SOME NUTRITIONAL PROPERTIES AND ANTIOXIDANT ACTIVITY OF AFRICAN LOCUST BEAN (*Parkia biglobosa*) PULP

Arinola Stephen Olanrewaju**, Oje** Opeyemi James and **Omowaye-Taiwo** Omolara Adenike
Department of Food Technology
Federal Polytechnic Ado-Ekiti, P.M.B. 5351 Ado-Ekiti, Ekiti State, Nigeria
Corresponding Author E-mail: lanrearinola@yahoo.com

Abstract
Amino acid and mineral compositions, vitamin A and C contents, and antioxidant activity of African locust bean (Parkia biglobosa) pulp were investigated in this study as a means of evaluating its food and nutraceutical potentials. The nutritional properties, that is, amino acid and mineral element compositions, vitamin A and C contents were determined using standard methods. Total phenolic content, Ferric Reducing Antioxidant Power (FRAP) and 1,1-diphenyl-2-picryl-hydrazyl (DPPH) free radical scavenging activity were used to assess the antioxidant activity of the pulp. All essential amino acids were present in the pulp with a total value (TEAA) of 29.50 mg/100g protein which constituted 34.82% of total amino acids present in the pulp. Sulphur containing amino acids (TSAA) constituted 2.58%; the concentration of total acidic amino acids (TAAA) (34.61%) was higher than that of total basic amino acids (TBAA) (13.49%). Predicted protein efficiency ratio (P-PER) of the pulp was 1.98. The pulp contained 88.09 mg/kg sodium, 10866.40 mg/kg potassium, 1457.64 mg/kg phosphorus, 595.35 mg/kg calcium, 45.86 mg/kg zinc, 70.66 mg/kg iron, 697.45 mg/kg magnesium, 72.60 mg/kg manganese, 1.17mg/100g vitamin A and 32.00 mg/100g vitamin C. Total phenol content of the pulp was 6.03 mg/g; the pulp exhibited considerable ferric reducing antioxidant power (14.08 mg/g) and DPPH free radical scavenging activity (87.07%). These results suggest that African locust bean pulp has potential to serve as a source of nutrients and as health promoting material in foods.

Keywords: African locust bean, amino acid composition, mineral element composition, antioxidant activity, nutraceutical potential.

Received: 07.02.2019   Reviewed: 20.06.2019   Accepted: 24.06.2019

1. INTRODUCTION

Food insecurity has remained a major challenge for many developing countries with the attendant effects on the diet and health of many rural and urban dwellers. Many approaches have been adopted to improve food availability and mitigate the problem of food insecurity. One of the approaches includes exploitation of some lesser known and underutilized plant materials and agricultural by-products (Abdullahi and Abdullahi, 2005). Some of these underutilized plants and agricultural by-products are cheap alternatives that could be used to make value added products because of their inherent chemical composition and functional properties (Bhat and Karim, 2009).
African locust bean (*Parkia biglobosa*) is a legume forest tree crop belonging to the family of *Leguminasae*. The trees are preserved as valuable economic plant because almost all parts of the tree are utilized. The tree is especially valuable for its seeds, which are embedded in yellow pulp inside a brown pod (Arinola *et. al.* 2018). African locust bean pulp is regarded as a by-product during the processing of African locust bean seed to *iru/dawadawa*, a local condiment. Mostly the pulp is washed off as unwanted material, only very few local communities utilized the pulp during the period of seasonal food insecurity. Extensive work has been done on the seed of this plant; however there is little information on the pulp. The nutritional and microbiological characterization of the pulp was reported by Dahouenon-Ahousse *et al* (2012). Arinola *et. al.* (2018) evaluated the physicochemical properties and phytochemical composition of the pulp. The pulp of African locust bean, being a leguminous plant, has appreciable quantity of protein; however the

amino acid composition of the pulp has not been evaluated. Likewise there is scanty information on the mineral composition and antioxidant activity of the pulp, this study was therefore carried out to provide this scientific base line information that could facilitate the use of the pulp as food resource.

2. MATERIALS AND METHODS

2.1 *Sample Preparation*

The pods of African locust bean were harvested from its tree at a location very close to Federal Polytechnic Ado-Ekiti, Nigeria. The pods were split open manually, the pulp with the attached seeds was oven dried at 50^oC for 6 hrs after which the pulp was separated from the seed manually. The pulp was milled, sieved using 0.5mm sieve, packaged in a well-labelled high density polythene bag and stored at refrigerated temperature (4^oC).

2.2 Determination of Amino Acids

The amino acid profile of the pulp was determined using the modified method 982.30 of AOAC (2006) and Danka *et. al.* (2012). The sample was defatted, hydrolysed and then derivatised. The derivatised sample solution that was free of derivatising reagents was made up to 1ml volume in a vial and then injected into Gas Chromatography (HP 6890 Powered with HP ChemStation Rev. A09.01 [1206] software) for amino acids analysis. Hydrogen was used as carrier gas with a flow rate of 1.0 ml/ minute, column dimension was 1.0 m x 0.2 mm x 0.25 µm, initial oven temperature was 110^oC, ramped at 27^oC/minutes to 320^oC and maintained at 320^oC for 5 minutes. The amount of each amino acid present in the pulp was calculated from the net weight and approximate area of each peak (representing each amino acid) and expressed as g/100g protein.

2.3 Estimation of Predicted Protein Efficiency Ratio (P-PER)

The Predicted Protein Efficiency Ratio (P-PER) was calculated from the amino acid composition using the equation developed by Alsmeyer *et. al.* (1974):

P-PER (Predicted Protein Efficiency Ratio) = - 0.468 + 0.454 (Leu) - 0.105 (Tyr).

2.4 Determination of Mineral Elements

Sample (0.5 g) was weighed in triplicate into Kjeldahl flasks and 10 ml concentrated HNO_3 was added and allowed to stand overnight. The samples were then heated carefully until the production of brown nitrogen (IV) oxide fume has ceased. The flasks were cooled and (2-4 ml) of 70% perchloric acid was added. Heating was continued until the solutions turned colourless. The solutions were transferred into 50 ml standard flasks and diluted to mark with distilled water. The mineral content was then analyzed by Atomic absorption spectrophotometer (Perkin-Elmer Model 403, Norwalk, CT, USA). (Falade *et. al.*, 2003)

2.5 Determination of Vitamin A and C

Vitamin A and C contents of the pulp were evaluated according to the procedures described by Rutkowski and Grzegorczyk (2007); absorbance was measured at 335 nm and 700nm respectively. Briefly, for vitamin A, mixture of 1 ml extract of the pulp and 1 ml of 1 M potassium hydroxide was heated in a water bath at 60^oC for 20 minutes and allowed to cool.1 ml of xylene was added, the mixture was shaken for 1 minute and centrifuged (1500xg) for 10 minutes. The absorbance of the supernatant was measured at 335 nm; the supernatant was thereafter exposed to UV light for 30 minutes and the absorbance was measured again at 335 nm. Vitamin A content of the pulp was calculated using multiplier factor of 22.23. For vitamin C, 1 ml extract of the pulp and 1 ml of phosphotungstate reagent were mixed together, allowed to stand for 30 minutes and centrifuged (7000xg) for 10 minutes. The absorbance of the supernatant was measured at 700nm and vitamin C content of the pulp was calculated with reference to the absorbance of standard sample prepared from 1 ml standard solution of vitamin C (56.8 µM).

2.6 Determination of Antioxidant Activity

Total phenol content was determined using the method described by Tesfay *et al*. (2011) with slight modifications**.** Briefly, 1 ml extract of the pulp, 5 ml distilled water, 10 ml of 7% sodium carbonate and 1 ml Folin Ciocalteu reagent were mixed together, the mixture was

incubated for 3 hours in a dark room and absorbance was read at 765 nm. Total phenol was expressed as mg/g gallic acid equivalents.
The method described by Nyau *et. al*. (2015) was used to determine the ferric reducing antioxidant power (FRAP) of African locust bean pulp. The method involves measurement of the ferric reducing ability of the antioxidant compounds in the aqueous extracts of the pulp. The amount of Fe^{2+} produced from the reduction of Fe^{3+} by the extract was calculated from the standard curve prepared from ferrous sulphate solution and result was expressed as mg Fe^{2+} / 100 g dry sample. DPPH (1,1-diphenyl-2-picryl-hydrazyl) radical scavenging assay was done as described by Moodley *et. al*. (2015) with slight modification. The ability to scavenge DPPH radical was calculated using the following equation:

$$\text{Radical scavenging activity (\%)} = [(Abs_{control} - Abs_{sample})/(Abs_{control})] \times 100$$

where: $Abs_{control}$ is the absorbance of DPPH radical + methanol;
Abs_{sample} is the absorbance of DPPH radical + sample extract/standard.

3. RESULTS AND DISCUSSION

3.1 Amino Acids Composition

The amino acid composition of African locust bean pulp is shown in Table 1. The pulp contained 18 amino acids; all essential amino acids are present in the pulp. The pulp contained higher amount of essential amino acids except methionine when compared with the FAO/WHO (2007) requirements for adult. However, comparison with FAO/WHO (2007) essential amino acids requirement for children (2 – 5years) shows that the pulp had lower amounts of threonine, leucine, methionine and phenylalanine. The presence of all essential amino acids in the pulp shows that it could be used to supplement local diet especially in the rural areas where protein malnutrition is endemic. The most abundant essential amino acid in the pulp was leucine with a value of 5.80 g/100g protein followed by lysine 5.73 g/100g protein. The leucine and lysine contents were however lower than the values reported for brown bambara groundnut (Oyeyinka, 2016). Leucine is a major essential amino acid because it stimulates protein synthesis and enhances the ability of the body to build muscle and spare muscle when dieting (Amaechi *et. al*., 2017; Layman and Walker, 2006). Lysine is important for its role in bone formation in children, production of hormone and reduction in serum triglyceride levels (Gersten, 2013). Lysine is a major limiting amino acid in cereals especially rice, millet, wheat; therefore incorporation of the pulp into cereal based foods will help to boost the lysine content of such food. Methionine had the lowest values of 1.00 g/100g protein among the essential amino acids. The most concentrated amino acid in the pulp was glutamic acid with a value of 15.26 g/100g protein followed by aspartic acid with a value of 14.06 g/100g protein. Oyeyinka (2016) and Ogunbusola *et. al*., (2008) reported glutamic acid as the most abundant amino acid in brown bambara groundnut (2.94 g/100g protein) and protein isolates of *Cucumeropsis mannii* seeds (143.20 mg/g protein) respectively.

Table 1: Amino Acid Composition of African Locust Bean Pulp (g/100g Protein)

Amino Acids	Values	FAO/WHO**	
		Child	Adult
Glycine	4.23	-	-
Alanine	4.55	-	-
Serine	4.25	-	-
Proline	4.36	-	-
Valine*	3.7	3.5	1.5
Threonine*	3.18	3.40	0.90
Isoleucine*	3.4	2.80	1.30
Leucine*	5.8	6.60	1.90
Aspartic acid	14.06	-	-
Lysine*	5.73	5.80	1.60
Methionine*	1.00	2.70	1.70
Glutamic acid	15.26	-	-
Phenylalanine*	3.31	6.30	1.90
Histidine*	2.18	1.90	1.60
Arginine	5.58	-	-
Tyrosine	1.74	-	-
Tryptophan*	1.2	1.10	0.50
Cystine	1.19	-	-

*Essential amino acids
**FAO/WHO (2007)

The summary of the amino acid composition is presented in Table 2. The total essential amino acids was 29.50 g/100g protein, this constituted 34.82% of the total amino acid (84.72 g/100g protein) present in African locust bean pulp. The percentage total essential amino acid of the pulp was lower than 52.32% reported for protein isolates of *Cucumeropsis mannii* seeds by Ogunbusola *et. al.* (2008). The total sulphur amino acids was 2.19g/100g protein, this constituted a marginal percentage (2.58%) of the total amino acid. The protein of *Parkia biglobosa* may be acidic due to the fact that acidic amino acids had higher percentage than basic amino acid. This is consistent with the low pH of the pulp reported by Arinola *et. al.* (2018). Acidic, basic and neutral amino acids constituted 34.61%, 15.92% and 49.47% respectively of the total amino acid. The predicted protein efficiency ratio was 1.98. Protein efficiency ratio indicates the capacity of food protein diets to meet the metabolic demand for amino acids and nitrogen in the body, a ratio below 1.5 indicates protein of low to poor quality (Friedman, 1996). With a protein efficiency ratio above 1.5, African locust bean pulp protein is therefore of good quality that can contribute positively to the nutritional needs of the body.

Table 2: Summary of Amino Acid Composition of Pulp (g/100g Protein)

Amino Acid Description	Values
Total Amino Acid (TAA)	84.72
Total Essential Amino Acid (TEAA)	29.50
% TEAA	34.82
Total Non Essential Amino Acid (TNEAA)	55.22
% TNEAA	65.18
Total Suphur Amino Acid (TSAA Met + Cys)	2.19
% TSAA	2.58
Total Acidic Amino Acid (TAAA Asp + Glu)	29.32
% TAAA	34.61
Total Basic Amino Acid (TBAA Arg + Lys + His)	13.49
% TBAA	15.92
Total Neutral Amino Acid (TNAA)	41.91
% TNAA	49.47
Predicted Protein Efficiency Ratio (PPER)	1.98

3.2 Mineral Elements Composition

The mineral composition of the pulp is presented in Table 3.

Table 3: Mineral Element Composition of African Locust Bean Pulp

Parameters	Values (mg/kg)*
Sodium	88.09±0.10
Potassium	10866.40±0.52
Phosphorus	1457.64±0.60
Calcium	595.35±0.32
Zinc	45.86±0.20
Iron	70.66±0.10
Magnesium	697.45±0.36
Manganese	72.60±0.10
Sodium/Potassium Ratio	0.01

**Values are means of triplicate determination ± standard deviation*

Potassium was the most abundant (10866.40 mg/kg) mineral element in African locust bean pulp. This value was however lower than the range reported for cowpea (Aremu *et. al.* 2006). The sodium content of the pulp was 88.09mg/kg. The sodium/potassium ratio in the body has important health implication in the prevention and management of high blood pressure. A ratio of less than one is recommended (Aremu *et. al.* 2006). The sodium/potassium ratio of the pulp was less than one, therefore the pulp may help to reduce incidence of high blood pressure. Potassium and sodium play important role in the maintenance of the osmotic balance of body fluid, regulation of pH of body fluid and blood, and normal function of nerve and muscle. Phosphorus was the second most abundant element. Aside its function in the maintenance of strong and healthy bones, and production of energy; phosphorus helps calcium in many metabolic reactions in the body. The calcium content of the pulp was 595.35 mg/kg; this was higher than the calcium content of brown bambara groundnut (Oyeyinka, 2016). Calcium is an important mineral constituent of bone and teeth; it also helps in blood clotting and in some enzymatic metabolic processes (Adeyeye and Fagbohun, 2005). The zinc and iron contents of the pulp were 45.86 and 70.66

mg/kg respectively; the two trace elements have some important metabolic functions in the body. Iron is the major mineral constituent of heamoglobin necessary for transportation of oxygen to body cells, zinc on the other hand is vital for the immune system of the body. Adequate intake of essential mineral elements including magnesium and manganese have been reported to promote health, growth and development of children and adults (Ivanovic *et. al*., 2002).

3.3 Vitamin A and C

Table 4 shows that African locust bean pulp contains 1.71 mg/100g vitamin A. This value was higher than 0.67 mg/100g reported for the leaves of sweet potato (Antia *et. al.*, 2006). When compared with recommended daily dietary reference intake African locust bean pulp can satisfactorily meet the daily vitamin A dietary need of both man and woman. Thus the pulp can help reduce incidence of deficiency of vitamin A which is of public health significance in the developing world. The vitamin C content of the pulp was higher than the range (4.5 – 12.7 mg/100g) reported for banana but lower than the range (45.3 – 55.6 mg/100g) reported for pawpaw (Wall, 2006). However, an average male would have to consume about 300g of the pulp to meet the dietary reference intake. Both vitamins, which are antioxidant vitamins, play various important metabolic roles in the body.

Table 4: Vitamin A and C Contents of African Locust Bean Pulp

Vitamins	Values (mg/100g) *	Dietary Reference Intake (DRI)** Adult Male	Dietary Reference Intake (DRI)** Adult Female
Vitamin A	1.71±0.03mg/100g	0.9mg/day	0.7mg/day
Vitamin C	32.00±0.72mg/100g	90 mg/day	75mg/day

**Values are means of triplicate determination ± standard deviation*
***Dietary recommendations established by the US Food and Nutrition Board of the IOM (2000, 2001).*
***Values are for ages 19–50 years.*

3.4 Antioxidant Activity

The total phenolic compounds of the pulp was 6.03 mg/g, phenolic compounds are generally known to possess antioxidant property due to the reactivity of the hydroxyl substituent on the aromatic rings, the hydrogen atoms from the hydroxyl groups can be given up to radicals to form a stable phenoxyl radicals (Rafat *et. al.* 2010). The antioxidant ability of the pulp may be linked to the phenolic compounds inherent in the pulp; determination of quantity of phenolic compounds is thus relevant in evaluating the antioxidant capacity of the pulp. Phenolic compounds can also inhibit auto-oxidation of unsaturated lipids, thus preventing the formation of oxidised low-density lipoprotein (LDL), which is considered to induce cardiovascular disease (Amic *et al.*, 2003). The FRAP result shows that the pulp has considerable ferric reducing ability (14.08 mg/g), this suggest that the sample may act as reducing agent by donating an electron to free radical and neutralize it, thereby reducing the capacity of the free radical to cause damage (Lobo *et. al*., 2010). The DPPH radical scavenging ability of the aqueous extract of the pulp was 87.07%, this indicate that the sample had good ability to convert DPPH radical to a stable diamagnetic DPPH-H molecules by donating an electron or hydrogen. DPPH assay is a preferred method of determining the radical scavenging ability of antioxidant due to its reliability and non laborious nature (Aksoy *et al*., 2013). The DPPH radical scavenging ability of African locust bean pulp was lower than 92.81% reported for vitamin E, a vitamin with a well known capacity to scavenge free radicals (Garcia *et. al.,* 2012). Reactive oxygen species and free radicals produced during body metabolism have been identified to cause some functional damages which have serious health implication in the body (Oboh *et. al*., 2015). Presence of antioxidants in food consumed by human will therefore help to scavenge these highly reactive pro-oxidants and protect the body against some of these damages. These results show the potential of African locust bean pulp to serve as a dietary source of antioxidants.

Table 5: Antioxidant Activity of African Locust Bean Pulp

Parameters	**Values***
Total Phenol (mg/g)	6.03±0.21
FRAP (mg/g)	14.08±0.15
DPPH (%)	87. 07±1.01

**Values are means of triplicate determination ± standard deviation.*

4. CONCLUSION

This study has shown that African locust bean pulp has appreciable amino acids and mineral elements profiles with good antioxidant activity that may qualify it for use in food application. The utilization of this agricultural by-product in the production of nutritious and health promoting foods should therefore be promoted.

Acknowledgements
The authors are grateful for the kind assistance of Oluwadare G. B. and Fele M. T.

5. REFERENCES

[1]. Abdullahi S. A. and Abdullahi G. M. Effect of boiling on the proximate, anti-nutrients and amino acid composition of raw Delonix regia seeds. Nigerian Food Journal, 2005; 23: 128-132.

[2]. Bhat R. and Karim A. A. Exploring the nutritional potential of wild and underutilized legumes. Compr. Rev. Food Sci. Food Saft, 2009; 8: 305–331.

[3]. Arinola S. O., Oje O. J. and Omowaye-Taiwo O. A. Evaluation of physicochemical properties and phytochemical composition of African locust bean (Parkia biglobosa) pulp. In the Proceedings of the 4th Regional Food Science and Technology Summit (ReFoSTS), Federal University of Technology, Akure, Ondo State, Nigeria. 6th – 7th June, 2018: 134.

[4]. Dahouenon-Ahoussi E., Adjou E. S., Lozes E., Yehouenou L. L., Hounye R., Famy N., Soumanou M. M. and Sohounhloue D. C. K. Nutritional and microbiological characterization of pulp powder of locust bean (Parkia biglobosa Benth.) used as a supplement in infant feeding in Northern Benin. African Journal of Food Science, 2012; 6(9): 232-238.

[5]. AOAC. Official Methods of Analysis of the Association of Analytical Chemists 18th edition. Association of Analytical Chemists, Washington D. C, USA. 2005.

[6]. Danka P. O., Dobrinna D. T. and Kalin T. I. Simultaneous identification and determination of total content of amino acids in a food supplements-tablets by gas chromatography. Asian Journal of Pharmaceutical and Clinical Research, 2012; 5: 57-68.

[7]. Alsmeyer R. H, Cunningham A. E. and Happich M. L. Equation to predict protein efficiency ratio (PER) from amino acid analysis. Food Technology, 1974; 28:34-38.

[8]. Falade O. S, Sowunmi O. R., Oladipo A, Tunbosun A. and Adewusi S. R. A. The Level of organic acids in some Nigerian fruits and their effect on mineral availability in composite diets. Pakistan Journal of Nutrition, 2003; 2: 82-88.

[9]. Rutkowski M. and Grzegorczyk K. Modifications of spectrophotometric methods for antioxidative vitamins determination convenient in analytic practice. Acta Sci. Pol., Technol. Aliment. 2007; 6(3): 17-28.

[10]. Tesfay S., Bertling I., Odindo A., Workneh T. S. and Mathaba N. Levels of anti-oxidants in different parts of moringa (Moringa oleifera) seedling. African Journal of Agricultural Research, 2011; 6 (22): 5123-5132.

[11]. Nyau V., Prakash S., Rodrigues J and Farrant J. Antioxidant activities of bambara groundnuts as assessed by FRAP and DPPH assays. American Journal of Food and Nutrition, 2011; 3 (1): 7-11.

[12]. Moodley T., Amonsou E. O. and Kumar S. Nutritional quality and acceptability of Buddleja saligna herbal tea. Journal of Food Science and Technology, 2015; 52 (11): 7519-7524.

[13]. FAO/WHO. Protein and amino acid requirements in human nutrition. Report of a Joint WHO/FAO/UNU Expert Consultation, WHO technical report series no. 935. Geneva, Switzerland: World Health Organization. 2007

[14]. Oyeyinka A. T. Nutritional, sensory and functional properties of a bambara groundnut complementary food. Ph.D. Thesis, Department of Dietetics and Human Nutrition. University of KwaZulu-Natal, Pietermaritzburg, South Africa. 2016.

[15]. Amaechi N. C., Okorie O. and Udeogu E. Amino acid composition, amino acid scores and predicted protein efficiency ratio of raw and cooked African yam bean (Sphenostylis sternocarpa). Journal of Applied Life Sciences International, 2017: 13(2): 1-10.

[16]. Layman D. K and Walker D. A. Potential importance of leucine in treatment of obesity and the metabolic syndrome. Journal of Nutrition. 2006; 136(1):319S–323S.

[17]. Gersten D. The 20 amino acids: What they are and how they keep you alive and vibrant. The Gersten Institute for Higher Medicine, San Diego, CA, USA. 2013.

[18]. Ogunbusola, E. M. Fagbemi, T.N. and Osundahunsi, O. F. Amino acid composition of Cucumeropsis mannii seed flour. In the Proceeding of 32nd Annual Conference of NIFST., 2008: 244-245.
[19]. Friedman M. C. Nutritional value of protein from different food sources: A review. Journal of Agriculture and Food Chemistry 1996; 44(1):6-29.
[20]. Aremu M. O., Olaofe O. and Akintayo E. T. Compositional evaluation of cowpea (Vigna unquiculata) and scarlet runner bean (Phaseolus coccineus) varieties grown in Nigeria. Journal of Food Agriculture and Environment, 2006; 4(2): 39-43.
[21]. Adeyeye, E. I. and Fagbohun, E. D. Proximate, mineral and phytate profiles of some selected spices found in Nigeria. Pakistan Journal of Science and Industrial Research 2005; 48(1):14-22.
[22]. Ivanovic DM, Leiva BP, Pérez HT, Almagia AF, Toro TD, Urrutia MSC, Inzunza NB, Bosch E. O. Nutritional status, brain development and scholastic achievement of Chilean high-school graduates from high and low intellectual quotient and socio-economic status. British Journal of Nutrition, 2002; 87 (01): 81-92.
[23]. Antia B. S., Akpan E. J., Okon P. A. and Umorn I. U. Nutritive and antinutritive evaluation of sweet potatoes (Ipeoma batatas) leaves. Pakistan Journal of Nutrition, 2006; 5 (2): 166 – 168.
[24]. Wall, M. M. Ascorbic acid, vitamin A, and mineral composition of banana (Musa spp) and papaya (Carica papaya) cultivars grown in Hawaii. Journal of Food Composition and Analysis, 2006; 19: 434–445.
[25]. Institute of Medicine (IOM). Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids. National Academy Press, Washington, DC. 2000.
[26]. Institute of Medicine (IOM). Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. National Academy Press, Washington, DC. 2001.
[27]. Rafat A., Philip K. and Muniandy S. Antioxidant potential and phenolic content of ethanolic extract of selected Malaysian plants. Research Journal of Biotechnology, 2010; 5, 16–19.
[28]. [28] Amic D., Davidovic-Amic D., Beslo D. and Trinajstic N. Structure-radical scavenging activity relationship of flavonoids. Croatia Chem. Acta., 2003; 76: 55-61.
[29]. Lobo V., Patil A. and Chandra N. Free radicals, antioxidants and functional foods: Impact on human Health. Pharmacognosy Reviews, 2010; 4(8): 118 – 126.
[30]. Aksoy L., Kolay E., Agilonu Y., Aslan Z. and Kagioglu M. Free radical scavenging activity, total phenolic content, total antioxidant status and total oxidant status of endemic Thermopsis turcica. Saudi Journal of Biological Sciences, 2013; 20: 235 – 239.
[31]. Garcia E. J., Oldoni T. L. C., Alencar S. M., Reis A., Loguercio A. D. and Grande R. H. M. (2012): Antioxidant activity by DPPH assay of potential solutions to be applied on bleached teeth. Brazilian Dentition Journal, 2012; 23 (1): 22 – 27.
[32]. Oboh G., Ademosun A. O., Akinleye M., Omojokun O. S., Boligon A. A. and Athayde M. L. (2015) Starch composition, glycemic indices, phenolic constituents, and antioxidative and antidiabetic properties of some common tropical fruits. Journal of Ethnic Foods, 2015; 2: 64 – 73.