The Bioscan 5(3) : 501-504, 2010
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES

DETERMINATION OF ANTIOXIDANT ACTIVITY AND VITAMIN C OF SOME WILD FRUITS OF MANIPUR

SUSHMA KHOMDRAM* AND G. A. SHANTIBALA DEVI
Life Science Department, Manipur Central University,
Canchipur -3 Imphal, Manipur -795 003
E-mail: sam.tamo@yahoo.com

KEY WORDS
Antioxidant
Vitamin C
Wild fruits

Received on :
17.05.2010
Accepted on :
19.07.2010

***Corresponding author**

ABSTRACT
Fruits are excellent sources of natural antioxidant and vitamins, especially ascorbic acid or vitamin C and fruits having very rich vitamin C are known to have very strong antioxidant properties. Vitamin C was estimated by using 2, 6-dichlorophenol indophenol reagent and antioxidant activity by DPPH (2, 2-diphenyl-1-picrylhydrazyl). The wild fruits of Manipur on analysis reveal that *Emblica officinalist* (amla) has very high amount of vitamin C (379.7 mg/100g) and simultaneously its antioxidant activity was also maximum (IC_{50} 181μg/mL) from among the selected samples. The present work is a brief screening of these wild fruits to explore their significant values as nutrition for mankind.

INTRODUCTION

About 5% or more of the inhaled oxygen (O_2) is converted to reactive oxygen species (ROS) by univalent reduction of O_2 (Maxwell, 1995). Antioxidant can act by scavenging reactive oxygen species (SOD removing O_2), by inhibiting their formation (e.g. by blocking activation of phagocytes), by binding transition metal ions and preventing formation of OH and or decomposition of lipid hydroperoxides, by repairing damage (e.g.α-tocopherol repairing peroxyl radicals and so terminating the chain reaction of lipid peroxidation) (Niwa *et al.,* 2001).

Recently, ROS have attracted a great deal of attention during metabolic processes of aerobic organisms, molecular oxygen accept electron to generate ROS such as superoxide, hydrogenperoxide and hydroxyl radical. ROS play important beneficial roles in living species, such as killing of bacteria and apoptosis of defective cells. On the other hand, ROS have been implicated in aging and a number of diseases, such as cancer, arteriosclerosis, neurodegenerative disorder, because they can alter lipids, proteins and DNA (Ritaro *et al*., 2008). Many living species have several antioxidative defence systems against oxidative stress induced by ROS. These systems include antioxidative enzymes such as catalase (CAT), superoxide dismutase (SOD), glutathion peroxidase (GPX), etc. SOD has been identified to play an important role in life span determination (Tolmasoff *et al.,* 1998).

Fruits are known to contain a variety of different antioxidant compounds such as ascorbic acid, tocopherol, glutathione and carotenoids, which may all contribute to protection against oxidative damage (Bloknina *et al.,* 2003).

Vitamin C is the popular antioxidants, which play a crucial role in preventing peroxidation damage in the biological systems (Fogliano *et al*., 1999, Mantene *et al.,* 2003). Glutathione (GSH) found in most tissues, cells and subcellular compartments (Simrnoff *et al.,* 2004) scavenges H_2O_2, reacts non-enzymetically with singlet oxygen, superoxide radical and hydroxyl radical. Due to environmental stresses the GSH concentration is found to be increased and protects the plants from heavy metals. Carotenoids (car) found in fruits and roots of plants (mango, tomatos, sweet potatoes, carrots etc), in photosynthetic chloroplast, function both as accessory light-harvesting pigments and as antioxidants (Larson, 1997).

Ascorbic acid (vitamin C) is the principal vitamin supplied by fruits in the diet. About 90% of a person's dietary vitamin C requirement is obtained from fruits and vegetables (Salunkhe *et al.,* 1991). An adult human being on average requires about 50mg of vitamin C per day, and many fruits contained this amount of ascorbic acid in less than 100g of tissues (Salunkhe *et al.,* 1991). Ascorbic acid performs a number of important functions in the body, like building and maintaining strong tissues especially connective tissues (bones, cartilage, dentin, collagen, etc), forming strong capillary walls for blood vessel tissue, building resistance to infection, helps in the absorption of calcium and ensures the health of bones, haemoglobin synthesis by aiding adsorption of iron, wound healing, infections and fever to help recovery. Naturally it is needed in growth stages of life and is important partner of protein for tissue synthesis. Being a strong reducing agent, it helps to tie up free radicals and thus protect the body from their deleterious effects (Sumati *et al.,* 2003). This shows that fruits having good source of vitamin C are a strong antioxidant.

Keeping the above significant values of vitamin C and antioxidant properties of fruits in human health, the present work is focus to investigate the vitamin C content and antioxidant activity of some wild fruits found in Manipur. It is one of the north-eastern states of India having rich bio-diversity. Further, this work can be useful to explore the potential nutrition of the above vitamin and antioxidant of these wild fruits for domestication by the mass people.

MATERIALS AND METHODS

Test samples are wild fruits harvested from various forests of Manipur. This wild fruits were washed and each fruit sample was shredded into small pieces and dried at 55ºC to 60ºC in hot air oven (Vaishali *et al*., 2003). After drying, the samples were ground in a mortar and stored in air tight container until they were requires for analysis. Samples used for ascorbic acid determination were harvested on the day of the analysis.

Ascorbic acid was determined titrimetrically by the modified Tillmann's method (Pauel and Pearon,1967) using 2, 6 - dichlorophenol, indophenol reagent. In all cases samples for analysis were prepared in 0.5% oxalic acid solution.

Antioxidant activity

The antioxidant activity of the wild fruit was examined by using the chemical assays of DPPH as described by Krings and Berger, 2001, using ascorbic acid as standard.

The reaction mixture consisted of 0.004% of 0.1mM DPPH methanol with 50-250 μg/mL of the fruits extract or 0.01mM of vitamin C. After 30 min incubation period in the dark at room temperature the absorbance was read against a blank at 517nm. Percentage inhibition was determined by comparison with a methanol treated control group. The percentage of DPPH decoloration was calculated as follows:

%DPPH decoloration = (1- O.D. sample/O.D. control) × 100

The degree of decoloration indicates the free radical scavenging efficiency of the fruits and the IC_{50} value shows the potential of antioxidant activity which was co-related by plotting graph of concentration sample vs the % of DPPH inhibition. Values are presented as mean ± standard deviation of three determinations.

RESULTS

The results of vitamin C content and antioxidant activity by different fruit extract are summarized in Table 1 and shown in Figs. 1 and 2. From the table the vitamin C content was found to be highest in *Emblica officinalis* 379.7 mg/100g followed by *Spondias pinata* 87. 5 mg/100g and lowest in *Prunus armeniaca* 6.9 mg/100g.

The change in colorization from violet to yellow and subsequent fall in absorbance of the stable radical. DPPH was measured at 517nm for various concentration *i.e.* 50-250 μg/mL and result were presented in Table 1. The IC_{50} value for each fruit extract defined as the concentration of extract causing 50% inhibition of absorbance was calculated, since IC_{50} is a measure of inhibitory concentration, a lower IC_{50} value would reflect greater antioxidant activity of the sample.

Antioxidant activity among the fruit samples was found to be maximum in *Emblica officinalis* having lowest IC_{50} value of 181 μg/mL and minimum in *Hodsonia macrocarpa* having highest IC_{50} 2717 μg/mL as lower IC_{50} value would reflect greater antioxidant activity of the sample.

Table 1: Table showing vitamin C content and anti oxidant activity (AOA) of some wild fruits of Manipur

S.N.	Sample	Vitamic C mg/100g ± SD	AOA IC_{50} μg/mL ± SD
1.	*Spondias pinnata* (L.f.) K.	87.4±12.0	518 ± 1.6
2.	*Emblica officinalis* G.	379.7 ± 20	181 ± 0.3
3.	*Averrhoa carambola* L.	16.4 ± 2	1179±113
4.	*Citrus medica* L.	11.7 ± 2.6	719 ± 144
5.	*Hodsonia macrocarpa* H.f.	13.2 ± 0.8	2717 ± 68
6.	*Zizyphus mauritiana* L.	11.9 ± 1.6	1378 ± 235
7.	*Docynia indica* D.	14.8 ± 2.7	1657 ± 74
8.	*Prunus armeniaca* L.	6.9 ± 0.4	755 ± 9
9.	*Punica garanatum* L.	14.4 ± 0.6	398 ± 4.8
10.	*Antidesma Bunius* S.	7.8 ± 0.9	1717 ± 78

DISCUSSION

Considering the importance of fruits in human health, that are recognised in treatment of diabetes mellitus and hypertension (Vaishali *et al.,* 2003) and in various treatment of deleterious effects in human body due to cheap and rich source of vitamins, mineral and antioxidant like vitamin C, the result obtained may have the potential sources in these wild fruits found in Manipur.

From the analysis, the vitamin c was found to be highest in sample *Emblica officinalis* 379.7 mg/100g and lowest in *Prunus armeniacea* 6.9 mg/100g. At the same time *Spondias pinnata* have much higher levels compared with values of grape juice (38mg/100g), oranges (50 mg/100g), strawberries (59 mg/100g) (Arnao *et al*., 2001), limes (63 mg/100g), papaya (57 mg/100g), mausambi (50 mg/100g), lemon sweet (45 mg/100g), pineapple (39 mg/100g) (Sumati *et al*., 2003) and Citrus fruit (50 mg/100g) (Smith and Somerset 1993). Garg *et al.,* (2008) reported that *Emblica officinalis* is nutritionally and medicinally important fruit due to high contents of vitamin C. Amla fruits are used as a principle ingredient in the preparation of famous ayurvedic tonic *chavyanpras*. Recently, consumption of amla juice has also increased. Mobasseri (2004) has reported that amla juice has 20 times more vitamin C than orange juice.

Majority of herbo - minerals and other proprietary preparation in Ayurveda contain ingredients like amalaki (*Emblica officinalis*) which is known for its 'rasaayana' (anti-aging) and corrective property. According to modern science, it is the fruit having highest content of ascorbic acid (600 mg/100g), one of the strong antioxidant vitamins. Three type of amalaki use in the preparation of *chavyanpras* showed variable content of ascorbic acid like, big amalaki 245 mg/100g, medium amalaki 275 mg/100g and small amalaki 350 mg/100g (Vaishali *et al.,* 2003) which correlate with our result of *Emblica officinalis* having more content of vitamin C that are grown wild in Manipur. The reason for this could be that Ascorbic acid content (AAC) increases as the fruit ripens (Lim *et al.,* 2006). The increase in AAC as the fruit mature is due to the breakdown of starch to glucose which is used in the bio-

synthesis of ascorbic acid. Report on the highest value of ascorbic acid in guava having 186 to 213 mg/100g (Singh *et al*., 2005) were also reported.

Nutritional quality of fruit tissue is in part a function of carbohydrate metabolism, color, pigment, flavour and antioxidative capacity. Antioxidants provide chemical protection for biological systems against harmful effects of reaction or processes that cause excessive oxidation, protein and DNA damage and cell death (Papas, 1999, Arnao *et al.,* 2001). Several studies have indicate that antioxidants prevent the onset of degenerative illness such as certain cancers, cardiovascular and neurodegenerative diseases, cataracts, oxidative stress dysfunction and aging (Schwartz, 1996; Papas, 1999; Deighton *et al.,* 2000; Arnao *et al.,* 2001). Recently, some studies have shown that a high intake of antioxidant food may decrease the risk of incidence of deadliest diseases (Ritaro *et al.,* 2008). Most of developed countries like UK and Germany encourage and specifically advise increased consumption of fruits (Smith and Somerset, 1993) as most of the nutritious values are easily available in them.

Primary antioxidant properties are generally measured by DPPH assay (expressed as IC_{50}). The DPPH assay measures the ability of the fruit extract to donate hydrogen to the DPPH radical resulting in bleaching of the DPPH solution. The greater the bleaching action the higher the antioxidant activity AEAC value (Ascorbic acid equivalent to antioxidant capacity), and this is reflected in lower IC_{50} value (Lim *et al.,* 2006). The same action is found in our sample *Emblica officinalis* having the lowest IC_{50} value 181 μg/mL from other fruits *Spondiaus pinnata, Averrhoa carambola, Citrus medica, Hodsonia macrocarpa, Zizyphus mauritiana, Docynia indica, Prunus armeniaca, Punica garanatum,* and *Antidesma bunius* showing lower IC_{50}. It is known that fruit ripening continues after harvest and this process leads to significant changes in the contents of the antioxidant (Lim *et al*., 2006). Sun *et al*., 2002 investigated antioxidant properties of 11 different fruits (including cranberry, appple, red grape, strawberry, pineapple, banana, peach, lemon, orange, pear and grape fruits).

Guava fruits contain ascorbic acid as much as ten times that of other fruits such as banana, dragon fruits and sugar apple and are healthy to consume from the antioxidant viewpoint as it has the richest content of AAC from other temperate fruit (Lim *et al*., 2006), that support our finding having *Emblica officinalis* the highest antioxidant activity as its vitamin C content was the highest from the rest of the samples *i*.e. higher vitamin C content, highest is the antioxidant activity.

Pomegranate (*Punica granatum* L.) of punieaceae family is the native of Iran and grown extensively with total production of 665,000 tons in 2003 (Sai, 2003). Pomegranate fruits contain considerable amount of seed, ranging between 40 and 100g/kg of fruit weight depending on fruit variety. And the antioxidant activity of pomegranate seed extract was higher ($p<0.05$) compared to that of the synthetic BHA (Butylated hydroxyanisole) (29). From our finding among the samples, pomegranate fruit that is found to be grown in wild also have considerable activity of antioxidant, lower IC_{50} 398 μg/mL though its vitamin C content was in reasonable level and at same time *Spondias pinnata* having high amount of vitamin C content next to *Emblica officinalis* according to our finding have enough antioxidant activity compared from other samples, these may be attributed to presence of some other antioxidant phytochemicals (Brahma *et al*., 2005). Several reports on blackberries were reported having excellent source of natural antioxidants (Halliwell and Gutteridge, 1989; Jiao and Wang, 2000) and vitamin C 43mg/100g fresh blackberries.

For a body to maintain antioxidant level, external supplementation is necessary for healthy living. In Manipur, a number of fruits based food, belonging to different families, possessing rich antioxidant properties and vitamin C are consumed by the people unaware of their nutritious significant and which perhaps may be the basis for low incidence of cancers. Thus, it can be concluded from the current investigation that exploring the source of natural antioxidant and vitamin C content in these wild fruits grown in Manipur will help to re-introduce their use as food supplements and encourage their cultivation, conservation in home garden or by state government authority before these fruits are almost going extinct due to deforestation and urbanization. At the same time these fruits can be used as alternative source of natural antioxidant rather than synthetic antioxidant like BHT (Butylated hydroxytoluene) and BHA (Butylated hydroxyanisole) because of carcinogenicity (Mahdavi and Salunkhe, 1995).

REFERENCES

Arnao, M. B., Cano, A. and Acosta, M. 2001. The hydrophilic and lipophilic contribution to total antioxidant activity. *Food chem*. **73**: 239-244.

Bloknina, O., Virolainen, E., and Fagerstedt, K. V. 2003. Antioxidants,oxidative damage and oxygen deprivation stress:a review. *Ann. Bot*. **91**: 179-194.

Brahma, N. Singh, Barthwal, J., Upadhyay, G., Singh, H. B., Dhan, P. and R.L. Singh. 2005. Antioxidant activity and nutraceutical potential of some soybean varieties. *Indian J. Agric. Biochem*. **18(2)**: 83-86.

Deighton, N. R., Brennan, C. Finn and Davies, H. V. 2000. Antioxidant properties of domesticated and wild *Rubus* species. *J. Sci. Food Agric*. **80:** 1307-1313.

Fogliano, V., Verde, V., Randazzo, G. and Ritieni, A. 1999. Method of measuring antioxidant activity and its application to monitoring the antioxidant capacity of wines. *J. Agric. Food Chem*. **47:**1035-1040.

Garg, N., Sonkar, P. and Bhriguvanshi, S. R. 2008. Nutritional and microbial quality evaluation of commercial samples of amla chavyanpras,amla preserve and amla juice. *J. Food Sci. Technol*. **45(2)**: 193-195.

Halliwell, B. and Gutteridge, J. M. C. 1989. Free radicals in biology and medicine. Clarendor Press. Oxford.

Jiao, H. and Wang, S. Y. 2000. Correlation of antioxidant capacities to oxygen radical scavenging enzymes avtivities in blackberries. *J. Agric. Food Chem*. **48**: 5672-5676.

Krings, U. and Berger, R. G. 2001. Antioxidant activity of some roasted foods. *Food chemistry*. **72:** 223 - 229.

Larson, R. A. 1997. In Naturally occurring antioxidants (RA Larson, Editor), CRC Press. Boca Raton. p. 83.

Lim Yau Yan, Lim Theng Teng and Tee Jing Jhi. 2006. Antioxidant properties of guava fruit: Comparision with some local fruits. *Sunway Academic J*. **3**: 9-20.

Mahdavi, D. L. and Salunkhe. 1995. Toxicological aspects of food antioxidant. In: Food antioxidants. Mahdavi D.L, Deshpande S. S, Salunkhe D. K (Ed), Marcel Dekker. *New York.*

Mantene, S. K., Jagdish, Badduri S. R., Siripurapu, K. B. and UniKrishnan, M. K. 2003. *In vitro* evaluation of antioxidant properties of *Cocos nucifera* Linn. water. *Nahrung Food.* **2:** 126-131.

Maxwell, S. R. J. 1995. Prospects for the use of antioxidant therapies. *Drugs*. **49(3):** 345-361.

Mobasseri, R. 2004. Amalaki-the wonder fruits of ayurveda. *Ayurveda News*. **5:** 2-3.

Niwa, T., Doi, U., Kato, Y. and Osawa, T. 2001. Antioxidant properties of phenolic antioxidants isolate from corn steep liquor. *J. Agric. Food chem*. **49:** 177-182.

Papas, A. M. 1999. Antioxidant status, diet nutrients and health CRC Press. Boca Raton. FL.

Pauel, G. and Pearson, W. N. 1967. *The vitamins*. 2nd Edn. **VII:** 31-32.

Ritaro Matsuura, Hironori Moriyama, Naruki Takeda, Kyoko Yamamoto, Yusuke Morita, Tomoko Shimamura and Hiroyuki Ukeda. 2008. Determination of antioxidant activity and characterization of antioxidant phenolics in the plume vinegar extract of cherry blossom (*Prunus lannesiana*). *J. Agric. Food chem*. **56:** 544-549.

Salunkhe, D. K., Bolin, H. R. and Reddy, N. R. 1991. Storage processing and nutritional quality of fruits and vegetables. **1:** Fresh fruits and vegetables. CRC Press.Boca Raton. FL.

Samadloiy, H. R., Azizi, M. H. and Barzegar, M. 2008. Physico-chemical quality of seeds of pomegranate cultivar (*Punica granatum* L.) grown in Iran and antioxidative activity of their phenolic component. *J. Food Sci. Technol*. **45(2):** 190-192.

Schwartz, J. L. 1996. The dual role of nutients as antioxidants and pro-oxidants: their effects on tumor cell growth. *J. Nutr*. **126S**: 1211-1227.

Simrnoff, N., Running, J. A. and Gatzek, S. 2004. In vitamin c: Its function and biochemistry in minerals and plants (H. Asard. J. M. Mary, N. Simroff, Edltors). *Bios Scientific. London*. p. 1.

Singh V., Pandey, S. and Sonkar, S. 2005. Comparative nutritive value of commercial varieties of fruits of Northern India. *Farm Sci. J*. **14(2)**: 53- 54.

Smith, L. G. and Somerset, S. M. 1993. Fruits of temperate climates: Commercial and dietary importance. *Encyclopaedia of Food Science, Food Technology and Nutrition*. (R.Ma Crae.R.K Robinsion and S.J. Sadlers Eds.) .Academic Press. London. p. 2083.

Sumati, R., Mudambi and Rajagopal, M. V. 2003. Fundamentals of foods and nutrition. Fourth Edition.Published K. K. Gupta. New Age International (p) Ltd. p.135.

Sun, J., Chu, Y. F., Wu, X. and Liu, R. H. 2002. Antioxidant and antiproliferative activities of common fruits. *J. Agr. Food Chem*. **50**: 7449-7454.

Tolmasoff, J. M., Ono, T. and Cutler, R. G. 1998. Superoxide dismutase; correlation with life-span and specific metabolic rate in primate species. *Proc. Natl. Acad. Sci. U. S. A*. **77(55)**: 2777-2781.

Vaishali, V. Agte, S., Mengale, S. Akkalkotkar, M., Paknikar, K. M. and Chiplonkar, S. A. 2003. Antioxidant and trace element potential of chyavanpraash and Ayurvedic preparations. *Indian J.Traditional Knowledege*. **2(3):** 215-223.