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Antioxidant Activity of Fruits Available in Aizawl Market of Mizoram, India

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Abstract: The antioxidant activity of the fruits available in the Aizawl market of Mizoram, India was estimated. A total of 20 fruits were evaluated for their antioxidant activity based on the ability of the fruit extracts to scavenge 1,1-diphenyl-2-picrlyhydrozyl free radicals, to reduce ferric ions determined by ferric reducing antioxidant potential assay and total phenolic content determination. The antioxidant activity was expressed as mg Trolox equivalents for 1,1-diphenyl-2-picrlyhydrozyl free radicals scavenging assay and FRAP ass ferric reducing antioxidant potential assay while the total phenolic content was expressed as mg Gallic acid equivalent per 100 gm of edible portion of the fruits. The antioxidant activity observed by the three methods does not vary markedly though the values obtained by 1,1-diphenyl-2-picrlyhydrozyl free radicals scavenging assay was highest among the three methods and least in total phenolic content determination. Among the fruits used in the present investigation, the highest antioxidant activity was observed in Amla and least in coconut water.

Key words: DPPH · FRAP · Total phenolic content · Antioxidant capacity · Fruits

INTRODUCTION

The biological antioxidants are compounds that protects biological systems against the potentially harmful effects of processes or reactions that causes excessive oxidation. Hydrophilic compounds, such as vitamin C, thiols and flavonoids, as well as lipophilic compounds, such as Vitamin E, Vitamin A, Carotenoids and ubiquinols are the best known natural antioxidants. Many of these compounds are of special interest due to their ability to reduce the hazard caused by reactive oxygen and nitrogen species (ROs and RNs). Some are free radicals and have been associated with lowered risks of cardiovascular diseases and other illnesses related to oxidative stress. Practically the natural antioxidants are obtained through ingestion of plant products such as fruits, vegetables, nuts, flours, vegetable oil, drinks and infusions, taken fresh or as processed foodstuffs [1].

The consumption of fruits and vegetables has been found to be associated with a lowered incidence of degenerative diseases including cancer, heart disease, inflammation, arthritis, immune system decline, brain dysfunction and cataracts [2-8]. These protective effects are considered, in large part, to be related to the various antioxidants contained in them. Free radicals cause oxidative damage to lipids, proteins and nucleic acids. Antioxidant which can inhibit or delay the oxidation of an oxidisable substrate in a chain reaction would therefore seem to be very important in the prevention of these diseases. [3, 9-14]. However, knowledge of the potential antioxidant compounds present in a food does not necessarily indicate its antioxidant capacity [15]. Epidemiological studies that analyze the health implications of dietary components rely on the intake estimates in sample populations found in databases that list the components content in commonly consumed foods. Therefore, the availability of appropriate and complete food composition data is crucial. Due to the chemical diversity of antioxidant compounds present in foods, complete databases on food antioxidant content are not yet available. Further, levels of single antioxidants in food do not necessarily reflect their total antioxidant capacity; this also depends on the synergic and redox interactions among the different molecules present in the food. Finally, geographical differences in food composition data should be considered when applying compositional databases to regional surveys [16].

The objective of this study was to measure the antioxidant activity of the fruits available in Aizawl market of Mizoram, India by 1.1-diphenyl -2-picrythydrozyl

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(DPPH) radical – scavenging, ferric reducing antioxidant potential (FRAP) and total phenolic content assay. Results from this preliminary study will provide information of the antioxidant capacity of the fruits available in the market and allow the identification of the fruits with higher antioxidant activity for further investigation.

MATERIALS AND METHODS

Materials

Fruits: Twenty different types of fruits were purchased from Aizawl market of Mizoram, India. The 20 fruits includes Amla (*Emblica officinalis*), Guava (*Psidium guajava* L), Pomegranate (*Punica granatum* L), Sweet lime (*Citrus limettioides*), Passion fruit (*Passiflora edulissims*), Tamarind (*Tamarindus india* L), Star fruit (*Averrhoa carambola* L), Orange (*Citrus sinensis* (L.), Papaya (*Carica papaya* L), Mango (*Mangifera indica* L), Butter fruit (*Persea americana*), Pummelo (*Citrus grandis* (L.) Osbeck), Lemon (*Citrus limon* (L) Burm f.), Pears (*Pyrus communis*), Pineapple (*Anans comosus* (L.), Apple (*Malus domestica)*, Grapes (*Vitis ninifera* L), Banana (*Musa paradisiaca*l), Watermelon (*citrullis lanatus*), Coconut water (*Cocos nucifera* L.)

Chemicals and Reagents: 1,1-Diphenyl-2-picrylhydrazyl (DPPH), 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) and 6-hydroxy-2,5,7,8–tetramethyl chromane-2-carboxylic acid (Trolox) were purchased from Sigma Chemicals Co. (St. Louis, USA); Methanol, Ethanol, Sodium acetate trihydrate, ferric chloride hexahydrate ($FeCl_3$. $6H_2O$), Folin-Ciocalteu Phenolic reagent, Sodium carbonate were from Merck (Darmstall, Germany). All the chemicals used were of analytical grade.

Sample Preparation: The edible portion of the fruits (4gm) was homogenized with 50% aqueous ethanol (8ml). The homogenate was allowed to stand at room temperature for 30 min with occasional agitation. The extract was centrifuged at 2,000xg for 15 min and the supernatant collected was used for DPPH free radical scavenging assay, Ferric reducing antioxidant potential assay (FRAP) and Total phenolic content determination.

Antioxidant Activity Determination

DPPH Free Radical Scavenging Assay: The DPPH free radical scavenging activity of fruits was determined using spectroscan 2600 UV/Vis Spectrophotometer (Chemito) according to the method described by Leong and Shui (15). A 0.1 mM Solution of DPPH was prepared in methanol. The initial absorbance of the DPPH was measured at 515 nm. An aliquot (40µl) of the fruit extracts (with appropriate dilution) was added to 3 ml of DPPH solution. The decrease in absorbance at 515nm was measured at different time intervals until the absorbance remains constant. The antioxidant activity based on the DPPH free radical scavenging ability of the fruit extracts was expressed as mg Trolox equivalents per 100 gm of edible portion of the fruits.

Ferric Reducing Antioxidant Potential (FRAP) Assay: The ability to reduce ferric ions was measured using a modified version of the method described by Benzic and Strain (17). An aliquot (50µl) of the fruit extracts (with appropriate dilution) was added to 3 ml of FRAP regaents (10 parts of 300 mM solution acetate buffer at pH 3.6, 1 part of TPTZ solution and 1 part of 20 mM $FeCl_3$. $6H_20$ solution) and reaction mixture was incubated at 37°C for 30 min. The increase in absorbance was measured at 593 nm using spectroscan 2600 UV/Vis Spectrophotometer (Chemito). The antioxidant activity of the fruit extracts based on the ability to reduce ferric ions was expressed as mg Trolox equivalents per 100 gm of edible portion of the fruits.

Total Phenolic Content Determination: The total phenolic content of the fruit extracts were estimated by the method described by Singleton and Rossi [18] downscaled to 2 ml final volume. An aliquot (100µl) of the appropriately diluted fruit extracts was added to 1000µl of 1:10 Folin-Ciocalteau's reagent and incubated at room temperature for 5 min followed by addition of 900 µl saturated (7.5%) sodium carbonate solution. After incubation for 1 hr at room temperature, the absorbance at 640 nm was measured using spectroscan 2600 UV/Vis Spectrophotometer (Chemito). The total phenolic content of the fruits were expressed as Gallic acid equivalents (GAE) mg/100 gm of edible portion of the fruits.

RESULTS

The antioxidant capacity of the fruits was evaluated by three different methods using 1,1-diphenyl-2-picrylhydrozyl (DPPH) radical–scavenging, ferric reducing antioxidant potential (FRAP) and total phenolic content assay. The fruit extracts had different antioxidant capacities in relation to the method of estimation. The antioxidant capacity of the different fruit extracts and the ranking order for each assay is shown in Table 1. Based on the values the antioxidant activity observed, the fruits are classified into four categories as extremely high, high, medium and low. On the basis of the wet weight of the fruits, alma is classified as extremely high, guava,

Table 1: Antioxidant activity of the fruits estimated by DPPH radical–scavenging assay, ferric reducing antioxidant potential (FRAP) assay and total phenolic content determination

		Antioxidant activity			
Sl. No.	Name of the Fruits	DPPH method(mg of Trolox equivalent/ 100 gm of fruit)[a]	FRAP method(mg of Trolox equivalent/ 100 gm of fruit)[b]	Total Phenolic contents (mg of Gallic Acid equivalent/ 100 gm of fruit)[c]	
01	Amla (*Emblica officinalis*)	2855.33±25.37	1577±4.56	1285.63±0.71	Extremely high
02	Guava (*Psidium guajava* L)	176.06±1.92	139.29±0.54	115.35±0.92	High
03	Promegranate (*Punica granatum* L)	156.37±0.48	131.63±0.39	94.94±0.06	
04	Sweet lime (*Citrus limettioides*)	105.40±0.99	100.95±0.16	72.82±0.61	
05	Passion fruit (*Passiflora edulissims*)	95.17±1.76	90.70±0.55	66.73±1.35	Medium
06	Tamarind (*Tamarindus india* L)	85.32±0.12	88.51±0.51	66.71±0.09	
07	Star fruit (*Averrhoa carambola* L)	81.03±1.97	78.770±0.35	54.45±0.43	
08	Orange (*Citrus sinensis* (L.)	61.39±5.50	64.32±0.39	52.58±0.44	
09	Papaya (*Carica papaya* L)	66.69±0.36	58.17±0.53	28.91±0.67	
10	Mango (*Mangifera indica* L)	65.24±0.61	57.33±1.01	28.15±0.17	
11	Butter fruit (*Persea americana*)	64.20±0.96	39.32±1.09	33.45±0.46	
12	Pummelo (*Citrus grandis* (L.) Osbeck)	57.81±6.50	47.58±0.48	71.26±2.07	
13	Lemon (*Citrus limon* (L) Burm f.)	60.02±0.31	42.31±0.52	25.23±0.28	
14	Pears (*Pyrus communis*)	58.59±0.69	41.02±0.03	22.96±0.30	
15	Pineapple (*Anans comosus* (L.)	57.06±0.40	31.66±0.44	19.01±0.29	
16	Apple (*Malus domestica)*	55.06±0.25	31.56±0.44	21.53±0.3	
17	Grapes (*Vitis ninifera* L)	44.61±0.31	26.42±0.01	21.81±0.04	Low
18	Banana (*Musa paradisiacal*)	28.67±0.09	27.23±0.81	28.13±0.77	
19	Watermelon (*citrullis lanatus*)	17.23±0.33	16.40±0.73	16.94±0.32	
20	Coconut water (*Cocos nucifera* L.)	16.60±0.38	16.22±0.36	22.79±0.63	

[a,b,c] Mean of three determinations ± S.D. (standard deviation)

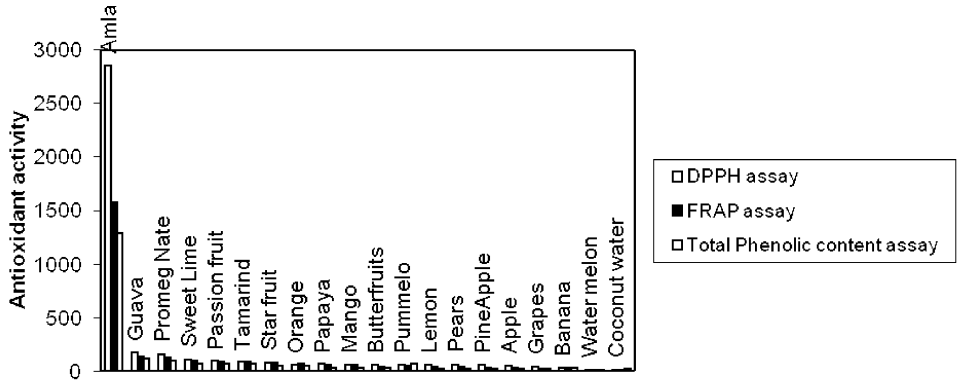

Fig. 1: Antioxidant activity of fruits estimated by DPPH, FRAP and Total Phenolic content assay

pomegranate and sweet lime as high, passion fruit, tamarind, star fruit, orange, papaya, mango, butter fruit, pummelo, lemon, pears, pineapple and apple as medium and grapes, banana, watermelon and coconut water as low. The antioxidant activity estimated by the three methods are almost the same, though the values obtained by DPPH free radical scavenging assay was highest among the three methods and least values were observed in total phenolic content determination. The level of antioxidant capacity of the fruits estimated is shown in Fig. 1.

DPPH Free Radical Scavenging Assay: The bleaching of DPPH absorption by a test compound is representative of its capacity to scavenge free radicals generated independently of any enzymatic or transition metal-based system. This is a widely used method to evaluate antioxidant activities in a relatively short time compared with other methods. Antioxidants react with DPPH which is a stable free radical and convert it to 1,1-diphenyl-2-(2,4,6-trinitrophenyl) hydrazine. The degree of decolourization indicates the scavenging potentials of the antioxidant compounds. As shown in the Table 1,

the antioxidant activity of the fruits tested, was found to vary over 170-fold from that at the lowest value. Amla shows the highest antioxidant activity followed by guava, pomegranate, sweet lime, passion fruit, tamarind, star fruit, orange, papaya, mango, butter fruit, pummelo, lemon, pears, pineapple, apple, grapes, banana, watermelon and coconut water. The antioxidant activity of the amla was found to be extremely high compared with other fruits in the present investigation.

Ferric Reducing Antioxidant Potential (FRAP) Assay: The ferric reducing antioxidant potential assay is based on the reducing power of a compound (antioxidant). It measures the reduction of Fe^{3+} (ferric iron) to Fe^{2+} (ferrous iron). As the ferric to ferrous ion reduction occurs rapidly with all reductants with half reaction reduction potentials above that of Fe^{3+}/ Fe^{2+}, the values in the FRAP assay expresses the corresponding concentration of electron donating antioxidants. As shown in Table 1, the antioxidant activity was found to vary over 97-fold. The highest activity was observed in amla followed by guava, pomegranate, sweet lime, passion fruit, tamarind, star fruit, orange, papaya, mango, pummelo, lemon, pears, butter fruit, pineapple, apple, banana, grapes, watermelon and coconut water.

Total Phenolic Content Determination: The plant phenolics constitute one of the major groups of compounds acting as primary antioxidants or free radical terminators, so it was reasonable to determine their total amount in the fruit extracts. The content of phenolic compounds (Gallic acid equivalent/ 100 gm of fruits) was determined from a standard plot prepared taking gallic acid as the standard. The antioxidant activity of the fruits was found to vary over 75-flod. Similar to estimation by DPPH free radical scavenging assay and Ferric reducing antioxidant potential (FRAP) assay, the highest activity was observed for amla followed by guava, pomegranate, sweet lime, pummelo, passion fruit, tamarind, star fruit, orange, butter fruit, papaya, mango, banana, lemon, pears, pineapple, coconut water, grapes, apple and watermelon.

DISCUSSION

The antioxidant activity estimated by the three methods *viz.* DPPH free radical scavenging assay, Ferric reducing antioxidant potential (FRAP) assay and total phenolic content determination, are almost same (as shown in Table 1.), though the values obtained by DPPH free radical scavenging assay was highest among the three methods and least values were observed in total phenolic content determination. A comparison of the values of the antioxidant activity observed in the present investigation with the data in the literature was problematic due to large variability and lack of standardization of the assay methods. Therefore, for discussion the ranking order of the antioxidant capacity of the fruits is used. The highest antioxidant activity was observed in amla followed by guava, pomegranate, sweet lime, passion fruit, tamarind, star fruit, orange, papaya, mango, butter fruit, pummelo, lemon, pears, pineapple, apple, grapes, banana, watermelon and coconut water. Amla is an important dietary source of vitamin C, minerals and amino acids and also contains phenolic compounds, tannins, phyllembelic acid, phyllemblin, rutin, curcuminoides and emblicol and the vitamin C content alone is about 2% [19]. Thus, the highest antioxidant activity observed for amla in the present investigation may be due to high content of vitamin C and other compounds which have antioxidant activity. The ranking order observed in this investigation is similar to the observation made by [15] although the values of antioxidant activity observed in the present study are lower. The most probable reason for observed lower values may be the low quality of the fruits and difference in the variety of fruits available in Aizawl market. [15], in their investigation of antioxidant capacity of fruits in Singapore market ranked Guava, Star fruit as high in their content of antioxidant capacity, Papaya, Orange, Pommelo, Pineapple, Avocado, Lemon, apple, mango are ranked as medium while Banana, watermelon and coconut water as low. [16] ranked the fruits according to their antioxidant capacity as orange> pineapple> pea> apple> grapes> banana> watermelon. The ranking of the fruits based on their antioxidant activity in the present investigation is similar with the observation made by [16].

It is known that majority of the antioxidant activity of the fruits are contributed by polyphenols, vitamin C, vitamin E, Maillard reaction products, â-carotene and lycopene. The differences in the antioxidant activities among the fruits could be attributed to their differences in phenolic contents and compositions and to other non-phenolic antioxidants present in the samples [20]. The vitamin C content of the fruits as available at *www.naturalhub.com/natural_food_guide_fruit_vitamin_c.htm*, is in the order of guava> papaya> orange> lemon> passion fruit> star fruit> pineapple> banana> avocardo> apple> grapes. This trend in the ranking is also almost similar to the ranking of the fruits in their

antioxidant activity in the present investigation. This observation suggest that one of the reason for difference in the antioxidant capacity of the fruits may be due to difference in the content of vitamin C besides the differences in total phenolic content and other non-phenolic antioxidants. In general, citrus fruits exhibited intermediate antioxidant capacity with orange as the most effective followed by grapefruit. This observation is in agreement with the higher concentration of phenolic compounds and vitamin C present in orange [16[. In the present investigation, citrus fruits exhibit intermediate antioxidant activity, with sweet lime as the most effective followed by orange, pummel, lemon etc. The finding is in agreement with the observation made by [16].

The probable reason for low antioxidant capacity observed in the present investigation from some of the values in the literature may be (i) low quality of the grapes fruit available in Aizawl market, (ii) long period of transportation from the place of production to Aizawl market for the fruits brought from other places, (iii) geographical differences and (iv) difference in variety of the fruits.

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REFERENCES

1. Halliwel, B. and J.M.C. Gutteridge, 2000. Free Radicals in Biology and Medice, 3rd Ed.; Oxford Univ. Press: New York.
2. Ames, B.N., 1983. Dietary carcinogens and anticarcinogens: oxygen radicals and degenerative diseases. Science, 221: 1256-1263.
3. Ames, B.N., M.K. Shigenaga and T.M. Hagwn, 1993. Oxidants, antioxidant and the degenerative diseases of aging. Proc. Natl. Acad. Sci. USA, 90: 7915-7922.
4. Feskanich, D ., R.G. Zeigler, D.S. Michaud, E.L. Giovannucci, F.E. Speizer, W.C. Willett and G.A. Colditz, 2000. Prospective study of fruit and vegetable consumption and risk of lung cancer among men and women. Journal of the National Cancer Institute, 92: 1812-1823.
5. Gordon, M.H., 1996. Dietary antioxidants in disease prevention. Natural Product Reports, 13: 265-273.
6. Heagele, A.D., C. Gillette, C. O'Neill, P. Wolfe, J. Heimendinger, S. Sedlacek and H.J. Thompson, 2000. Plasma xanthophylls carotenoids correlate inversely with indices of oxidative DNA damage and lipid peroxidation. Cancer Epidemiology Biomarkers and Prevention, 9: 421-425.
7. Halliwell, B., 1996. Antioxidants in human health and disease. Annual Review of Nutrition, 16: 33-50.
8. Michels, K.B., E. Giovannucci, K.J. Joshipura, B.A. Rosner, M.J. Stampfer, C.S. Fuchs, G.A. Colditz, F.E. Speizer and W.C. Willet, 2000. Prospective study of fruit and vegetable consumption and incidence of colon and rectal cancers. Journal of the National Cancer Institute, 92: 1740-1752.
9. Aruoma, O.I., 1998. Free radicals, oxidative stress and antioxidants in human health and disease. Journal of the American Oil Chemists' Society, 75: 199-212.
10. Jacob, R.A. and B.J. Burri, 1996. Oxidative damage and defense. American Journal of Clinical Nutrition, 63: S985-S990.
11. Steinberg, D., 1991. Antioxidants and atherosclerosis: a current assessment. Circulation, 84: 1420-1425.
12. Maxwell, S.R.J. and G.Y.H. Lip, 1997. Free radicals and antioxidants in cardiovascular disease. British Journal of Clinical Pharmacology, 44: 307-317.
13. Pratico, D. and N. Delanty, 2000. Oxidative injury in disease of the central nervous system: focus on Alzheimer's disease. American Journal of Medicine, 109: 577-585.
14. Wang, H., G. Cao and R.L. Prior, 1996. Total antioxidant capacity of fruits. Journal of Agriculture Food Chemistry, 44: 701-705.
15. Leong, L.P. and G. Shui, 2001. An investigation of antioxidant capacity of fruits in Singapore markets. Food Chemistry, 76: 69-75.
16. Pellegrini, N., M. Serafini, B. Colombi, D.D. Rio, S. Salvatore, M. Bianchi and F. Brighenti, 2003. Total Antioxidant Capacity of Plant Foods, Beverages and Oils Consumed in Italy Assessed by Three Different In Vitro Assays. Journal of Nutrition, 133: 2812-2819.
17. Benzie, I.F.F. and J.J. Strain, 1999. Ferric reducing/ antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power. Methods of Enzymology, 299: 15-27.

18. Singleton, V.L. and J.A. Rossi Jr., 1965. Colorimetry of total phenolics with phosphomolybdic- phosphotungstic acid reagents. American Journal of Enology and Viticulture, 16: 144-158.
19. Yokozawa, T., H.Y. Kim, H.J. Kim, T. Okubo and L.R. Juneja, 2007. Amla (Emblica officinalis Gaertn.) prevents dyslipidaemia and oxidative stress in the ageing process. British Journal of Nutrition, 97: 1187-1195.
20. Hassanien, M.A.R., 2008. Total antioxidant potential of juices, beverages and hot drinks consumed in Egypt screened by DPPH in vitro assay. GRASAS Y ACEITES, 59(3): 254-259.