International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 3, Issue 1, 2011

Research Article

IN VITRO ANTIOXIDANT STUDIES IN SOME COMMON FRUITS

JAMUNA KS, RAMESH CK*, SRINIVASA TR AND RAGHU KL

P.G. Dept of Biotechnology, Sahyadri Science College (Autonomous), Kuvempu University, Shivamogga - 577 203, Karnataka, India
Email: ckramck@yahoo.co.in

Received: 04 Sep 2010, Revised and Accepted: 10 Oct 2010

ABSTRACT

The present study evaluates the *in vitro* antioxidant activity of eleven different fruits *viz. Ananas comosus*, *Artocarpus heterophyllus*, *Carica papaya*, *Citrullus vulgaris*, *Citrus sinensis*, *Malus domestica*, *Manilkara zapota*, *Musa paradisiaca*, *Phyllanthus emblica*, *Psidium guajava* and *Pyrus communis*. The methanol extract of the fruits was assessed for their antioxidant activity using three different assays like DPPH, reducing power and total antioxidant capacity at different concentrations. In all the three assays, *P. emblica* has exhibited highest antioxidant activity among the various fruits studied. The results on the antioxidant potentiality of various fruits are discussed.

Keywords: *In vitro* antioxidant activity, DPPH, Reducing power, Total antioxidant capacity, Fruits.

INTRODUCTION

In recent times, there is an increasing interest in the role of free-radical-mediated damage in the etiology of human diseases. In the status of normal metabolism, the levels of oxidants and antioxidants in humans are maintained in balance, which is important for sustaining optimal physiological conditions[1-2]. Overproduction of oxidants in certain conditions can cause an imbalance, leading to oxidative damage to large biomolecules such as lipids, DNA, and proteins[3]. Oxidative damage to body cells and molecules has been widely postulated to be involved in the causation and progression of a range of chronic diseases, such as cardiovascular disease, neuronal disease, cataracts, and several forms of cancer[4]. Human metabolism counts on an antioxidant defensive system involving enzymes and proteins to prevent these effects. However, the defenses can be overwhelmed in certain circumstances so that harmful effects occur. It is accepted that the intake of antioxidant substances reinforces defenses against free radicals. The use of synthetic antioxidants has been limited because of their toxicity[5]. Therefore, it is of great significant and necessity that research focuses on discovering potential natural, effective antioxidants to replace the synthetic ones.

It is widely accepted that fruits and vegetables have many healthful properties[6]. Consumption of fruits is beneficial to health and contributes to decrease of the mortality rate of cardiovascular and other diseases[7-9]. This positive influence is attributed to some natural antioxidant phytonutrients[10-13]. The majority of the antioxidant capacity of a fruit may be from polyphenols- flavonoids, tannins, along with vitamin A, B, C, and E and carotenoids[14].

In view of huge importance of fruits as antioxidant sources, in the present research programme, a comparison of their antioxidant property of commonly consumed fruits was investigated in order to evaluate their extent antioxidant capacity. Three *in vitro* antioxidant methods have been used to compare the activity utilizing the methanol extracts of eleven different fruit samples *viz.* DPPH, reducing power and total antioxidant capacity.

MATERIALS AND METHODS

Plant materials

Eleven different commonly consumed fruits were selected. Samples of fresh ripe fruits were purchased from a local market of Shivamogga - Bhadravathi, Karnataka, when they were most available, during the year 2009. The fruits comprised of *Ananas comosus* (Pineapple), *Artocarpus heterophyllus* (Jackfruit), *Carica papaya* (Papaya), *Citrullus vulgaris* (Watermelon), *Citrus sinensis* (Sweet orange), *Malus domestica* (Apple), *Manilkara zapota* (Sapota), *Musa paradisiaca* (Banana), *Phyllanthus emblica* (Indian Gooseberry), *Psidium guajava* (Guava) and *Pyrus communis* (Pear). The fruit samples were authenticated by the taxonomist from the Dept of Botany, Sahyadri Science College, Shivamogga.

Extraction

After selection, each fresh fruit was washed under running tap water followed by washing with distilled water to remove the surface debris. Exactly 500g of peeled fruit pulps were weighed and were minced using a mixer grinder for fine maceration. After homogenization, it was extracted in 500ml methanol solvent for 7 days in dark under room temperature with intermittent shaking. After 7 days, the whole extracts are filtered using muslin cloth at first and then through filter paper. To the marc, 300ml fresh solvent was added and refluxed for 90min followed by filtration and finally both the filtrate were mixed together and concentrated. The yield of crude extracts were noted and stored in desiccators for maximum of 3 days; later preserved in a deep freezer (-20^{o}C) for further use.

Qualitative phytochemical analysis

The preliminary qualitative phytochemical studies were performed for testing the different chemical groups present in methanol extracts of eleven different fruit extracts[15-16].

Evaluation of *in vitro* antioxidant activity

General chemicals and instruments

All chemicals and solvents used in the study were of analytical grade. 2, 2-diphenyl-1-picryl hydrazyl (DPPH), methanol, trichloro acetic acid (TCA) are purchased from Himedia, India. Ascorbic acid, monobasic and dibasic sodium phosphate, potassium ferri cyanide, ferric chloride, sulphuric acid, sodium phosphate, ammonium molybdate is procured from Sd Fine chem. Ltd, India.

UV-Vis Spectrophotometer (Elico SL 159, India), centrifuge (Remi RM12C, India), low deep freezer (Modern Industrial Corporation, India), vacuum rotary evaporator (Shivam Instruments, India), weighing balance (Sartorius, India) and pH meter (Systronics, India) were the instruments used for the study.

DPPH (2, 2 - diphenyl-1-picryl hydrazyl) radical scavenging activity

DPPH free radical scavenging assay was measured using the method of Wong *et al.* 2006 [17]. The different concentrations of each extracts prepared in methanol were added to 3ml of 0.1mM methanolic solution of DPPH. The tubes were shaken vigorously and allowed to stand for 30min at room temperature in dark. Changes in absorbance of samples were measured at 517nm. A control reading was obtained using methanol instead of the extract. Ascorbic acid was used as the standard control. All the tests were performed in triplicates.

Free radical scavenging activity was expressed as inhibition percentage and was calculated using the following formula:

$$\% \quad \text{Inhibition} \quad = \left[\frac{(A_0 - A_1)}{A_0} \right] \quad X\ 100$$

Where, A_0 is the absorbance of the control (without test samples)

A_1 is the absorbance of test samples.

Results are expressed as IC_{50}, which is the amount of antioxidant necessary to decrease the initial DPPH• concentration by 50%.

Reducing power assay

The reducing power of the extracts was evaluated according to Oyaizu, 1986 [18]. Different amounts of methanol extracts were perched in methanol solvent and diverse with 2.5ml of 0.2M phosphate buffer (pH 6.6), and 2.5ml of 1% $K_3Fe(CN)_6$. This mixture was incubated at 50°C for 20 min, 2.5ml of 10% TCA was added and centrifuged at 3000rpm for 10min. The upper layer of the solution (2.5ml) was assorted with methanol (2.5ml) and $FeCl_3$ (0.5ml, 0.1%), and the absorbance was measured at 700nm. Increase in absorbance of the reaction mixture indicated increased reducing power. The experiment was conducted in triplicates and the reducing power was expressed as equivalents of ascorbic acid (µg) / mg of extract.

Total antioxidant capacity (Phosphomolybdenum method)

The total antioxidant capacity was measured by spectrophotometeric method of Prieto *et al.* 1999 [19]. At different concentration, methanol extracts were prepared in their respective solvents and combined in an eppendorf tube with 1ml of reagent solution (0.6M H_2SO_4, 28mM sodium phosphate, 4mM ammonium molybdate mixture). The tubes were incubated for 90min at 95ºC. The mixture was cooled to room temperature and the absorbance was read at 695nm against blank. The experiment was conducted in triplicates and values are expressed as equivalents of ascorbic acid (µg) /mg of extract.

RESULTS

Qualitative phytochemical analysis

The preliminary qualitative phytochemical analysis revealed that all the eleven methanol fruit extracts showed the presence of carbohydrates, proteins, amino acids, steroids, glycosides, flavonoids, tannins and polyphenols. *Phyllanthus emblica, Psidium guajava, Ananas comosus, Citrus sinensis, Malus domestica, Pyrus communis* and *Artocarpus heterophyllus* revealed the presence of alkaloids whereas alkaloids were absent in other fruits viz., *Citrullus lanatus, Carica papaya, Manilkara zapota, Musa paradisiaca.* Analysis also revealed that none of the fruits under study gave positive results for saponins in the methanol extract (Table 1).

Table 1: Results of qualitative phytochemical analysis of eleven methanol fruit extracts

TESTS	Fruit extracts										
	Ananas comosus	*Artocarpus heterophyllus*	*Carica papaya*	*Citrullus lanatus*	*Citrus sinensis*	*Malus domestica*	*Manilkara zapota*	*Musa paradisiaca*	*Phyllanthus emblica*	*Psidium guajava*	*Pyrus communis*
Carbohydrates	+	+	+	+	+	+	+	+	+	+	+
Proteins	+	+	+	+	+	+	+	+	+	+	+
Amino acids	+	+	+	+	+	+	+	+	+	+	+
Steroids	+	+	+	+	+	+	+	+	+	+	+
Glycosides	+	+	+	+	+	+	+	+	+	+	+
Saponins	-	-	-	-	-	-	-	-	-	-	-
Alkaloids	+	+	-	-	+	+	-	-	+	+	+
Flavonoids	+	+	+	+	+	+	+	+	+	+	+
Tannins and Polyphenols	+	+	+	+	+	+	+	+	+	+	+

DPPH Radical scavenging activity

The IC_{50} for methanol extracts were found to be highest activity in *Phyllanthus emblica* followed by *Psidium guajava > Carica papaya, Artocarpus heterophyllus > Malus domestica > Citrus sinensis > Citrullus lanatus, Ananas comosus > Manilkara zapota > Musa paradisiaca > Pyrus communis* and the values were 8.50µg/ml, 276.20µg/ml, 785.70mg/ml, 1.44mg/ml, 2.34mg/ml, 2.92mg/ml, 3.45mg/ml, 7.60mg/ml, 10.02mg/ml, 15.97mg/ml and 16.63mg/ml, respectively while, the similar activity was 2.45µg/ml for standard **(Fig.1)**. The results revealed that, dose dependent radical scavenging activity in terms of IC_{50} values.

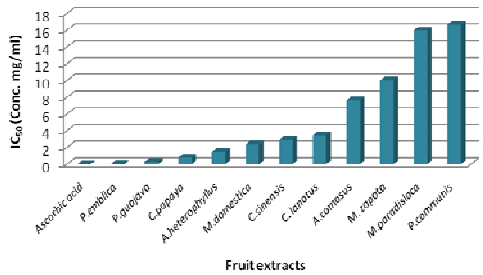

Fig 1: DPPH radical scavenging activity (IC_{50}) of eleven methanol fruit extracts

Reducing power assay

The ascorbic acid equivalents for reducing power were 595.00, 23.60, 11.90, 11.60, 9.10, 8.50, 8.20, 6.70, 6.20, 3.00 and 2.10 µg per mg of extract in *Phyllanthus emblica* followed by *Psidium guajava, Carica papaya, Citrus sinensis, Malus domestica, Artocarpus heterophyllus, Manilkara zapota, Citrullus lanatus, Ananas comosus, Pyrus communis, Musa paradisiaca* respectively (Fig.2).

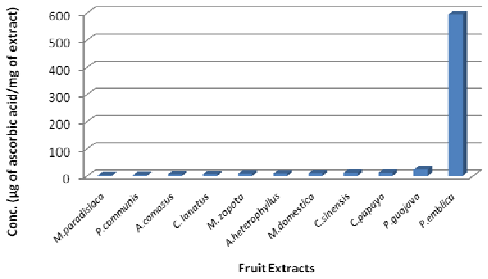

Fig 2: Reducing power assay of eleven methanol fruit extracts (Equivalents of ascorbic acid)

Total antioxidant capacity

The total antioxidant capacity was found to be highest in *Phyllanthus emblica* followed by *Citrullus lanatus*, *Psidium guajava*, *Carica*

papaya, *Artocarpus heterophyllus, Citrus sinensis*, *Musa paradisiaca*, *Malus domestica*, *Manilkara zapota*, *Ananas comosus* and *Pyrus communis* and the values were 248.50, 59.30, 57.30, 39.30, 26.50, 19.00, 17.25, 14.60, 10.85, 10.32 and 2.22 µg of ascorbic acid/mg of extract respectively (Fig.3).

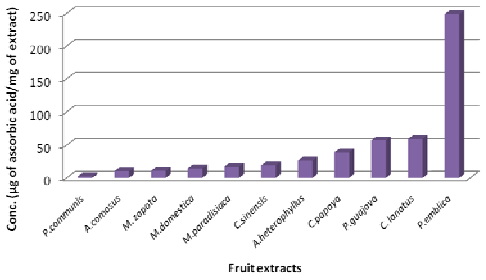

Fig. 3: Total antioxidant capacity of eleven methanol fruit extracts

(Equivalents of ascorbic acid)

DISCUSSION

Qualitative phytochemical analysis

The result of present investigation revealed that, the preliminary phytochemical analysis of the plant extracts are bestowed with the presence of several bioactive compounds *viz.* polyphenols, tannins, steroids, flavonoids and alkaloids in fruit extracts which therefore encourages antioxidant studies.

DPPH radical scavenging activity

DPPH assay is based on the concept that a hydrogen donor is an antioxidant. DPPH• is one of the few stable and commercially available organic nitrogen radicals[20-21]. The antioxidant effect is proportional to the disappearance of DPPH• in test samples. A freshly prepared DPPH• solution exhibit a deep purple color with absorption maximum at 517nm. The purple color generally fades or disappears when an antioxidant is present in the medium[22]. Thus, antioxidant molecules can quench DPPH free radicals *i.e.* by providing hydrogen atoms or by electron donation, conceivably *via* free radical attack on the DPPH molecule and converted them to a colorless stable molecule 2,2-diphenyl-1-hydrazine, or a substituted analogous hydrazine, resulting in a decrease in absorbance at 517nm. Hence absorbance decreases; the more potent the antioxidant more decrease in absorbance is seen[23]. In the present study the methanolic fruit extracts were evaluated for the DPPH radical scavenging activity. Among eleven methanol fruit extracts, *P. emblica* exhibited the highest radical-scavenging activity whereas *A. heterophyllus* showed lowest activity.

Reducing power assay

The reducing capacity of extracts Fe^{3+}/ ferricyanide complex to the ferrous form may serve as a significant indicator of its antioxidant capacity[24-25]. The existence of reductones are the key of the reducing power, which exhibit their antioxidant activities through the action of breaking the free radical chain by donating a hydrogen atom. The reduction of the Fe^{3+} / ferricyanide complex to the ferrous form occurs due to the presence of reductants in the solution. Absorbance of Fe^{3+} can be observed by measuring the O.D. values at 700nm the reduction power of the extract increases with increase in concentration[26]. In the present study the methanolic fruit extracts were evaluated for the reducing power ability. Among eleven methanol fruit extracts, *P. emblica* exhibited the highest reducing activity whereas *M. paradisiaca* showed lowest activity in terms of ascorbic acid equivalents.

Total antioxidant capacity

Total Antioxidant Capacity by phosphomolybdenum method assay is based on the reduction of Mo (VI) to Mo (V) by the sample analyte and the subsequent formation of green phosphate/Mo (V) complex at acidic pH. The phosphomolybdenum method is quantitative since the total antioxidant activity is expressed as the number of equivalents of ascorbic acid[19]. In the present study the methanolic fruit extracts were evaluated for the total antioxidant capacity. Among the eleven methanol fruit extracts, total antioxidant capacity was found to be high in *Phyllanthus emblica* extract and low in *Pyrus communis* extract in terms of ascorbic acid equivalents.

A preponderance of epidemiological studies provides convincing evidence of the beneficial role of fruits and vegetables in the diet for the maintenance of health and prevention of disease[27]. The presence of phytochemicals, in addition to vitamins and provitamins, in fruits and vegetables has been recently considered of crucial nutritional importance in the prevention of chronic diseases, such as cancer, cardiovascular disease, and diabetes[28]. Many of these phytochemicals have been found to provide a much stronger antioxidant activity than vitamins C and E and *β*-carotene within the same food[29]. Synergistically or additively, these dietary antioxidants provide bioactive mechanisms to reduce free radical induced oxidative stress. Prevention is a more effective strategy than treatment for chronic diseases, a constant supply of phytochemical-containing plants with desirable health benefits beyond basic nutrition is essential to furnish the defensive mechanism to reduce the risk of chronic diseases in humans[3]. Recent research has also shown that, through overlapping or complementary effects, the complex mixture of phytochemicals in fruits and vegetables provides a better protective effect on health than single phytochemicals[29]. This perspective has been strengthened by the occurrence of inconsistent results in human clinical trials using single antioxidants[30]. Although 5,000 plant phytochemicals have been identified, a large proportion remains unknown[31]. Different plants have different contents of phytochemicals with different structures and thus offer different protective mechanisms to different levels.

Natural antioxidants, particularly in fruits, can be phenolic compounds (tannins, flavonoids, phenolic acids and tocopherols), nitrogen compounds (alkaloids, chlorophyll derivatives, amino acids, and amines), or carotenoids as well as ascorbic acid[32]. Tannins are known to inhibit lipid peroxidation and lipoxygenases *in vitro*, and information has been accumulated over the past few years demonstrating their ability to scavenge free radicals which are known to be important in cellular prooxidant states[33]. Several researchers have investigated the antioxidative activity of flavonoids compounds and have attempted to define the structural characteristics of flavonoids that contribute to their activity[34]. Phenolic acids, such as caffeic, chlorogenic, ferulic, sinapic, and *p*-coumaric acids, appear to be more active antioxidants than the hydroxy derivatives of benzoic acid such as *p*-hydroxybenzoic, vanillic, and syringic acids[35]. α-tocopherol is one of the most active in vitro chain-breaking antioxidants[36]. Vitamin C is a hydrophilic antioxidant, and is considered to be a poor antioxidant within the lipophilic plasma membrane[37]. Vitamin C plays a valuable role in the regeneration of vitamin E and thereby acts to reduce the rate of oxidative consumption of vitamin E[38]. Carotenoids also have a protective function against oxidative damage, and singlet oxygen is very powerfully quenched by *β*-carotene[39].

CONCLUSION

Considering the three assays, it can be concluded that the *Phyllanthus emblica* is the best fruit followed by *Psidium guajava* and *Carica papaya* in terms of antioxidant potential of the fruits tested. It is also interesting to note that the other fruits cannot be ranked due to their varied results in different assays. The present research programme establishes the antioxidant ability the fruit extracts, even though extent potential varies from case to case. The results and inferences from different methods in other fruits under study differ substantially because each complex chemical reaction generates unique values. Hence, authors are of the opinion that an appropriate index needs to be developed which does not represent a specific antioxidant property but can rank the antioxidant capacity of the fruits.

ACKNOWLEDGEMENT

The authors wish to thank Prof. Siddaramappa BR for providing laboratory facilities and encouragement. Our sincere thanks also to

Dr. Prabhakar BT and Dr. Paramesha M for their help rendered during the study.

REFERENCES

1. Temple NJ. Antioxidants and disease: More questions than answers. Nutr Res. 2000; 20: 449-459.
2. Thompson LU. Antioxidant and hormone-mediated health benefits of whole grains. Food Sci Nutr. 1994; 34: 473-497.
3. Liu RH. Supplement quick fix fails to deliver. Food Technol. Int. 2002; 1: 71-72.
4. Halliwell B. Antioxidants and human disease: a general introduction. Nutr Rev. 1997; 55:S44–S52.
5. Valentao P, Fernandes E, Carvalho F, Andrade PB, Seabra RM, Bastos M. Antioxidative properties of cardoon (*Cynara cardunculus* L.) infusion against superoxide radical, hydroxyl radical and hypochlorous acid. J. Agric. Food. Chem. 2002; 50: 4989–4993
6. Hertog MGL, Kromhout D, Aravanis C, Blackburn H, Buzina R, Fidanza F, Flavonoid intake and long-term risk of coronary heart disease and cancer in the seven countries study. Archives of Internal Medicine. 1995; 155: 381–386.
7. Vinson JA, Su X, Zubik L, Bose P. Phenol antioxidant quantity and quality in foods: Fruits. J. Agric. Food. Chem. 2001; 49: 5315–5321.
8. Wang H, Cao G, Prior RL. Total antioxidant capacity of fruits. J. Agric. Food. Chem. 1996; 44: 701–705.
9. Joshipura KJ, Ascherio A, Manson JE, Stampfer MJ, Rimm EB, Speizer FE, Fruit and vegetable intake in relation to risk of ischemic stroke. Journal of American Medical Association. 1999; 282: 1233–1239.
10. Rice-Evans C, Diplock AT. Current status of antioxidant therapy. Free Radicals in Biology and Medicine. 1993; 15: 77–96.
11. Halliwell B. Free radicals and antioxidants: A personal view. Nutr Rev. 1994; 52: 253–265.
12. Rice-Evans C, Miller NJ. Total antioxidant status in plasma and body fluids. Methods in Enzymology, 1994; 234: 279–293.
13. Rice-Evans C, Miller NJ, Paganga G. Structure antioxidant activity relationships of flavonoids and phenolic acids. Free Radicals in Biology and Medicine. 1996; 20: 933–956.
14. Frei B, Higdon JV. Antioxidant activity of tea polyphenols in vivo: evidence from animal studies. J Nutr. 2003; 133: 3275S–3284S.
15. Trease GE, Evans WC. A Text book of Pharmacognosy. 11th edition, Bailliere Tidall, London; 1978.p. 530.
16. Kokate CK, Purohith AP, Gokhale SB. Pharmacognosy. Nirali Prakashan, Pune; 1990.p.120.
17. Wong SP, Lai PL, Jen HWK, Antioxidant activities of aqueous extracts of selected plants, Food Chem, 99, 2006, 775-783.
18. Oyaizu M. Studies on products of browning reaction prepared from glucosamine. Jpn J of Nutr. 1986; 44: 307–15.
19. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of Vitamin E. Anal Biochem.1999; 269: 337–341.
20. Burits M, Bucar F. Antioxidant activity of *Nigella sativa* essential oil, Phytother Res. 2000; 14: 323–28.
21. MacDonald-Wicks LK, Wood LG, Garg ML, Methodology for the determination of biological antioxidant capacity in vitro: a review. J. Sci. Food Agric. 2006; 86: 2046.
22. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. Lebensm Wiss Technol. 1995; 28: 25-30.
23. Singh R, Jain SC, Jain R. Antioxidant activity of some medicinally important arid zone plants. Asian J Exp Sci. 2009; 23: 215-221.
24. Meir S, Kanner J. Determination and involvement of aqueous reducing compounds in oxidative defense systems of various senescing leaves. J. Agric. Food. Chem. 1995; 43: 1813-1819.
25. Yildirim A, Mavi A. Comparison of antioxidant and antimicrobial activities of *Tilia* (*Tilia Argentea Desf* Ex DC), Sage (*Salvia triloba* L.) and Black tea (*Camellia sinensis*) extracts. J. Agric. Food. Chem. 2000; 48: 5030-5034.
26. Zou YP, Lu YH. Antioxidant activity of a flavonoid rich extract of *Hypericum perforatum* L. *in vitro.* J. Agric. Food. Chem. 2004; 52: 5032-5039.
27. Ames BN, Shigenaga MK, Hagen TM. Oxidants, antioxidants, and the degenerative diseases of aging. Proc Natl Acad Sci. U.S.A. 1993: 90: 7915-7922.
28. Willet WC. Diet and Health: what should we eat. Science. 1994; 254: 532-537.
29. Eberhardt MV, Lee CY, Liu RH. Nutritions Antioxidant activity of fresh apples. Nature. 2000; 405: 903-904.
30. Rapola JM, Virtamo J, Ripatti S, Huttunen JK, Albanes D, Taylor PR, ET AL. Randomised trial of R-tocopherol and β-carotene supplements on incidence of major coronary events in men with previous myocardial infarction. Lancet. 1997; 349: 1715-1720.
31. Shahidi F, Naczk M. Phenolic compounds in grains. In Food Phenolics: Sources, Chemistry, Effects, Applications; Technomic Publishing: Lancaster, PA; 1995.p. 3-39.
32. Hall CA, Cuppett SL. Structure-activities of natural antioxidants. In Antioxidant ethodology *in vivo* and *in vitro* Concepts; Aruoma, O. I., Cuppett, S. L., Eds.; AOCS Press: Champaign, IL; 1997.p. 2-29.
33. Gyamfi MA, Aniya Y. Antioxidant properties of Thon-ningianin A, isolated from the African medicinal herb, *Thonningia sanguine.* Biochem Pharmacol. 2002; 63:1725 –1737.
34. Foti M, Piattelli M, Baratta MT, Ruberto G. Flavonoids, coumarins and cinnamic acids as antioxidants in a micellar system. Structure-activity relationship. J. Agric. Food. Chem. 1996; 44: 497-501.
35. Larson RA. The antioxidants of higher plants. Phytochemistry. 1988; 27 (4): 969-978.
36. Burton GW, Ingold KU. Antioxidation of biological molecules: The antioxidant activity of vitamin E and related chain-breaking phenolic antioxidants in vitro. J Am Chem Soc. 1981; 103: 6472-6477.
37. Doba T, Burton G, Ingold K. Antioxidant and co-antioxidant activity of vitamin C. The effect of vitamin C, either alone or in the presence of vitamin E or a water-soluble vitamin E analogue, upon the peroxidantion of aqueous multilamellar phospholipid liposomes. Biochim Biophys Acta. 1985; 835: 298–303.
38. Sies H, Stahl W. Vitamin E and C, β-Carotene, and other carotenoids as antioxidants. Am J Clin Nutr. 1995; 62:1315S-21S.
39. Foote CS, Denny RW, Weaver L, Chang Y, Peters J. Quenching of singlet oxygen. Ann N Y Acad Sci. 1971; 139-145.