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COMPARATIVE NUTRITIONAL STUDIES ON THREE VARIETIES OF *HEINSIA CRINITA*

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ABSTRACT

The paper presents chemical data on three varieties of *Heinsia crinita*, a leafy green vegetable commonly consumed in Southeastern Nigeria. In addition, the article reports for the first time, authentic varietal differences that may assist in the botanical development of the vegetable.

Of the three varieties studied, the White one was found to have highest protein and organic matter, but lowest lipids, fiber, and caloric value (per cent dry matter: 14.7, 96, 1.4, 12.5 and 391 Kcals respectively). Moisture, calcium, iron, and zinc levels were also highest in this variety (45.2% wet weight, 105.6 mg/100 g, 19.9 mg/100 g and 2.9 mg/100 g respectively) while toxic substances were minimal. (Total oxalate: 17.1 mg/100 g; HCN: 8.1 mg/100 g; tannins: 5.0 mg/100 g). These levels were far below toxic limits. Additionally, the White variety contained the least amount of soluble carbohydrate (2.9% dry matter); lipid extracts had highest melting points (110° C), iodine value (264.7), least saponification value (112.2), and linolenic acid was the lipid of prominence.

Major lipids of the Black and Ekoi varieties were identified as lecithins. Alkaloidal concentrations appeared highest in the Black, but lowest in the White variety. The findings and their implications are discussed with special reference to possible nutrient interactions, dietetic and nutritional values of the leafy vegetable to humans.

KEY WORDS: *Heinsia crinita*, varieties, chemical composition, lipid characterization, dietary fiber, micronutrients, antinutrients

INTRODUCTION

Heinsia crinita (Afzel.) G. Taylor (Rubiaceae) is a leafy vegetable widely consumed among the people of Southeastern Nigeria. It is cheap, harvested from the rain forest and many regard its characteristic, slightly bitter flavour as a delicacy. It has antimicrobial properties, justifying its use by native herbalists as a remedy for infections of the gastro-intestinal tract (Ekpa *et al.* 1991). It is rich in lipids, fiber, calcium, and iron (Ekpa *et al.* 1991).

There is a virtual absence of published information on the chemical composition of leafy green vegetables commonly consumed in this region of Nigeria, and even less exists as regards their diverse varieties. A spattering of literature does however exist on *Heinsia crinita*, but of unspecified varieties (Ekpa *et al.* 1991; Eyo *et al.* 1983; Ifon & Bassir 1979). This work is novel because it presents chemical data on three specified varieties that have been hitherto unreported.

It is undisputed that the vegetable has a number of varieties; several have been botanically identified in this paper but there is an absence of botanical development. Many varieties are edible, others not, and yet others preferred for their alleged medicinal values. The more common edible varieties include the White (Ibibio: "afia atama"), Black (Ibibio: "obubit atama"), and "Ekoi". The latter originates from the northern regions of Southeastern Nigeria. The White variety has glossy leathery, light green leaves with scanty hairs along the nerves. The leaves appear fairly succulent and are about 8.0 cm long and 3.2 cm wide on the average. It is preferentially cultivated by natives at subsistence levels. The Black variety has dull leathery, dark green leaves with dense brown hairs along the nerves; 7.5 cm long and 3 cm wide on the average. It is because of the very dark green color of the leaves that it is called black, as compared with the former which has characteristic light green colored leaves. This Black variety is harvested wild from fast disappearing forests for food, but is more popular for its supposed medicinal uses. For instance, it is used by natives as an enema and abortifacient as well as a remedy for diarrhea, peptic ulcer, and pustulation (U.J. Ekott, personal communication, 1990). It has a very bitter taste and must be thoroughly processed before used as food (includes boiling with crude bicarbonate). The Ekoi variety has papery, glossy green leaves, densely hairy along the nerves beneath, and leaves are 13.5 cm long and 4.5 cm wide on the average.

Vegetables from this region have been both unresearched and underexploited nutritionally. Studies such as this one are therefore needful and should be conducted with the view of inclusion in existing food composition tables for the region. With the growing awareness of the crucial role played by diet in the prevention and therapy of many of the ailments that assail man, it has become more critical to establish the chemical compositions of local food varieties used for human nutrition in order to assist their exploitation for health management as well as calculations required by dieticians.

MATERIALS AND METHODS

Sample collection and post harvest treatment.

The leaves, fruits, and flowers of the three varieties (White, Black, and Ekoi) were freshly harvested in July and botanically identified. Each sample composite consisted of an average of sixteen to twenty leaves. About ten of such composites were subjected to post harvest treatment which comprised the following steps: leaves were destalked, washed, and oven-dried at 50° C for 48 hrs. The low temperature was a quality control measure to minimize volatilization of characteristic odiferous volatile oils. Fine particulation followed, using a mortar and samples were stored in air-tight, brown bottles.

Proximate analysis

Methods used for proximate analysis were as recommended by the AOAC (1975: Nos. 7.007 - 7.100). Crude fat determination involved exhaustive Soxhlet extraction of a known weight of sample with petroleum ether (bp. 40-60° C). The microkjeldahl nitrogen method was employed for crude protein (N $\times$ 6.25) while crude fiber was obtained from the loss in weight on ignition of dried residue remaining after digestion of fat-free samples with 1.25% each of sulfuric acid and sodium hydroxide solutions under specified conditions. Although the AOAC method employed for crude fiber determination in this study has been superseded by more contemporary procedures such as the AOAC method 991.43 for dietary fiber, the former one was chosen deliberately for two reasons. First, it provides sufficient perspective for the comparison of the three varieties; an absolute measure was not deemed a critical factor here. Secondly, the method was considered relatively simpler in view of the fact that contemporary and expensive equipment are unavailable in laboratories situated in many developing nations.

Quantitation of ash involved incineration in a muffle furnace at 600° C for 24 hrs. Carbohydrates estimates were by difference, while caloric values were obtained by summing the multiplied mean values for protein, fat, and carbohydrate by their respective Atwater factors (4,9,4).

Micronutrients and antinutrients

Mineral element composition was assayed using the atomic absorption spectrophotometer after acid digestion of the samples (AOAC 1975). Ascorbate estimates were by the method of Scharafert & Kingsley (1955), hydrocyanic acid by the alkaline titration method of the AOAC (1984: No. 26.151) and oxalate by permanganate titration method of Dye (1956). Tannins were measured by the method of Price & Beutler (1978).

Lipid characterization and other analyses

The second phase of the analysis involved soluble carbohydrate determination as glucose using the Anthrone method (Joslyn 1970). Here again, this relatively simpler method was preferred over current chromatographic ones because of limited equipment. Moreover, the procedure is adequate for the purposes of the present study which is more of a comparative than absolute nature. True protein determination involved bovine albumin as a standard in the Biuret method (AOAC 1975: Nos. 22.012-22.013), while lipid analysis including melting point determination was by the method of AOAC (1984: No. 28.014). Gravimetric iodine value determination was by the method of Devine & Williams (1961) and saponification number determination by the method of Vogel (1962). Lipid extracts were separated by thin layer chromatography using chloroform, petroleum ether, and methanol (20:60:20; v/v/v) as developing solvent. Infra-red spectroscopy of the extracts were performed. Alkaloids were screened for, using routine Meyer's, Wagner's, and Dragendorff's tests.

Statistical Analysis

Standard deviations were calculated for triplicate determinations. The student's 't' test was applied at the 99% confidence level for each analysis, as well as between varieties.

RESULTS AND DISCUSSION

Results of the study have been summarized in Tables 1-6. Ash contents of all three varieties (4-5% dry matter) compare well with those of other leafy vegetables commonly consumed in Nigeria (Bassir & Fafunso 1975). Strikingly, the highest ash content was found in the Black variety (Table 1) which also had the highest quantity of alkaloids (Table 2). Alkaloids are known to cause a selective uptake of minerals for chelation (Pelletier 1970). They also elicit diverse pharmacological action and are therefore prized phytocomponents. Infra-red spectroscopy in this study indicated the alkaloids to contain mostly aromatic amines (Table 3 a). Further investigation was however not attempted.

Levels of iron and zinc in all the three varieties were significant (13-20 mg/100 g and 2-3 mg/100 g respectively). The White variety in particular had superior quantities of both iron and zinc. The Recommended Daily Allowances (RDA) for both micronutrients are respectively 10 and 15 mg/d (Davidson *et al.* 1975); all three varieties can thus adequately meet dietary needs for iron. This finding is significant because the Southeastern Nigeria region is malaria-endemic resulting in widespread anemia. Anemic patients may therefore benefit from diets comprising *Heinsia crinita*. Iron bioavailability is favorably modulated by high levels of ascorbic acid and most leafy vegetables are good sources of the vitamin (Dagnelie 1989). All three varieties however show low ascorbate readings (Table 4) compared with reported average values (25 mg/100 g) for other vegetables (Davidson *et al.* 1975).

Table 1. Proximate analysis of the three varieties of *Heinsia crinita* (% dry matter).*

Varieties	Moisture (% fresh wt.)	Ash	Ether extract	Crude Protein	Crude Fibre	Total Carbo-hydrate	Calorie Value/ Kcals
White	45.2 ± 8.6	4.0 ± 0.6	1.0 ± 0.2	14.7 ± 1.2	12.5 ± 1.3	79.9	391
Black	42.4 ± 9.2	5.0 ± 0.6	4.2 ± 0.3	11.8 ± 1.0	14.8 ± 1.6	79.0	401
Ekoi	33.0 ± 6.8	4.5 ± 0.3	3.4 ± 0.3	9.45 ± 0.8	13.9 ± 0.9	82.7	399

* Mean ± S.D. of three determination ($p < 0.1$).

Table 2. Nutrient analysis of three varieties of *Heinsia crinita**(g/100 ml).

Varieties	Soluble Carbohydrate	True Protein	Alkaloids
White	29.0 ± 0.0	96.0 ± 0.5	+
Black	34.0 ± 0.1	88.0 ± 0.1	+++
Ekoi	49.0 ± 0.0	60.0 ± 0.3	++

* Mean ± S.D. of triplicate determinations ($p < 0.1$).
+ = present; ++ = present in higher quantity; +++ = present in highest quantities.

Table 3. Infra-red spectroscopy of (a) alkaloids and (b) lipid extracts from *Heinsia crinita*.

ALKALOIDS

Peaks (cm^{-1})	Assignment	White	Black	Ekoi
3500-3200	N-H stretch of NH_2	v.w	b	v.w
2900-2800	C-H stretch of alkyl groups	w	s	w
1700-1680	C=O stretch of amides	w	w	v.w
1460-1420	C-H deformation	-	v.w	-

*b = broad; s = strong; w = weak; v.w = very weak

LIPIDS

Peaks (cm^{-1})	Assignment	White	Black	Ekoi
3600-3100	O-H stretch	w	v.w	s
2900-2800 (doublet)	C-H stretch of alkyl groups	s	b	w
1730-1680 (centered 1700)	C=O stretch of acids	s	s	v.w
1430-1400	C-H deformation of CH_2=CH-(S) or CH_3C=O(M)	w	w	v.w

*b = broad; s = strong; w = weak; v.w = very weak

Table 4. Ascorbate* and minerals** in three varieties of *Heinsia crinita* (mg/100 g).

Varieties	Ascorbate	Fe	Mg	Zn	Ca	Cd
White	1.0 ± 0.1	19.9	38.3	2.9	105.6	0.2
Black	0.5 ± 0.2	13.3	79.4	2.2	64.6	0.1
Ekoi	1.0 ± 0.0	14.6	81.4	2.0	62.3	0.1

*Mean ± S.D. of three determinations ($p < 0.1$).
** automated analysis

Table 5. Antinutrients in three varieties of *Heinsia crinita**.

Varieties	HCN (mg/100 g)	Tannin (mg/g catechin)	Total Oxalate (mg/100 g)
White	8.1 ± 1.2	5.0 ± 0.5	17.1 ± 2.1
Black	10.8 ± 1.0	11.5 ± 1.0	13.3 ± 1.8
Ekoi	10.0 ± 2.1	6.2 ± 0.2	29.2 ± 3.3

*Mean ± S.D. of duplicate determinations ($p < 0.1$).

The Black variety had the lowest ascorbate reading of the three. In view of the rigorous food processing methods needed for the culinary preparation of the vegetable, ascorbic levels may be ultimately compromised since it is easily lost during even the mildest of food processings (Davidson *et al.* 1975). Regarding calcium, the White variety was found to contain amounts that were almost twice that found in the other varieties (*i.e.*, 105 mg/100 g). The RDA for calcium is 800 mg/d for an adult man. Cow milk, one of nature's richest sources of calcium contains about 120 mg/100 g of calcium, while human milk contains 20-40 mg/100 g (Davidson *et al.* 1975). Thus, the White variety compares well with these and may be recommended over the other two for growing children or elderly people who require substantial amounts of the nutrient for adequate bone metabolism. It may be noted that the pervading economic depression in this developing nation makes milk unaffordable to a majority of the people, and *Heinsia crinita* could thus provide a significant source of calcium.

Strikingly, while the Ekoi variety presented the lowest calcium levels, it also had highest levels of oxalate (29.2 mg/100 g), which were nearly double the levels found in the other two varieties (Table 5). Oxalic acid is an established mitigator of calcium uptake (Davidson *et al.* 1975). The observed levels are below toxic limits (Oke 1969; Munro & Bassir 1969), but may however be sufficient enough to interfere with the nutritive value of the Ekoi variety *vis-à-vis* calcium availability.

Table 6. Lipid analysis of *Heinsia crinita* ether extract.*

Varieties	Saponification Identification	Iodine Number	Melting Point °C	TLC spot
White	112.2 ± 19.3	264.7 ± 18.2	110	Linolenic
Black	202.6 ± 18.5	102.8 ± 21.1	105	Lecithin
Ekoi	168.3 ± 9.6	194.0 ± 16.3	75	Lecithin

* Mean ± S.D. of triplicate determinations ($p < 0.1$).

Lipids

Fat levels were relatively low in the three samples as expected (Table 1). The lipid compositions of the three varieties were significantly different from each other ($p < 0.1$) and this is indicative of authentic botanical variation. Results of lipid analysis of the White variety suggest high molecular weight, long chain, polyunsaturated fatty acids and indeed, chromatography verified this by indicating linolenic acid to be the lipid of prominence. The Ekoi variety is the least culinarily aromatic of the three varieties. Its lipids had the lowest melting point, but high unsaturation and lecithin was identified as the most prominent of its lipids. On the other hand, the Black variety is known to be the most aromatic of the three and its lipid analysis, while indicating lecithins also as most prominent, suggested that the fatty acids are short-chained and the least unsaturated (Table 6).

Infra-red spectroscopy of the crude lipid extract showed four major peaks of varying intensities across the three varieties (Table 3b). The Ekoi variety had a unique peak in the OH stretch region, indicating a possible presence of fatty alcohols. Further identification using HPLC and NMR techniques will be necessary to substantiate this. The lipid content of vegetables is often less than 1% by weight (Beare-Rogers 1989), but in this case, the Black variety had up to 4% in the ether extract. Vegetable lipids often consist mainly of essential PUFA particularly from chloroplasts of green leaves. Seasonal differences may occur in absolute contents of linolenic and linolenic acids, and also may vary according to time of harvest, stage of growth, and method of analysis (Beare-Rogers 1989). Currently, the RDA for linolenic acid is 0.5-1.0% of daily calorie intake (Galli & Simopoulos 1989). Further investigation may establish whether levels of linolenic acid in the leaves will meet significantly recommended allowances.

Dietary Fiber

Crude fiber content of the three varieties were comparable and ranged between 12.5 and 14.8% dry matter. This is substantial, considering the fact that crude fiber contents of vegetables commonly consumed in this region have documented crude fiber estimates ranging from 10 to 13% dry matter (Odutola & Carl 1983; Ifon & Bassir 1979; Oyenuga 1968). Non-starchy vegetables are the richest sources of dietary fiber (Agostoni *et al.* 1995) and they are employed in the prevention and treatment of such diseases as obesity, diabetes, cancer, and gastrointestinal disorders (Saldanha 1995). The crude fiber assay is currently considered unrepresentative of contemporary definitions of dietary fiber (Hillemeier 1995). However, in developing nations where sophisticated equipment are unavailable, the method still finds usefulness in certain studies, especially those that emphasize relativeness over absoluteness. Future research may be directed at estimating both soluble and insoluble fiber contents of this vegetable. Such studies will assist in the proper exploitation of the vegetable for health purposes; 12 g dietary fiber is the RDA required for every 100 Kcal of food consumed (Nishimune *et al.* 1993). Therefore, 100 g of the vegetable can meet this requirement adequately.

Soluble carbohydrate concentrations ranged between 29 and 49 g/100 ml (Table 2). Low concentrations of soluble carbohydrates often reflect a complementary high fiber content, but the White variety strikingly had both the lowest soluble carbohydrate as well as lowest crude fiber levels (Tables 1 and 2). This suggests that the variety may contain appreciable amounts of soluble fibers such as hemicelluloses, pectin, and/or other hydrocolloids (Agostoni *et al.* 1995).

Protein and Tannins

Finally, the White variety was observed to have the highest protein, but least tannin content (Tables 1 and 5). Tannins mitigate the bioavailability of protein (Davidson *et al.* 1975). Thus, the significant levels of leaf protein in this variety are favorably complemented by its low tannin levels. Highest tannin levels were found in the Black variety, which may account for its characteristic astringent taste. The biological value of fiber foods is considered low because plant proteins lack the full complement of essential amino acids (Young & Pellett 1994). A vegetable such as the Black variety of *Heinsia crinita*, with low protein and high tannin would therefore be considered as nutritionally poor.

CONCLUSIONS

Conclusively, the nutritional assay of three commonly consumed varieties of the *Heinsia crinita* leaf is novel, and seen here to establish authentic varietal differences. The White one appears to be most nutritive of the three because its highest protein content is complemented with least tannins, highest calcium complemented with low oxalate, and highest moisture complemented with least fiber for succulence. It further possesses a significant presence of the essential fatty acid, linolenic acid. In addition, it had more iron, zinc and calcium than the other varieties as well as least all around antinutritional principles. The Black variety, on the hand, was found to have the highest ash, ether extract, crude fiber, hydrocyanic acid, tannin, and alkaloids. All these constitute distinct "pluses" for plants with high medicinal potential. This variety actually is that used by native herbalists for medicinal concoctions. Future research could therefore be directed towards the pharmacological fingerprinting of this Black crinita. For proper maintenance of health and nutrition, use of the White variety should be encouraged over the other two. It could find usefulness in the dietetic management of several disease conditions as described in the text.

REFERENCES

Agostoni, C., E. Riva, & M. Giovannini. 1995. Dietary fiber in weaning foods of young children. Pediatrics 96(5/2):1002-1005.

AOAC, Association of Official Analytical Chemists. 1975. *Official Methods of Analysis*, 10th ed. Williams, S. (editor), USA.

AOAC, Association of Official Analytical Chemists. 1984. *Official Methods of Analysis*, 14th ed. Williams, S. (editor), USA. pp. 152-164.

Bassir, O. & M. Fafunso. 1975. Variations in the protein, carbohydrate, lipid and ash contents of six tropical vegetables. Plant Foods Manual 1:209-216

Beare-Rogers, J.L. 1989. Some aspects of omega -3- fatty acids from different foods. *In*: Galli, C. & A.P. Simopoulos (editors). *Dietary Omega -3- and Omega -6- Fatty Acids: Biological Effects and Nutritional Essentiality.* Plenum Publishers, New York, New York. pp. 21-32.

Dagnelie, P.C. 1989. Nutritional status of infants on a vegetarian diet. J. Amer. Diet. Assoc. 89:1661-1663.

Davidson, S., R. Passmore, J.F. Brock, & A.S. Truswell. 1975. *Human Nutrition and Dietetics*, 6th ed. Churchill, Livingstone, Longman Group, Ltd., Great Britain. pp. 108-118, 162, 187.

Devine, J. & P.N. Williams. 1961. *The Chemistry and Technology of Edible Oils and Fats*, 1st ed. Pergamon Press, London, Great Britain.

Dye, W.B. 1956. Studies on *Halogeton glomerulus*. Weeds 4:55-59.

Ekott, U.J. 1990. Personal communication: renowned native herbalist, AKS-Nigeria.

Ekpa, O. & R. Ebana. 1991. Chemical composition of antimicrobial of leaves of four Nigerian plants with flavoring principles. Tropical J. Appl. Sci. 1:30-34.

Eyo, E.S., E. Mohme, & H.J. Abel. 1983. Chemical compositions and amino acid contents of *Gnetum africanum, Heinsia crinita* and *Piper guineensis*. Nig. J. Nutr. Sci. 4(1):57-62.

Galli, C. & A.C. Simopoulos (eds). 1989. Panel Statement. General recommendations on dietary fats for human consumption. *In*: *Dietary Omega -3- and Omega -6- Fatty Acids: Biological Effects and Nutritional Essentiality*. Plenum Publishers, New York, New York. pp. 403-404.

Hillemeier, C. 1995. An overview of the effects of dietary fiber on gastro-intestinal transit. Pediatrics 96(5/2):997-998.

Ifon, E.T. & O. Bassir. 1979. The nutritive value of some Nigerian leafy green vegetables. Part 3: Distribution of protein, carbohydrate, fats, etc. Food Chem. 5:231-235.

Joslyn, R.A. 1970. *Methods of Food Analysis*, 2nd ed. Academic Press, New York, New York.

Munro, A. & O. Bassir. 1969. Oxalate in Nigerian vegetables. W. African J. Biol. Appl. Chem. 12:14-17.

Nishimune, T., T. Sumimoto, & Y. Konishi. 1993. Dietary fiber intake of Japanese younger generations and the recommended daily allowance. J. Nutr. Sci. Vitaminol. (Tokyo) 39:263-278.

Odutola, O. & M.F. Carl. 1983. Dietary fiber composition of some Nigerian foods as influenced by cooking and energy. Nutr. Res. 4:236-239.

Oke, O.L. 1969. Role of hydrocyanic in nutrition. World Review of Nutrition and Dietetics 11:170-174.

Oyenuga, V.A. 1968. *Nigerian Foods and Feeding Stuffs, Their Chemistry and Nutritive Value*. Ibadan University Press, Ibadan - Nigeria.

Pelletier, S.W. (ed.). 1970. *Chemistry of the Alkaloids*. Van Nostrade Reinhold Publ., New York, New York.

Price, M.L. & L.G. Beutler. 1978. Rapid visual estimation and spectrophotometric determination of tannin content of sorghum grains. J. Agric. Food Chem. 25:1268-1271.

Saldanha, L.G. 1995. Fiber in the diet of U.S. children: results of national surveys. Pediatrics 96(5/2):994-996.

Scharafert, R.S. & G.A. Kingsley. 1955. A rapid method for the determination of reduced dehydroascorbic acid and total ascorbic acid in biological membranes. J. Biol. Chem. 212:59-68.

Vogel, A.I. 1962. *A Textbook of Quantitative Inorganic Analysis Including Elementary Instrumental Analysis*, 3rd ed. Longman Group, Ltd., New York, New York.

Young, V.R. & P.C. Pellet. 1994. Plant proteins in relation to human proteins and amino acid nutrition. Amer. J. Clin. Nutr. 59 (suppl.):1203-1212S.