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# *Cola parchycarpa* K. Schum: Chemical Evaluation of Amino Acids, Vitamins and Other Nutritional Factors in Seed, Fruit Mesocarp and Epicarp

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**Abstract**

*Cola parchycarpa* is one of the under-utilized monkey kola plants that yield edible tasty fruits. The amino acids, vitamins, mineral elements, proximate and anti-nutrients composition of the white aril, seed and fruit epicarp were evaluated using standard procedures. The total essential amino acids ranged from 31.84-55.70 g/100 g, predominated in lysine, leucine and cysteine. The fruit pulp and epicarp contained substantial amount of ascorbic acid (6.310 and 6.646 mg/100 g) and tocopherols (7.328 and 5.314 mg/100 g), respectively including vitamin B1, B2, B3, B5, B6, B9 and K1. Potassium, zinc and manganese were relatively high in the seed while calcium, magnesium and iron dominated in the fruit epicarp. The proximate analysis data of the fruit pulp and epicarp were similar except in protein and lipid content. Anti-nutritional factors (phytate, oxalates, cyanide and tannins) were below permissible limits. This is the first report on amino acids and detailed vitamins composition of *C. parchycarpa*. These findings indicate the rich nutritional potential of this tasty fruit and further processing into other value added products would encourage conservation and subversion of its impending extinction.

## 1 Introduction

*Cola parchycarpa* K. Schum (Malvaceae) is a perennial tree commonly described as monkey kola[1]. Monkey kola is a popular nomenclature for the lesser known members of the *Cola* species that yield edible tasty fruits. They are a close relative to the familiar West African kola nuts (*C. nitida* and *C. acuminata*), cultivated for their masticatory and stimulating nuts[1,2]. In southern Nigeria and the Cameron, the fruit pulp is eaten by humans as well as some wild primate animals especially monkeys, baboons and other species. The regular cylindrical caulescent follicles of *C. parchycarpa* consist of one to eight nuts which correspond to the fruit length. The follicles are beaked and ribbed with rough and light brown epicarp; seeds (greenish or reddish brown) are obliquely ovate with two flat rough surfaces. The whitish aril (waxy mesocarp) consist the sweet edible portion of the follicle[3] (Fig. 1).

Proximate, anti-nutrients, mineral elements analyses and antioxidant activity of *C. lepidota, C. parchycarpa* and *C. lateritia* fruits' pulp have been reported[4-8]. Research has shown that juice and jam can be developed from the pulp of the monkey kola[9,10]. Fabunmi and Arotupin[11], suggested from their findings that the husk and white shell of slimy kola nut (*C. verticillata*) could serve as a blend in animal feed. *In vivo* studies also revealed that about 50% of kola nut husk meal could replace maize diets of rabbits[12].

Several valuable fruit species in Africa are not yet domesticated. However, substantial economic produce are obtained from their wild or gardens, farms and forest reserves[13-15]. Dearth of scientific research inputs on these indigenous plants have led to concepts such as neglected and underutilized species[16,17]. As part of the systematic analysis of the poorly studied fruit plants for their nutritional potentials[18], we present the first report on amino acids and detailed vitamins composition of this endangered species of monkey kola; as well as the mineral elements, proximate and anti-nutrients profiles of the aril, seed and fruit epicarp.

**Fig 1: *Cola parchycarpa* fruit, fruit pulp and epicarp**

**2 Materials and Methods**

*2.1 Sample collection and preparation*

The fruits of *C. parchycarpa* were purchased from a local market in Essien Udim Local Government Area, Akwa Ibom State, Nigeria, in July 2015. The plants were identified and authenticated by a taxonomist, M. E. Bassey, Department of Botany and Ecological Studies, University of Uyo, where voucher specimens were deposited. The seed, fruit pulp and epicarp were separated, chopped into small pieces, oven dried at 40 ºC to constant weight and stored in air tight containers.

*2.2 Extraction and analysis of amino acids*

The methods of AOAC[19] and Obreshkova *et al*.[20] with slight modifications were employed for this determination. The sample (10 g) was defatted with petroleum spirit in a Soxhlet extractor. The sample was macerated in KOH (30 mL, 1M) and incubated for 48 hr at 110 ºC in hermetically closed borosilicate glass container. After the alkaline hydrolysis, the hydrolysate was neutralized to pH 2.5-5.0. The solution was purified by cation-exchange solid phase extraction. The amino acids in purified solutions were derivatized with ethyl chloroformate.

The derivatized amino acids were evaluated by Gas Liquid Chromatography on a HP 6890 Powered with HP ChemStation Rev. A09.01 [1206] Software and equipped with a PFPD detector. Separation was performed using a fused capillary column (HP EZ, 10 m x 0.2 mm x 0.25 µm) as stationary phase. The oven temperature was programmed as follows: initial temperature at 110 ºC, first ramping at 27 ºC/min to 320 ºC; second ramping, constant 5 min at 320 ºC. The injector and detector temperatures were 250 ºC and 320 ºC respectively. The carrier gas was hydrogen and a split ratio of 20:1 was used. The amino acids were identified by comparing their retention times to those of a standard mixture of amino acids and the peak areas were integrated.

*2.3 Vitamins determination*

The vitamins profile of the samples were analysed by methods of AOAC[19] 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 ºC for 2 mins; first ramp at 10 ºC /min for 20 mins, maintain for 4 mins; second ramp at 15 ºC/min for 4 mins, constant for 2 mins; injector temperature: 250 ºC, 20:1 split ratio; temperature of PFPD detector: 320 ºC; 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.

*2.4 Mineral element composition*

The minerals were determined after the ground samples were subjected to dry ashing. Triplicate sample of one gram each were weighed into porcelain crucible and placed in muffle furnace. The temperature was raised gradually to 450 ºC. The sample was ashed at 550 ºC for 5-6 hours. After cooling to room temperature, the ash was dissolved in one millilitre (l ml) 0.5% $HNO_3$. The sample volume was made up to 100 mL and the level of mineral elements, calcium (Ca), magnesium (Mg), manganese (Mn), iron (Fe), copper (Cu) and zinc (Zn), was analyzed by atomic absorption spectrophotometer (UNICAM 959). Sodium (Na) and potassium (K) were determined using flame atomic emission spectrometer[21].

*2.5 Proximate compositional analysis*

Moisture content was obtained from fresh samples; total lipid, protein, ash, crude fibre and carbohydrate were determined from oven-dried powder using standard procedures[19,22]. The moisture content was obtained by drying in a moisture determination apparatus (Precisa HA60) at 110 ºC until circulation was complete; ash, from the incinerated residue was obtained at 550 °C after 3 h; crude protein content was established by the Kjeldahl method with a conversion factor of 6.25; while the crude fat was gravimetrically determined after Soxhlet extraction with petroleum ether. The crude fat was converted into fatty acids by multiplying with conversion factor of 0.8. The total carbohydrate was calculated as 100% - (% moisture+ % ash+ % crude protein+ % fat+ % fibre). The total energy values were calculated by multiplying the amounts of protein and carbohydrate by the factor of 4 Kcal/g and lipid by the factor of 9 kcal/g. Data points represent mean of three

determinations and proximate values were reported in percentage.

*2.6 Determination of anti-nutritional factors*

*2.6.1 Phytate determination*

Extraction and precipitation of phytate were done through phytic acid determination using the procedure described by Lucas and Markaka[23]. This entails the weighing of sample (2g) into a 250 mL conical flask. 2% conc. HCl (100 mL) was used to soak the samples in the conical flask for 3 h and then filtered through a double layer filter paper. Sample filtrate (50 mL) was placed in a 250 mL beaker and distilled water (107 mL) added to give/ improve proper acidity. 0.3% ammonium thiocyanate solution (10 mL) was added to each sample solution as indicator and titrated with standard iron chloride solution which contained 0.00195 g iron/mL and the end point was signified by brownish-yellow colouration that persisted for 5 min. The percentage phytic acid was calculated.

*2.6.2 Tannins determination*

Tannin values were obtained by adopting the method of Jaffe[24]. Each sample (1g) was dissolved in distilled water (10 mL) and agitated, left to stand for 30 min. at room temperature. The samples were centrifuged and the extracts recovered; the supernatant (2.5 mL each) were dispersed into 50 mL volumetric flask. Similarly, standard tannic acid solution (2.5 mL) was dispersed into separate 50 mL flasks. Folin-dennis reagent (1.0 mL) was measured into each flask followed by the addition of saturated $Na_2CO_3$ solution (2.5 mL). The mixture was diluted to 50 mL in the flask and incubated for 90 min at room temperature. The absorbance of each sample was measured at 250 nm with the reagent blank at zero. The % tannin was calculated.

*2.6.3 Cyanogenic glycoside determination*

The alkaline picrate method[25] was used for cyanogenic glycoside determination. The samples (5 g each) in conical flasks were added distilled water (50 mL) and allowed to stand overnight. Alkaline picrate (4 mL) was added to sample filtrate (1 mL) in a corked test tube and incubated in a water bath for 5 min. A colour change from yellow to reddish brown after incubation for 5 min in a water bath indicated the presence of cyanides. The absorbance of the samples was taken at 490 nm and that of a blank containing distilled water (1 mL) and alkaline picrate solution (4 mL) before the preparation of cyanide standard curve.

*2.6.4 Oxalates determination*

The oxalates content of the samples was determined using titration method[26]. The samples (2 g each) were placed in a 250 mL volumetric flask suspended in distilled water (190 mL) for soluble oxalate determination; 6 M HCl solution (190 mL) was added to the samples (2 g each). The suspensions were digested at 100 ºC for 1h. The samples were then cooled and made up to 250 mL mark of the flask. The samples were iltered, triplicate portions of the filtrate (50 mL) were measured into beaker and four drops of methyl red indicator was added, followed by the addition of concentrated $NH_4OH$ solution (drop wise) until the solution changed from pink to yellow colour. Each portion was then heated to 90 ºC, cooled and filtered to remove the precipitate containing ferrous ion. The filtrates were again heated to 90 ºC and 5% $CaCl_2$ (10 mL) solution was added to each of the samples with consistent stirring. After cooling, the samples were left overnight. The solutions were then centrifuged at 2500 rpm for 5 min. The supernatant were decanted and the precipitates completely dissolved in 20% $H_2SO_4$ (10 mL). The total filtrates resulting from digestion of the samples (2 g each) were made up to 200 mL. Aliquots of the filtrate (125 mL) were heated until near boiling and then titrated against 0.05 M standardized $KMnO_4$ solution to a pink colour which persisted for 30 sec. The oxalate contents of each sample were calculated. All determinations were performed in triplicates and presented in mg/100 g.

**3 Results and Discussions**

The amino acids content of the seed, fruit pulp and epicarp of *C. parchycarpa* is shown in table 1. Results indicated that the dominant essential amino acids are lysine (5.07-14.64 g/100 g), leucine (7.17-9.29 g/100 g) and cysteine (0.54-9.19 g/100 g); total essential amino acids ranged from 31.84-55.70 g/100 g. Glutamic acid (5.59-14.74 g/100 g) and aspartic acid (7.40-10.37 g/100 g) were the major non-essential amino acids identified. The aril contained relative higher amount of the essential amino acids while both seeds and fruit pericarp showed predominance in the non-essential components in comparative amount. Eleyinmi *et al*.[27] reported that lysine, leucine and valine in the seed, and valine, leucine and lysine in the hull of *Garcinia kola* were the dominant essential amino acids. The samples in this study contained higher amount of essential amino acids than *C. acuminata* and *G. kola* (356.24 mg/g and 112.90 mg/g respectively) and *G. kola* seed and hull (11. 10 g/kg and 28.0 g/kg respectively)[27,28]. Amino acid profile is significant in two aspects, namely nutrition and flavour[29]. Glycine and analine bestow sweetness, while valine is bitter and glutamin furnish umami[30]. This may account in part for the relative low content of valine (1.98 g/100 g) in the sweet pulp of *C. parchycarpa* in this study compared with the seed and fruit epicarp (4.4 and 4.56 g/100 g respectively). The essential amino acids (EAA) to non-essential amino acids (NEAA) ratio was 1.62, 0.65 and 0.79 respectively for aril, seed and fruit epicarp; this is higher than sea urchin, *Paracentrotus lividus*, EAA:NEAA, 0.58. Usually in marine foods, EAA:NEAA greater than 0.5 indicates a useful source of dietary proteins[31].

The white aril of *C. parchycarpa* contained higher amount of vitamins compared to the seed and fruit pericarp, except the

ascorbic acid content of fruit pulp and pericarp which were relatively similar (6.310 and 6.646 mg/100 g respectively) (Table 2). Lower concentrations of vitamin B1 and B2 (0.01 mg/100 g) are shown for juice obtained from *C. parchycarpa* pulp[9]. Processing may account for the observed differences in vitamins content of *C. parchycarpa* pulp juice and unprocessed pulp. 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[9]. Vitamins are a broad class 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 ailments[32].

**Table 1: Amino acids composition (g/100 g) of *Cola parchycarpa***

| Amino acid | Fruit pulp | Seed | Fruit epicarp |
|---|---|---|---|
| Lysine | 14.64450 | 5.07578 | 6.93420 |
| Threonine | 3.08649 | 1.22664 | 3.96042 |
| Cysteine | 9.19446 | 0.53643 | 0.32101 |
| Valine | 1.97742 | 4.40194 | 4.56269 |
| Methionine | 3.42068 | 0.50968 | 0.76437 |
| Isoleucine | 6.84774 | 4.38702 | 3.62706 |
| Leucine | 9.28887 | 7.95312 | 7.16841 |
| Tyrosine | 3.62786 | 2.41878 | 3.25083 |
| Phenylalanine | 2.63233 | 4.04416 | 4.80659 |
| Tryptophan | 0.97738 | 1.29065 | 0.51005 |
| Total (EAA) | 55.69773 | 31.84420 | 35.90563 |
| Histidine | 2.50188 | 2.28163 | 4.34090 |
| Arginine | 5.26105 | 4.36494 | 6.02845 |
| Aspartic acid | 7.40121 | 10.36936 | 8.11549 |
| Serine | 2.40693 | 5.95879 | 2.78044 |
| Glutamic acid | 5.59426 | 14.74402 | 11.67527 |
| Proline | 3.54296 | 4.53876 | 4.52287 |
| Glycine | 3.05760 | 3.61983 | 3.13948 |
| Alanine | 4.66057 | 3.40487 | 4.70192 |
| Total (NEAA) | 34.42646 | 49.28220 | 45.30482 |
| Total Amino Acids | 90.12420 | 81.12640 | 81.21045 |
| EAA/NEAA | 1.62 | 0.65 | 0.79 |

EAA = essential amino acids; NEAA = non-essential amino acids

**Table 2: Vitamins composition (mg/100 g) of *Cola parchycarpa***

| Vitamin | Fruit pulp | Seed | Fruit epicarp |
|---|---|---|---|
| Retinol (vitamin A) | 0.000288 | 0.004588 | 0.000212 |
| Thiamine (vitamin B1) | 0.192687 | 0.117670 | 0.119610 |
| Riboflavin (vitamin B2) | 0.091560 | 0.050766 | 0.051396 |
| Nicotinamide (vitamin B3) | 0.966717 | 0.050008 | 0.717315 |
| Pantothenic acid (vitamin B5) | 0.000004 | 0.448437 | 0.000003 |
| Pyridoxine/pyridoxal hydrochloride (vitamin B6 ) | 0.264659 | 0.111234 | 0.203060 |
| Folic acid (vitamin B9) | 0.133600 | 0.000035 | 0.096790 |
| Ascorbic acid (vitamin C) | 6.309850 | 0.557099 | 6.646090 |
| Tocopherol (vitamin E) | 7.328250 | 3.170090 | 5.313950 |
| Phylloquinone (vitamin K1) | 0.000009 | 0.001249 | 0.000006 |

The mineral elements content of *C. parchycarpa* aril, seed and fruit pericarp are presented in Table 3. High amount of potassium (4005 and 6285 mg/Kg) in the fruit pulp and seed were identified compared with the sodium content. The concentration of sodium (1430 mg/Kg) in the fruit pulp was relatively lower compared to the seed and fruit pericarp. Interestingly, the fruit pulp is cherished as food which is advantageous due to the direct correlation of sodium intake with hypertension in human[33]. The seeds have a relative high content of potassium, zinc and manganese whereas calcium, magnesium and iron were predominant in the fruit pericarp**.** The relative high content zinc in the seed could be implicated in the management of diabetes, which results from insulin malfunctioning. Zinc is significant for the production of insulin, a hormone and carbonic anhydrase[34]. This observed high calcium content in the fruit pericarp could be implicated in the maintenance of fruit firmness[35] and is required in fruits to enhance cell wall and membrane stability[36]. The rich Ca and Mg content of *C. parchycarpa* fruit epicarp could be exploited in animal feed blends with nutrient requirements in Ca and Mg. Eneobong *et al*.[8] reported that calcium and magnesium were the most abundant minerals in the fruit pulp of *C. parchycarpa* and *C. lepidota*. High amount of some essential minerals in the endocarp of *C. lepidota* relative to the exocarp was also documented by Osabor *et al*.[7].

**Table 3: Mineral elements composition (mg/Kg) of *Cola parchycarpa***

| Mineral | Fruit pulp | Seed | Fruit pericarp |
|---|---|---|---|
| Na | 1430 | 2965 | 1580 |
| K | 4005 | 6285 | 1430 |
| Ca | 2800±0.013 | 3.00±0.028 | 136250±0.029 |
| Mg | 140850±0.021 | 73800±0.026 | 151200±0.019 |
| Zn | 1550±0.019 | 6000±0.075 | 60.0±0.102 |
| Cu | 19.0±0.014 | 56±0.002 | 10.5±0.006 |
| Mn | 50.5±0.009 | 62.5±0.490 | 38.5±0.045 |
| Fe | 198±0.012 | 267±0.105 | 289±0.102 |

Results are mean ± standard deviation of three determinations

Table 4 is a presentation of the proximate analysis of *C. parchycarpa* seed, aril and fruit epicarp. Moisture content is highest in the pulp (65.05%) and ash content in both pulp and fruit epicarp which indicates the presence of some nutritionally important mineral elements. Ogbu *et al*.[4] showed that *C. parchycarpa* waxy aril contain moisture (80.15 g/100g) and ash (1.76 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 are related to factors such as, harvesting time, plant maturity, environmental, and storage conditions[38]. The result also revealed a relative high carbohydrate content (67.79%) in the seeds, lipid (2.73%) in the fruit pulp and protein (14.88%) in the fruit epicarp. Calculated energy values for the samples varied from 321.28-349.81 kCal/100 g. Lipid (free fatty acids, tri-, di- and monoglycerides, phospholipids, tocopherols, sterols and derivatives) are isolated from these plant parts as crude fat[38]. Nwiisuator *et al*.[37] showed that proximate contents were higher in the yellow arils compared to the seeds of *C. lepidota*, except for fats and carbohydrates. A relative lower proximate content is also documented for juice developed from *C. parchycarpa* and *C. lepidota* pulp[9] compared to the unprocessed fruit pulps.

The anti-nutrients analysis revealed the presence of tannins (6.81-17.82 mg/100 g), cyanides (1.76-8.32 mg/100 g), phytates (1.14-9.69 mg/100 g), and total oxalates (14.52-42.24 mg/100 g). A relative higher content of cyanide and phytates were found in the seed, however with lower tannins and oxalate values (Table 5). Osabor *et al*.[7] reported lower levels of cyanide in the fruit endocarp and exocarp of *C. lepidota* and our studies revealed low phytate content compared with the yellow pulp specie of monkey kola. The levels of anti-nutrients in the seeds, aril and fruit epicarp of *C. parchycarpa* in this study, were below the permissible toxic levels[39] and indicate probable lack of interference with the availability of mineral elements.

**Table 4: Proximate composition of *Cola parchycarpa***

| Parameter | Fruit pulp | Seed | Fruit epicarp |
|---|---|---|---|
| Moisture (%) | 65.05±0.02 | 18.80±0.01 | 15.50±0.15 |
| Ash (%) | 12.84±0.028 | 8.58±0.010 | 12.97±0.007 |
| Fibre (%) | 7.55±0.015 | 4.73±0.00 | 7.67±0.021 |
| Protein (%) | 9.10±0.495 | 7.70±0.400 | 14.88±0.247 |
| Lipid (%) | 2.73±0.028 | 0.61±0.014 | 0.76±0.003 |
| Carbohydrates (%) | 67.79±0.544 | 78.38±0.495 | 63.49±0.120 |
| Caloric value (kCal/100 g) | 332.11±0.057 | 349.81±0.127 | 321.28±0.184 |

All values are the mean of triplicate determinations expressed in dry weight basis ± standard deviation.

**Table 5: Anti-nutrients composition (mg/100 g) of *Cola parchycarpa***

| Anti-nutrient | Fruit pulp | Seed | Fruit epicarp |
|---|---|---|---|
| Cyanide | 4.31±0.091 | 8.32±0.001 | 1.76±0.028 |
| Phytates | 1.14±0.013 | 9.69±0.086 | 1.52±0.031 |
| Tannins | 13.35±0.014 | 6.81±0.016 | 17.82±0.049 |
| Oxalates | 42.24±1.20 | 14.52±0.620 | 38.28±0.80 |

All values are the mean of triplicate determinations expressed in dry weight basis ± standard deviation

## 4 Conclusions

*Cola parchycarpa* aril, seeds and fruit epicarp contain substantial amount of amino acids, vitamins and mineral elements required for nutrition; the fruit pulp showed high amount of these nutrients. The anti-nutritional factors were below permissible toxic levels to allow for bioavailability of mineral elements. The results also provide value-added potential to the seeds and fruit epicarp of *C. parchycarpa* (for exploitation in the formulation or fortification of animal feeds) which hitherto were less appropriated compared to the white aril. Furthermore, intensified scientific research on this underutilized, economic and nutritional viable plant would serve as a necessary step towards its conservation and revert the likelihood of its extinction.

## 5 Conflicts of Interest

The authors declare no conflict of interest.

**6 Author Contributions**

EEE and IIU conceived, designed and performed the experiments, and wrote the manuscript.

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