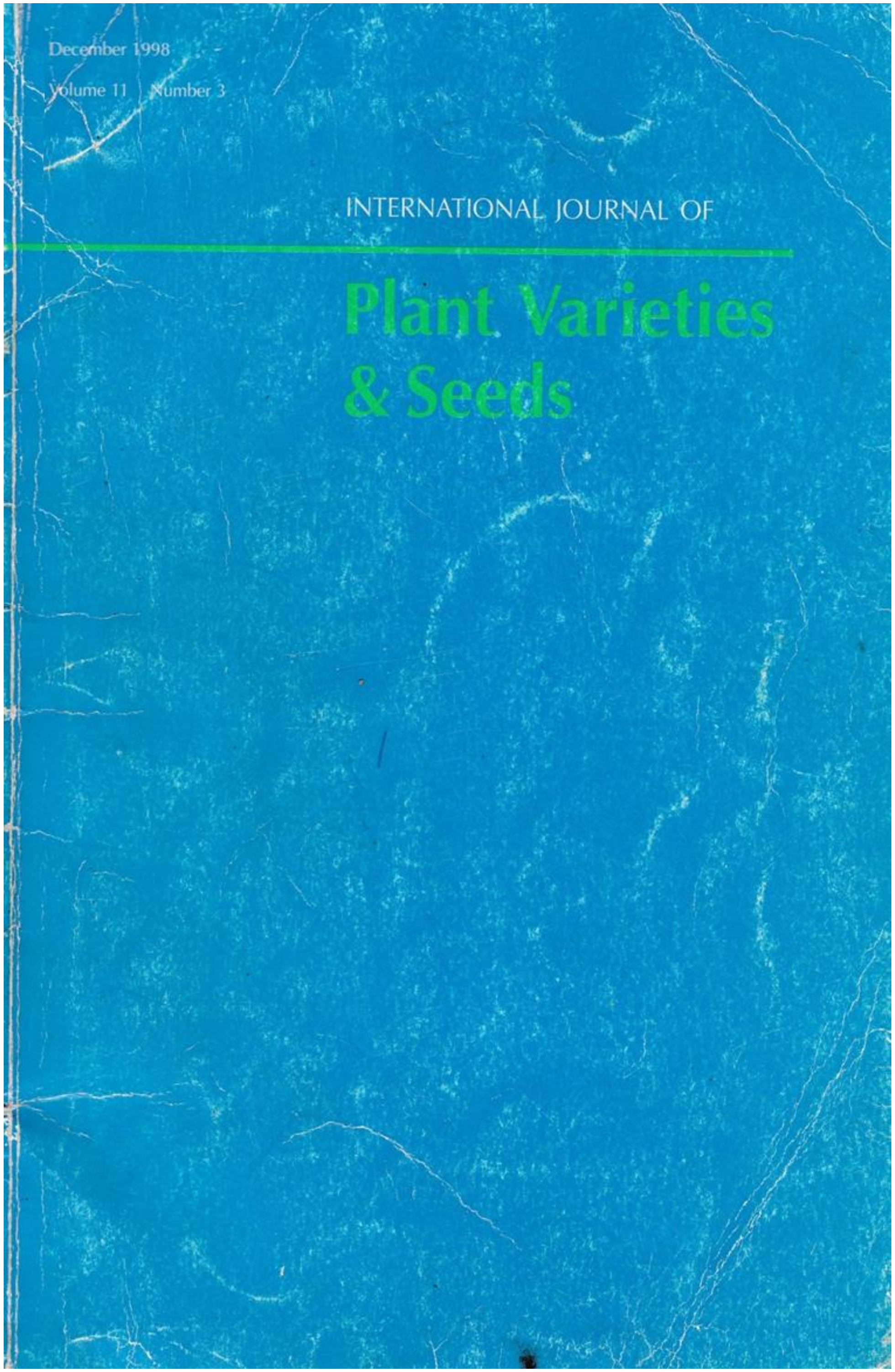

Comparative nutritional studies on three varieties of *Heinsia crinita*

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Comparative nutritional studies on three local varieties of *Heinsia crinita*

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The paper presents chemical data on three local varieties of Heinsia crinita, *a leafy green vegetable commonly consumed in South Eastern 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, fibre and calorific 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.

INTRODUCTION

Heinsia crinita (Rubiaceae) is a shrub widely consumed among the people of South Eastern Nigeria. It is cheap, harvested wild from the rain forest or selectively cultivated in homesteads and many regard its characteristic, slightly bitter flavour as delicatessen. It has antimicrobial properties, justifying its use by native herbalists as a remedy for infections of the gastrointestinal tract (Ekpa & Ebana 1991). It is rich in lipids, fibre, calcium and iron (Ekpa & Ebana 1991).

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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 unspecific varieties (Ekpa & Ebana 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.

About six or more 'varieties' are recognized by consumers in the region. The argument as to whether these may be landraces rather than local varieties is the

subject of ongoing research in this laboratory. The paucity of established botanical development in this regard may perhaps stem from the fact that this slow-growing shrub takes about three to four years to mature. There appears, however, to be a strong probability that they are local varieties because the various *Heinsia crinita* plants grown in the same geographical locality still retain their characteristic colour, taste and medicinal properties. The present paper will therefore refer to the plants under discussion as local varieties.

Several of the varieties are edible, others not, and yet others preferred for their alleged medicinal properties. The more common edible ones include the White (Ibibio: 'afia atama'), Black (Ibibio: 'obubit atama') and 'Ekoi' (refers to the ethnic region in which it is found, i.e. the northern part of South Eastern Nigeria). The White variety has glossy, leathery, light green leaves with dense brown hairs along nerves. They are opposite, acuminate, obtuse and elliptical in shape and appear fairly succulent, averaging about 8.0 cm long and 3.2 cm wide. Stems are clearly quadrangular in younger branchlets, flowers are epigynous and syncarpous, while fruits are globose, reaching up to 1.2 cm long on average. It is selectively cultivated by subsistence farmers because of its preferred taste.

The Black variety has dull leathery, dark green leaves with scanty brown hairs along nerves; 7.5 cm long and 3 cm wide on average. It is because of the very dark green colour of the leaves that it is called black, as compared with the white which has characteristic light green coloured leaves. Leaves are opposite, acuminate, cuneate and lanceolate in shape. Stems are more cylindrical than angular in younger branchlets, flowers are epigynous and syncarpous, while fruits are elliptic in shape, averaging up to 1.5 cm long. 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 as an enema and abortifacient as well as a remedy for diarrhoea, 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, which are obovate, cuspidate cuneate at base and 10.5 cm long; 4.5 cm wide on average. They are pubescent on the nerves beneath: hairs are whitish, not brown. The inflorescence is a compound dichasium as in the other types, but sometimes flowers appear solitary at the end of short branchlets. Flowers are also epigynous and syncarpous; fruits are globose and up to 1.2 cm long.

Vegetables from this region have been both unresearched and underexploited nutritionally. Studies such as this one are therefore needed 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 plant species and their 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 from various shrubs in the forest or homesteads as relevant. All collections were made from the same eco-region and botanically identified. Each sample composite (twigs containing several branchlets of 6–8 pairs of leaves) from different plants in the same location consisted of 16–20 leaves. About 10 of such composites were subjected to post harvest treatment which comprised the following steps: leaves were destalked, washed and oven-dried (Gallenkamp, Blue M, SW 17 TC) at 50°C for 48 h. The low temperature was a quality control measure to minimize volatilization of characteristic odiferous volatile oils. Fine participation 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). Crude fat determination (AOAC No. 7.061) involved exhaustive Soxhlet extraction of a known weight of sample with petroleum ether (bp. 40–60°C). The microKjeldahl nitrogen method (AOAC No. 7.015) was employed for crude protein (N × 6.25) while crude fibre was obtained from the loss in weight on ignition of dried residue remaining after digestion of fat-free samples with 1.25% each of sulphuric acid and sodium hydroxide solutions under specified conditions. Although the AOAC method employed for crude fibre determination in this study has been superseded by more contemporary procedures such as AOAC method 991.43 for dietary fibre, the former 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 is unavailable in laboratories situated in many developing nations.

Quantification of ash involved incineration in a muffle furnace (Gallenkamp FR 510) at 600°C for 24 h (AOAC, No. 7.099). Carbohydrate estimates were by difference while calorific values were obtained by summing the multiplied mean values for protein, fat and carbohydrate by their respective Atwater factors (4, 9, 4).

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 was considered adequate for the purposes of the present study, which is more of a comparative than absolute nature. True protein determination involved bovine albumin as standard in the Biuret method (AOAC 1975, Nos 22.012 and 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 value determined 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 was performed. Alkaloids were screened using routine Meyer's, Wagner's and Dragendorff's tests.

Micronutrients and antinutrients

Mineral element composition was assayed using the atomic absorption spectrophotometer (Unicam 919, UN 1749) after acid digestion of the samples (AOAC 1975, No. 3.013). Ascorbate estimates were made by the method of Scharafert & Kingsley (1955), hydrocyanic acid by alkaline titration method of AOAC (1984, No. 26.151) and oxalate by the permanganate titration method of Dye (1956). Tannins were measured by the method of Price & Beutler (1978).

Statistical analysis

Standard deviations were calculated for triplicate determinations. The Student's *t*-test

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

Varieties	*Moisture (% fresh wt)*	*Ash*	*Ether extract*	*Crude protein*	*Crude fibre*	*Total carbohydrate*	*Energy (kcal/100 g)*
White	45.2 ± 0.86	4.0 ± 0.6	1.0 ± 0.2	14.7 ± 0.12	12.5 ± 1.3	79.9	391
Black	42.4 ± 0.92	5.0 ± 0.6	4.2 ± 0.3	11.8 ± 0.10	14.8 ± 1.6	79.0	401
Ekoi	33.0 ± 0.68	4.5 ± 0.3	3.4 ± 0.3	9.45 ± 0.08	13.9 ± 0.9	82.7	399

*Mean ± S.D. of three determinations. All pairs were significant at $P<0.1$.

was applied at 99% confidence level for each analysis, as well as between varieties.

RESULTS AND DISCUSSION

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 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 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 7).

Infra-red spectroscopy of the crude lipid extract showed four major peaks of varying intensities across the three varieties (Table 4). The Ekoi variety had a unique peak in the OH stretch region, indicating a possible presence of long-chain alcohols. Further identification using HPLC and NMR techniques will be necessary to elucidate this. The lipid content of vegetables is often inferior to 1% by weight (Beare-Rogers 1989) but, in this case, the Black variety had up to 4% ether extract. Vegetable lipids often consist mainly of essential PUFA particularly from chloroplasts of green leaves. Seasonal differences may occur in absolute contents of linoleic 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% of daily calorie intake (Galli & Simopoulos 1989). Further investigation may establish whether levels of linolenic acid in the leaves will make relevant contributions to recommended allowances.

Carbohydrates

Soluble carbohydrate concentrations ranged between 2.9 and 4.9 g% dry matter (Table 2). Low concentrations of soluble carbohydrates often reflect a complementary high fibre content but the White variety strikingly had both the lowest soluble carbohydrate as well as lowest crude fibre levels (Tables 1 and 2). This suggests that the variety may contain appreciable amounts of soluble fibres such as hemicelluloses, pectin and/or other hydrocolloids (Agostoni *et al.* 1995).

Crude fibre

Crude fibre contents of the three varieties were comparable and ranged between 12.5

Table 2. Nutrient (g% dry matter) and alkaloid analysis* of three varieties of *H. crinita***

Varieties	*Soluble carbohydrate*	*True protein*	*Alkaloids*
White	2.9 ± 0.6	9.6 ± 0.5	+
Black	3.4 ± 0.1	8.8 ± 0.1	+++
Ekoi	4.9 ± 0.6	6.0 ± 0.3	++

*Relative concentration: +low; ++moderate, +++very high.
**Mean ± S.D. of triplicate determinations. All pairs significant at $P<0.01$.

Table 3. Infra-red spectroscopy of alkaloid extracts from *H. crinita*

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 gps	w	s	w
1700–1680	C=0 stretch of amides	w	w	v.w
1460–1420	C – H deformation	—	v.w	—

Table 4. Infra-red spectroscopy of lipid extracts from *H. crinita*

Peaks (cm-1)	*Assignment*	*White*	*Black*	*Ekoi*
3600–3100	O – H stretch	w	v.w	s
2900–2800	C=H stretch of alkyl gps (doublet)	s	b	w
1730–1680	C=O stretch of acids (centred 1700)	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.

and 14.8% dry matter. This is substantial, considering the fact that crude fibre contents of vegetables commonly consumed in this region have documented crude fibre 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 fibre (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 fibre assay is currently considered unrepresentative of contemporary definitions of dietary fibre (Hillemeier 1995). However, in developing nations where sophisticated equipment is not available, 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 fibre contents of this vegetable. Such studies will assist in the proper exploitation of the vegetable for health purposes.

Protein and tannins

The White variety was observed to have the highest protein, but the lowest tannin content (Table 1 and 6). Tannins inhibit the bioavailability of protein and minerals (Davidson *et al.* 1975). Thus the significant

Table 5. Ascorbate* and minerals** in three varieties of *H. crinita* (mg/100 g, on dry matter)

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. All pairs were significant at $P<0.1$.
**Automated; significant at $P<0.01$.

level of leaf protein in this variety is favourably complemented by its low tannin. The highest tannin levels were rather found in the Black variety, which may account for its characteristic astringent taste. The biological value of fibre foods is considered low because plant proteins lack the full complement of essential amino acids (Young & Pellet 1994). A vegetable such as the Black variety of *Heinsia crinita*, with low protein and high tannin, would therefore be considered as nutritionally poor.

Minerals and alkaloids

Results of the study have been summarized in Tables 1–7. 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.

Table 6. Antinutrients in three varieties of *H. crinita* (on dry matter)*

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 three determinations. All pairs were significant at $P<0.1$.

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 Allowance (RDA) for both micronutrients are respectively 10 and 15 mg/d (Davidson *et al.* 1975); all three varieties can thus contribute relevantly to dietary iron. This observation is significant because the South Eastern Nigeria region is malaria-endemic, resulting in widespread anaemia. Anaemic patients may therefore benefit from diets containing *Heinsia crinita*. Iron bioavailability is favourably enhanced by high levels of ascorbic acid; most leafy vegetables are good sources of the vitamin (Dagnelie 1989). All three varieties, however, show low ascorbate readings (Table 5) compared with reported average values (25 mg/100 g) for other vegetables (Davidson *et al.* 1975).

The Black variety had the lowest ascorbate content 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 those found in the other varieties (i.e. 105 mg/100 g). The RDA for calcium is

Table 7. Lipid analysis of *H. crinita* ether extract*

Varieties	*Saponification #*	*Iodine value*	*Melting point (°C)*	*TLC spot identification*
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 three determinations. All pairs were significant at $P<0.1$.

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 varieties for growing children or elderly people, who require substantial amounts of the nutrient for adequate bone metabolism. It may be noted that the prevalent economic depression in this developing nation makes milk unaffordable to a majority of the population and *Heinsia crinita* could thus provide a significant source of calcium. Strikingly, while the Ekoi variety showed the least calcium levels, it also had the highest level of oxalate (29.2 mg/100 g), which was nearly double the levels found in the other two (Table 6). Oxalic acid is an established inhibitor of calcium uptake (Davidson *et al.* 1975). The observed levels are below toxic limits (Oke 1969; Munro & Bassir 1969), but may however be sufficient to interfere with the nutritive value of the Ekoi variety *vis-à-vis* calcium availability.

CONCLUSION

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 variety appears to be the most nutritive of the three because its highest protein content is complemented with the least tannins, highest calcium complemented with low oxalate and highest moisture complemented with least fibre 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-round antinutritional principles. On the other hand, the Black variety was found to have the highest ash, ether extract, crude fibre, hydrocyanic acid, tannin and alkaloids. All these constitute distinct 'pluses' for plants with high medicinal potential. This is the variety used by native herbalists for medicinal concoctions. Future research could therefore be directed towards the pharmacological finger-printing of this black variety of *Heinsia 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.

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