Original Research Article

Evaluation of selected vitamins and antioxidant potential of *Persea americana* seed

ABSTRACT

Persea americana seeds have been used in ethnomedicine as a potent remedy for putative health conditions such as muscular and menstrual pains, hypertension, diabetes mellitus, anaemia, insomnia, hyperlipidaemia, diarrhoea, dysentery, gastric and peptic ulcers. This study investigated the antioxidant scavenging activities of the methanol extract of *Persea americana* seed. The seed samples were collected, dried, ground into powder and extracted in methanol by cold maceration. The antioxidant activity was determined using 2,2-diphenyl-1-picryhydrazyl, lipid peroxidation and reducing power assays. Antioxidant vitamins (A, E and C) and enzymes (superoxide dismutase, peroxidase and catalase) were assayed using absorption spectroscopic methods. There was a significant increase ($p \leq 0.05$) in the DPPH scavenging effect and inhibition of lipid peroxidation activity with increasing extract concentration. There is an increase in spectroscopic absorbance value as the concentration of the sample extract, in the reaction mixture, increases indicating its reducing power ability. The EC_{50} values for DPPH, lipid peroxidation and reducing power assays were 610µg/ml, 640.51µg/ml and 580µg/ml respectively. Vitamin E (267.73 ± 0.07mg/100g) content was the highest among the antioxidant vitamins investigated (Vitamin A (7.60 ± 0.01mg/100g) and C (3.88 ± 0.03mg/100g). The chemical compositions of the investigated samples might be responsible for their medicinal values in phytomedicine. This study shows that *Persea americana* seeds are adequate in maintaining healthy nutrition for preventing the accumulation of destructive free radicals. The radical scavenging abilities exhibited by the seed extract may be attributed to the high polyphenolic content and antioxidant enzymes present.

Keywords: *Persea americana* seeds, antioxidant enzymes, vitamins, DPPH, SOD and peroxidase activities, reactive oxygen species.

INTRODUCTION

Persea is an evergreen tree belonging to the laurel family, Lauraceae (Kopp, 1966). The best-known member of the genus is the avocado, *Persea americana*, widely cultivated in subtropical regions for its large, edible fruit. Avocado seeds have traditionally been used to treat mycoses and parasitic infections, its seed preparations are known to have local anaesthetic effects that decrease muscle pain (Ramos *et al.,* 2004). In Mexico and parts of Africa, decoctions of avocado seeds are used in ethnomedicine as a potent remedy against different diseases such as muscular and menstrual pains, hypertension, diabetes mellitus, anaemia, insomnia, hyperlipidaemia, diarrhoea, dysentery, gastric and peptic ulcers (Adeboye *et al.,* 1999).

Antioxidants are compounds that inactivate the oxygen species/free radicals and, thus, prevent oxidative damage to the cells and body tissues. Plant foods confer numerous health benefits as they combat oxidative stress in the body by maintaining a balance between oxidants and antioxidants. Plant/animal foods contain a variety of nutrient/non-nutrient antioxidants, such as glutathione, vitamin C, vitamin A, vitamin E and phenolic compounds. Humans have evolved highly complex antioxidant systems (enzymatic and non-enzymatic) that work synergistically, and in combination with each other, to protect cells and organ systems against free radical-induced damage (Saeid *et al.,* 2011).

In addition, (Joao *et al.,* 2009) and (Arukwe *et al.,* 2012) have shown that avocado seeds contain bioactive compounds such as flavonoids, phenols, alkaloids, saponins and phytosterols that have tremendous health benefits. These compounds have antioxidant properties that help in preventing and treating putative health diseases such as cancer, atherosclerosis, diabetes, hypertension, Alzheimer's disease and ulcer (Deepti *et al.,* 2013). The seed of *P. americana* has been reported to contain higher antioxidant properties than its pulp and this is due to the presence of high content of phenolic compounds, such as flavonoids, and ascorbic acid (Bertling *et al.,* 2007). This served as a basis for investigating the antioxidant properties of avocado seed with a focus on the analysis of extractable natural products concerning their potential use for pharmaceutical and food applications.

MATERIALS AND METHODS

Sample collection and identification

Avocado pear samples were purchased from Eke Awka Market in Awka, Anambra State. The pear samples were identified by a taxonomist in the Department of Botany, Nnamdi Azikiwe University Awka, Anambra State.

Preparation of sample extract

The seeds were separated from the fruit, washed, chopped in bits, dried at room temperature for ten days, and ground into a fine powder using a manual grinder. The powdered sample was stored in an air-tight container until further analysis. The sample extraction was done by cold maceration as described by (Kumar *et al.,* 2010). The powdered sample (10g) was dissolved in 100% methanol (100 ml) for 24 hours. The mixture was filtered through Whatman paper No. 4 and the filtrate was concentrated over a water bath at 40^{o}C. The concentrated extract was weighed and redissolved in methanol at a concentration of 100mg/ml and stored at 4^{o}C for further analysis.

Determination of antioxidant activity

DPPH scavenging activity

The stable 2,2-diphenyl-1-picryl hydrazyl radical (DPPH) was used for the determination of the free radical scavenging activity of the methanolic extract of *P. americana* seeds. This was assayed using the method of (Ebrahimzadem *et al.*, 2009).

Inhibition of lipid peroxidation using thiobarbituric acid (TBA) reactive substance.

This was determined by the method of (Barros *et al.,* 2008) where the inhibition ratio (%) was calculated using the following formula:

Inhibition ratio (%) = [(A-B)/A] × 100%.

Reducing power assay

The reducing power was determined according to the method of (Oyaizu, 1986). This method is based on the principle of an increase in the absorbance of the reaction mixture. BHA was used as the standard (Barros *et al.,* 2007).

Antioxidant Enzymes Assay

Determination of superoxide dismutase (SOD) activity

Superoxide dismutase activity was determined by its ability to inhibit the auto-oxidation of epinephrine determined by the increase in absorbance at 480nm as described by (Sun and Zigma, 1978).

Determination of catalase activity

Sample catalase activity was determined according to the method of Beers and Sizer as described by (Usoh et al., 2005) by measuring the decrease in absorbance at 240 nm due to the decomposition of H_2O_2 in a UV recording spectrophotometer.

Peroxidase

The method proposed by (Reddy *et al.,* 1995) was adopted for assaying the activity of peroxidase. In the presence of a hydrogen donor, (pyrogallol or dianisidine), peroxidase converts H_2O_2 to H_2O and O_2.

Antioxidant vitamin assay

Analysis of vitamin A was done by the absorption spectroscopic method (Rutkowski *et al.,* 2006).

Determination of vitamin E was done by absorption spectroscopy method (Rutkowski *et al.,* 2005).

Procedure for vitamin C assay: Ascorbic acid content of *P. americana* seed was determined according to (Klein and Perry, 1982).

RESULTS

Result for antioxidant scavenging activity

The result below (fig 1a, 1b, and 1c) showed that methanol extract of *Persea americana* (MEPA) seeds exhibited relatively good antioxidant scavenging potentials. The scavenging potential increase as the concentration of the MEPA increases.

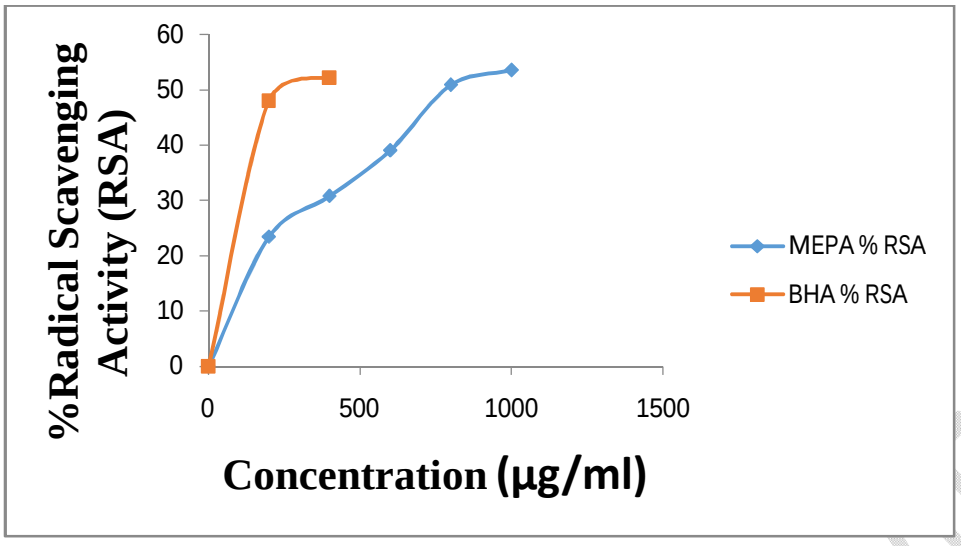

Figure 1a Radical scavenging activity (%) of methanol extract of *P. americana* seed on DPPH radicals.

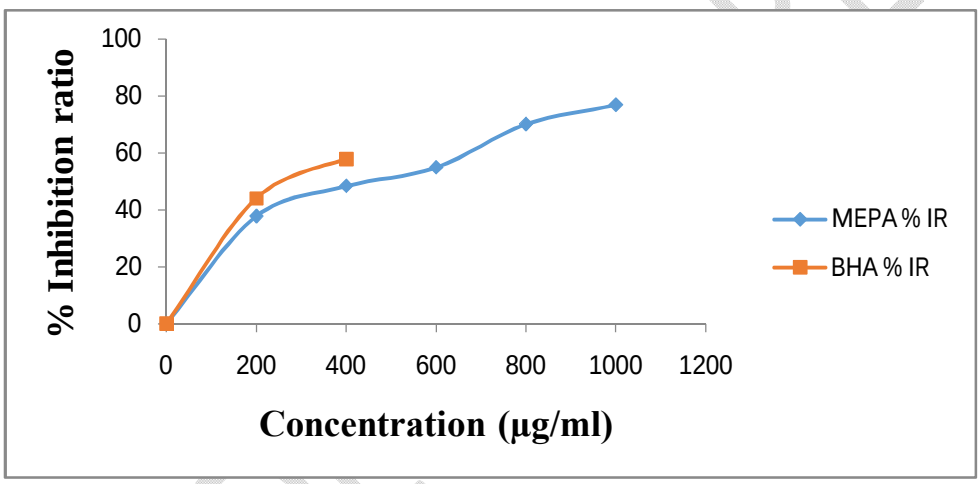

Figure 1b: Inhibition of lipid peroxidation activity (%) of methanol extract of *P. Americana* seed.

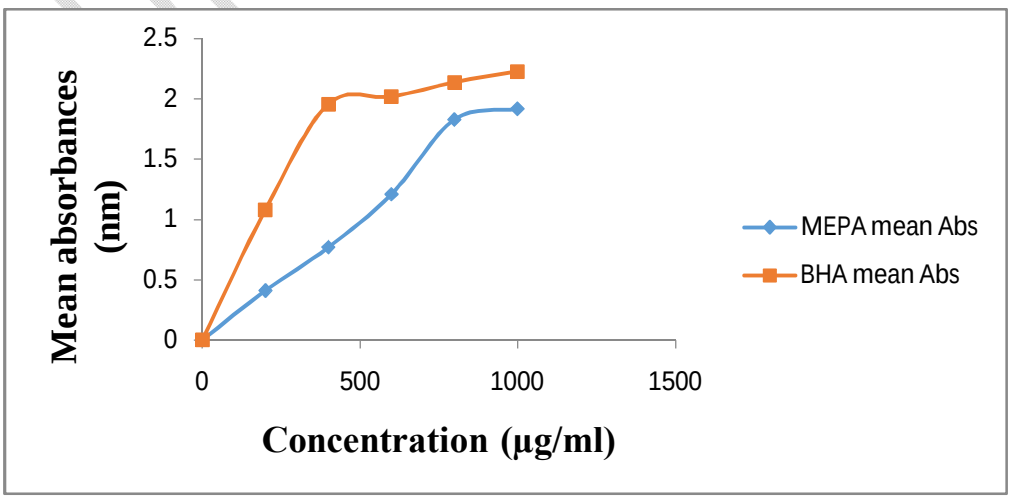

Figure 1c: Reducing Power activity (%) of methanol extract *P. americana* seed.

Result of Antioxidant Enzyme assay

The result as shown below (fig 2) reveals that methanol extract *P. americana* seeds have good peroxidase enzyme activity followed by catalase and SOD has the least enzyme activity.

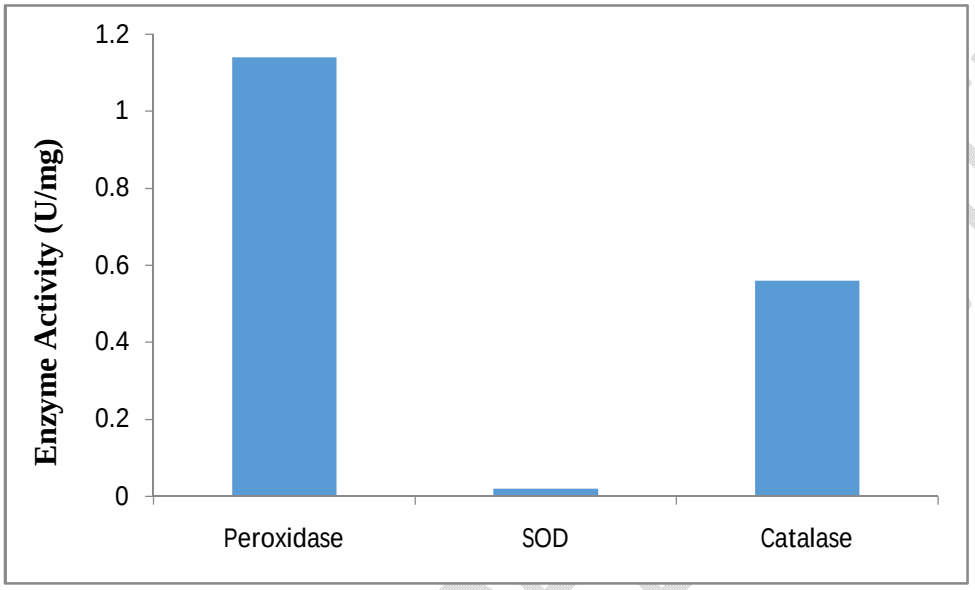

Figure 2: Bar Chart of Enzymatic activity of *P. americana* seed.

Result of the antioxidant Vitamins (A, C, and E) in methanol extract *P. americana* seed

The result (fig 3) reveals that P. americana seed is highly packed with vitamin E and there is also the presence of vitamins A and C.

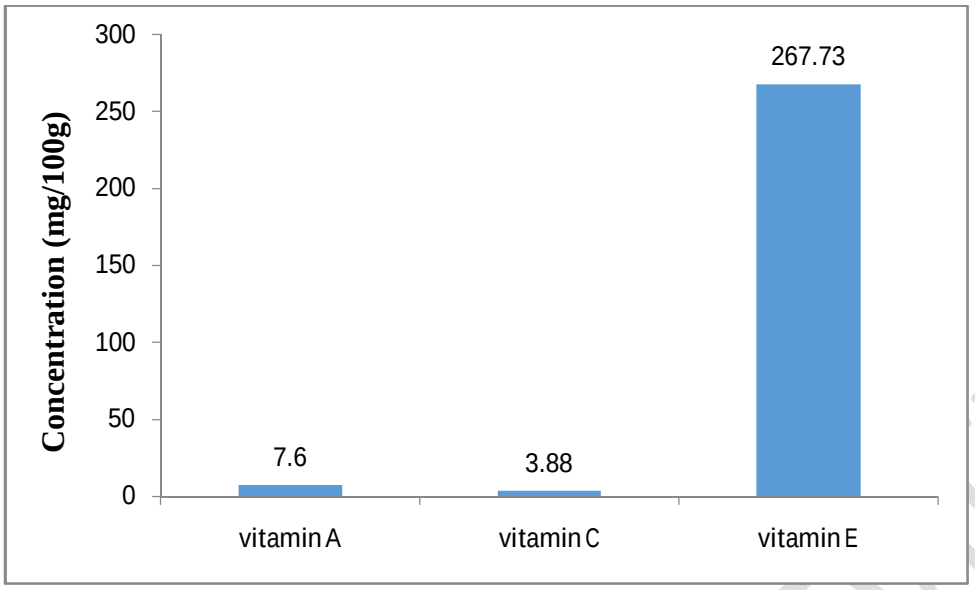

Figure 3: Bar Chart of Vitamin A, C, and E in *P. americana* seed.

DISCUSSION

It was observed in this study, Figure 1a, that the radical scavenging effect of methanol extract of *P. americana* seed on DPPH radicals increased with increasing concentration. Methanol extract of *P. americana* seed presented moderate RSA values with an EC_{50} value of 610µg/ml. This significant scavenging ability in the above extract could be attributed to the presence of phenolic and flavonoids in the methanol extract of *Persea americana* seed. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triple oxygen or decomposing peroxides (Mohammad *et al.*, 2007).

The methanol extract was an effective inhibitor of lipid peroxides generated by membrane lipid peroxidation of goat brain using TBA reactive substance (as shown in fig. 1b). The extract was able to inhibit lipid peroxide formation and the inhibition ratio (IR) for inhibition of lipid peroxidation assay increased with the increased concentration. The methanol extract of *P. americana* seed presented high %Inhibition Ratio values with an EC_{50} value of 640.51µg/ml. For the measurements of reductive ability, it has been found that the Fe^{3+} to Fe^{2+} transformation occurred in the presence of the extracted sample (Oyaizu, 1986).

The reducing properties are usually associated with the presence of reductones (Duh *et al.*, 1999), which have been shown to exert antioxidant action by breaking the free radical chain by

donating a hydrogen atom (Gordon, 1990). The reducing power of the methanol extract of *P. americana* is excellent as it increased steadily with concentration increase (Figure 1c). The high reducing power exhibited by the extract might be indicative of the hydrogen-donating ability of the active species present in the extract (Shimada *et al*., 1992). The EC_{50} value obtained for reducing power was better than the EC_{50} values for RSA and inhibition of lipid peroxidation.

Also, the result as shown in Figure 2 depicts the peroxidase, superoxide dismutase (SODs) and catalase (CATs) activities of *P. americana* seed extract. The presence of these enzymes may explain the traditional use of *P. americana* seed extract for treating ulcers, diabetes, hypertension and cancer (Deepti *et al.,* 2013). The CATs and peroxidases remove H_2O_2 very efficiently (Scandalios, 1993) and SODs scavenge the superoxide anion away from cells. The CATs and SODs are the most efficient antioxidant enzymes. Their combined action converts the potentially dangerous superoxide radical (O^{2-}) and hydrogen peroxide (H_2O_2) to water (H_2O) and molecular oxygen (O_2), thus averting cellular damage (Scandalios, 1993). Thus, the combined action of SOD and CAT abates the formation of the most toxic and highly reactive oxidant, the hydroxyl radical (OH^-), which can react indiscriminately with all macromolecules (Scandalios, 1993).

The result of the Vitamin assay shown in Figure 3 showed that the seed sample also contains an appreciable amount of vitamin E. Vitamin C content value obtained for *Persea americana* raw seed falls within the range of values obtained for its pulp (Morton and Dowling, 1987). Vitamin C is a water-soluble, antioxidant vitamin and heat-labile. The low ascorbic acid value of avocado seeds indicates that the seeds may be a poor source of ascorbic acid when compared to citrus fruits (Adeboye *et al.*, 1999). The vitamin A (β-carotene equivalent) content of the seed sample was reasonable and this may be due to the presence of a good amount of beta-carotene. Both carotenoids and retinoic acids (RAs) are good antioxidants and are capable of regulating transcription factors (El-Agamey *et al.,* 2009). β-Carotene inhibits the oxidant-induced NF-κB activation and interleukin (IL)-6 and tumour necrosis factor-α production. Carotenoids also affect the apoptosis of cells. Antioxidants such as ascorbic acid, carotenoids and tocopherol have been associated with the prevention of nutritionally associated diseases such as cancer, coronary heart disease and obesity (Larrauri *et al.,* 1996). Vitamins A and E are oil-soluble vitamins that are mostly contained within the dry matter of the seed. This is the reason they are not leached into the water used in processing them (Justina *et al.,* 2016).

CONCLUSION

The appreciable antioxidant activities exhibited by *Persea americana* seed extract can be attributed to the presence of antioxidant vitamins (A, E and C) as well as the enzymatic activities of peroxidase and catalase. The study also demonstrated high DPPH scavenging and lipid peroxidation activities relative to standard vitamin C. Therefore, *Persea americana* seeds could be potential sources of natural antioxidants that could have great importance as a therapeutic agent and in preventing or slowing down the process of ageing and age-associated oxidative stress-related diseases.

REFERENCES

Adeboye, J. O., Fajonyomi, M. O., Makinde, J. M. and Taiwo, O. B. (1999). A preliminary study on the hypotensive activity of *Persea americana* leaf extracts in anaesthetized normotensive rats. *Fitoterapia*. **70**(1): 15-20.

Adepoju, O.T. (2004). Studies on nutritional characteristics of shea butter (*Butyrospernum paradox*) fruit pulp. PhD Thesis, Department of Human Nutrition, University of Ibadan, Ibadan, Nigeria.

Arukwe, U., Amadi, B.A., Duru, M. K.C., Agomuo, E.N., Adindu, E. A., Odika, P.C., Lele, K.C., Egejuru, L., and Anudike, J. (2012). Chemical Composition of *Persea americana* leaf, fruit and seed. *International Journal of Research and Reviews in Applied Sciences.* **11**(2): 2-10.

Barros, L., Ferreira, M. J., Queiros, B., Ferreira, I. C. F. R and Batista, P., (2007).Total phenols, ascorbic acid, á-carotene and lycopene in Portuguese wild edible mushrooms and their antioxidant activities, *Food Chemistry*. **103:** 413-419.

Barros, L., Falcâo, S., Baptista, P., Freire, C., Vilas-Boas, M. and Ferreira, I.C.F.R. (2008). Antioxidant activity of *Agaricus sp*. Mushrooms by chemical, biochemical and electrochemical assays. *Food Chemistry*. **111**: 61.

Bertling, I., Tesfay S.Z. and Bower, J.P. (2007). The antioxidant in Hass Avocado. In: South African Avocado Grower Association. pp 17-19.

Deepti, D., Rachel, M. S., Gregory, R. Z. and Joshua, D. L. (2013). Avocado (*Persea americana*) seed is a source of bioactive phytochemicals. *Current Pharmaceutical Design.* **19:** 6133-6140.

Duh, P. D., Tu, Y. Y. and Yen, G. C. (1999). Antioxidant activities of the aqueous extract of harn jyur (*Chrysanthemummorifolium Ramat*). *Lebensmittel-Wissenschaft and Tehnologie.* **32**:269-277.

Ebrahimzadem, M. A., Seyed, M. N., Seyed, F. N., Fatemeh B. and Ahmad, R. B. (2009). Antioxidant and free radical scavenging activity of *H. officinalis, L.angustifolius, V. odorata, B. hyrcana and C. speciosum. Pakistan Journal of Pharmaceutical Sciences.* **23**(1): 29-34.

El-Agamey, A., Lowe, G.M., McGarvey, D.J., Mortensen, A., Phillip, D.M., Truscott, T.G. (2004). Carotenoid radical chemistry and antioxidant/pro-oxidant properties. *Archives of Biochemistry and Biophysics.* **430***:* 37-48.

Gordon, M.H. (1990). The mechanism of antioxidant action in *in-vitro*. In : BJF Hudson Edition, Food antioxidants Elsevier Applied Science, London.

João, J.L., Érika, H.B., Rossana, A.C., Raimunda, S.N., José, J.C., Luciana, M.B., Selene, M.M. and Marcos, G.R. (2009). Chemical composition, toxicity and larvicidal and antifungal activities of *Persea americana* (avocado) seed extracts. *Revista da Sociedade Brasileira de Medicina Tropical.* **42**(2):110-113.

Justina, Y. T., Olukemi, A. O., Ajayi, O. O. and Adegoke, G. O. (2016). Nutritional and antinutritional compositions of processed Avocado (*Persea americana* Mill) seeds. *Journal of Plant Science and Research***. 6**(2):6-12.

Klein, B.P. and Perry, A.K. (1982). Ascorbic acid and vitamin A activity in selected vegetables from different geographical areas of the United States. *Journal of Food Sci*ence. **47**: 941-945.

Kopp, L. E. (1966). A taxonomic revision of the genus *Persea* in the western hemisphere. (*Perseae-Lauraceae*). *Memoirs of The New York Botanical Garden*. **14**(1):1-117.

Kumar, A.K., Ramachandra, S.S. and Narsu, L. (2010). Pharmacognostic and Phytochemical investigation of roots of *Hibiscus micranthus* Linn. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*. **1**(4): 324-337.

Larrauri, J.A., Goni, I., Martin-Carron, N., Ruperez P. and Saura-Calixto, F. (1996). Measurement of health-promoting properties in fruit dietary fibres: antioxidant capacity, fermentability and glucose retardation index. *Journal of the Science of Food and Agriculture*. **71**:515-519.

Mohammad, A., Koji, Y., Toshiki, M., Yomi, N., Katsumi, S., Hiroaki, S. and Yoshifumi, T. (2007). Superoxide anion radical scavenging activities of herbs and pastures in northern Japan were determined using electron spin resonance spectrometry. *International Journal of Biological Sciences*. **3**:349-355.

Morton, J. F. and Dowling, C.F. (1987). Avocado in: Fruits of Warm Climate. Winterville, N.C. pp 91:102.

Oyaizu, M. (1986). Studies on products of browning reactions: antioxidative activities of products of browning reaction prepared from glucosamine. *Japanese Journal of Nutrition*. **44**: 307–315.

Ramos, M.R., Jerz, G., Villanueva, S., Lopez-Dellamary, F., Waibe, R. and Winterhalter, P. (2004). Two glucosylated abscisic acid derivates from avocado seeds (*Persea americana* Mill. Lauraceae cv. Hass). *Phytochemistry*. **65**: 955-962.

Reddy, K.P., Subhani, S.M., Khan, P.A. and Kumar, K.B. (1995). Effect of light and benzyl adenine and dark-treated graving rice (*Oryza sativa*) leaves –changes in peroxidase activity. *Plant Cell Physiology*. **26**: 987-994.

Rutkowski, M., Grzegorczyk, K. and Paradowski M.T. (2005). Colourimetric method of blood plasma total vitamin E determination – the own modification of Tsen method]. *Diagnostic Laboratory*. **41**: 375.

Rutkowski, M., Grzegorczyk, K., Gendek, E. and Kedziora, J. (2006). Laboratory convenient modification of Bessey method for vitamin A determination in blood plasma. *Journal of Physiological Pharmacy*. **57**: 221.

Saeid, G., Alireza, E. and Ismail, L. (2011). Antioxidants in the treatment of diabetes. *Current Diabetes Reviews*. **7**: 106-125.

Scandalios, J.G. (1993). Oxygen stress and superoxide dismutases. *Plant Physiology*. **101**: 7-12.

Shimada, K., Fujikawa, K., Yahara, K., & Nakamura, T. (1992). Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. *Journal of Agriculture and Food Chemistry*. **40**: 945–948.

Sun, M. and Zigma, S. (1978). An improved spectrophotometer assay of superoxide dismutase based on epinephrine antioxidation. *Analytic Biochemistry*. **90**: 81-89.

Usoh, F.I., Akpan, E.J., Etim, E.O. and Farombi, E.O. (2005). Antioxidant actions of dried flower of *Hibiscus sabidariffa* L. on sodium arsenite-induced oxidative stress. *Pakistan Journal of Nutrition*. **4**:135-141.