

Proximate Analysis, Mineral Contents and Amino Acid Composition of *Antrocaryon micraster* Stem Bark

Akinsola Abiodun Folasade¹ and Omotayo Felix Olorunfemi²

Abstract

In the present study, the proximate, mineral and amino acid composition of the stem bark of *Antrocaryon micraster* was determined. Results of the proximate analysis revealed the presence of 12.4 ± 0.0 % moisture; 9.01 ± 0.01 % ash; 13.8 ± 0.1 % crude protein; 1.20 ± 0.04 % fat and 51.9 ± 0.1 % carbohydrate. The mineral composition showed high level of K (889 ± 1.0 mg/100g), Ca (628 ± 3.0 mg/100g), P (104 ± 1.0 mg/100g) and low levels of Mn (6.71 ± 0.03 mg/100g), Zn (5.29 ± 0.10 mg/100g) and Cr (0.1 ± 0.0 mg/100g). The antinutrients studied revealed lower range of values when compared with most vegetables. Two acidic amino acids (glutamic and aspartic acid) were the highest concentrated amino acids (15.2 ± 0.0 and 10.0 ± 0.1 g/100g crude protein). The total amino acid was 81.6 g/100g crude protein, while the total essential and non-essential amino acids were 36.4 and 45.2 g/100g crude protein respectively. The predicted protein efficiency ratio (P-PER) of *Antrocaryon micraster* stem bark was 1.55 and the calculated isoelectric point (pI) was 4.67.

Keywords: *Antrocaryon micraster*, stem bark, proximate analysis, mineral composition, amino acids

1.0 Introduction

Plants have been utilized by human being for their medicinal and edible values since the dawn of civilization. These medicinal plants are available in many parts of Africa. Rain forest contain no less than 60 % of all higher plant species known on earth and they provide all that is needed for human survival including remedies against diseases (FAO, 1997). Many of these medicinal plants are becoming extinct due to over exploitation resulting from excessive commercialization, habitat destruction and other man made destructive influences; one of such plant is *Antrocaryon micraster* A. Chev. & Guill.

Antrocaryon micraster (*A. micraster*) is a forest tree occurring in tropical West Africa- moister regions from Sierra Leone east through the Congo basin to Uganda. It is a deciduous tree with medium size to large up to 45 -50 m tall (Ayarkwa, 2011). The bole is straight and cylindrical, scarcely buttressed or with a shallow buttresses (Keay, 1989). It is known in English as forest plum, sauce or soup bark. In Nigeria, it is commonly known as awogba, igi obe, eepo obe, eso esi, ifa okete (Yoruba), egin, egbo (Itsekiri), ekhuen (Edo) (Keay *et al.*, 1964; Omotayo, 1997). The tree is collected for timber and is classified as "vulnerable" in the Red List of threatened species of the International Union for Nature Conservation (IUCN) (IUCN, 2011).

A. micraster bark is thick and rough; it is used locally to prepare soup in Nigeria (Burkill, 1985). The bark is also used as an enema to treat impotence and in mixtures to treat threatened abortion (Ayarkwa, 2011). The fruits which have a strong mango like smell are sometimes called plums because of their similarities to the temperate fruit and are around 37 mm long by 50 mm in diameter. The flesh of the fruit is locally eaten and sometimes it is fermented to prepare alcoholic drinks (Ayarkwa, 2011; Burkill, 1985). The fruit apart from being taken for stomach ache is equally used for cough and heart ache. The hard endocarp is hard to open to get at the seeds but will burst if put in hot cylinders. The oil content of the kernel is over 70 % (Burkill, 1985).

¹ Department of Chemistry, Ekiti State University, PMB 5363, Ado Ekiti, Nigeria. Email: akinsolaf87@gmail.com Phone No: +2348034962515

² Department of Plant Science, Ekiti State University, PMB 5363, Ado Ekiti, Nigeria.

Considering the fact that there is paucity of information on the nutritional composition of *A. micraster* stem bark, the present investigation was carried out to study the proximate, mineral, antinutrient and amino acid compositions of *A. micraster* stem bark.

2.0 Materials and methods

2.1 Sample collection and preparation

A. micraster stem bark was collected from a farmland at Ikere Ekiti in Ekiti State, Nigeria. It was duly authenticated at the Herbarium in Faculty of Science, Ekiti State University, Ado-Ekiti. It was washed, dried and milled into fine powdered form and kept in an air tight plastic bottle prior analysis.

2.2 Proximate analysis

The moisture, ash and fat contents were determined using the methods of Association of Official Chemists (AOAC, 2005). Nitrogen was determined by micro-Kjedahl method as described by Pearson (1976) and the percentage nitrogen was converted to crude protein by multiplying the value with 6.25. Crude fibre content was determined by using the method of Joslyn (1970). Carbohydrate was determined by difference;

$$\{100 - (\text{ash} + \text{moisture} + \text{crude protein} + \text{crude fibre} + \text{crude fat contents})\}$$

The energy value was obtained by multiplying carbohydrate, crude protein and crude fat values by the Atwater factors of 17, 17 and 37 respectively.

2.3 Mineral analysis

The minerals were analysed from solutions obtained by first dry-ashing the powdered sample at 550 °C for 5 hr and dissolving the ash in 10 % HNO₃; warming, filtering and transferring to 100 ml standard flask using distilled deionised water to make it up. Mineral analysis for the Na and K contents of the resulting solution was determined by flame photometry (Jenway Ltd, Dunmow, Essex, UK) and Ca, Mg, Fe, Zn, Cu, Mn, Cr and Pb were determined using Atomic Absorption Spectrometer (Buck Scientific East Norwalk, CT, USA). Phosphorus was determined colorimetrically using a spectronic 20 (Gallen kemp, London, UK) instrument with KH₂PO₄ as a standard.

2.4 Antinutrient Analysis

2.4.1 Determination of oxalate content

1 g of the sample was weighed into 100 ml conical flask. 75 ml of 1.5 N H₂SO₄ was added and the solution was carefully stirred intermittently with a magnetic stirrer for about 1 hr and then filtered using Whatman filter paper. 25 ml of the filtrate was collected and titrated hot (80-90 °C) against 0.1N KMnO₄ solution to the point when a faint pink colour persisted for at least 30 s (Day and Underwood, 1986).

2.4.2 Determination of tannin content

Tannin content was determined using Van-burden and Robinson (1981) method. 500 mg of the sample was weighed into a 50 ml plastic bottle; 50 ml of distilled water was added and shaken for 1hr in a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark. Then 5 ml of the filtrate was pipetted out into a test tube and mixed with 2 ml of 0.1M ferric chloride in 0.1 M hydrochloric acid and 0.008 M potassium ferrocyanide, and the absorbance was measured at 220 nm within 10 min. The tannin content for the sample was determined from a standard calibration plot.

2.4.3 Determination of alkaloid content

The method used for the determination of alkaloid was that of Harborne (1973). 5.0 g of the sample was weighed into a 250 ml beaker and 200 ml of 10 % acetic acid in ethanol was added. The beaker was covered and allowed to stand for 4 hr. This was filtered and the extract was concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wisely to the extract until precipitation was complete. The precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue alkaloid was dried and weighed.

2.4.4 Determination of saponin content

Saponin content determination was carried out following the procedure of Obadoni and Ochuko (2001). 5 g of the sample was put into a conical flask and 100 cm³ of 20 % aqueous ethanol was added. The sample was heated over a hot water bath for 4 hr with continuous stirring at about 55 °C.

The mixture was filtered and the residue re-extracted with another 200 ml 20 % ethanol. The combined extracts were reduced to 40 ml over water bath at about 90 °C. The concentrate was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. Thereafter, 60 ml *n*-butanol was added. The combined *n*-butanol extracts were washed twice with 10 ml of 5 % aqueous sodium chloride. The remaining solution was heated in a water bath after evaporation; the sample was dried in the oven to a constant weight. The saponin content was calculated as percentage.

2.4.5 Determination of cyanide content

The cyanide level of *A. micraster* was determined using the modified alkaline picrate method of Onwuka (2005) as described by Eleazu and Eleazu (2012). 5 g of the sample was dissolved in 50 ml distilled water and allowed to stay overnight. The sample was filtered and the filtrate was used for the cyanide determination. To 1 ml of the aqueous extract was added, 4 ml of alkaline picrate (obtained by dissolving 1 g of picrate and 5 g of Na₂CO₃ in 200 ml of distilled water) and the whole setup was incubated in a water bath at a temperature of 50 °C for 5 min. The formation of a dark red color was read spectrophotometrically at 490 nm against a reagent blank which contained 1 ml of distilled water and 4 ml of alkaline picrate solution. The cyanide content of the sample was extrapolated from a standard curve that was prepared by diluting potassium cyanide (KCN) standard (in water, acidified with HCl) to varying concentrations of 0.01 to 0.05 µg ml⁻¹ in 0.01 increments. The cyanide concentration was calculated from the equation of the calibration curve.

2.5 Sample preparation for amino acid analysis

About 2.0 g of the sample was weighed into the extraction thimble and the fat extracted with chloroform/methanol (2:1 v/v) mixture using a Soxhlet apparatus (AOAC, 2005). The extraction lasted for 15 hr. About 30 mg of the defatted sample was weighed into glass ampoule. 7 ml of 6 M HCl was added and oxygen expelled by passing nitrogen gas into the ampoule (This is to avoid possible oxidation of some amino acids during hydrolysis). The glass ampoule was sealed with a Bunsen burner flame and put into an oven at 105 ± 5 °C for 22 hr. The ampoule was allowed to cool; the content was filtered to remove the humins. The filtrate was then evaporated to dryness at 40 °C under vacuum in a rotary evaporator. The residue was dissolved with 5 ml acetate buffer (pH 2.0) and stored in a plastic specimen bottle and kept in the deep freezer.

2.5.1 Amino acid analysis

Amino acid analysis was by ion exchange chromatography (IEC) (FAO/WHO, 1991) using the Technicon Sequential Multisample (TSM) Amino Acid Analyser (Technicon Instrument Corporation, New York). The period of analysis was 76 min for the sample. The gas flow rate was 0.50 ml/min at 60 °C with reproducibility consistent within ± 3 %. The net height of each peak produced by the chart recorder of the TSM (each representing an amino acid) was measured and calculated. The amino acid values reported were the averages of two determinations.

2.5.2 Determination of quality parameters

2.5.2.1 Determination of amino acid scores

The amino acid scores were calculated using three different procedures:

- Scores based on essential amino acid scoring pattern (FAO/WHO, 1973).
- Scores for both essential and non essential amino acids based on whole hen's egg (Paul *et al.* 1976).
- Scores based on essential amino acid suggested pattern of requirements for pre-school child (FAO/WHO/UNU, 1985).

2.5.2.2 Determination of the predicted protein efficiency ratio

The predicted protein efficiency ratio (P-PER) was determined using one of the equations derived by Alsmeyer *et al.* (1974) i.e. P-PER = - 0.468 + 0.454 (Leu) - 0.105 (Tyr)

2.5.2.3 Other determinations

The Total amino acid (TAA), total essential amino acid (TEAA), total non-essential amino acid (TNEAA), total acidic amino acid (TAAA), total basic amino acid (TBAA), total neutral amino acid (TNAA) total sulphur amino acid (TSAA) and total aromatic amino acid (TArAA) and their percentage values, percentage cystine in TSAA (% Cys/TSAA) were calculated.

The isoelectric point (pI) was calculated using the equation of the form (Olaofe and Akintayo, 2000):

$$IP_m = \sum_{i=1}^n IP_i X_i$$

Where IP_m is the isoelectric point of the mixture of amino acids, IP_i is the isoelectric point of the i^{th} amino acid in the mixture and X_i is the mass or mole fraction of the i^{th} amino acid in the mixture (Finar, 1975).

3.0 Results and Discussion

3.1 Proximate composition

The result of the proximate composition of *A. micraster* stem bark is shown in Table 1. The moisture content (12.4%) fell within the range of reasonable amount of moisture in most vegetables 6 % to 15 % (Rishi *et al.*, 2012). The low level of moisture in *A. micraster* stem bark will not enhance the growth and development of micro organisms, thus it can be stored easily. The ash value is comparable to the percentage ash present in *Alchornea cordifolia* stem bark (10.35 %; Ngaha *et al.*, 2016) but higher than that of *Bridelia ferruginea* stem bark (6.54 %; Adesina and Akomolafe, 2014). Ash content of any food substance gives an idea of the minerals present in the food. The percentage crude protein content of *A. micraster* (13.8 %) was higher than 6.0 % reported for the stem bark of *Bombax buonopozense* (Edem *et al.*, 2016) and 10.51 % for *Cassia nigricans* stem bark (Gbekele-Oluwa Ayo, 2013) but comparable with the values for some legumes such as kersting's groundnut (12.9 %) cowpea (12.1 %) and bambara groundnut (11.6 %) as reported by Aremu *et al.* (2006). According to Pearson (1976), a plant with more than 12 % of calorific value from protein can be a good source of protein. The evidence from this study reveals that an appreciable amount of protein can be obtained from *A. micraster* stem bark when consumed.

The low value of crude fat obtained in the current study does not qualify *A. micraster* as oil rich food compared with oil seeds such as groundnut and melon with 40.83 % and 53.04 % crude fat respectively (Onyeike and Acheru, 2002). The observation in the present study was consistent with those of Abu *et al.* (2016) who reported low fat content for *Alstonia boonei* stem bark (0.404%). *A. micraster* stem bark contains 11.7 % crude fibre content. Studies have shown that increase in fibre consumption in foods reduces the incidence of obesity, cardiovascular disease, type 2 diabetes, digestive disorders and some cancers (Turner and Lupton, 2011). Carbohydrates are the main energy source of the human diet (Jequier, 1994). The sample contained moderate carbohydrate content which makes it a good source of energy. Calculated metabolisable energy value of *A. micraster* was 1161 kJ/100 g an indication that it could be an important source of dietary calorie.

Table 1: Proximate composition of *A. micraster* stem bark (%) and calculated energy (kJ/100g)

Parameters	Mean Composition
Moisture	12.4 ± 0.0
Ash	9.01 ± 0.01
Crude protein	13.8 ± 0.1
Crude fat	1.20 ± 0.04
Crude fibre	11.7 ± 0.1
Carbohydrate	51.9 ± 0.1
Energy	1161

3.2 Mineral Composition

Table 2 presents the results of the mineral composition of *A. micraster* stem bark. Potassium was found to be the most abundant mineral in the sample. This result corroborates the report of Olaofe and Sanni (1988) where it was noted that potassium is the most abundant mineral in some agricultural products. Evidence suggests that high intake of potassium protect against strokes and cardiovascular diseases (Hendler and Rovik, 2008). The concentration of potassium was closely followed by calcium with a value of 628 mg/100g. Calcium is the principal mineral of bones and teeth. It also acts in normal muscle contraction and relaxation, nerve functioning, regulation of cell activities, blood clotting, blood pressure and immune deficiencies (Sizer and Whitney, 2008).

The concentrations of the other macro minerals investigated in the present study are 104, 67.8 and 23.8 mg/100g for phosphorus, magnesium and sodium respectively. Phosphorus is essential for the process of bone mineralization. It also makes up the structure of nucleic acids and nucleotides including adenosine triphosphate (Owens and Harriet, 2009). Magnesium is important for nerve and heart functions and aids many enzyme reactions while sodium is important for maintaining fluid balance and conducting nerve impulses (Wardlaw and Smith, 2009).

The Na/k and the Ca/P ratios are nutritionally important parameters. It has been noted by Shills *et al.* (1992) that modern diets which are rich in animal protein and phosphorus may promote the loss of calcium in the urine, this has led to the concept of calcium to phosphorus ratio. If the ratio is low (low calcium, high phosphorus intake) more than the normal amount of calcium may be lost in the urine. If the Ca/P ratio of a food is above 1, it is considered 'good' but if the ratio is less than 0.5, it is 'poor' (Nieman *et al.*, 1992). Ca/P ratio above 2 helps to increase the absorption of calcium in the small intestine (Audu and Aremu, 2011). The result of Ca/P ratio in *A. micraster* stem bark was 6.04; this makes it a good source of these minerals.

Also, to prevent high blood pressure, the sodium to potassium ratio in the body should be put into consideration. Na/K ratio of 0.027 was obtained for the sample and this was found to be within the range recommended (less than 1) for the prevention of high blood pressure by Nieman *et al.* (1992). This result suggests that *A. micraster* stem bark has the capacity to prevent hypertension.

The concentration of micro elements in the present study are in the order of Fe > Mn > Zn > Cu > Pb > Cr. Iron requirement by human is 10-15 mg for children, 18 mg for women and 12 mg for men (Fleck, 1976). Iron is required in a number of biological functions including proper functioning of the immune system, electron reactions, gene regulation, cell growth and differentiation as well as binding and transport of oxygen (Siddiqui *et al.*, 2014). Zinc plays a vital role in proper functioning of the reproductive system (Hambidge, 2000). The presence of lead in the sample indicates the onset of pollution in environs where the sample was collected.

Table 2: Mineral Composition of *A. micraster* stem bark (mg/100g)

Mineral	Concentration
Sodium	23.8 ± 0.4
Potassium	889 ± 1.0
Calcium	628 ± 3.0
Magnesium	67.8 ± 0.3
Phosphorus	104 ± 1.0
Zinc	5.29 ± 0.10
Iron	9.46 ± 0.20
Lead	0.18 ± 0.0
Chromium	0.1 ± 0.0
Manganese	6.71 ± 0.03
Copper	1.43 ± 0.06
Ca/P	6.04
Na/K	0.027

3.3 Anti-nutrients

The results of some of the anti-nutrients present in *A. micraster* stem bark are shown in Table 3. Oxalate content was low compared to that of *Senna alata* Linn leaves and flowers 8.00 and 3.50 mg/100g (Abdulwaliyu *et al.*, 2013). Oxalate binds to calcium to form calcium oxalate crystals; these prevent the absorption and utilization of calcium by the body thereby causing diseases such as ricket and osteomalacia (Ladeji *et al.*, 2004). The sample contain relatively small amount of tannins, alkaloid and saponin. Tannins chelate metals such as iron and zinc and reduce the absorption of these nutrients (Karamac, 2009). Alkaloids can block ion channels, inhibit enzymes, or interfere with neurotransmission, producing hallucinations, loss of coordination, convulsions, vomiting, and death (Tiwari and Rana, 2015). High saponin levels have been associated with gastroenteritis manifested by diarrhoea and dysentery (Awe and Sodipo, 2001). The cyanide content obtained in the present study was lower than the reported lethal dose of cyanide in human which ranges between 50 to 300 mg kg⁻¹ body weights (Bolhuis, 1954; Akiyama *et al.*, 2006). The low anti-nutrients level obtained in the present study implies that *A. micraster* is suitable for consumption.

Table 3: Anti-nutritional contents of *A. micraster* stem bark

Anti-nutrients	Level ± SD
Oxalate (mg/100g)	2.37 ± 0.02
Tannins (%)	0.012 ± 0.0
Alkaloid (g/100g)	0.018 ± 0.0
Saponin (%)	0.025 ± 0.0
Cyanide (mg/kg)	4.5 ± 0.1

3.4 Amino acid composition

Result of the amino acid composition of *A. micraster* stem bark is presented in Table 4. The result showed that glutamic acid was the most concentrated amino acid (AA), followed by aspartic acid. It has been observed by some researchers that glutamic and aspartic acids were the most abundant amino acids in agricultural products such as legumes (Olafe and Akintayo, 2000); *Amaranthus hybridus* leaves (Akubugwo *et al.*, 2007); *Celosia spicata* leaves (Ogungbenle and Otemuyiwa, 2015) and *Bridelia ferruginea* stem bark (Adesina and Akomolafe, 2014). Moreover, in the present study methionine had the least value of 0.6 g/100g cp. The amount of total amino acid (TAA) was 81.6g/100g cp. The value was comparable to the values reported for raw and cooked groundnut seeds (83.5 and 85.9 g/100g cp) as reported by Adeyeye (2010). All the essential amino acids were present in the sample with arginine having the highest concentration of 7.19g/100g cp.

Table 4: Amino acid concentrations of *A. micraster* stem bark (g/100g crude protein)

Amino acid	Concentration
Glycine (Gly)	3.88 ± 0.00
Alanine (Ala)	2.85 ± 0.01
Serine (Ser)	2.36 ± 0.02
Proline (Pro)	4.53 ± 0.04
Valine (Val)*	2.78 ± 0.04
Threonine (Thr)*	3.30 ± 0.02
Isoleucine (Ile)*	3.07 ± 0.05
Leucine (Leu)*	5.52 ± 0.18
Aspartic acid (Asp)	10.0 ± 0.1
Methionine (Met)*	0.60 ± 0.04
Glutamic acid (Glu)	15.2 ± 0.0
Phenylalanine (Phe)*	4.79 ± 0.15
Histidine (His)*	3.40 ± 0.02
Arginine (Arg)*	7.19 ± 0.03
Tyrosine (Tyr)	4.67 ± 0.06
Cystine (Cys)	1.75 ± 0.01
Lysine (Lys)*	4.50 ± 0.14
Tryptophan (Try)*	1.24 ± 0.04
Total (TAA)	81.6

*Essential amino acid

Table 5 reveals the concentration of total amino acid (TAA), total essential amino acid (TEAA), total non-essential amino acid (TNEAA), total acidic amino acid (TAAA), total basic amino acid (TBAA), total neutral amino acid (TNAA) total sulphur amino acid (TSAA) and total aromatic amino acid (TArAA) and their percentage values. The predicted protein efficiency ratio (P-PER) and isoelectric point (pI) are also shown in Table 5. Investigation into the amino acid concentration of *A. micraster* stem bark showed that the sample contains higher percentage of TNEAA (55.4) when compared with that of TEAA with histidine (44.6). This observation is similar to the report of the amino acids present in *Bridelia ferruginea* stem bark (Adesina and Akomolafe, 2014); raw and cooked groundnut seeds (Adeyeye, 2010) where the % TNEAA of the plant parts reported on were higher than % TEAA with histidine. The content of TEAA of 36.4 g/100g cp of *A. micraster* stem bark was close to the value for egg reference protein (56.6g/100g cp) (Paul *et al.*; 1976) and soya bean (44.4g/100g cp) (Altschul, 1968).

Table 5: Summary of the calculated essential, non- essential, acidic, basic, neutral, total sulphur and aromatic amino acid composition of *A. micraster* stem bark sample (g/100g crude protein)

Parameter	Level
Total Amino Acid (TAA)	81.6
Percent total amino acid (% TAA)	100
Total non-essential amino acid (TNEAA)	45.2
Percent total non-essential amino acid (% TNEAA)	55.4
Total essential amino acid (TEAA)	36.4
Percent total essential amino acid (% TEAA)	44.6
Total essential amino acid (TEAA) with Histidine	36.4
Percent total essential amino acid (% TEAA) with Histidine	44.6
Total essential amino acid (TEAA) without histidine	33.0
Percent total essential amino acid (% TEAA) without histidine	40.4
Total neutral amino acid (TNAA)	41.3
Percent total neutral amino acid (% TNAA)	50.6
Total acidic amino acid (TAAA)	25.2
Percent total acidic amino acid (% TAAA)	30.9
Total basic amino acid (TBAA)	15.1
Percent total basic amino acid (% TBAA)	18.5
Total sulphur amino acid (TSAA)	2.35
Percent total sulphur amino acid (% TSAA)	2.88
Percent cystine in TSAA	74.5
Total aromatic amino acid (TArAA)	10.7
Percent total aromatic amino acid (% TArAA)	13.1
Calculated isoelectric point (pI)	4.67
Predicted protein efficiency ratio (P-PER)	1.55

The percentage TNAA, TAAA and TBAA values followed the trend $50.6 > 30.9 > 18.5$ in *A. micraster* stem bark. The TArAA obtained in the present study (10.7g/100g cp) fell within the range suggested for ideal infant protein (6.8-11.8g/100g cp) (FAO/WHO/UNU, 1985). The ArAA are the precursors of epinephrine and thyroxine (Robinson, 1987). Result of the % Cys/TSAA in *A. micraster* stem bark corroborates the result obtained by other researchers where it has been observed that many vegetable proteins contain substantially more cystine than methionine. For example % Cys/TSAA in coconut endosperm was 62.9 (Adeyeye, 2004), while that of *Anacardium occidentale* was 50.5 (Adeyeye *et al.*, 2007). Most animal protein are low in Cys and hence Cys in TSAA (Adeyeye and Adamu, 2005). Cystine can spare methionine in improving protein quality and also has effect on mineral absorption, particularly zinc (Mendoza, 2002).

Table 6: Essential amino acid scores of *A. micraster* stem bark based on FAO/WHO (1973) standards

Amino acid	^a Suggested level of scoring pattern (mg/g)	standard	Amino acid content of sample (mg/g)	Sample Score
Ile	40		30.7	0.77
Leu	70		55.2	0.79
Lys	55		45.0	0.82
Met+ Cys	35		23.5	0.67
Phe + Tyr	60		94.6	1.58
Thr	40		33.0	0.83
Try	10		12.4	1.24
Val	50		27.8	0.56
Total	360		322	0.89

^a(FAO/ WHO, 1973)

Table 7: Amino acid score of *A. micraster* with respect to whole hen's egg scoring pattern

Amino Acid	^a Whole Hen's egg (g/100g)	Sample(g/100g)	Sample Score
ys	6.20	4.50	0.73
His	2.40	3.40	1.42
Arg	6.10	7.19	1.18
Asp	10.7	10.0	0.94
Thr	5.10	3.30	0.65
Ser	7.90	2.36	0.30
Glu	12.0	15.2	1.27
Pro	3.80	4.53	1.19
Gly	3.00	3.88	1.29
Ala	5.40	2.85	0.53
Cys	1.80	1.75	0.97
Val	7.50	2.78	0.37
Met	3.20	0.60	0.19
Ile	5.60	3.07	0.55
Leu	8.30	5.52	0.67
Tyr	4.00	4.67	1.17
Phe	5.10	4.79	0.94
Try	1.80	1.24	0.69

^aPaul *et al.*, 1976

The predicted isoelectric point (pI) is a good starting point in predicting the pI for protein in order to enhance a quick precipitation of protein isolate from biological samples (Olaofe and Akintayo, 2000). The pI of *A. micraster* was 4.67 (Table 5). The predicted protein efficiency ratio (P-PER) was calculated to be 1.55. The value was within the range of literature levels of cowpea (1.21) and pigeon pea (1.82) reported by (Salunkhe and Kadam, 1989); millet ogi (1.62) and sorghum ogi (0.27) reported by Oyarekua and Eleyinmi (2004). The experimentally P-PER usually ranged from 0.0 for a very poor protein food to a maximum of about 4.0 in very rich protein source (Muller and Tobin, 1980).

The results of the essential amino acid (EAA) scores based on provisional essential amino acid scoring pattern (FAO/WHO, 1973) are shown in Table 6. Trp (1.24) and Phe + Tyr (1.58) had values greater than 1.0. Val had the lowest score with a value of 0.56. To correct for the AA needs from the sample, it becomes 100/56 or 1.79 times as much sample protein of *A. micraster* stem bark to be taken (eaten) when it is the sole source of protein in the diet (Bingham, 1977). Table 7 reveals the AA scores (AAs) of *A. micraster* based on the whole Hen's egg profile. Values greater than 1.0 were obtained in His (1.42), Arg (1.18), Glu (1.27), Pro (1.19) Gly (1.29) and Tyr (1.17). The least score was methionine (0.19).

The scoring of the EAA of *A. micraster* stem bark based on the suggested requirement of the EAA of a pre-school child (FAO/WHO/UNU, 1985) are shown in Table 8. Ile, Phe + Tyr, Try and His had values greater than 1.0., while the other EAA scores were lower than 1.0. Lys had the lowest score which makes it the limiting amino acid based on this scoring profile. To correct for the limiting AA, it is 100/78 or 1.28 multiply by the protein of *A. micraster*.

Table 8: The scoring of the essential amino acid of *A. micraster* stem bark based on the suggested requirement of a pre-school child

Amino acid	^a Pre-school (g/100g)	Amino acid content of sample (g/100g)	Sample Score
Leu	6.60	5.52	0.84
Ile	2.80	3.07	1.10
Lys	5.80	4.50	0.78
Met+ Cys	2.50	2.35	0.94
Phe + Tyr	6.30	9.46	1.50
Try	1.10	1.24	1.13
Thr	3.40	3.30	0.97
Val	3.50	2.78	0.79
His	1.90	3.40	1.79

^aFAO/WHO/UNU (1985)

Conclusion

Our result in this study revealed that *A. micraster* stem bark contains high carbohydrate content and appreciable amount of protein and fibre. The sample is a very good source of many of the essential and non-essential amino acids. In addition to these, the presence of high mineral content of *A. micraster* revealed that it can serve as a source of these valuable nutrients when included in diets.

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