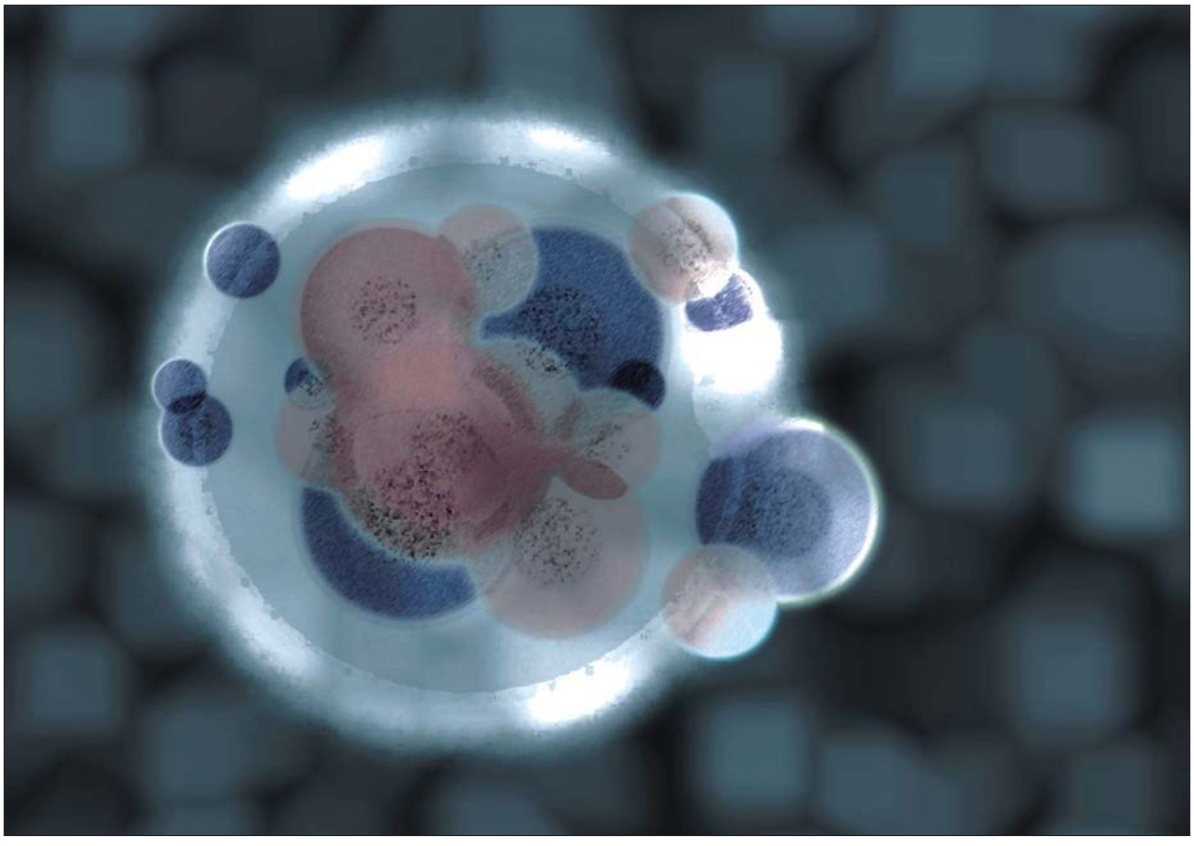

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Amino Acids and Amionic Components of *Sacoglottis gabonenis* Stem Bark Extract, A Nigerian Alcoholic Beverage Additive

H.C.C. Maduka, [1]Z.S.C. Okoye and M. Mahmood
Department of Biochemistry, College of Medical Sciences, University of Maiduguri, Maiduguri, Nigeria
[1]Department of Biochemistry, Faculty of Medical Sciences, University of Jos, Jos, Nigeria

Abstract: The amino acid profile and anionic composition of three aqueous preparations of *Sacoglottis gabonensis* stem bark were determined. Curde extract of *Sacoglottis gabonensis* stem bark prepared in 4% ethanol (1:10^{w}/v), the deffated fraction of the ethanol crude extract and methanol extract (1:10^{w}/v) were analyzed. The amionic composition analysis showed that in ethanolic extract, Cl^{-} was present in highest amount followed by NO^{-}_{3} then F^{-} and lastly SO_4^{2-} being 27.7±1.25, 6.82±0.11, 5.10±0.30 and 3.08±0.11 ppm respectively. The deffated ethanol extract contained Cl^{-} in highest quantity followed by SO_4^{2-} and then NO^{-}_{3} and finally F-being 24.8±0.98, 12.34±0.15, 9.14±0.38 and 5.10±0.30 ppm respectively. The methanolic extract contained Cl^{-}, SO_4^{2-}, NO_3^{-} and F^{-} to the extent of 30.3±1.08, 6.77±0.11, 4.77±0.20 and 4.12±0.18 ppm respectively of the amions. Results showed that while the ethanol crude extract contained eleven amino acids including seven essential amino acids (1 ys, Leu, Ile, Tyr, Thr, His and Val), the defatted extract and methanolic extract contained 13 and 17 amino acids respectively including all the nine essential amino acids. Both the defatted portion and methanolic extract contained Cys thus suggesting a role in detoxification of autooxidizable drugs. The bark extract can serve as alternative source of essential amino acids and hence relevant in nutrition. Toxic species like CN, Cd and Hg that could inhibit drug metabolizing enzymes were absent. Methanol is the best extractor while defatting unmasked Phe and Met which were not detected in the crude extract.

Key words: Amino acids, anions, ethanol, methanol and *Sacoglottis gabonensis*

INTRODUCTION

Food and Agricultural Organization of the UN observed that the food consumed in local communities is deficient in quality and quantity causing widespread malnutrition and under nutrition (Ladeji *et al*., 1995). The remedy was to consume more of the conventional protein rich foods such as meat, eggs and fish, but the poor state of economy has made these protein rich foods out of the reach of over 70% of the population (Ladeji *et al*., 1995). It was then recommended that people should eat unconventional sources of leaf proteins as supplement especially if they do not contain some toxic chemical species that can cause metabolic and biochemical inhibitions.

Natural plant products have also been known to contain cytoporotective properties against drug or chemical induced-cytotoxicities. Among such reports are Parasakthy *et al*., (1993) on the hepato-protective property of eugenol, an active principle of ocimum and nut meg, of *Cyperus scariosus* on acetaminophen and CCl_4-induced hepatotoxicicy by Gilani and Janbaz (1995), of Aliyu (1995) on hepatoprotective property of *Cochlospermum planchoni* against Ccl_4 jaundice, of Ladeji and Okoye (1995) on the anti-hepatotoxic properties of Vitex doniana bark extract, of Sugiyama *et al.* (1993) on purpurogallin as an antioxidant against lysis of human erythrocytes by CCl_4 and those of Maduka and Okoye (2001 and 2002a) on *Sacoglottis gabonensis* as an antioxidant *In vivo* and *In vitro* against doxorubicin and 2, 4-dinitrophepyl hydrazine induced membrane per oxidation.

The above reports not withstanding, there have been paucity of information on the proximate, elemental, amino acids and other biochemical components of most of the plants being implicated in cytoporotective responses to further justify their use in folkoric medicine practices. Some of the few reports in this regard that have shown the potentials of these natural plant products from their biochemical components include that on *Sacoglottis gabonensis* stem bark extract by Maduka and Okoye (2002c), by Onwuliri and Anekwe (1993a) on *Bryophyllum pinnatum,* Onwuliri and Anekwe (2001) on *Ricinus commucunis* (variety miner), an anticonceptive seed.
Sacoglottis gabonensis is a tropical rain forest free that grows along the coastal region of west African from Gambia down to Nigeria. Its stem bark has been commonly used in Southern Nigeria especially among the rural

communities in Abia, Akwa Ibom, Cross River, Delta, Edo, Imo and Rivers States as an additive to palmwine. The inner layer when bitten to a red fibrous mesh is dipped into the palmwine to which it imparts an amber colour and bitter taste to the sweet sugary white palmwine. The amber colour and bitter taste make the palmwine more acceptable and testable to the local consumers. From surveys conducted over the years among the local consumers of *Sacoglottis gabonensis* stem bark treated palmwine, claims are unanimous that it improves the shelf-life and reduces foaming and fermentation of the palmwine. There were further claims that the bark extract ameliorates the intoxicating power of palmwine suggesting that it contains elements that may be antitoxic against alcohol-induced toxicity.

Scientific evidence have been provided to back the reduction of alcohol induced toxicity in the brain (Maduka, 2000) by bark extract. The bark extract has been shown to affect disposition of acetaminophen in human volunteers (Madusolumuo *et al*., 1993). Recent report showed that the bark extract contains elements that form part of the composition of key antioxidant elements like catalase, superoxide dismutase and glutathione peroxidase. These are the three primary antioxidant enzymes that act to detoxify free radicals in biological systems (Kurmar *et al*., 1988). The above reports suggest a possible role in nutrition, electrolyte balance functions and management of hypertension for *S. gabonensis* stem bark extract. The present study was designed to determine the amino acid profiles and anion components of three aqueous extracts of the stem bark of *S. gabonensis* as part of its nutritional and toxicological evaluation.

Another objective was to know the best solvent extractor of the three systems used.

MATERIALS AND METHODS

Preparation of *Sacoglottis gabonensis* stem bark extract: Freshly harvested samples of *Sacoglottis gobonensis* stem bark were purchased from Ngwa in Aba, Abia state, Nigeria and wrapped in polythene bag and refrigerated prior to use immediately upon return to Maiduguri. The outer coatings were scrapped clean and rinsed with distilled water. The pinkish material was pounded to a fibrous mesh in a mortar, weighed and transferred quantitatively to a large breaker and stepped in a known volume of an aqueous solution containing 4 ml ethanol in 100 ml in ratio of 1:10 w/v bark:aqueous ethanol. The beaker was covered and transferred to a cupboard and left for three nights. This was filtered and the filtrate refrigerated as the stock ethanol extract for quantitation of amino acids and anion components.

Preparation of defatted ethanol extract: Ethanol extract was prepared by the procedure of Maduka (2000) which is an adaptation of the local method of preparation by consumers of bark extract treated palmwine earlier described by Madusolumuo (1993). The aqueous ethanol extract was divided into two and one half was extracted with petroleum spirit in order to remove unwanted lipids and fats in the extract and then with methylated spirit by the method of Faparusi and Bassir (1972). The methylated spirit extract was removed with the help of a separation funnel and refrigerated for determination of amino acids and anion components in the same way as the ethanol crude extract.

Preparation of methanol extract of *Sacoglottis gabonensis*: Methanol extract of *S. gabonensis* stem bark was prepared using the same procedure as for ethanol extract except that pure methanol instead of 4% ethanol was used as the solvent of extraction. The methanol extract was prepared 1:10 bark weight to pure methanol and refrigerated for determination of the amino acids and anions.

Determination of amino acid profiles in *S. gobonensis* stem bark extracts: The amino acid profile of the crude extract of *Sacoglottis gabonensis* stem bark extract was determined by the method of Spackman and Moore (1958) using amino acids analyzer (Technicom TSM-1 model DNA 0209 Ireland).

Three different samples of the bark extract namely; 1:10 w/v *Sacoglottis gabonensis* bark extract in 4% ethanol, defatted 1:10 w/v *S. gabonensis* bark extract and 1:10 w/v methanolic extract of *S. gabonensis* stem bark prepared as described above were used for comparison purposes.

The samples were dried to constant weight, defatted, hydrolyzed and evaporated in a rotary evaporator and handed into technicon sequential multisample amino acid analyzer (TSM). A known weight of the dried sample was weighed into extraction thimble and the fat was extract with methanol mixture using soxhlet extraction apparatus as described by AOAC (1983). The extract lasted for 8 h. Between 30-50 mg of the defatted samples were weighed into glass ampule and 7 ml of NH_4Cl was added with the expulsion of oxygen by passing nitrogen into the empoule (to prevent possible oxidation of some of the amino acids during hydrolysis). The glass empoule was sealed with bunsen burner flame and put in an oven preset at 105°C for 22 h. The ampoule was allowed to cool before being broken at the tip and the content filtered to remove the humids. The filtrated was then evaporated to dryness at 40°C under vacuum in a rotary evaporator. The residues

were dissolved with 54 ml of acetate buffer (pH 2.0) and stored in plastic specimen bottles which were kept in deep freezer. The amount loaded was between 5 to 10 μl which was then dispensed into the cartridge of analyzer. The TSM analyzer was designed to separate and analyze free acidic, neutral and basic amino acids of the hydrolysate. The period of analysis lasted for 76 min. The analysis was done with each of the three different bark extract solutions.

Calculation of amino acid values from the chromatogram peaks: The net heights of each peak produced by the chart recorder of the TSM (each representing an amino acid) were measured. The half height of the peaks were found and the widths of the peaks at the half heights of the measured, read and recorded. The approximate area of each peak was then obtained by multiplying the height with the width at half height.

The Norleucine Equivalent (NE) for each amino acid in the standard mixture was calculated using the following formula (Spackman and More, 1958):

$$NE = \frac{\text{Area of norleucine peak}}{\text{Area of each amino}}$$

a constant ,s, was calculated for each amino acid in the standard mixture as follow:

$$S^{std} = \text{NestdxMol weight}$$

Finally, the amount of each amino acid present in the sample was calculated in g/16gN using the following formula (Spackman and More, 1958):

$$\text{Concentration (g) (6gN)} = NH \times \frac{NH}{2} \times S^{std} \times C$$

Where, C= Dilution x 16

$$\frac{\text{Sample wt (g) x N2\% x 10 x Vol-loaded}}{NH \times W \text{ (NLue)}}$$

Where, NH=net height, W=width, Nleu= Norleucine

Determination of anion components in the *S. gabonensis* stem bark extract: The ion chromatograph used is the YOKOGAWA ion chromatographic analyzer (YEW, ICA) model IC 100-25 equipped with a conductivity and uv detector. The IC 100-25 has dual column system comprising a suppressor and a separator column (SAX-I, YEW x 4.6 mm ID) which uses a strong base anion exchanger resin and concentrator (SAX-2, 100 x 4.6 mm ID). The conductivity cell and separator column were placed in a temperature controlled oven set at 40°C. Standard solution of anions NO_2, NO_3, PO_4^{2-} and SO_4 were usedd. The scavenger and element were prepared with distilled deionized water which had a conductivity of <1 M_s cm^{-1}, element contraction was 30 mMol $Na\,CO_2$, 3 scavenger contraction was 0.05 M H_2SO_4 while the injector volume was 100 ml at temperature of 40°C.

The amion components were quantitatively determined in the ethanol, defatted and methanol extracts of the bark extract following the procedure of Hanaoka *et al.* (1982) and Ubom and Tsuchiya (1988). Results were presented in ppm as means ±S.D. of triplicate determinations ($P<0.05$).

RESULTS

Amino acid profile of *Sacoglottis gabonenis* stem bark extract: About eleven amino acids made of six essential amino acids and five non essential ones were present. The essential amino acids present include Lys, His, lle, Leu, Tyr and Val while the none essential amino acids detected were Arg, Asp, Ser and Glu. Phe, Thr, Met and Cys were absent (Table 1). The results would suggested that the bark extract contains both essential and non essential amino acids.

The amino acid profile of the defatted ethanol extract of the bark showed that fifteen amino acids were present including seven essential amino acids namely Lys, His, Val, Leu, IIe, Tyr and Phe, The absent amino acids were Thr and Met. The non-essential amino acid components detected were Arg, Asp, Ser Glu, Ala and Cys. This would suggest that defattening unmasked Phe and yielded Cys, a sulphur-containing amino acid (Table 2).

As can be seen from the results a total of nine essential amino acids namely lys. His. Thr. Val. Met, Ile, Leu. Tyr and Phe and the non-essential acids namely Arg, Asp, Ser, Glu, Pro, Gly, Ala and Cys were detected suggesting that methanol extracted and unmasked Thr, Met and Phe unlike ethanol (Table 3).

N-Leucine was present in all the three preparations. Overall, methanol was the best extractor of the three solvents.

Anion components of *Sacoglottis gabonensis* stem bark extract: Fluoride, (F^-) chloride (CL^-) Nitrate (NO_3^-) and Sulphate (SO_4^{2-}) were detected in the three aqueous extracts of the bark as shown on Table 5.

In the ethanol extract of the bark, Cl was present in highest amount followed by Nitrate (NO_3^-) and then fluroide and lastly (SO_4^{2-}) being 27-7±1.75, 682±0.11, 5.10±0.30 and 3.08±0.010 ppm respectively (Table 5). The defatted ethanol fraction showed that chloride was the highest anion present at 24.8±0.98 ppm followed by

Table 1: Amino acid profile of crude extract of *S. gabonensis* in 4% ethanol

Amino acids	Net height	Nh/Z (mm)	Width NH/z (mm)	S. std	g/100g of protein	FAO performanceS. sts
Lys	34	17.0	5	11.20	1.45	4.20
His	13	6.5	4	12.13	0.48	
Amm						
Arg	20	10.0	11	8.35	1.39	
Asp	44	22.0	4	8.16	2.19	
Thr	-	-	-	8.26		2.80
Ser	9	4.5	3	5.23	0.21	
Glu	29	14.5	7	9.06	2.79	
Pro	-	-	-	14.44		
Gly	27	13.5	4	6.40	1.01	
Cys	-		10.84		2.0	
Met	-	-	-	7.76		2.20
Ileu	30	15.0	4	6.29	1.15	4.20
Leu	40	20.0	4	6.52	1.59	4.20
Nleu	80	40.0	7	3.28	2.79	
Tyr	4	2.0	5	12.29	0.37	2.80
Phe	-	-	-	9.49		2.80
Val	5	2.5	3	8.25	0.19	4.20

[b]FAO, 1970 %N=1.225% C=0.000759712 (basic) Wt=1.6327 C=0.000151942 (acidic/neutral)

Table 2: Amino acid profile of crude extract of *S. gabonensis* in defatted ethanol

Amino acids	Net height	Nh/Z (mm)	Width NH/z (mm)	S. std	g/100g of protein
Lys	37	18.5	3.0	11.20	0.09
His	9	4.5	3.0	12.13	0.02
Amm					
Arg	19	9.5	13.0	8.35	0.16
Asp	43	21.5	4.0	8.16	0.21
Thr	-	-	-	8.26	
Ser	10	5	3.5	5.23	0.03
Glu	30	19	5.5	9.06	0.29
Pro	-	-	-	14.44	
Gly	29	14.5	3.5	4.21	0.06
Ala	27	13.5	3.5	6.40	0.09
Cys	4	2	5.0	10.84	0.03
Val	5	2.5	4.0	8.25	0.03
Met	-	-	-	7.76	
Ileu	29	14.5	4.0	6.29	0.11
Leu	43	21.5	4.0	6.52	0.17
Ileu	79	39.5	6.0	3.28	0.24
Tyr	4	2	6.0	12.29	0.24
Phe	3	1.5	5.0	9.49	0.02

%N= 1.233%, C= 0.000075971 (basic), Wt.= 1.6221, C= 0.000151943 (acidic/neutral), 7 = (2 x 4 x 6 x c) = g/100g of protein

Table 3: Amino acid profile of methanolic extract of *S. gabonensis*

Amino acid	Net height	NH/z (mm)	Width NH/Z (mm)	S. std	g/100g of protein
LYS	43	21.5	4.0	11.20	0.15
His	13	6.5	4.0	12.13	0.05
Amm					
Arg	30	15.0	13.0	8.35	0.25
Asp	44	22.0	4.0	8.16	0.22
Thr	5	2.5	3.0	9.26	0.02
Ser	12	6.0	3.5	5.23	0.03
Glu	40	20.0	6.0	9.06	0.33
Pro	5	2.5	4.0	14.44	0.04
Gly	30	15.0	3.0	4.21	0.06
Ala	28	14.0	3.5	6.40	0.09
Cys	5	2.5	5.0	10.84	0.04
Val	6	3.0	3.0	8.24	0.02
Met	4	2.0	5.0	10.84	0.02
Ileu	34	17.0	3.5	6.29	0.11
Leu	46	23.0	4.0	6.52	0.18
n leu	90	45.0	6.0	3.28	0.27
Tyr	5	7.5	7.0	12.29	0.07
Phe	3	1.5	5.0	9.49	0.02

%N= 1.251% C=0.000079574 (basic) Wt= 1.5987 C=0.0000151948 (acid/neutral)

Table 4: Amino acid profile of standard amino acid preparation

Amino acid	Net height	NH/z (mm)	Width NH/z (mm)	NH x 10	g/100g of protein	Std.
Lys	90	45.0	5.0	450	182.65	11.20
His	79	39.5	6.5	477	209.63	12.13
Amm					53.49	
Arg	48	24.0	12.0	576	174.20	8.35
Asp	90	45.0	5.0	450	133.11	8.16
Thr	98	49.0	4.5	441	119.11	8.26
Ser	111	55.5	5.0	555	105.09	5.23
Glu	64	32.0	7.0	4	147.13	9.06
Pro	22	11.0	10.0	220	115.13	14.44
Gly	82	41.0	6.0	462	75.07	4.21
Ala	64	32.0	6.0	384	89.09	6.40
Cys	36	18.0	17.0	612	240.30	10.84
Val	112	56.0	3.5	392	117.15	8.25
Met	118	59.0	4.5	531	149.21	7.76
Ileu	96	48.0	6.0	576	149.21	6.29
Leu	111	55.5	5.0	555	131.18	6.52
Nleu	138	69.0	8.0	1104	131.18	3.28
Tyr	37	18.5	11.0	407	181.19	12.29
Phe	40	20.0	12.0	408	165.19	9.49

$$\text{NE std} = \frac{\text{NH x w (n leu)}}{\text{NH x w (AA)}} \qquad \text{u moles A.A} = 0.025 \quad S = \text{NE std x mol wt x um A Astd}$$

Table 5: Anion components of crude extract, defatted extract and methanoic extract of S. *gabonenis* components (ppm)

Sample	Fluoride (F^-)	Chloride (Cl^-)	Nitrate (NO_3^-)	Sulphate (SO_4^{2-})
Ethanol extract	5.10±0.30^{eb}	27.7±1.25^{ef}	6.28±0.11^{eb}	3.08±0.010cb
Defatted ethanol Extract	25.51±0.18^{de}	24.8±0.98^{c}	9.14±0.38^{de}	12.31±015de
Methanol extract	4.72±0.18^{ab}	30.3±1.08^{df}	4.77±0.20^{ab}	6.77±0.11^{bd}

Values are means of five determinations (± S.D.)
[a]Statistically lower than ethanol extract (P<0.05)
[b]Statistically lower than defatted ethanol extract (P<0.05)
[c]Statistical lower than methanol extract (P<0.05)
[d]Statistical higher than ethanol extract (P<0.05)
[e]Statistical higher than methanol extract (P<0.05)
statistically higher than defatted ethanol extract (P<0.05)

Sulphate12.34±0.1, 15 ppm and then Nitrate 9.14±0.38 pmm and finally fluroide 5.10±0.30 ppm respectively (Table 5). The methanolic extract showed that Chloride was 30.3±1.08 ppm, Sulphate 6.77±0.11, Nitrate 4.77±0.02 ppm and lastly Fluroide 4.12±0.18ppm. While Fluroide, Nitrate and Sulphate were extracted most in the defatted ethanol extract, the highest Cl^- was extracted by the pure methanol solution suggesting that defattening will make the bark extract more relevant in management of dental carries due to Flouride (F^-) deficiency. All the extracts extracted Cl^- quantitatively. Results also showed that while methanol extracted Cl^- and SO_4^{2-} more than aqueous ethanol, the later extracted more NO_3^- and F^- than the former. The results suggest that the bark extract may serve as a source of Flouride required for tooth stability, play electrolyte balance functions as well as aid in Nitrate and Sulphate conjugations.

DISCUSSION

The level of work done on Sacoglottis gabonensis have shown that if possesses essential inorganic elements of the primary antioxidant enzymes Cu, Mn, Fe, Zn and hence may find ready application in ameliorating lipid per oxidation in free radical medicated reactions as well as being relevant in the management of hypertension and other electrolyte imbalances related disorders. The results being presented in this study have shown that Fluoride (F), Chloride(Cl), Nitrate (NO_3^-) and Sulphate (SO_4^{2-}) are quantitatively present in nitro and ethereal conjugations in the detoxification of xenobiotic oxidations. This result is consistent with result of Madusolumuo *et al.* (1993) that the bark extract influenced the disposition of acetaminophen in human volunteers consuming S. gabonensis stem bark treated palmwine.

In the body, K^+, Na^+, Cl^- and Mg^{2+} are encountered on a daily basis in dealing with patients with electrolyte imbalances. In addition to the macro elements C, H, O, N the body contains up to 25 order mineral element such as S, P, Cl, F, Br, I, B, S, Hs, Mg, K, Ca, Na, Fe, Cu, Zn, Nr, Co, Mn, AL, Pb, Sn, Mo, V and F. While the role of some elements in human metabolism are known, the role of others (As, B, T, Pb) is not clear yet. These elements are needed for electrolyte, acid base balance, osmotic equilibrium functions in extracellular and intracellular fluids, structural roles in bones and teeth components and as cofactors of enzymes in electron transport chain. Some of the aboveroles were highlighted in an earlier report (Maduka and Okoye, 2002c). This study revealed that chloride which plays important role in fluid and electrolyte

balance is present in copious amounts in the bark extract. Fluroide increases hardness of bones and teeth and stabilizes these tissues against toxicity and dental fluorosis. Fluoride though not proved to be strictly essential for human growth, plays well defined roles in prevention and treatment of dental carries and has been accepted as being able to affect the crystal structure in animals by forming a fluoro appetite. The presence of large amount of Cl^- and F^- in the bark extract serves as scientific basis to justify the claims by local consumers of bark extract treated palmwine that the extract is used in management of hypertension as well as teeth problems.

Earlier reports of antioxidant properties of the bark (Ekong and Ejike, 1974; Maduka and Okoye, 2002a, b, d) suggested that the bark extract can be useful additive in the pharmaceutical preparations. The presence of all essential and non-essential amino acids in S. gabonensis in this study is forcing a conclusion that the extract is potential in food industries as a novel source of proteins. The importance of the above findings is further emphasized by the fact that amino acids are the building blocks of proteins. Though as simple molecules, they serve as substrates for protein synthesis and other diverse roles as tissue repairs, hormone synthesis and precursors of hemes which are compounds of biological importance as well as enzymes involved in energy transduction in the mitochondria.

It is worthy of note that Glu and Asp represent a storage form of nitrogen in addition to being starting materials for the backbone of amino acids (Onwuliri and Anekwe, 1993 b).

During acid hydrolysis step, Gln and Asn were converted to Glu acid and Asp acid with the liberation of ammonium NH^+_4 ions. This seems to account for the concentrations of the two amino acids in the profiles of all the extracts examined. While methanol was the best extractor, a fact consistent with earlier report (Mauka and Okoye, 2002c), defattenning unmasked more essential amino acids than detected in the untreated crude extract. We conclude a possible role for the bark extract in detoxification, nutrition and management of electrolyte-balance disorders.

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REFERENCES

Aliyu, R., 1995. Studies on the medicinal and biochemical properties of *Cochlospermum planchonii*. Ph.D thesis, University of Jos, Nigeria.

AOAC, 1983. Official methods of Analysis, 13th edn. Association of official Analytical Chemists, Washington D.C., pp: 768-800.

Ekong, D.E. and C. Ejike, 1974. Antioxidant principles from the bark of *Spomdianthus preussi* and *Sacoglottis gabonensis* (family hummiriacea). J. West African Sci. Asso., 19: 63-68.

Faparusi, S.I. and O. Bassir, 1972. Effect of extracts of the bark of *Sacoglottis gabonensis* on the microflora of palmwine. Appl. Microbiol., 24: 853-856.

Gilani, A.H. and K.H. Janbaz, 1995. Studies on the protective effect of *Cyperus scariosus* extract on acetamionophen and Ccl_4-induced hepatotoxicity. General Pharmacol., 26: 627-631.

Hanaoka, Y., T. Murayama, S. Muramoto, T. Muramoto, T. Mutsure and A. Nanba, 1982. Ion chematography, 239: 537-548.

Kumar, K.S., Y.N. Vaishnav and J.F. Weiss, 1988. Radioprotection by antioxidant enzymes and ezyme mimetics. Pharmaceutical Therapeutics, 39: 301-309.

Ladeji, O., Z.S.C. Okoye and T. Ojobe, 1995. Chemical ecaluation of the nutritive calue of leaf of fluited pumpkin (*Teferia occidentalis*). Food Chemistry, 53: 353-355.

Maduka, H.C.C. and Z.S.C. Okoye, 2001. The effect of *Sacoglottis gabonensis* and its isolate bergenin on doxorubicin-Ferric ions (Fe^{3+})- induced degradation of deoxyribose. The Science, 1: 316-319.

Maduka, H.C.C. and Z.S.C. Okoye, 2002a. Enhanced protective role of *Sacoglottis gabonensis* stem bark extract, a Nigerian alcoholic beverage additive on experimental membrane lipid peroxidation induced by 2,4- dinitrophenyl hydrazine. J. Ethnopharmac. (In press)

Maduka, H.C.C. and Z.S.C. Okoye, 2002b. Effect of bergenin isolated from *Sacoglottis gabonensis* stem bark on 2,4-dinitrophenyl hydrazine induced lipid peroxidation. J. Toxic. Sci.,(in Press).

Maduka, H.C.C., 2000. Biochemical antioxidant properties of *Sacoglottis gabonensis* stem bark extract. Ph.D thesis, University of Jos, Nigeria.

Maduka, H.C.C. and Z.S.C. Okoye, 2002c. Elemental Composition of Sacoglottis gabonensis, a Nigeria alcoholic beverage additive. Pak. J. Biol. Sci., 5: 66-68.

Maduka, H.C.C. and Z.S.C. Okoye, 2002d. The effect of *Sacoglottis gabonensis* a Nigerian alcoholic beverage additive on the natural antioxidant befences during 2,4-dinitrophenyl hydrazine-induced membrane peroxidation. Vascular Pharmacology, 39: 21-31.

Madusolumuo, M.A., Z.S.C. Okoye and E.O. Ayalogu, 1993. Effect of consumption of *Sacoglottis gabonensis* stem bark trated palmwine on disposition of acetaminophen in human colunteers. Bioscience Research Communications, 5: 109-111.

Madusolumuo, M.A., 1993. Biochemical assessment of the metabolism and biological effects of two common analgesics. PhD thesis, University Jos, Nigeria.

Onwuliri, V.A. and G.E. Anekwe, 1993a. Proximate and elemental composition of *Bryophyllum pinnatum* lim. Med. Sci. Res., 20: 103-104.

Onwuliri, V.A. and G.E. Anekwe, 1993b. Amino acid composition of *Bryophyllum pinnatum* (lim). Med. Sci. Res., 21: 507-508.

Onwuliri, V.A. and G.E. Anekwe, 2001. Amino acids and other biochemical components of *Ricinus cummunis* (variety minor), an anticonceptive seed. Pak. J. Biol. Sci., 4: 866-868.

Parasakthy, K., Depalakshmi, S. Shanttny and S.N. Deraraj, 1993. The hepatoprotective action of eugenol. Med. Sci. Res., 21: 611-612.

Spackman, D.H. and S. Moore, 1958. Automatic recording apparatus for use in the ehromatography of amino acids. Analytical Chemistry, 30: 1190-1191.

Sugiyama, H., K.P. Fung and T.W. Wu, 1993. Purpurogallin as an antixidant protector of human erythrocytes against lysis by peroxyl radicals. Life Sciences. Accelerated communication. Pharmacology letter, 53: 39-43.

Ubom, G. and Y. Tsuchiya, 1988. Detrmination of amions in natural water by ion chromatography. Water Resources, 22: 1455-1458.