

Amino Acids, Vitamins and Other Nutritional and Anti-nutritional Components of *Cola lepidota* (Monkey Kola)

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Received: April 28, 2017; **Accepted:** June 2, 2017; **Published:** August 30, 2017

Keywords

Malvaceae, Monkey Kola, *Cola lepidota*, Amino Acids, Vitamins, Mineral Elements, Proximate Composition, Anti-nutrients

The study examined, for the first time, amino acids and vitamins composition of the yellow aril, seed and fruit epicarp of *Cola lepidota*. The mineral elements, proximate and anti-nutritional factors were also determined using standard procedures. The total essential amino acids ranged from 20.91-33.77 g/100 g, predominated in leucine, valine, phenyl alanine and lysine. The fruit pulp and epicarp contained substantial amount of ascorbic acid (6.50 and 6.14 mg/100 g) and tocopherols (5.94 and 4.83 mg/100 g) respectively including B and K vitamins. Analysis revealed higher levels of Na, Ca, Mg, Zn, Cu and Mn in the fruit epicarp; iron and potassium in aril and seed respectively. The samples were enriched in proximate content and anti-nutritional factors (phytate, oxalates, cyanide and tannins) were below permissible limits. These findings indicate the rich nutritional potential of this tasty fruit and further processing into other value added products would encourage conservation, preservation and reversal of imminent extinction of this under-utilized *Cola* specie.

Introduction

Fruit is an important component of a balanced diet as they are natural sources of food nutrient needed by human and animals. Such food nutrient includes protein, vitamins, carbohydrate, minerals and dietary fiber. There is global attraction on increased food production and impressed on provision of nutritive food for the world population [1], it is of essence to consider our locally available fruits and to determine their nutrient composition for the integral aim of increasing the production of such fruits. Edible seeds are endowed with high contents of lipids, proteins, dietary fiber and ashes (minerals); they also contain a good essential amino acids profile, usually with a low lysine deficiency [2]. African native fruits can aid greater contribution to nutrition, health and economic development of several countries of the world, given renewed scientific and institutional framework [3, 4].

The plant under investigation, *Cola lepidota*, is commonly known as “Monkey kola”. Monkey kola is a collective nomenclature for a number of minor relatives of the *Cola* spp that bear edible tasty fruits. Native people of southern Nigeria and the Cameroon eat the fruits, as well as some wild primate animals such as monkeys and baboons. They are members of the Malvaceae and sub-family Sterculioideae alongside the familiar West African kola nuts (*Cola nitida*), grown for their masticatory and stimulating nuts [5, 6].

The caulescent follicles of *C. lepidota* is comprised of one or two seeds hinge on the fruit length (3.2-8.2 cm), ovoid or semi-spheroid shape. Finely pubescent and scaly rough dark brown exocarp characterized follicle. Seeds (reddish brown or green in colour) are obliquely ovate with two flattered rough surfaces. The yellowish aril (waxy mesocarp) form the edible part of the follicle (Figure 1). *C. parchycarpa* possess relatively the most prominent fruit sweetness taste, followed by *C. lepidota* and *C. lateritia* [7]. Proximate, antinutrients, mineral elements analyses and antioxidant activity of *C. lepidota*, *C. parchycarpa* and *C. lateritia* fruits' pulp have been reported [8-12]. The nutritive value of juice and jam produced from the pulp of the monkey kola fruits have been investigated [13, 14]. Fabunmi & Arotupin [15] suggested from their findings that the husk and

white shell of slimy kola nut (*C. verticillata*) could be used as a blend in animal feed. Research also revealed that about 50% of kola nut husk meal could replace maize diets of rabbits [16].

The assessment, identification, characterization, and domestication of various neglected and underutilized species have been highlighted as significant toward the conservation and prevention of continuous exploitation as well as extinction of rich plant genetic diversity [17]. Therefore, in continuation of our research on the nutritional composition of the poorly studied and underutilized plants [18], this work provides the first report on the characterization of the amino acids and vitamins, in addition to the mineral elements, proximate and anti-nutrients profiles of aril, seed and fruit epicarp of the endangered plant, *C. lepidota*.



Figure 1. *Cola lepidota* fruit pulp and epicarp.

Materials and Methods

Sample Collection and Identification

The fruits of *C. lepidota* were purchased from a local market in Essien Udim Local Government Area, Akwa Ibom State, in July 2015. The plants were identified and authenticated by M. E. Bassey, a taxonomist in the Department of Botany and Ecological Studies, University of Uyo, Nigeria where voucher specimens were deposited.

Determination of Amino Acid

The amino acid profile was determined using the method described by Benitez [19]. The sample was dried to constant weight and defatted using Soxhlet extractor. The defatted sample (2 g) was weighed into a glass ampoule; 7 mL of 6 M HCl was added and oxygen was expelled by passing nitrogen into the glass ampoule sealed with Bunsen burner flame and placed in an oven at $105\pm 5^{\circ}\text{C}$ for 22 hours. The ampoule was allowed to cool before broken at the tip and the content was filtered to remove the organic matters. The filtrate was then evaporated to dryness at 40°C in a hot air oven. The residue was dissolved in 5 ml of acetate buffer (pH 2.0) and stored in specimen bottles which were kept in the freezer. The hydrolysate (7.5 μL) was dispensed into the cartridge of the Technicon Sequential Multi-Analyser (TSM) using a syringe. The TSM analyser is designed to separate and analyse neutral, acidic and basic amino acids of hydrolysate. The amount of amino acids was obtained from the chromatogram peaks. The whole analysis lasted for 76 minutes and the gas flow rate was 0.50 mL per minute at 60°C with reproducibility consistent within $\pm 3\%$.

Vitamins Determination

The vitamins profile of the samples were analysed by methods of AOAC [20] with slight modifications. The sample (0.1 g) was extracted and concentrated to 1.0 ml for chromatographic analysis.

Chromatographic conditions: Analytical column, 30 m x 0.25 mm x 0.25 μm HP 5; oven program, initial at 50°C for 2 mins; first ramp at $10^{\circ}\text{C}/\text{min}$ for 20 mins, maintain for 4 mins; second ramp at $15^{\circ}\text{C}/\text{min}$ for 4 mins, constant for 2 mins; injector temperature: 250°C , 20:1 split ratio; temperature of PFPD detector: 320°C ; carrier gas, nitrogen; flow rate: 1.0 ml/min. The vitamins were identified by comparing their retention times to those of a standard mixture of vitamins and the peak areas were integrated.

Mineral Element Composition

The elemental composition of the samples was determined following the method of Idouraine *et al.* [21]. The sample (1 g) was dried-ashed in a muffle furnace at 550°C for 5 h until a white ash was obtained. The minerals were extracted from ash by adding 3 ml of concentrated HNO₃ (63%). The digest was carefully filtered into 100 mL standard bottle and made up to mark with distilled water. Minerals elements (Na, K, Ca, Mg, Zn, Cu, Mn and Fe) were estimated with the use of a flame photometer (Jenway model PFP7) and atomic absorption spectrophotometer (Perkin Elmer model 703, USA). The instruments were calibrated with standard solutions containing known amounts of the minerals being determined, using analytical reagents.

Proximate Compositional Analysis

Protein, crude fibre, fat, moisture and ash contents of the samples were determined by the method of AOAC [20]. Carbohydrate content was calculated by difference [22].

Determination of Anti-nutritional Factors

Extraction and precipitation of phytates were carried out through phytic acid determination using the procedure described by Lucas & Markaka [23]. Tannin values were obtained by adopting the method of Jaffe [24]. The alkaline picrate method [25] was used for cyanogenic glycoside determination while oxalates content was determined using titration methods [26].

Results and Discussion

The amino acid profile of *C. lepidota* is presented in Table 1. Analysis revealed the relative abundance of essential amino acids in the seeds and fruit epicarp. The dominant occurrence of constituents followed the order: leucine (3.81-6.98 g/100 g), valine (3.59-4.5 g/100 g), phenyl alanine (3.08-5.11 g/100g) and lysine (2.94-4.51 g/100 g). Glutamic acid and aspartic acid were the major non-essential amino acids in the samples. Eleyinmi *et al.* [27] reported that *Garcinia kola* hulls contain higher amount of the essential amino acids, lysine, leucine and valine, compared with the seeds. The identified content of essential amino acids in this study is comparable with the reported content in *C. acuminata* [28], however higher in content than *G. kola* (112.90 mg/g) [28] and *G. kola* seed and hull (11. 10 g/kg and 28.0 g/kg respectively) [27]. Amino acids are the building blocks for proteins, and in the free state, they play a significant function in the taste and sensory expression of numerous foods [29]. Glycine and alanine bestow sweetness, while valine is bitter and glutamine furnish umami [30]. This may account in part for the relative low content of valine in the sweet pulp of *C. lepidota* in this study compared with the seed. The essential amino acids (EAA) to non-essential amino acids (NEAA) ratio was 0.43, 0.41 and 0.40 respectively for aril, seed and fruit epicarp; this is comparable with gonads of sea urchin, *Stomopneustes variolaris*, (EAA:NEAA, 0.5). Usually in marine foods, EAA:NEAA greater than 0.5 indicates a useful source of dietary proteins [31].

The yellow aril of *C. lepidota* contained high amount of vitamin B1 (0.249 mg/100g), B2 (0.125 mg/100 g), B3 (0.940 mg/100 g), B6 (0.235 mg/100 g), B9 (0.174 mg/100 g), C (6.497 mg/100 g) and E (5.936 mg/100 g) than the seed and epicarp; vitamin A (0.004 mg/100 g), B5 (0.49 mg/100 g) and K (0.002 mg/100 g) were found predominant in the seed compared with other samples. However, the fruit pericarp showed major constituents in vitamin B3 (0.654 mg/100 g), B6 (0.164 mg/100 g), B9 (0.103 mg/100 g), C (6.137 mg/100 g) and E (4.826 mg/100 g) compared with the seed (Table 2). A relative narrow spectrum of vitamins profile of juice and jam developed from *C. lepidota* and *C. parhycarpa* fruit pulps [13, 14] reveal lower concentrations of vitamin B1 and B2 compared to the unprocessed fruit in this study. The observed differences in vitamins level in both studies, may be attributed to processing. Vitamins are relatively labile and can be destroyed during processing and storage of food [32]. The juice obtained from the yellow pulp specie was also reported to contain higher levels of vitamins than the white pulp juice [13]. Vitamins are a broad group of organic compounds that are minor, but significant components of food required for normal growth, self-maintenance, and functioning of human and animal systems. They play diverse specific and indispensable functions in metabolism, and their deficiency produces specific diseases [32].

Table 1. Amino acids composition (g/100 g) of *Cola lepidota*.

Amino acid	Fruit pulp	Seed	Fruit pericarp
Lysine	2.94	4.12	4.51
Threonine	2.61	3.52	3.58
Cysteine	0.97	1.52	1.52
Valine	3.59	4.50	3.65
Methionine	0.59	1.69	1.34
Isoleucine	1.83	3.29	3.59
Leucine	3.81	6.98	6.81
Tyrosine	1.49	3.31	2.65
Phenylalanine	3.08	4.84	5.11
Total (EAA)	20.91	33.77	32.76
Histidine	1.30	2.98	2.86
Arginine	3.66	5.62	7.40
Aspartic acid	5.65	9.53	10.10
Serine	3.00	3.20	3.41
Glutamic acid	6.11	15.21	13.57
Proline	1.97	3.48	3.36
Glycine	2.51	4.20	4.20
Alanine	2.98	3.48	4.10
Total (NEAA)	27.18	47.7	49.0
Total Amino Acids	48.09	81.47	81.76
EAA/NEAA	0.43	0.41	0.40

EAA, essential amino acids; NEAA, non-essential amino acids; ° Values are means of three replicate readings.

Table 2. Vitamins composition (mg/100 g) of *Cola lepidota*.

Vitamin	Fruit pulp	Seed	Fruit pericarp
Retinol (vitamin A)	0.000269	0.004305	0.000201
Thiamine (vitamin B1)	0.249287	0.152234	0.108706
Riboflavin (vitamin B2)	0.124707	0.069144	0.046952
Nicotinamide (vitamin B3)	0.939537	0.547934	0.654485
Pantothenic acid (vitamin B5)	0.000003	0.489768	0.000003
Pyridoxine/pyridoxal hydrochloride (vitamin B6)	0.235147	0.138230	0.163988
Folic acid (vitamin B9)	0.174020	0.000046	0.103248
Ascorbic acid (vitamin C)	6.496900	0.527443	6.136570
Tocopherol (vitamin E)	5.935880	3.713580	4.826340
Phylloquinone (vitamin K1)	0.000012	0.001924	0.000006

The mineral elements content of *C. lepidota* aril, seed and fruit pericarp are presented in Table 3. Analysis revealed higher levels of Na, Ca, Mg, Zn, Cu and Mn in the fruit epicarp compared with the pulp and seed; however, potassium was below detection limit. Interestingly, the aril contained significant amount of essential mineral elements (especially Fe and Mg), and a relative low amount of sodium (1725 mg/Kg); this fruit part is cherished as food which is an added advantage due to the direct relationship of sodium intake with hypertension in human [33]. The zinc content could mean that the plants can play a valuable role in the management of diabetes, which results from insulin malfunctioning. Zinc is significant for the production of insulin, a hormone and carbonic anhydrase, an enzyme in the body [34]. The relative high concentration of calcium (5000 mg/Kg) in the fruit epicarp could be implicated in the maintenance of firmness of fruits [35] and its requirements in fruits are related to cell wall stability and membrane integrity [36]. The rich Ca and Mg content of *C. lepidota* fruit epicarp could be exploited in animal feed blends with nutrient requirements in Ca and Mg. Eneobong *et al.* [12] also indicated that calcium and magnesium were the most abundant minerals in the fruit pulp of *C. parhycarpa* and *C. lepidota*. High amount of some essential minerals in the endocarp of *C. lepidota* relative to the exocarp has been documented by Osabor *et al.* [11].

Table 3. Mineral elements composition of *Cola lepidota* (mg/Kg).

Minerals	Fruit pulp	Seed	Fruit pericarp
Na	1725	1135	1835
K	730	1375	BDL
Ca	250±0.03	125±0.007	5000±0.03
Mg	59400±0.02	59850±0.02	88650±0.013
Zn	41.0±0.07	23.5±0.12	55.5±0.13
Cu	11.0±0.006	7.50±0.005	18.0±0.003
Mn	29.5±0.61	30.0±0.003	36.5±0.04
Fe	337±0.18	87.5±0.02	170±0.004

BDL=Below Detection Limit

The proximate analysis of *C. lepidota* seed, fruit pulp and epicarp is indicated in Table 4. Moisture content was highest in

the pulp (55.0%) and ash content in both pulp and fruit epicarp which indicates the presence of some nutritionally important mineral elements. The result also revealed a significant amount of carbohydrate (80.68-86.07%) and protein (6.12-8.28%) compared with previous reported data [8, 37]. Ogbu *et al.* [8] showed that *C. lepidota* waxy aril contain moisture (82.6 g/100 g), fibre ash (1.58 g/100 g), carbohydrate (25.8 g/100 g) and crude protein (1.75 g/100 g). The yellow aril and seed of *C. lepidota* contain moisture (10.14 & 6.08%) and ash (3.87 & 2.48%) respectively [37]. Research has shown that the moisture content of plant foods depends on factors such as, harvesting time, maturation period and environmental conditions: humidity and temperature in growing period, and storage conditions [38]. Lipid content varied from 0.73% (fruit epicarp) to 1.53% (fruit pulp) and calculated energy values (362.27-381.81 kCal/100 g) in the present study. Lipid compounds such as free fatty acids, tri-, di- and monoglycerides, phospholipids, tocopherols, sterols and derivatives can be isolated from plant tissues as crude fat [38]. Nwuisuator *et al.* [37] observed that proximate contents were higher in the yellow arils compared with the seeds of *C. lepidota*, except for fats and carbohydrates. A relative lower proximate content is also documented for juice developed from *C. paryhycarpa* and *C. lepidota* pulp [13].

Table 4. Proximate composition of *Cola lepidota*.

Parameter	Fruit pulp	Seed	Fruit pericarp
Moisture (%)	55.00±0.50	24.80±0.22	30.50±0.05
Ash (%)	4.53±0.025	2.84±0.015	5.90±0.00
Fibre (%)	3.47±0.025	2.44±0.038	4.41±0.021
Protein (%)	6.12±0.185	8.05±0.350	8.28±0.404
Lipid (%)	1.53±0.026	0.59±0.021	0.73±0.115
Carbohydrates (%)	84.33±0.165	86.07±0.385	80.68±0.230
Caloric value (kCal/100 g)	375.31±0.719	381.81±0.141	362.27±0.580

The anti-nutrients analysis revealed the presence of phytates (14.2-30.77 mg/100 g) and total oxalates (66.0-133.8 mg/100 g); the cyanide (0.2-0.81 mg/100 g) and tannins (0.15-1.52 mg/100 g) levels were relatively low (Table 5). The total oxalate matrix was found to contain more soluble portions compared with the insoluble components. A number of researchers report soluble and insoluble oxalate as separately measurable components of the oxalate content of foods [39, 40]. In foodstuff, oxalic acid is essentially found as either sodium or potassium oxalate, which are water soluble, or calcium oxalate, which is insoluble. Magnesium oxalate is also poorly soluble in water, although the contribution of this salt to the insoluble fraction of oxalate in food is uncertain. The tendency of a specific food to raise urinary oxalate rely both on oxalate content and efficiency of absorption, because it has been verified that little oxalate catabolism occurs after absorption and >90% of absorbed oxalate can be recovered in the urine within 24–36 h [41]. Osabor *et al.* [11] reported lower levels of cyanide, phytates and soluble oxalates in the fruit endocarp and exocarp of *C. lepidota* compared with our present findings; however, below the permissible toxic levels [42] and indicate probable lack of interference with the availability of mineral elements.

Table 5. Anti-nutrients composition (mg/100 g) of *Cola lepidota*

Anti-nutrient	Fruit pulp	Seed	Fruit pericarp
Cyanide	0.80±0.001	0.81±0.00	0.20±0.001
Phytates	14.20±0.006	17.59±0.002	30.77±0.021
Tannins	0.63±0.002	1.52±0.004	0.15±0.00
Soluble oxalates	79.20±0.00	39.60±6.222	74.80±6.222
Insoluble oxalates	22.00±0.020	26.40±0.00	59.00±14.99
Total oxalates	101.20±0.020	66.00±6.222	133.80±14.99

Conclusions

Cola lepidota seed, fruit endocarp and epicarp contain substantial amount of amino acids, vitamins and mineral elements required for nutrition. The anti-nutritional factors were below permissible toxic levels. The results also provide value-added potential to the seeds and fruit epicarp of *C. lepidota* (for exploitation in the formulation or fortification of animal feeds) which hitherto were less appropriated compared to the yellow aril. Furthermore, intensified scientific research on this underutilized, economic and nutritional viable specie would serve as a necessary step towards its conservation and reverse the likelihood of its extinction. ■



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References

- [1] Sadiq, I. S., Izuagie, T., Shuaibu, M., Dogoyaro, A. I., Garba, A., Abubakar, S. The nutritional evaluation and medicinal value of date palm (*Phoenix dactylifera*). *Inter. J. Modern Chem.* 2013, 4(3): 147-154.
- [2] Venkatachalam, M., Sathe, S. K. (2006). Chemical composition of selected edible nut seeds. *J. Agric. Food Chem.* 2006, 54(13): 4705-4714.
- [3] National Academy of Sciences. *Lost Crops of Africa: Fruits*. Vol. 3. Washington D.C. USA: National Academies Press; 2008, 381.
- [4] Akinnifesi, F. K., Sileshi, G., Ajayi, O. C., Tchoundjeu, Z. Indigenous Fruit Tree Domestication. In: Akinnifesi, F. K., Leaky, R. B., Ajayi, O. C., Sileshi, G., Tchoundjeu, Z., Matalala, P. (Eds.), *Indigenous fruit trees in the tropics: domestication, utilization and commercialization*. Wallingford, UK: CABI; 2007.
- [5] Keay, R. W. J. *Trees of Nigeria*. New York: Oxford University Press; 1998.
- [6] Bosch, C. H., Siemonsma, J. S., Lemmens, R. H. M J., Oyen, L. P. A. *Plant resources of tropical Africa. Basic list of species and commodity grouping*. Wageningen, the Netherlands: PROTA Programme; 2002.
- [7] Ogbu, J. U., Umeokechukwu, C. E. Aspects of fruit biology of three wild edible monkey kola species fruits (*Cola* spp: Malvaceae). *Annual Res. Rev. Bio.* 2014, 4: 2007-2014.
- [8] Ogbu, J. U., Essien, B. A., Kadurumba, C. H. Nutritive value of wild edible species of monkey kola (*Cola* spp.) *Nigerian J. Horticult. Sci.* 2007, 12: 113-117.
- [9] Kolawole, S. E., Obueh, H. O. Proximate and micronutrient compositions of some selected foods and diets in South-South Nigeria. *Scholarly J. Biotech.* 2012, 1(3): 45-48.
- [10] Essien, E. E., Peter, N. S., Akpan, S. M. Chemical composition and antioxidant property of two species of monkey kola (*Cola rostrata* and *Cola lepidota* K. Schum) extracts. *Europ. J. Med. Plants* 2015, 7(1): 31-37.
- [11] Osabor, V. N., Bassey, F. I., Ibe, K. A. Chemical profile of the endocarp and exocarp of yellow monkey kola (*Cola lepidota*). *Glob. J. Pure Appl. Sci.* 2015, 21(1): 33-39.
- [12] Ene-Obong, H. N., Okudu, H. O., Asumugha, U. V. Nutrient and phytochemical composition of two varieties of monkey kola (*Cola panchycarpa* and *C. lepidota*): An underutilized fruit. *Food Chem.* 2016; 193: 154-9.
- [13] Okudu, H. E., Ene-Obong, H. N., Asumugha, V. U. The chemical and sensory properties of juice developed from two varieties of monkey kola (*Cola panchycarpa* and *Cola lepidota*). *Afri. J. Food Sci. Techn.* 2015, 6(5): 149-155.
- [14] Okudu, H. O., Ene-Obong, H. N. The chemical and sensory properties of jam developed from two varieties of monkey kola (*Cola panchycarpa* and *Cola lepidota*). *Am. J. Food Nutr.* 2015; 5(1): 16-22.
- [15] Fabunmi, T. B., Arotupin, D. J. Proximate, mineral and antinutritional composition of fermented slimy kola nut (*Cola verticillata*) husk and white shell. *British J. Appl. Sci. Tech.* 2015, 6(5): 550-556.
- [16] Babatunde, B. B., Hamzat, R. A., Adejinmi, O. O. Replacement value of kola nut husk meal for maize in rabbit diets. *Trop. J. Anim. Sci.* 2010, 4(2): 127-133.
- [17] IPGRI. *Neglected and Underutilized Plant Species: Strategic Action Plan of the International Plant Genetic Resources Institute*. Rome: International Plant Genetic Resource Institute; 2002, p. 27.
- [18] Antia, B. S., Essien, E. E., Udonkanga, E. D. Nutritional composition and acute toxicity potentials of *Archontophoenix tukeri* and *Adonia merrilli* kernels. *UK J. Pharmaceut. Biosci.* 2017, 5(2):17-24.

- [19] Benitez, L. V. Amino acid and fatty acid profiles in aquaculture nutrition studies. In: De Silva, S. S. (Ed.), *Fish nutrition research in Asia: Proceedings of the Third Asian Fish Nutrition Network Meeting*. Bangkok, Thailand. Manila, Philippines: Asian Fisheries Society. AFS Spec. Publ. 4; 1989, pp. 23-35.
- [20] AOAC. *Official Methods of Analysis*. 15th Ed. Association of Official Analytical Chemists. Washington DC. USA; 1990.
- [21] Idouraine, A., Kohlhepp, E. A., Weber, C. W. Nutrient constituents from eight lines of naked seed squash (*Cucurbita pepo* L.). *J. Agri. Food Chem.* 1996, 44: 721-724.
- [22] Pearson, D. *The Chemical Analysis of Foods*, 7th Edition. Churchill LivingStone, London; 1976.
- [23] Lucas, G. M., Markaka, P. Phytic acid and other phosphorus compounds of bean (*Phaseolus vulgaris*). *J. Agri. Edu. Chem.* 1975, 23: 13-15.
- [24] Jaffe, C. S. *Analytical Chemistry of Food*, Vol. 1. Blackie Academic and Professional, New York; 2003.
- [25] Onwuka, G. *Food Analysis and instrumentation*, 3rd Ed. Naphohla Prints, A Division of HG Support Nigeria Ltd.; 2005.
- [26] Munro, A. B., Bassiro, W. A. Oxalate in Nigerian vegetables. *J. Bio. Appl. Chem.* 2000, 12(1): 14-18.
- [27] Eleyinmi, A. F., Bressler, D. C., Amoo, I. A., Sporns, P., Oshodi, A. A. Chemical composition of bitter cola (*Garcinia kola*) seed and hulls. *Polish J. Food Nutr. Sci.* 2006, 15/56(4): 395-400.
- [28] Adeyeye, E. I., Asaolu, S. S., Aluko, A. O. Amino acid composition of two masticatory nuts (*Cola acuminata* and *Garcinia kola*) and a snack nut (*Anacardium occidentale*). *Inter. J. Food Sci. Nutr.* 2007, 58(4): 241-9.
- [29] Osako, K., Kiriya, T., Ruttanapornvaressakul, Y., Kuwahara, K., Okamoto, A., Nagano, N. Free amino acid composition of the gonad of the wild and cultured sea urchins *Anthocidaris crassispina*. *Aquacult. Sci.* 2006, 54: 301-304.
- [30] Lindermann, B. A taste for unami. *Nature Neurosci.* 2000, 3(2): 99-100.
- [31] Archana A., Babu, K. R. Nutrient composition and antioxidant activity of gonads of sea urchin *Stomopneustes variolaris*. *Food Chem.* 2016, 197: 597-602.
- [32] Ottaway, P. B. *The Technology of Vitamins in Food*. Chapman and Hall, New York; 1993.
- [33] Dahl, L. K. Salt and Hypertension. *Am. J. Clin. Nutr.* 1972, 25: 231-238.
- [34] Okwu, D. E. Phytochemicals and vitamin content of indigenous spices of South Eastern Nigeria. *J. Sustainable Agri. Environ.* 2004, 6: 30-34.
- [35] Soetan, K. O., Olaiya, C. O., Oyewole, O. E. The importance of mineral elements for humans, domestic animals and plants: A review. *Afri. J. Food Sci.* 2010, 4(5): 200-222.
- [36] Belakbir, A., Ruiz, J. M., Romero, L. Yield and fruit quality of pepper (*Capsicum annum* L.) in response to bio-regulators. *Horticult. Sci.* 1998, 33: 8587.
- [37] Nwiiisuator, D., Oddo, E., Emerhi, E. A., Owuno, F., Sangh, P. Mineral composition of *Cola parchycarpa* (K. Schum) arils and seeds. *Am. J. Food and Nutr.* 2012, 2(2): 37-41.
- [38] Crisan, E. V., Sands, A. *The Biology and Cultivation of Edible Mushrooms*. Academic Press, New York; 1978.
- [39] Hönow, R., Hesse, A. Comparison of extraction methods for the determination of soluble and total oxalate in foods by HPLC-enzyme-reactor. *Food Chem.* 2002, 78: 511-521.
- [40] Savage, G. P., Vanhanen, L., Mason, S. M., Ross, A. B. Effect of cooking on the soluble and insoluble oxalate content of some New Zealand foods. *J. Food Compos. Anal.* 2000, 13(3): 201-206.
- [41] Elder, T. D., Wyngaarden, J. B. The biosynthesis and turnover of oxalate in normal and hyperoxaluric subjects. *J. Clin. Investi.* 1960, 39: 1337-1344.
- [42] Birgitta, G., Gullick, C. Exploring the Potential of Indigenous Wild Food Plants in Southern Sudan. In Proceeding of a Workshop held in Lokichoggio, Kenya; 2000, pp. 22-25.