TOXICOLOGICAL AND HISTOLOGICAL EVALUATION OF *Psychotria sp, C. aconitifolius* AND *T. occidentalis* ON WISTAR ALBINO RATS

Supported by tetfund TERTIARY EDUCATION TRUST FUND

O. Otitoju[1], G.T.O. Otitoju[2]*, H.N. Ene-Obong[3], J.U. Nwamara[2] and I.P. Aniekan[4]
[1]Department of Biochemistry, Federal University Wukari, PMB 1020, Taraba State Nigeria
[2]Home Science, Nutrition & Dietetics, University of Nigeria, Nsukka, Enugu State, Nigeria
[3]Department of Biochemistry, University of Calabar, Cross-River State, Nigeria
[4]Department of Anatomy, University of Uyo, Akwa-Ibom State, Nigeria
*Corresponding author: otitojugrace.to@gmail.com

Received: July 18, 2016 **Accepted:** September 11, 2016

Abstract: The study examined the toxicological effects of some Nigerian indigenous green leafy vegetables (*Cnidoscolus aconitifolius, Psychotria* sp. and *T. occidentalis*) on adult albino rats. Different diets were formulated using the individual green leafy vegetable (GLV) and rats food. Groups 1 – 3 consist of rats fed with 5 g (5%) each of *Psychotria* sp, *Cnidoscolus aconitifolius* and *Telfairia occidentalis* pulverized leaves per 100 g (95 g of rat food + 5 g vegetable) of rat food and Groups 4 – 6 consist of rats fed 10 g (10%) each. However, Group 7 (control) was fed with rat food only. Biochemical analysis (liver function test) was determined using standard methods. Histological analysis was carried out according to the procedure recommended by Drury and Wallington. Biochemical results showed that the activity of alkaline amino transferase (ALT) decreased generally in all the groups, except for *Cnidoscolius* group at 10% concentration that had increased activity relative to the control group. However the decreases/increases in ALT activity werenot significant ($p>0.05$) relative to the control. There was a significant decrease ($p<0.05$) in alkaline phosphatase (ALP) activity in all the test groups relative to the control. Aspartate aminotransferase (AST) activity increased in groups fed *Psychotria sp and Telfaria* (5% and 10%) diet. The liver tissues of rats fed *Psychotria* and *Cnidoscolus* revealed moderate to severe histopathological lesions which implied that they are toxic to the liver. Hence, their consumption should be regulated.

Keywords: Nutrition, vegetables, toxicity, liver function, *Cnidoscolus aconitifolius, Psychotria* sp

Introduction

Nigeria is rich in plant biodiversity possessing nutritional and medicinal values. Many of these plants are either being consumed in different forms by different people but they are in most cases being underutilized by the communities where they are located.

Vegetables are those herbaceous plants whose part or parts are eaten as supporting food or main dishes and they may be aromatic, bitter or tasteless (Edema, 1987; Mensah *et al*., 2008; Ekpenyong *et al*., 2012). The utilization of leafy vegetable is part of Africa's cultural heritage and they play important roles in the customs, traditions and food culture of the African household. Nigeria is endowed with a variety of traditional vegetables and different types are consumed by the various ethnic groups for different reasons. The nutrient content of different types of vegetables varies considerably and they are not major sources of carbohydrates compared to the starchy foods which form the bulk of food eaten, but contain vitamins, essential amino acids, as well as minerals and antioxidants (Fasuyi, 2006; Punna and Paruchuri, 2004; Mnzava, 1997). According to Mensah *et al.* (2008), vegetables are the cheapest and most available sources of important proteins, vitamins, minerals and essential amino acids. Vegetables are included in meals mainly for their nutritional value. However, some are reserved for the sick and convalescence because of their medicinal properties. Most tropical countries are blessed with a diversity of foodstuffs which play a basic role in nutrition and healthy development. Unfortunately an estimated 789 million people in developing countries still suffer from malnutrition especially infants and children from rural areas (World Hunger Factor, 2005). Malnutrition can be tremendously reduced with an increase use of foods rich in energy, proteins, iron and vitamin A.

The problem of anemia in most rural communities in Nigeria is enormous especially among children and pregnant women. Therefore, communities tend to explore alternative and cheaper means in treating anaemic disease condition when they occur. *Cnidoscolus aconitifolius* and *Psychotria sp* are two of such vegetables. During the documentation of indigenous food of the Igbo cultural area, Okeke *et al.* (2008) identified *Psychotria sp* as a vegetable used in soup preparation and believed to have several health benefits, including the treatment of anaemia. Also in a personal communication, *Cnidoscolus aconitifolius* was also mentioned as a vegetable that is used in the treatment of anaemia and is popularly referred to as "hospital too far", meaning that it prevents one from visiting the hospital regularly.

The genus *Psychotria* contains about 2,000 species of shrubs, small tress and herbs, but rarely epiphytes, and is the largest genus in the family Rubiaceae. Most species of *Psychotria* are common in the understory of tropical and subtropical forest. Majority of them are found in west tropical Africa, but few are indigenous to Nigeria. Some are found in the Northern, middle and Northeast of Nigeria while some are located in the south, southeast and middle belt of Nigeria (Lachenaud & Harris, 2010; Otitoju *et al*., 2014). However, there are about five different species of *Psychotria* in Nigeria. These are *Psychotranevosa* found in southern Nigeria, *Psychotria articulata* in southern Nigeria, *Psychotria succulenta* in northeast Nigeria *Psychotria fernandopoesis* in south-East and lastly *Psychotria leptophylla* in the middle belt of Nigeria had shown that, despite the large number of *Psychotria spp* very few studies have been carried out on them.

The lack of nutritional information and inadequate development of nutritionally improved products from local raw materials have direct bearing on nutrition. Leafy vegetables are ideal for weight management as they are typically low in calories. They are useful in reducing the risk of cancer and heart disease since they are low in fat, high in dietary fiber, and rich in folic acid, vitamin C,

potassium and magnesium, as well as containing a host of phytochemicals, such as lutein, beta-cryptoxanthin, zeaxanthin, and beta-carotene (Maisarah *et al.*, 2014). Because of their high magnesium content and low glycemic index, green leafy vegetables are also valuable for persons with Type 2 diabetes. The risk of hip fracture in middle-aged women was decreased 45% for one or more servings/day of green leafy vegetables compared to fewer servings (Craig, 2008).

In Nigeria, some lesser known vegetables have been implicated for possessing hematopoietic properties in the treatment of anemia mostly in children and pregnant women. The nutritional content of some lesser known vegetables make them desirable as dietary supplement for humans in most rural communities in Nigeria. Consumption of green leafy vegetables (GLVs) among the rural populace is a daily part of most diets because of their relative availability and cheap cost. Although consumption of GLVs has been associated with numerous health benefits, the toxicological effects are less explored. Therefore the aim of this study is to investigate the toxicological effects of some lesser known indigenous green leafy vegetables (*Psychotriasp, C. aconitifolius and T. occidentalis*) on Wistar rats.

Materials and Methods

Experimental design

Wistar strain albino rats weighing between 65 – 87 g and 9-12 weeks of age were used. The rats were purchased from the animal house of the College of Medical Sciences, Department of Biochemistry University of Uyo. The rats were housed in the laboratory in wire mesh cages under standard conditions (Temperature, 25 - 29°C; 12 h light and 12 h dark cycle). Fifty-six (56) Wistar albino rats were randomly distributed into seven groups of eight (8) animals per group. Different diets were formulated using individual leafy vegetable and ratsfood (Table 1). Groups 1, 2 and 3 consist of rats fed with 5 g each of *Psychotria sp, Cnidoscolus aconitifolius* and *Telfairia occidentalis* pulverized leaves per 100 g of rat food and Groups 4, 5 and 6 consist of rats fed 10 g each of P*sychotria sp, Cnidoscolus aconitifolius* and *Telfairia occidentalis* pulverized leaves per 100 g of feed. However, Group 7 (Control) was fed with rat food only. Water and food were fed *ad libitum* to all the groups.

Table 1: Composition of experimental diet

Group	Diet formulation
Group 1	5 g *Psychotria sp* + 95 g of rat food
Group 2	5 g *C. aconitifolius* + 95 g of rat food
Group 3	5 g *T. occidentalis* + 95 g of rat food
Group 4	10 g *Psychotria sp* + 90 g of rat food
Group 5	10 g *C. aconitifolius* + 90 g of rat food
Group 6	10 g *T. occidentalis* + 90 g of rat food
Group 7	Rat food only

Blood collection

Blood samples were collected from the animals through cardiac puncture into non-EDTA coated bottles after anaesthetizing the animals with chloroform on the 14th day of the experiment. The samples were allowed to clot prior to centrifugation at 350 g for 10 min to obtain serum sample for assay of alkaline phosphatase (ALP), alanine aminotransaminase (ALT) and aspartate aminotrasaminase (AST). This was determined using Randox laboratory reagent kit, UK, BT 29 4QY based on method by Reitman and Frankel (1957). Aspartate aminotransferase (AST) catalyses the transfer of the amino group from aspartate to 2-oxoglutarate forming oxaloacetate. Glutamate AST was measured spectrophotometrically by monitoring the concentration of oxaloacetate hydrazone with 2,4-dinitrophenylhydrazine at the wavelength of 405nm.

Histopathological examination

The liver tissues were extracted by the use of forceps and scissors. They were placed on clean filter paper to mop up excess blood and weighed. These tissues were separately stored in labeled screw tight containers containing 10% buffered formaldehyde solution. They were used for histological analysis according to the procedure recommended by Drury and Wallington (1967). The slides (duplicate) were examined using a light microscope and the histopathological changes were recorded.

Statistical analysis

The design of this experiment was completely randomized design (CRD). The animals were grouped into five rats per group. All data collected were subjected to analysis of variance (ANOVA) according to Obi (1986). Duncan's studentized new multiple range tests and Fishers' Least significant difference was utilized to detect significance of treatment means at 5% probability level (Steel and Torrie, 1980).

Results and Discussion

Total food intake of the experimental rats is presented in Table 2. The result showed that the total food consumed by the groups fed 5% *Psychotria sp, C. aconitifolius* and *T. occidentalis* were 1242, 1129 and 877 g, respectively for the whole duration of the study. Similarly, total food consumed by the 10% treated groups were 961, 997 and 1028 g, respectively. In general, there was a general significant increase ($p<0.05$) in total feed consumption rate in groups fed 5% or 10% vegetables with respect to the control group. The liver function enzymes (ALP, ALT and AST) are presented in Table 3. The activity of ALT decreased generally in all treatment groups, except for *Cnidoscolius* 10% group that had increased activity relative to the control group. However the decreases/increases in ALT activity were insignificant ($p>0.05$) relative to the control. The ALP activity decreased in all the test groups, these decreases were significant ($p<0.05$) relative to the control except for Ps10% group, whose value was similar to the control ($p>0.05$). The AST activity increased in groups fed *Psychotria sp and T. occidentalis* (5% and 10%) diet. These values were comparable to the control ($p>0.05$). On the other hand, the AST activity significantly ($p<0.05$) decreased in the rats fed *Cnidoscolus* at 5% (47.56IU/L) and 10% (50.04IU/L) levels relative to the control (68.42IU/L).

Table 2: Total daily food intake by rats fed *Psychotria sp, Cnidoscolus aconitifolius* and *Telfaria occidentalis*

Group	Average Food Intake (g)
C. aconitifolius 5%	80.64±13.79
Psychotria 5%	88.71±15.75
T.occidentalis 5%	62.64±20.47
C. aconitifolius 10%	71.21±15.79
Psychotria 10%	68.64±15.24
T. occidentalis 10%	73.43±13.99
Control	60.14±19.27

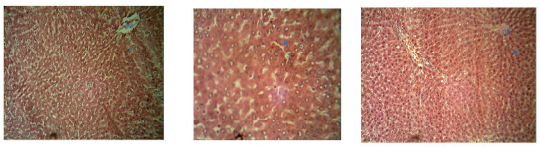
n ± SD

Table 3: Effect of *Psychotria sp, Cnidoscolus aconitifolius* and *Telfaria occidentalis* onliver function enzymes in Wistar rats (IU/L)

Group	Alanin amino transferase (ALT)	Alkaline phosphatase (ALP)	Aspartate aminotransferase (AST)
Psychotria 5%	19.64	162.38[b]	70.5
Cnidoscolus 5%	20.33	184.96[b]	47.56[b]
*Telfaria*5%	21.92	119.14[b]	82.74
Psychotria 10%	18.92	247.52	74.08
Cnidoscolus 10%	23.51	161.57[b]	50.04[b]
Telfaria 10%	22.80	125.12[b]	70.01
Control	22.93[a]	255.41[a]	68.42[a]
LSD(P<0.05)	**6.82**	**59.69**	**14.48**

Values in the same column bearing different superscript letters were significantly different (p<0.05).

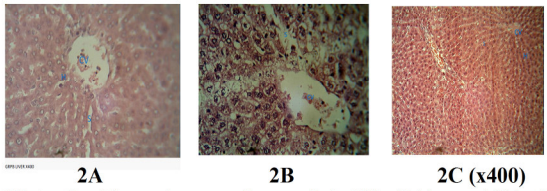
Histological evaluations of the liver tissues **(**Plate **1A, B, and C**) shows the result of liver histopathology of rats fed 5%, 10% *Psychotria* and control respectively. The result showed that rats fed with 5% and 10% *Psychotria* had mild liver derangement and cellular architecture was maintained when compared with the control. The mild derangement was more in Plate 1B (5%), showing a dose dependent effect. Plate 2(**A, B,** and **C**) shows the result of liver histopathology of rats fed 5%, 10% *Cnidoscolus* and control, respectively. The result showed that rats fed with 5% *Cnidoscolus* had moderate liver derangement and cellular architecture was maintained when compared with the control but rat liver tissue fed 10% *Cnidoscolus* had severe cellular distortion with complete derangement of cellular architecture when compared with the control. Plate 3 (**A, B**, and **C**) showed the result of liver histopathology of rats fed 5%, 10% *Telfaria* and control. The result showed that rats fed with 5% *Telfaria* showed moderate derangement of hepatocytes and cellular architecture was maintained while rats fed 10% *Telfaria* showed moderate cellular derangement with dilated sinusoid, and cellular architecture was maintained.



1A **1B** **1C (x400)**

Plate 1: Liver tissues of rats fed 5% (**1A**) or 10% (**1B**) *Psychotria* compared with control (**1C**)

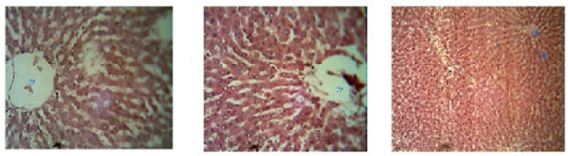
Leafy vegetables such as *Psychotria* and *Telfairia occidentalis* have been reported to possess unique nutritional and phytochemical properties which can affect physiological and biochemical effects (Otitoju *et al.,* 2014; Emeka and Onyechi, 2009; Longe *et al.,* 1983). In this study, the long term feeding of *Psychotria, Cnidoscolus* and *T. occidentalis* diets caused a significant increase in weight of the animals which may be due to the presence of high nutrient components present in the vegetable samples. These vegetables contain nutrients such as proteins, minerals and vitamins which are in agreement with the report of Fagbemi (2007). The increase in weight may also be related to increase food intake. The animals fed with 5% diet consumed more than those fed with 10%. This variation may be as a result of additional palatability added to the composed feed by different ration of *Psychotria, Cnidoscolus* and *T. occidentalis.* However, at higher concentration of 10% this palatability was lost probably because of excess fiber content in the diet. The three vegetables under study are highly palatable and consumed largely in a variety of diets in South-Eastern Nigeria for both nutrients and therapeutic purposes. Although there is paucity of knowledge on their toxicity they are been consumed mainly for their high nutrient values.



2A **2B** **2C (x400)**

Plate 2: Liver tissues of rats fed 5% (**2A**) or 10% (**2B**) *Cnidoscolus* compared with control (**2C**)

The most commonly used markers of hepatocyte injury are AST, ALT and ALP. Injury to hepatocytes causes these enzymes to leak into the blood circulationby raising the serum levels of some of these enzymes. ALT elevation is most specific for hepatocyte injury as its concentration in the liver far exceeds that in any other organ. AST is less specific as elevation is also caused by damage to skeletal muscle, kidney, brain and red blood cells (Hasan & Owyed, 2003; Crook, 2006). The observed decrease in the activities of ALT and ALP for all the groups except for *C. aconitifolius* 10% group may be attributed to the dose-dependent protective effect of some antioxidants and flavonoids present in the lesser known vegetables. Nada *et al.* (1997) and Udosen and Ojong, (1998) have reported changes in the levels of these enzymes in animals treated with phytochemicals. Catechin, a flavonoid from *Uncariagambri has* been reported to have a lowering effect on plasma aminotransferase activity compared with the control (Thabrew and Hughes, 1996). However, serum AST activity slightly increased in all the groups except for the group fed with 10% *C. aconitifolius*. The non-significant changes in the enzymes namely Alanine amino transaminase (ALT) and Aspartate Transaminase (AST) may suggest a reversible toxicity on the hepatocytes. Bamidele *et al*. (2010) suggested that there is some level of hepatoprotective effects of *Telfaria* on rats fed with vegetable supplemented diets.



3A **3B** **3C**

Plate 3: Liver tissues of rat fed 5% (**3A**) or 10% (**3B**) *Telfaria* compared with control (**3C**)

The liver is a vital organ in the body with specific role in drug and xenobiotic metabolism. In this study the liver tissues of the experimental animals were histologically processed and examined under light microscope to evaluate the concentration effects of the different GLVs on their architectural integrity. There was mild to severe derangement in the hepatic tissues when compared to the control. Liver histology of rats fed *Psychotria* 5% or 10% showed mild derangement in hepatic cells with liver architecture being maintained. Since cellular architecture was maintained, it shows that hepatocyte functions are not adversely affected. This may not be an obvious toxicological index because the hepatic cells could recover after ingestion. The animals fed with 5% or 10% *Cnidoscolus* (Plates **2A** and **2B**), respectively showed that rats fed with 5% *Cnidoscolus* had moderate liver derangement and cellular architecture was maintained when compared with the control but rats liver tissue fed 10% *Cnidoscolus* had severe cellular distortion with complete derangement of cellular architecture when compared with the

control. This degeneration of hypatocytes showed that *Cnidoscolus* altered cellular integrity which could result in cellular malfunctioning, this is an obvious toxicity effect of *Cnidoscolus* on the liver cells and so its consumption will require some level of processing to remove or reduce some toxic alkaloids and or saponins that are present in this vegetable before consumption. The moderate to severe hepatic derangement observed in this work is an obvious toxicological index for *Cnidoscolus*. Histological plates (3A and 3B) shows the liver cells of rats fed with Telfaria 5% or 10% with moderate derangement of hepatocytes and cellular architecture been maintained. This shows that *Telfaria* may not significantly affect hepatic function. Toxicity of *Telfaria* has been reported by some researchers (Salman *et al.,* 2008; Eseyin *et al*., 2011). In terms of severity of liver derangement, *Cnidoscolus*at 10% showed the highest level of toxicity even in the liver function test result where significant elevation of serum ALT was observed.

Liver function enzymes ALT and ALP showed a general decrease except *Cnidoscolus* 10% which showed a slight increase. However, AST result showed a general increase except for the 10% *Cnidoscolus* group which showed decrease AST values. The liver tissues of rats fed *Psychotria and Cnidoscolus* revealed moderate to severe histopathological lesions which implied that they may be harmful to the liver and can lead to cirrhosis and other degenerative changes in the liver as such their usage should be regulated or discouraged.

In conclusion, despite the fact that these vegetables contain so many nutritional benefits, the study also showed that *Psychotria and Cnidoscolus* have some toxicological effects. Therefore caution should be exercised in their use for nutritional and medicinal purposes. More importantly is the fact that continuous or regular consumption of these vegetable may lead to serious health challenges.

References

Bamidele O, Akinnuga AM, Olorunfemi JO, Odetola OA, Oparaji CK & Ezeigbo N 2010. Effects of aqueous extract of Basellaalba leaves on haematological and biochemical parameters in albino rats. *Afri. J. Biotech*., 9(41): 6952-6955.

Craig C 2008. Health Benefits of Green Leafy Vegetables. http://www.vegetarian-nutrition.info/updates/benefits-of-green-leafy-vegetables.php (Retrieved 12/09/2011).

Crook MA 2006. *Clinical Chemistry and Metabolic Medicine* (7th ed.). New Delhi: Edward Arnold.

Drury RAB & Willington EA 1967. *Carleton's Histological Technique* (4th ed.). London: Oxford University Press.

Edema AO 1987. Production of Some Common Vegetables, *Horticultural Research Institute* Ibadan Nigeria, pp. 1-5.

Ekpenyong CE, Akpan EE & Udoh NS 2012. Phytochemistry and toxicity studies of *Telfairia occidentalis* aqueous leaves extract on liver biochemical indices in Wistar rats. *Amer. J. Med. & Med. Sci.*. 2(5): 103-110.

Emeka EJI & Onyechi O 2009. Some biochemical, haematological and histological responses to long tern consumption of *Telferiria occidententalis* – supplemented diets in rats. *Pak. J. Nutr*., 8(8): 1199-1203.

Eseyin OA, Ebong P, Eyong E, Awofisayo O & Agboke A 2010. Effect of *Telfairia occidentalis* on oral glucose tolerance in rats. *Afri. J. Pharmacy & Pharmac.*, 4(6): 368-372.

Fagbemi TN 2007. Effects of processing on the nutritional composition of fluted pumpkin (*Telfairia occidentalis*) seed flour. *Nig. Food J*., 25: 1–22.

Fasuyi AO 2006. Nutritional potentials of some tropical vegetable leaf meals: Chemical characterization and functional properties. *Afri. J. Biotech.*, 5(1): 49-53.

Hasan FA & Owyed S 2003. Interpretation of liver chemistry test. *Bull. Kuwait Inst. Med. Specialization*, 2: 27-31.

Horder M & Rej R 1983. Alanine Transaminase. In: *Methods in Enzymatic Analysis* (3rd ed.). Weinheim: Verlag Chemic.

Longe OG, Farimu GO & Fetuga GL 1983. Nutritional value of fluted pumpkin. *J. Agric. Food Chem*., 31: 989-992.

Maisarah AM, Asmah R & Fauziah O 2014. Proximate analysis, antioxidant and antiproliferative activities of different parts of *Carica papaya*. *J. Nutr. And Food Sci*., 4(2): 1-7.

Mensah JK, Okoli RI, Ohaju-Obodo JO & EifediyiK 2008. Phytochemical, nutritional and medical properties of some leafy vegetables consumed by Edo people of Nigeria. *Afr. J. Biotech*., 7: 2304-2308.

Mnzava NA 1997. Comparing nutritional values of exotic and indigenous vegetables. In: Proceedings of a Workshop on Indigenous Vegetables. Limbe Cameroon, January 1997. Schippers RR, Budd L (eds). Chatham. UK; NRI/IPGRI/CPRO workshop.

Musa TY & Isah FM 2012. Liver and kidney functional indices of pregnant rats following the administration of the crude alkaloids from *Senna a lata* (Linn. Roxb) Leaves. *Iranian J. Toxicol.*, 6(16): 615-625.

Nada SA, Bashandy SAE & Negam SA 1997. Evaluation of the hypoglycaemie activity of traditional herbal preparation on male diabetic rats. *Fitoterapia*, 68(3): 240-244.

Obi IU 1986. Statistical Methods of Detecting Differences between Treatment Means. Snapp Press (Nig.) Ltd., Enugu.

Otitoju GTO, Nwamarah JU, Otitoju O & Iyeghe LU 2014. Nutrient composition of some lesser known green leafy vegetables in Nsukka LGA of Enugu State. *J. Biodiversity & Envtal. Sci. (JBES)*. 4 (4): 233-239.

Punna R & Paruchuri RU 2004. Effect of maturity and processing on total, insoluble and soluble dietary fiber contents of Indian green leafy vegetables. *Int. J. Food Sci. & Nutr.*, 55(7): 561-567.

Reitman S & Frankel S 1957. Serum-transaminase levels. *Amer. J. Clin. Patholo.*, 28: 56-63.

Salman TM, Olayaki LA & Oyeyemi WA 2008. Aqueous extract of *Telfairia occidentalis* leaves reduces blood sugar and increases haematological and reproductive indices in male rats. *Afr. J. Biotech*., 7: 2299-2303.

Sillanaukee P 1996. Laboratory markers of alcohol abuse. *Alcohol & Alcoholism*, 1: 613–616.

Steel RG & Torrie GH 1980. Principles and procedures in statistics. *Biometrical Approach*, 2nd Ed. McGraw-Hill Book Co. Inc, New York.

Thabrew MI & Hughes RD 1996. Phytogenic agents in the therapy of liver disease. *Phytotherapy Res*., 10(6): 461–467.

Udosen EO & Ojong AS 1998. Hepatotoxic activity of *Sacoglottisgabonensis* in rats. *Pharmaceut. Biol.*, 36(5): 368-371.

World Hunger Facts (WHF) 2005. World Hunger Education Service. In: FAO World Food Summit Progress Report September 2004. FAO Publications, Rome.