Australian Journal of Basic and Applied Sciences, 7(10): 244-247, 2013
ISSN 1991-8178

Proximate Composition and Toxicant Levels of Ethanolic Extracts of of *Cnidoscolus carumbium* and *Spigelia marilandica*

[1]Nsor Odo Alobi, [2]Emmanuel Maunday Ikpeme, [2]Kingsley Hovana Enyi-Idoh, [2]Arikpo Ikpi Okoi, [2]Matthew Egbobor Eja.

[1]Department of Chemical Sciences, Cross River University of Technology, P.M.B. 1123, Calabar, Nigeria.
[2]Department of Biological Sciences, Cross River University of Technology, P.M.B. 1123, Calabar, Nigeria.

Abstract: There has been a growing concern over the use of *Cnidoscolus carumbium* and *Spigelia marilandica* as vegetable foods and for boosting red blood cells. Therefore, this study aimed at determining the levels of toxicants (hydrocyanate, phytate and oxalate) and proximate composition of the plants so as to establish their safe use by man. The toxicant and proximate values were determined in 10g portions of the powder of each plant extract using standard procedures. These procedures were described by the Association of Official Analytical Chemists (AOAC), while crude protein was determined using Leco-N nitrogen determinator (Model FP-428, Leco Corporate MI USA). Toxicant levels for *C. carumbium* were 0.20mg/ml, 1.50mg/ml and 1.1mg/ml of extract for hydrocyanate, phytate and oxalate respectively, while those of *S. marilandica* were 1.62mg/ml 0.30mg/ml and 0.05mg/ml for hydrocyanate, phytate and oxalate respectively. Toxicant levels were observed to be too low as to pose a nutritional problem. On the other hand, the proximate values were appreciably high (22.33% moisture, 34.35% fibre, 0.67% crude protein, 14.22% fat, 4.37% ash, 18.40% carbohydrate for *C. carumbium* and 37.36% protein, 6.50% fibre, 1.28% protein, 5.20% fat, 6.60% ash and 17.20% carbohydrate for *S. marilandica*), which make the plants safe for consumption and for medicinal use. Although the proximate values of *C. carumbium* were significantly higher ($P<0.01$) than *S. marilandica*, the nutritional profiles of the plants are equally high.

Key words: Toxicants, proximate values, *C. carumbium, S. Marilandica,* plant extracts.

INTRODUCTION

Cnidoscolus carumbium and *Spigelia marilandica* are tropical plants found abundantly in the tropical rainforest of Cross River State of Nigeria, where they are used for medicinal and food purposes. *C. carumbium* is in the family Euphorbiaceae and commonly called "Hospital no far" in pidgin English, by the local population of Okuni in Ikom Local Government Area of Cross River State (or Ulo Ogwu di anya, in Igbo dialect). This has worked so successfully for them that the plant is now being domesticated (Alobi *et. al*., 2013).

Spigelia marilandica is in the family Loganiaceae and commonly called "pink root" plant. It is a flowering plant that grows 18-24 inches tall in sun or shady landscape environment. The herb is known locally as "worm grass" because of its high efficacy in the treatment of intestinal worms, especially roundworms (Sofowora, 1984; Odugbemi, 2006).

There is evidence that *C. carumbium* and *S. marilandica* have high food and medicinal potentials (Obot, 1996). Some of the fresh leaves of *C. carumbium*, for example, are used for the treatment of malaria (Gbile and Sofowora, 1986). It is also evident that some medicinal plants contain some toxicants (Davis and Warrington, 1986). Evidently too, the nutritional components of most plants taken by man as foods are well reported (Udosen *et al*., 1999; Oku, 2001; Atangwho *et al*., 2009; Alobi *et al*., 2012; Al-Tawaha et al, 2013.).

However, so many plants used by man for medicinal and nutritional purposes, have not been investigated with respect to their toxicant levels and nutritional composition. Some researches have been carried out focusing on the toxic levels of hydrocyate, phytate and oxalate, and the significance of their molar ratios in predicting the bioavailability of dietary zinc and calcium (Aremu and Abara, 1992). The contributions of phytate, oxalate and hydrocyanate in Nigerian foods have been investigated (Chakraborty, 1978; Aremu, 1989). However, there appears to be little research on phytate, oxalate and hydrocyanate levels in medicinal and food plants especially *C. carumbium* and *S. marilandica*.

MATERIALS AND METHODS

Collection of Leaf Materials:

The fresh leaves of *C. carumbium* and *S. marilandica* were obtained from domesticated species at the Cross River University of Technology Staff Quarters in Calabar, and transported to the Herbarium of Botany Department of the University of Calabar, for identification.

Corresponding Author: Nsor Odo Alobi, Department of Chemical Sciences, Cross River University of Technology, P.M.B. 1123, Calabar, Nigeria.

Preparation of Plants Extracts:

The leaves of the plants were chopped into smaller bits and air-dried separately for fourteen days to constant weight. The dried leaves were then separately ground to powder in a mortar using the method of Mukhtar and Turkur (2000). The crude extracts of each of the leaves was then prepared using the methods of Fatope *et al.* (1999) and Mukhtar and Huda (2005). This involved soaking 50g of the powder of each of the leaf samples in 95% ethanol for 48hrs at room temperature to allow for maximum extraction of the components (Alobi *et al*., 2012). The filtrate was then evaporated using rotary evaporator (Stuarc Scientific, England). The residue was retained as a crude extract for each of the test plants, stored in reagent bottles and maintained in the freezer at 0^oC until they were used for analyses (Alobi *et al*., 2012).

Proximate Analysis:

Methods Nos. 930.09, 930.10 and 930.05 as described by AOAC (1990), were respectively used in analyzing the plants extracts for fat, crude fibre and ash, while crude protein was determined using Leco-N nitrogen determinator (Model FP-428, Leco Corporates MI, USA). This involved extracting the fat from 5.0g of sample weighed into a thimble and placed in a soxlet extractor with 130ml petroleum ether (boiling point 40-60%) placed in a previously dry-weighted round bottomed flask, which was held on the hot plate for 10hrs as the fat was extracted. The fat free extract was later used for fibre determination. After distilling off the petroleum ether in the flask and collected for subsequent use, the flask and its content was again dried to a constant weight. The amount of lipid extracted was obtained as the difference between the weight of the flask before and after extraction. The fat free extract was acid digested with 50ml of 1.25% H_2S0_4 and filtered to produce a residue which was digested with a base (50ml of 1.25% Na0H). This was filtered and washed several times with hot water until it was free from NaOH. After twice washing with 95% of methanol, it was transferred into a porelain crucible and dried at 100^0c. The percent crude fibre was equal to the content of crucible after washing with methanol, minus dried crucible x 100, divided by the weight of thimble. The ash was determined by igniting the residue in the crucible after drying at 100^oC. The difference between the weights of sample before and after ignition at 550^0c is the weight of the ash.

Using the Leco-N nitrogen determinator (Model FP-428, Leco Corporate, M1, USA), crude protein was determined. By difference, the nitrogen free extractive (NFE) was obtained.

The moisture content was determined by introducing 2.0g of fresh leaf sample into a beaker with a known weight. The sample was then air-dried to a constant weight in an air circulating oven at 70-90^oC. It was cooled in a desicator and weighed. The difference in weight of the leaf sample before and after drying, multiplied by 100 gave the percentage moisture content.

The total carbohydrate was determined by subtracting the percentages of fat, ash, moisture and protein from 100.

Determination Of Hydrocyanate, Phytate And Oxalate Contents:

The hydrocyanate content of each of the plant extracts was determined using the method of AOAC (1990). This involved soaking 10g portion of each plant extract in distilled/deionized water for 4hrs. The suspension was steam-distilled into a dilute NaOH solution, followed by treating the distillate with dilute KI and titrated against $AgNO_3$. Hydrocyate was calculated taking 0.02N $AgNO_3$ as equivalent to 1.08mg HCN.

The modification of the method of McCance and Widdowson (1935) as described by Aremu (1989) was adopted in the determination of phytate. This involved extracting the phytate from the powdered sample with dilute HCL, and precipitated from the solution as ferric phytate by addition of ferric chloride solution. This was followed by solubilization of the precipitate by adding dilute NaOH with heating to give a Na phytate solution already treated with a mixture of concentrated H_2SO_4 and 65% perchloric acid to liberate phytate phosphorus. The inorganic phosphorus was determined using the AOAC (1990) method. The phytate content was calculated from inorganic phosphorus taking the molecular weight of phytic acid to be 660.

Oxalate was determined on 2.2g of each sample (Aremu and Abara, 1992). This involved digesting the sample for 4hrs at 50^oC using dilute HCL, followed by evaporation of an aliquot to a brownish suspension which was filtered and the filtrate treated with concentrated ammonia. By treating the solution with dilute $CaCl_2$ solution at 90^oC, oxalate was precipitated. Hot dilute H_2SO_4 was used to solubilize the precipitate and titrated against a dilute $KMnO_4$ solution. The oxalate content was calculated by taking 1ml of 0.05$KMnO_4$ as equivalent to 2.2mg of oxalate.

Statistical Analysis:

A T-test analysis (Miller and Miller, 1986) was done on the data on proximate and toxicant values for comparison between *C. carumbium* and *S. marilandica*. Data were expressed as mean of 3 determinations and difference between groups considered significant at $P<0.05$, $P<0.01$ or $P<0.001$.

Results:

The proximate values of the ethanolic extracts of *C. carumbium* and *S. marilandica* leaves are presented in Table 1. This shows high significant difference (P<0.01) between the values of *C. carumbium* and *S. marilandica* at a value of t = 3.7921. Fibre (34.35 ± 0.02%), crude fat (14.22 ± 0.10%) and carbohydrate (18.40 ± 0.10%) in *C. carumbium* were higher than their corresponding values in *S. marilandica* with its higher moisture content (37.36 ± 0.26%).

Table 2 shows the toxicant levels in *C. carumbium* and *S. marilandica*. There was no significant difference (P>0.10) in toxicant levels between *C. carumbium* and *S. marilandica* at t = 0.3416.

Table 1: Proximate Composition of *Cnidoscolus carumbium* and *Spigelia marilandica.*

Medicinal plants	Moisture (%)	Fibre (%)	Crude protein (Nx 6.25)%	Crude fat (%)	Ash (%)	Carbohydrate (%)
C. carumbium	22.33±0.13	34.35±0.02	0.67±0.01	14.22±0.10	4.37±0.08	18.40±0.10
S. marilandica	37.36±0.26	6.50±0.16	1.28±0.10	5.20±0.05	6.60±0.10	17.20±0.15

Table 2: Toxicant levels in *Cnidoscollus carumbium* and *S. marilandica.*

Toxicant	Concentration (mg/ml of extract)	
	C. carumbium	*S. marilandica*
Hydrocyanic acid (HCN)	0.20	1.62
Phytic acid	1.50	0.30
Oxalate precipitation	1.1	0.05

Discussion:

In this study, there was no significant difference (P > 0.10) between the toxicant levels in *C. carumbium* and *S. morilandica*. The toxicant levels observed to be present in *C. carumbium* were 0.20mg (hydrocyanic acid), 1.50mg (phytic acid) and 1.10mg (oxalate); and for *S. marilandica*, the toxicant levels were 1.62mg (hydrocyanic acid), 0.30mg (phytic acid) and 0.05mg (oxalate), which were considerably low compared to the levels observed in cocoa beverage (Aremu and Abara, 1992) that is rather consumed in higher dosages and more frequently. Oyenuga and Amazigo (1957) indicate that the lethal dose of hydrocyanate is about 60mg/head/day in adult man, suggesting that hydrocyanate poisoning from common foods is generally unlikely.

Also, the threshold of oxalate toxicity has been estimated to be between 2 and 5g (Aremu and Abara, 1992). This implies that oxalate poisoning is insignificant in *C. carumbium* and *S. marilandica*, judging their levels in the two plants.

Although the threshold of dietary phytate toxicity in humans and animals is unknown (Aremu and Abara, 1992), but it has been established that high dietary phytate is deleterious (Ferguson *et al*., 1988). For instance, zinc availability to humans is reduced when the zinc molar ratios are about 15:1. However, the phytate levels in *C. carumbium* and *S. marilandica* are significantly low compared to levels found in other foods (Chakraborty and Eka, 1978; Aremu, 1989).

The low levels of these toxicants combined with the high levels of nutrients in the plants indicate why the plants are used as food and as medicines. Nutritionally, most plants taken as food and as medicines by man have high levels of nutrients and reasonable levels of relevant bioactive substances and minerals (Udosen *et al*., 1999; Oku, 2001; Atangwho *et al*., 2009; Alobi *et al*., 2012). The signficiantly higher levels of fibre, crude fat and carbohydrate in *C. carumbium* than *S. marilandica* (P < 0.01) indicate a higher calorific value in *C. carumbium*. Of course, the two plants contain appreciable levels of other proximate substances conducive for blood formation. However, the literature on the proximate values of *C. carumbium* and *S. marilandica* is not available. Some works on other plants such as *Azadirachta indica*, *Vernonia amygdalina* and *Gongronema latifolium* have been done (Atangwho *et al*., 2009) which showed varying but lower levels of proximate values, indicating that *C. carumbium* and *S. marilandica* are nutritionally better than a good number of other plants locally used as foods and as medicines.

Conclusion:

With low toxicant levels and high proximate values, *C. carumbium* and *S. marilandica* are safe to be used as vegetable food and as medicines.

REFERENCES

Al-Tawaha, A., G. Al-Karaki, A. Massadeh, 2013. Antioxidant activity, total phenols and variation of chemical composition from essential oil in sage (*Salvia officinalis* L.) grown under protected soilless condition and open field conditions. Advances in Environmental Biology, 7(5): 894-901.

Alobi, N.O., E.M. Ikpeme, A.I. Okoi, K.D. Etim and M.E. Eja, 2012. Phytochemical and Nutritional Profiles of *Lasianthera africana*, *Heinsia crinata* and *Gongronema latifolium*. *New York Science Journal*, 5(3): 45-48.

AOAC, 1990. *Official methods of analysis.* 15th Ed. Washington, DC. Association of Official Analytical Chemists.

Aremu, C.Y., 1989. Quantitative estimation of the dietary contributions of phytate, oxalate and hydrocyanate by six popular Nigerian Foods. *N. J. Nutr. Sc.*, 10: 79-84.

Aremu, C.Y. and A.E. Abara, 1992. Hydrocyanate, oxalate, phytate, calcium and zinc in selected brands of Nigerian cocoa beverage. *Plant Foods for Human Nutrition*, 42: 231-237.

Atangwho, I.J., P.E. Ebong, E.U. Eyong, I.O. Williams, M.U. Eteng and G.E. Egbung, 2009. Comparative chemical composition of leaves of some antidiabetic medicinal plants: *Azadirachta indica*, *Vernonia amygdalina* and *Gongronema latifolium*. *African Journal of Biotechnology*, 8(18): 4685-4689.

Chakraborty, R. and O.U. Eka, 1978. Studies on Hydrocyanic, oxalic and phytic acid contents of foodstuffs. *West African Journal of Biology and Applied Chemistry*, 21: 50-55.

Davis, N.T. and S. Warrington, 1986. The phytic acid, mineral, trace element, protein and moisture of U.K. Asian immigrant food. *Human Journal of Nutrition and Applied Nutrition*, 40A: 49-59.

Fatope, M.O., H. Ibrahim and Y. Takeda, 1999. Screening of higher plants reputed as pesticides using brine shrimp lethality assay. *International Journal of Pharmacology*, 3(1): 250-260.

Ferguson, E.L., R.S. Gibson, L.U. Thompson, S. Ounpuu and M. Berry, 1988. Phytate, zinc and calcium of 30 East African foods and their calculated phytate: Zn, Ca: phytate, and [Ca] [phytate]/[Zn] molar ratios. *Journal of Food Composition and Analyses*, 1: 316-325.

Gbile, Z.O. and A. Sofowora, 1986. *Ethnobotany: Taxonomy and conservation of plants*. University of Ibadan Press, Ibadan.

McCance, R. and E.M. Widdowson, 1935. Phytin in human nutrition. *Biochemistry Journal*, 29:2694-3699.

Miller, J.C. and J.N. Miller, 1986. *Statistics for Analytical Chemistry*. Ellis Horwood Ltd., New York, pp: 202.

Mukhtar, M.D. and M. Huda, 2005. Prevalence of Tinea capitis in primary schools and sensitivity of etiological Agents to *Pistia stratiotes* Extract. *Nigerian Journal of Microbiology*, 19(1-2): 418-419.

Mukhtar, M.D. and A. Tukur, 2000. Biology of *Pistia stratiotes* and its toxicity effects in rat. *Journal of Applied Zoology and Environmental Biology*, 49(2): 39-49.

Obot, E.A., 1996. Ethnobotanical survey of Okwangwo Division, Cross River National Park, Progress Report 1994-1996, Okwangwo Programme, Obudu.

Odugbemi, T., 2006. *Outlines and pictures of medicinal plants from Nigeria*. University of Lagos Press, Lagos. 283pp.

Oku, D.E., 2001. Evaluation of the chemical composition of indigenous spices and flavouring agents. *Global Journal of Pure and Applied Science*, 7(3): 455-459.

Oyenuga, V.A. and E.O. Amazigo, 1957. A note on the hydrocyanic acid content of some local varieties of cassava (*Manihot esculenta*) and some traditional cassava food products. *East African Agricultural and Forestry Journal*, 40: 161-167.

Sofowora, E.A., 1984. *Medicinal plants and traditional medicine in Africa*. (4th edition), John Willey and Sons, New York, pp: 26-105.

Udosen, E.O., U.E. Udok and O.S. Unuigbe, 1999. The comparison of the nutrient composition of *Lasianthera africana* and *Heinsia crinata*. *Journal of Food Biochemistry*, 23: 571-576.