous extract the clove exhibited most potent antioxidant activity with average FRAP value of 633,315μM/g (table 3). The garlic showed lowest values of 80μM/g. Among various ethanolic extracts, again the clove showed most potent antioxidant activity with FRAP values of 291,922μM/g. The cucumber manifested lowest values of 27μM/g. Among various aqueous ethanolic extracts, the clove exhibited most potent antioxidant activity with FRAP values of 86,240μM/g. The cucumber showed lowest values of 115μM/g. The potency order for average total antioxidant activity (TAC) exhibited by various herbs / spices extracts is clove > Rosemary >Thyme >Oregano >Cinnamon >Cumin > Kalonji >Paprika >Neem (Flower) >Fennel >Black Cardamom >Turmeric >Coriander >Ginger >Garlic Among all extracts, the aqueous clove extract exhibited the most potent antioxidant activity (633,315μM/g) while the garlic ethanolic extract showed lowest TAC (27 μM/g).

DISCUSSION

The current study was aimed at investigating the total antioxidant activity (FRAP assay) of various commonly used fruit, vegetables, herbs and spices extract (aqueous, ethanol and aqueous-ethanolic) grown in Pakistan. Oxidative stress i.e. imbalance between oxidant and antioxidant in the body underlies pathogenesis of several ailments (Halliwell, 1996). Naturally occurring fruits provide best supplement to normalize the balance in population living in rural areas with limited access to allopathic supplementation. Our data showed that among various fruits, the guava possesses highest average antioxidant activity (1061±154μM/g, table 1). In case of vegetables, spinach appeared to be the most potent antioxidant (750±105μM/g, table 2). In herbs and spices group, the clove displayed most potent antioxidant action (337159±276329μM/g, table 3). Among all the thirty-six samples analyzed for TAC, clove shown the highest value (337159μM/g) and second to it was rosemary.

Our data revealed that the type of solvent for extraction has substantially contributed to the TAC of fruits/ vegetable/herbs and spices. In certain instances, aqueous extract exhibited highest TAC while in other, the major antioxidant moieties were extracted in ethanolic or aqueous ethanolic extracts. This is suggestive of the fact that there is a diversity of constituents, apart from phenolics (Rice-Evans *et al*., 1997), underlying the antioxidant capacity. Literature also revealed that there is significant effect of type of solvent on the antioxidant action of the substances (Spigno *et al*., 2007, Sousa *et al*., 2008). However, further work is required to measure the phenolic contents of the extracts in order to ensure whether the differences observed in TAC is due to diversity of extracted contents or varied concentrations of the phenolics.

Hence, aforementioned dietary substances provide the best natural dietary intervention for treatment / prevention of oxidative stress. These outcomes are especially helpful in remote areas where sufficient quantity of antioxidant supplements cannot be supplied or the acceptance for the natural remedies is high. Additionally, people working in hazardous environment can adopt prophylactic approach by taking these fruits, vegetables, herbs and spices (depending upon the availability) to avoid oxidative stress.

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